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Salmonella enteritidis-Associated Thoracic Spondylodiscitis with Paraspinal Abscess in an Immunocompetent Adult: First Case Report from Türkiye

İmmünkompetan Bir Erişkinde *Salmonella enteritidis* ile İlişkili Torasik Spondilodiskit ve Paraspinal Apse: Türkiye'den Bildirilen İlk Olgu

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Abstract

Salmonella enteritidis, a non-typhoidal Salmonella (NTS) serotype, rarely causes spondylodiscitis and typically affects immunocompromised individuals. We report the first documented case of *Salmonella enteritidis*-associated thoracic spondylodiscitis in an immunocompetent adult from Türkiye. A 32-year-old previously healthy male presented with chronic back pain. Magnetic resonance imaging revealed T7–T8 spondylodiscitis with a right-sided paraspinal abscess. Blood cultures were sterile; however, cultures from the drained abscess grew *Salmonella* spp., identified as *Salmonella enteritidis,* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and serotyping. Histopathological examination showed abundant polymorphonuclear leukocytes. The patient received eight weeks of antibiotic therapy: two weeks of intravenous ceftriaxone followed by six weeks of oral ciprofloxacin, resulting in complete clinical and laboratory recovery. This case underscores the importance of considering NTS in the differential diagnosis of vertebral infections, particularly in regions endemic for tuberculosis and brucellosis.

Keywords: Immunocompetent, *Salmonella enteritidis,* spondylodiscitis, thoracic osteomyelitis, Türkiye

Öz

Tifo dışı Salmonella (NTS) serotiplerinden biri olan *Salmonella enteritidis*, nadiren spondilodiskite neden olmakta ve genellikle immünkompromize bireylerde görülmektedir. Bu raporda, Türkiye'den immünkompetan bir erişkinde *Salmonella enteritidis* ilişkili torasik spondilodiskit olgusu ilk kez sunulmaktadır. Otuz iki yaşındaki, önceden sağlıklı erkek hasta kronik sırt ağrısı ile başvurmuştur. Manyetik rezonans görüntülemede T7–T8 düzeyinde spondilodiskit ve sağ paraspinal alanda apse saptanmıştır. Kan kültürleri steril olmakla birlikte, drene edilen apse kültüründe *Salmonella* spp. üremiş, tür düzeyinde *Salmonella enteritidis* olarak tanımlanmıştır. Histopatolojik incelemede yoğun polimorfonükleer lökosit infiltrasyonu izlenmiştir. Hasta iki haftalık intravenöz seftriakson ve ardından altı haftalık oral siprofloksasin tedavisi olmak üzere toplam sekiz haftalık antibiyotik tedavisiyle tamamen klinik ve laboratuvar iyileşmesi göstermiştir. Bu olgu, özellikle tüberküloz ve brusellozun endemik olduğu bölgelerde, vertebral enfeksiyonların ayırıcı tanısında NTS etkenlerinin de göz önünde bulundurulması gerektiğini vurgulamaktadır.

Anahtar Kelimeler: İmmünkompetan, *Salmonella enteritidis*, spondilodiskit, torasik osteomyelit, Türkiye

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Introduction

Salmonella enteritidis, a non-typhoidal Salmonella (NTS) serotype, is primarily associated with foodborne gastroenteritis. Rarely, it can cause focal infections, including spondylodiscitis, via hematogenous dissemination. These infections typically occur in patients with predisposing conditions such as sickle cell disease, leukemia, immunosuppression, aortic aneurysms, or solid organ malignancies[1–4].

Several cases of vertebral osteomyelitis and epidural abscess due to *Salmonella enteritidis* have been reported, mainly from the United States, Europe, and Asia, and most involved immunocompromised hosts[1–9]. In Türkiye, only one case has been reported, involving a patient receiving immunosuppressive therapy for rheumatoid arthritis[10].

We present the first documented case of *Salmonella enteritidis*-associated thoracic spondylodiscitis with paraspinal abscess in an immunocompetent adult from Türkiye, contributing to the limited literature on this uncommon clinical presentation.

Case Report

A 32-year-old previously healthy male presented with a three-month history of progressive thoracic back pain. The pain was mechanical in character and was not associated with fever, weight loss, night sweats, or gastrointestinal symptoms. Physical examination revealed mild tenderness over the thoracic spine without deformity or scoliosis. Neurological examination was unremarkable, and other systemic findings were within normal limits.

Laboratory investigations showed a white blood cell count of 8,300/µL, erythrocyte sedimentation rate of 17 mm/h (reference < 20 mm/h), and C-reactive protein level of 8.7 mg/L (reference < 5 mg/L). Thoracic magnetic resonance imaging (MRI) demonstrated spondylodiscitis at the T7–T8 level with a right-sided paraspinal abscess. Imaging showed T1 hypointense and T2/short tau inversion recovery (STIR) hyperintense signal changes predominantly involving the T7 and T8 endplates, with increased T2/STIR signal intensity in the intervertebral disc space. Post-contrast images revealed marked enhancement of the endplates and disc. A right-sided paraspinal lesion measuring approximately 9 mm, consistent with an abscess extending anteriorly, was also observed. These findings were compatible with spondylodiscitis and paraspinal abscess.

Given the endemic nature of tuberculosis and brucellosis in Türkiye, these infections were initially considered; however, both Quantiferon-TB Gold and Brucella agglutination tests were negative. Blood cultures remained sterile.

The patient underwent surgical drainage, debridement, and posterior stabilization due to progressive symptoms and the presence of the paraspinal abscess. Cultures of intraoperatively obtained abscess material yielded bacterial growth. Colonies were identified as *Salmonella* spp. using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, USA), and serotyping according to the Kauffmann–White–Le Minor scheme (SSI Diagnostica, Denmark) confirmed the isolate as *Salmonella enteritidis*. Histopathological examination of the abscess wall revealed acute inflammatory infiltrates with abundant polymorphonuclear leukocytes. Antimicrobial susceptibility testing showed the isolate was susceptible to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, ceftriaxone, and ciprofloxacin.

Further evaluation excluded hematologic disorders, immunosuppressive conditions, and vascular pathologies, including infected aortic aneurysm. Transthoracic echocardiography showed no evidence of endocarditis or cardiac involvement.

The patient received intravenous ceftriaxone for two weeks, followed by oral ciprofloxacin for six weeks, totaling eight weeks of targeted antimicrobial therapy according to the 2015 Infectious Diseases Society of America (IDSA) guidelines. This treatment resulted in full clinical, laboratory, and radiological resolution. Follow-up MRI demonstrated complete resolution, and no recurrence was observed during one year of follow-up.

Discussion

Spondylodiscitis is a severe spinal infection associated with significant morbidity, particularly when the thoracic spine is involved. Globally, the most common causative agents are *Staphylococcus aureus*, *Mycobacterium tuberculosis*, and *Brucella* species, with geographical and epidemiological factors influencing this distribution[1,11]. In Türkiye, both tuberculosis and brucellosis remain endemic, with Brucella seroprevalence estimated at approximately 4.5%[12]. Accordingly, these pathogens are typically prioritized in the initial differential diagnosis of spinal infections.

The 2015 IDSA Clinical Practice Guidelines for Native Vertebral Osteomyelitis recommend serologic testing for Brucella and interferon-gamma release assays for *Mycobacterium tuberculosis* in patients presenting with vertebral infections, particularly in endemic regions[1]. However, when these tests are negative or the clinical presentation is atypical, less common pathogens, including NTS, should be considered.

In our case, both blood cultures and standard serological tests for Brucella and tuberculosis were negative. The pathogen was identified only after surgical drainage of a paraspinal abscess, highlighting the limitations of relying solely on blood cultures and endemic pathogens for diagnosis. This emphasizes the importance of direct sampling from the infected site for microbiological confirmation.

Vertebral involvement due to *Salmonella enteritidis* is extremely rare, particularly in immunocompetent individuals. Most reported cases involve immunosuppressed patients or those with significant comorbidities such as hematologic malignancies, sickle cell disease, or vascular infections[2–9]. For example, Ikejiri et al.[8] described a case of *Salmonella enteritidis* vertebral osteomyelitis complicated by meningitis following influenza A infection, while Tomek et al.[5] reported a case associated with a mycotic aortic aneurysm in a patient with chronic lymphocytic leukemia. In contrast, our patient had no underlying disease or predisposing condition, making this presentation particularly unusual.

Diagnostic confirmation was achieved by MALDI-TOF MS and serotyping after specimen collection from the infection site. This approach aligns with the 2015 IDSA guidelines and the comprehensive review by Lew and Waldvogel, both emphasizing early MRI-based imaging and microbiological confirmation for pathogen-specific diagnosis[1,11].

The patient received two weeks of intravenous ceftriaxone followed by six weeks of oral ciprofloxacin, totaling eight weeks of targeted antimicrobial therapy in accordance with IDSA recommendations. This regimen led to full clinical, laboratory, and radiological recovery and is consistent with IDSA guidance suggesting 6–12 weeks of pathogen-specific therapy for vertebral osteomyelitis, depending on clinical response[1].

To our knowledge, this is the first reported case of *Salmonella enteritidis* spondylodiscitis in an immunocompetent adult from Türkiye, specifically involving the thoracic spine with a paraspinal abscess.

Clinical implications of this case include:

• In endemic regions, Brucella and *Mycobacterium tuberculosis* should be ruled out first; however, rare pathogens such as *Salmonella enteritidis* should be considered in atypical or culture-negative cases.

• Blood cultures may not always detect the causative pathogen; therefore, image-guided biopsy or surgical sampling is essential.

• Treatment should follow pathogen-specific guidelines with an adequate duration of antibiotics.

• Clinicians must recognize that invasive Salmonella infections can occur even in immunocompetent individuals.

This case broadens the clinical spectrum of *Salmonella enteritidis* infections and highlights the importance of guideline-based diagnostic and therapeutic strategies in spinal infections.

Ethics

Informed Consent: Written informed consent was obtained from the patient for publication of this case and accompanying clinical data.

Footnotes

Conflict of Interest: The author declare no conflict of interest.

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Pyogenic Spondylodiscitis: A 9-year Analysis

Piyojenik Spondilodiskit: 9 Yıllık Analiz

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Abstract

Introduction: The management of pyogenic spondylodiscitis (PS) remains challenging due to the absence of clear, evidence-based guidelines. This study aimed to assess the clinical characteristics, diagnostic and follow-up challenges, and treatment outcomes of patients with PS.

Materials and Methods: The clinical, laboratory, and radiological data of all patients aged ≥18 years who were hospitalized with PS between January 2015 and June 2024 were retrospectively analyzed.

Results: Among 25 patients diagnosed with PS, 60% were male, with a mean age of 61 ± 10.6 years (range: 41–78). The most common symptoms were back or neck pain (88%), difficulty walking (24%), and fever (20%). The mean symptom duration was 2 months. The lumbosacral (60%), thoracic (44%), and cervical (12%) regions were the most frequently affected. A total of 84% of patients had at least one comorbidity, and 80% had a predisposing risk factor. Blood cultures were positive in 60% of patients. Among the 23 patients who underwent tissue and/or abscess culture, 43.7% and 30% yielded positive results, respectively. The most frequently isolated pathogen was methicillin-susceptible *Staphylococcus aureus* (MSSA) (48%). Among patients followed with contrast-enhanced magnetic resonance imaging (MRI), 41.2% demonstrated persistent contrast enhancement without significant change. The total treatment duration was 12 ± 4.1 weeks (range: 7–24). Treatment success was achieved in 86.3% of cases, while 3 (13.6%) patients experienced recurrence. In all recurrent cases, *Staphylococcus aureus* was the causative agent, and paraspinal abscess and bacteremia were present concomitantly. All recurrent cases had received at least 12 weeks of pathogen-targeted therapy.

Conclusion: Hospitalization and invasive procedures appear to be significant risk factors for PS. Obtaining blood and tissue/abscess cultures before initiating antimicrobial therapy enhances the likelihood of pathogen identification. Despite adequate treatment, MRI findings may persist without complete radiological resolution. Close monitoring is warranted for potential recurrence when *Staphylococcus aureus* is the causative pathogen, especially in the presence of abscess or bacteremia.

Keywords: Pyogenic spondylodiscitis, vertebrae, *Staphylococcus aureus*, recurrence

Öz

Giriş: Piyojenik spondilodiskitin (PS) tanısı, takibi ve tedavisi ile ilgili önerilerin sınırlı olması yönetimini güçleştirmektedir. Bu çalışmanın amacı PS olgularının klinik özelliklerinin, tanı ve takip sürecinde karşılaşılan zorlukların ve sonuçlarının değerlendirilmesidir.

Gereç ve Yöntem: Ocak 2015 ile Haziran 2024 tarihleri arasında PS tanısıyla hastaneye yatırılan ≥18 yaşındaki tüm hastaların klinik, laboratuvar ve radyolojik özellikleri retrospektif olarak analiz edildi.

Bulgular: PS tanısı alan 25 hastanın %60'ı erkekti ve yaş ortalaması 61±10,6 (41–78) idi. En sık görülen semptomlar sırt/boyun ağrısı (%88), yürüme güçlüğü (%24) ve ateşti (%20). Semptom süresi ortalama iki aydı. En sık tutulan bölgeler lumbosakral (%60), torakal (%44) ve servikaldi (%12). Hastaların %84'ünün en az bir ek hastalığı, %80'inin ise risk faktörü vardı. Kan kültürleri hastaların %60'ında pozitifti. Doku ve/veya apse kültürü alınan 23 hastanın sırasıyla %43,7'sinde ve %30'unda pozitif sonuç elde edildi. Tüm kültürlerde en sık izole edilen etken metisiline duyarlı *Staphylococcus aureus*'tu (%48). Kontrastlı manyetik rezonans görünteleme (MRI) ile izlenen hastaların %41,2'sinde belirgin iyileşme olmaksızın kontrast tutulumu devam etmekteydi. Toplam tedavi süresi ortalama 12±4,1 (7–24) haftaydı. Vakaların %86,3'ünde tedavi başarılıydı. Üç hastada (%13.6) nüks gelişti.

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Öz

Nüks vakalarının tamamında etken *Staphylococcus aureus*'tu ve eşlik eden paraspinal apse ile bakteremi mevcuttu. Nüks vakalarının tamamı, etkene yönelik en az 12 haftalık tedavi almıştı.

Sonuç: Hastanede yatış ve invaziv girişimler, PS için önemli risk faktörleridir. Tedavi öncesi kan ve doku/apse kültürü alınması, etkenin belirlenme olasılığını artırır. Uygun tedaviye rağmen MRI bulguları tamamen düzelmeyebilir. *Staphylococcus aureus* kaynaklı, özellikle apse veya bakteremi ile seyreden vakalarda, nüks riski açısından yakın takip gereklidir.

Anahtar Kelimeler: Pyojenik spondilodiskit, vertebra, *Staphylococcus aureus*, nüks

Introduction

Spondylodiscitis is an infection of the spinal column caused by various pathogens, potentially involving the vertebral body, intervertebral discs, spinal canal, and paravertebral structures[1]. The etiology may be pyogenic (bacterial), granulomatous (tuberculous, brucellar, or fungal) or rarely parasitic; however, the majority of cases are bacterial in origin[1–3]. Pathogens may reach the vertebrae through hematogenous dissemination, direct inoculation (most commonly during spinal surgery), or contiguous spread from adjacent structures. A distant focus of infection is identified in about half of the cases, and infective endocarditis accompanies approximately 12% of them[3].

Spondylodiscitis accounts for 0.15%–7% of all osteomyelitis cases[1,2,4]. In Western countries, its annual incidence ranges from 0.4 to 2.4 per 100,000 population[5]. It is 1.5–3 times more common in males and occurs across all age groups, with a higher prevalence among individuals aged 50–70 years[1,2,4,6].

The incidence of spondylodiscitis has shown a concerning rise over the past two decades. This trend is attributed to increased life expectancy, the presence of comorbidities, greater use of invasive procedures and immunosuppressive therapies, expanding indications for spinal surgery, a growing vulnerable population, and advancements in diagnostic methods[2,6–8].

The clinical spectrum of the disease ranges from mild to rapidly progressive, potentially leading to severe morbidity and mortality, including vertebral collapse, permanent neurological deficits, and even death. Its nonspecific early symptoms often result in diagnostic delays of 30 to 90 days[6]. Early identification of the causative pathogen and prompt initiation of targeted antimicrobial therapy are therefore crucial[9]. However, diagnosis, follow-up, and treatment remain challenging due to the limited availability of evidence-based management guidelines. This study aimed to evaluate the clinical characteristics and challenges associated with microbiological and radiological assessments during diagnosis and follow-up, as well as the treatment outcomes of pyogenic spondylodiscitis (PS).

Materials and Methods

This study was designed as a single-center retrospective cohort study conducted at a tertiary care facility in Türkiye. Data were retrospectively collected from the hospital information system and patient records for all patients aged ≥ 18 years who were hospitalized with a diagnosis of PS between January 2015 and June 2024. Patients with tuberculous or brucellar spondylodiscitis were excluded.

The diagnosis of spondylodiscitis was based on typical clinical findings, characteristic changes on magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography–CT (PET–CT), and microbiological evidence of infection.

Demographic, clinical, microbiological, and radiological data of the patients were retrospectively reviewed. Microorganism identification and antimicrobial susceptibility testing (AST) were performed using fully automated systems (VITEK2 Compact, bioMérieux, France, and VITEK MS, bioMérieux, France). The AST results were interpreted according to the recommendations of the European Committee on AST.

The durations of intravenous and oral treatments were recorded. All patients received a total treatment duration of at least 6 weeks. Therapy was extended in patients with inadequate clinical or laboratory response or those with insufficient source control. Owing to the retrospective design of the study, the frequency of follow-up imaging was not standardized. Therefore, radiology reports obtained at the initiation and completion of therapy were compared for all patients.

Treatment success was defined as the absence of clinical or laboratory deterioration during a one-year follow-up. Patients who demonstrated clinical, laboratory, or radiological worsening after completion of therapy were classified as recurrent cases.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 26 (IBM SPSS, USA). The normality of parameter distribution was assessed using the Shapiro–Wilk test. Descriptive statistics were calculated for numerical data, including median, mean, and standard deviation values.

Data collection was conducted with the approval of the University of Health Sciences Türkiye, Haydarpaşa Numune Training and Research Hospital (approval number: 711/06/2024, dated: 05.06.2024).

Results

Between January 2015 and June 2024, 25 patients were diagnosed with PS. Of these, 15 (60%) were male and 10 (40%) were female, with a mean age of 61 ± 10.6 years (range: 41–78). At least one chronic disease was present in 21 (84%) patients. The most common comorbidities were diabetes mellitus in 14 (56%), hypertension in 12 (48%), hyperlipidemia in eight (32%), and chronic kidney failure in six (24%) patients (Table 1).

The most frequent presenting symptoms were back or neck pain in 22 (88%) patients, difficulty walking in six (24%), fever in five (20%), leg weakness in two (8%), incontinence in two (8%), weight loss in two (8%), night sweats in two (8%), and numbness in one (4%) at admission. The mean duration of symptoms before diagnosis was 2 months (range: 1 week–6 months). Motor deficit was detected in 11 (44%) patients, and a cardiac murmur in one (4%). No abnormal physical findings were observed in 13 (52%) patients. Pretreatment laboratory findings showed a mean white blood cell (WBC) count of 9,132 ± 3,986 cells/mm^3 (range: 1,780–19,690), C-reactive protein (CRP) level of 116 ± 92 mg/L (range: 3–317), and erythrocyte sedimentation rate (ESR) of 72 ± 22 mm/h (range: 26–104). By the end of treatment, the mean ESR had decreased to 29 ± 18.7 mm/h (range: 2–83) (Table 2). In recurrent cases, initial WBC counts were 7,700–15,300 and 19,690 cells/mm^3, the CRP levels were 10–190 and 29 mg/L, and the ESR values were 76–63 and 95 mm/h, respectively. At the end of treatment, WBC counts decreased to 4,890–8,170 and 8,120 cells/mm^3, the CRP levels to 0.4–4 mg/L, and the ESR values to 23–36 and 29 mm/h, respectively.

Discitis was detected in 20 (80%) patients, paravertebral soft tissue involvement in 21 (84%), and abscess formation in 12 (48%). The most frequently affected regions were the lumbosacral (60%), thoracic (44%), and cervical (12%) spine.

Table 1. Baseline characteristics of the study patients.

	n (%)		n (%)
Male/female	15 (60)/10 (40)	**Known risk factors**	20 (80)
Age (mean ± SD[§] (min.–max.)	61 ± 10.6 (41–78)	Hospitalization in the last 3 months	13 (52)
Chronic disease	21 (84)	ICU[†] admission in the last 3 months	4 (16)
Diabetes mellitus	14 (56)	Catheter infection	1 (4)
Hyperlipidemia	10 (40)	ERCP[‡]	2 (8)
Hypertension	8 (32)	Spinal procedure	3 (12)
Chronic renal failure	6 (24)	Other invasive procedures and operations	2 (8)
Chronic heart diseases	5 (20)	Hemodialysis	3 (12)
Pulmonary diseases	2 (8)		
Malignancy	1 (4)		

[†]ICU; [‡]ERCP; [§]SD; min.–max., minimum–maximum.

Table 2. Clinical characteristics and laboratory results.

	n (%)		Mean ± SD[||] (min.-max.)
Localization		**Laboratory results**	
Cervical	3 (12)	Pretreatment WBC[§] (cell/mm^3)	9,132 ± 3,986 (1,780–19,690)
Thoracal	11 (44)	Pretreatment CRP[‡] (mg/L)	116 ± 92 (3–317)
Lumbosacral	15 (60)	Pretreatment ESR[§] (mm/h)	72 ± 22 (26–104)
Paraspinal/psoas abscess	12 (40)	End treatment ESR (mm/h)	29 ± 18.7 (2–83)
Complications			
Spinal cord pressure	11 (44)		
Neurodeficit	2 (8)		
Height loss	10 (40)		
Endocarditis	2 (8)		

[†]WBC; [‡]CRP; [§]ESR; [||]SD.

As potential risk factors, 13 (52%) patients had a hospital admission within the past 3 months, and four (16%) had a history of intensive care unit stay. Three patients developed infection following vertebral surgery; two had undergone endoscopic retrograde cholangiopancreatography (ERCP), one had a catheter-related infection, and three were on hemodialysis. No risk factors were identified in 5 (20%) patients (Table 1).

Blood cultures were obtained before the initiation of antibiotic therapy in all patients, and 23 (92%) underwent tissue or abscess culture. Blood cultures were positive in 15 (60%) patients. Tissue cultures were obtained from 16 patients, with positive results in seven (43.7%). Abscess cultures showed bacterial growth in three of 10 (30%) patients. In seven (77.7%) of the tissue culture–negative cases and six (85.7%) of the abscess culture–negative cases, samples were collected after the initiation of empirical antibiotic therapy. No organism was isolated from any culture in 5 (20%) patients, who were treated empirically (Table 3).

The most frequently isolated pathogen was *Staphylococcus aureus*, identified in 12 (48%) cases with positive blood or tissue/abscess cultures. All isolates were methicillin-susceptible (MSSA); no methicillin-resistant *Staphylococcus aureus* (MRSA) was detected. Other identified pathogens included *Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecium, Streptococcus pneumoniae, Streptococcus parasanguinis, Staphylococcus epidermidis, Bacteroides fragilis*, and *Enterobacter cloacae* (each in one case). Blood cultures were positive in 10 (83.3%) of the cases, where *Staphylococcus aureus* was the causative agent (Table 3).

Complications included spinal cord compression in 11 (44%) patients, vertebral height loss in 10 (40%), and endocarditis in two (8%).

The mean duration of intravenous antibiotic therapy was eight weeks (range: 4–24, standard deviation [SD]: 4.2). Oral sequential therapy lasted a mean of five weeks (range: 1–12, SD: 2.4), with a total treatment duration averaging 12 weeks (range: 7–24, SD: 4.1). The recurrent cases received total antibiotic therapy for 13, 12, and 24 weeks, respectively, with intravenous administration for the first 6, 8, and 24 weeks.

A total of 17 (68%) patients were followed with contrast-enhanced MRI, one (4%) with noncontrast MRI, two (8%) with CT, and one (4%) with PET–CT. Follow-up imaging data were unavailable for 4 (16%) patients. At the end of treatment, complete resolution of contrast enhancement was observed in 3 (17.6%) patients, partial regression in seven (41.2%), and no significant change in seven (41.2%). Among the three recurrent cases, regression on MRI was observed in two, while no change was detected in one at the end of treatment.

Of the 22 patients who completed treatment, three (13.6%) developed recurrence, and two (9%) required surgical intervention. Treatment was successful in 19 (86.3%) patients. Treatment outcomes could not be evaluated in 3 patients due to unavailable follow-up data. All recurrent cases were caused by *Staphylococcus aureus* and were accompanied by bacteremia and paraspinal abscess.

Discussion

The rising incidence of PS over the past 2 decades, along with the complexity of its management and the lack of standardized treatment guidelines, prompted this study to evaluate the clinical and etiological characteristics, diagnostic process, and prognosis of patients treated in our center.

A review of previous studies reported a mean patient age of approximately 60 years and a predominance of male (56%) patients with spondylodiscitis[5]. Our findings are consistent with these reports, with a mean age of 61 years and 60% of patients being male.

Table 3. Microbiological tests and results.

	n (%)		n (%)
Blood cultures obtained	25 (100)	**Organisms isolated from all cultures**	
Positive results	15 (60)	MSSA	12 (48)
Abscess and/or tissue culture obtained	23 (92)	*Staphylococcus epidermidis*	1 (4)
Abscess cultures obtained	10 (40)	*Enterococcus faecium*	1 (4)
Positive results	3 (30)	*Streptococcus pneumoniae*	1 (4)
Negative results	7 (70)	*Streptococcus parasanguinis*	1 (4)
Tissue cultures obtained	16 (64)	*Pseudomonas aeruginosa*	1 (4)
Positive results	7 (43.7)	*Escherichia coli*	1 (4)
Negative results	9 (57.3)	*Enterobacter cloacae*	1 (4)
		Bacteroides fragilis	1 (4)

The increasing prevalence of spondylodiscitis has been associated with longer life expectancy and the rise in comorbid conditions[3,10,11]. In support of this, 84% of our patients had at least one chronic disease. Furthermore, 52% of the cohort had been hospitalized in the preceding 3 months, indicating that hospitalization is a major risk factor. Catheter-related infections, hemodialysis, and prior spinal surgeries were common underlying causes, as expected. Notably, two cases of PS caused by gram-negative pathogens following ERCP highlight ERCP as a potential risk factor for gram-negative spondylodiscitis, a relationship described in only a few previous reports[12–14]. In 20% of our cases, no identifiable cause was detected, emphasizing that PS can occur even in patients without apparent risk factors.

Consistent with the literature, the lumbosacral region was the most frequently affected site (60%), followed by the thoracic (44%) and cervical (12%) regions[5]. Multilevel involvement was observed in 16% of all cases. Nearly half of the patients presented with concurrent abscess formation, consistent with the 19%–68% range reported in the literature[5,15,16]. Since abscesses can significantly influence both treatment approach and prognosis, screening for additional foci and the early detection of abscess formation are essential steps in clinical evaluation.

Identifying the causative pathogen is critical for ensuring targeted and effective treatment, especially for a disease such as spondylodiscitis, which requires prolonged therapy. Blood cultures yielded a high positivity rate of 60%, underscoring the value of this simple diagnostic tool and reaffirming the importance of obtaining blood cultures before initiating therapy[3]. Positive blood culture rates can reach up to 70% in patients without prior antibiotic exposure[1].

Tissue and abscess cultures are also valuable diagnostic tools. In our study, 44% of tissue cultures and 30% of abscess cultures yielded growth, which aligns with previously reported rates of 43%–78%[3]. The lower positivity rates observed in our study may be attributed to the exclusive use of percutaneous biopsies and the fact that most samples were obtained after empirical antibiotic therapy had begun. Tissue cultures obtained after antimicrobial initiation were negative in 77% of cases, while abscess cultures were negative in 85%. Antibiotic exposure significantly reduces culture positivity rates, and even without prior antibiotic use, biopsy cultures can remain negative in up to 39% of cases[17]. Moreover, CT-guided percutaneous biopsies may yield limited tissue, identifying the pathogen in only about half of cases[1]. Because the procedure is invasive, delays in sampling can also pose challenges in clinical practice. When the causative organism remains unidentified, determining optimal therapy becomes difficult, often necessitating broad-spectrum parenteral antibiotics and prolonged hospital stays. Therefore, obtaining appropriate cultures before initiating therapy is crucial. In nonemergent cases without neurological deficits or sepsis, treatment should be deferred until adequate samples are obtained[9].

The prevalence of *Staphylococcus aureus* in PS ranges from 20% to 84%, accounting for approximately half of all cases[18]. Consistent with the literature, *Staphylococcus aureus* was the most common causative pathogen in our study (48%). Methicillin susceptibility among *Staphylococcus aureus* isolates varies geographically and depends on patient risk factors and disease etiology[6]. Although most community-acquired strains are MSSA, the rising rate of MRSA raises concerns regarding its inclusion in empirical therapy[18]. A review of 14 studies in 2009 reported an MRSA prevalence of 2.6%[19], while more recent studies, particularly from high-income countries, have reported rates as high as 25%–30%[20–22]. In studies conducted in Türkiye, the prevalence ranges from 3.7% to 5.5%[4,23,24], and one study reported a rate of 12.5% in nosocomial cases[25]. Notably, all *Staphylococcus aureus* strains in our study were MSSA, despite a significant proportion of patients having prior hospitalizations or invasive procedures. This finding suggests that MRSA is not yet a major concern in PS cases in our region and that routine MRSA coverage in empirical therapy may not be necessary for most patients.

Spinal cord compression and vertebral height loss were the most common complications, while 8% of cases had concomitant endocarditis. This finding aligns with prior reports describing endocarditis in up to 12% of PS cases, highlighting the importance of screening for endocarditis in these patients[19].

MRI remains the gold standard for diagnosing spondylodiscitis, although its role in treatment follow-up remains debated[26]. In our study, 68% of patients underwent contrast-enhanced MRI. At the end of treatment, only 17% of patients demonstrated complete resolution of contrast enhancement, while 41% showed no significant change. As shown in previous studies, imaging abnormalities may persist despite clinical and laboratory improvement[26,27].

Treatment was successful in 86.3% of cases; however, 13% of patients experienced recurrence. All recurrent cases were caused by *Staphylococcus aureus* infections with concurrent paraspinal abscess and bacteremia. *Staphylococcus aureus* has been identified as an independent risk factor for treatment failure[6,15]. Bacteremia and paraspinal abscess have also been identified in some studies as predictors of recurrence and treatment failure[7,20,21]. One study reported that patients with bacteremia required longer treatment durations (> 8 weeks) [28], while another showed a significant decrease in recurrence among patients treated for more than eight weeks[22]. In our study, recurrent cases received at least 12 weeks of treatment, including a minimum of six weeks of intravenous therapy

targeting the causative agent. Despite the prolonged duration, recurrence still occurred. Furthermore, in these patients, WBC, CRP, and ESR values normalized by the end of treatment, and two patients demonstrated regression on contrast-enhanced MRI. However, neither laboratory nor imaging findings were reliable predictors of relapse. Therefore, close monitoring of patients with *Staphylococcus aureus* infections, particularly those with bacteremia and paraspinal abscess formation, is essential to minimize recurrence.

Study Limitations

This study has several limitations that should be considered when interpreting the findings. As a retrospective, single-center study with a relatively small sample size, the generalizability of the results is limited, and the statistical power may be insufficient to detect less common associations or outcomes. The analysis relied on hospital records, which may have lacked uniformity or completeness, particularly in documenting clinical symptoms and follow-up details. Moreover, follow-up imaging was not standardized in terms of timing or modality (MRI, CT, or PET–CT), introducing potential variability in the evaluation of radiological treatment response. Owing to the retrospective nature of the study, treatment decisions—including antibiotic duration and follow-up strategies—were not standardized and could have influenced treatment outcomes and recurrence rates.

Conclusion

In conclusion, recent hospitalization and invasive procedures remain significant risk factors for the development of PS. Obtaining blood, tissue, or abscess cultures before initiating antibiotic therapy substantially increases the likelihood of identifying the causative pathogen; at the very least, blood cultures should be obtained as a simple yet valuable diagnostic step before treatment. Despite appropriate therapy, MRI abnormalities may persist and should not be used in isolation to determine treatment success. *Staphylococcus aureus* continues to be the most frequently isolated pathogen. While methicillin resistance did not emerge as a concern in this cohort, all recurrent cases involved *Staphylococcus aureus* infections accompanied by abscess formation and bacteremia. Therefore, patients with *Staphylococcus aureus* spondylodiscitis, particularly those with concurrent bacteremia or paraspinal abscess, should be closely monitored for potential recurrence even after completion of therapy.

Ethics

Ethics Committee Approval: Data collection was conducted with the approval of the University of Health Sciences Türkiye, Haydarpaşa Numune Training and Research Hospital (approval number: 711/06/2024, dated: 05.06.2024).

Informed Consent: Not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.K., S.E., N.C., A.İ., S.Ş., C.A., Concept: M.K., S.E., N.C., A.İ., S.Ş., C.A., Design: M.K., S.E., Data Collection or Processing: M.K., C.A., Analysis or Interpretation: M.K., S.E., Literature Search: M.K., S.E., Writing: M.K., S.E., N.C., A.İ., S.Ş., C.A.

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Improvements in Surgical Site Infection Control in Albania: Impact of Infrastructure, Sterilization Standards, and Clinical Practices

Arnavutluk'ta Cerrahi Alan Enfeksiyonunun Kontrolünde İyileştirmeler: Altyapı, Sterilizasyon Standartları ve Klinik Uygulamaların Etkisi

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Abstract

Introduction: Surgical site infections (SSIs) are a major cause of postoperative morbidity, prolonged hospitalization, and increased healthcare costs worldwide. In Albania, SSI rates have historically been high; however, recent infrastructural and organizational reforms in tertiary hospitals have led to measurable progress. This study evaluated the effects of infrastructure modernization, centralization of sterilization services, and the introduction of disposable materials and laparoscopic techniques on SSI incidence at the University Hospital Center "Mother Teresa" in Albania.

Materials and Methods: A prospective observational study was conducted in the Department of General and Digestive Surgery from October 2023 to October 2024. Data were collected on patient demographics (age, sex), surgical characteristics (upper or lower gastrointestinal; elective or emergency), comorbidities (hypertension, diabetes mellitus, malignancy), and SSI occurrence. Microbiological analyses included pathogen identification and antimicrobial susceptibility testing (AST) in accordance with the 2024 European Committee on AST guidelines.

Results: Among 1,179 patients (51.2% male; mean age, 57.8 years), 5.4% developed SSIs after abdominal surgery. Infection rates were significantly higher in lower gastrointestinal procedures (57.8%) than in upper gastrointestinal procedures (42.2%). Patients aged ≥ 50 years exhibited a greater risk of SSI ($p = 0.01$), as did those with comorbidities ($p = 0.0007$) and diabetes mellitus ($p = 0.0006$). Mean hospital stay was markedly longer among infected patients (4.4 vs. 2.0 days; $p < 0.0001$). *Escherichia coli* (39%) and *Enterococcus faecalis* (22%) were the most common isolates, demonstrating notable resistance to ciprofloxacin (34%) and trimethoprim–sulfamethoxazole (31%). Reductions in infection rates were closely linked to enhanced operating room ventilation, improved sterilization practices, and the use of single-use materials.

Conclusion: Albania has achieved substantial progress in SSI prevention through targeted infrastructural and procedural reforms. Nonetheless, persistent challenges—particularly antimicrobial resistance and the lack of a national SSI surveillance system—underscore the need for a coordinated, multidisciplinary strategy. Strengthening antimicrobial stewardship, standardizing perioperative protocols, and expanding the use of minimally invasive surgery are key priorities for sustaining improvements.

Keywords: Surgical site infection, Albania, abdominal surgery, hospital infrastructure, antimicrobial resistance, infection control

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Öz

Giriş: Cerrahi alan enfeksiyonları (CAE), dünya çapında ameliyat sonrası morbidite, uzun süreli hastanede kalış ve artan sağlık hizmetleri maliyetlerinin başlıca nedenidir. Arnavutluk'ta CAE oranları tarihsel olarak yüksek olmuştur; ancak, üçüncü basamak hastanelerdeki son altyapısal ve organizasyonel reformlar ölçülebilir bir ilerleme sağlamıştır. Bu çalışmada, Arnavutluk'taki "Rahibe Teresa" Üniversite Hastanesi Merkezi'nde altyapı modernizasyonu, sterilizasyon hizmetlerinin merkezileştirilmesi ve tek kullanımlık malzemeler ile laparoskopik tekniklerin kullanılmaya başlanmasının CAE insidansı üzerindeki etkileri değerlendirilmiştir.

Gereç ve Yöntem: Ekim 2023 ile Ekim 2024 tarihleri arasında Genel ve Sindirim Sistemi Cerrahisi Anabilim Dalı'nda prospektif bir gözlemsel çalışma yürütüldü. Hastaların demografik özellikleri (yaş, cinsiyet), cerrahi özellikleri (üst veya alt gastrointestinal; elektif veya acil), eşlik eden hastalıklar (hipertansiyon, diabetes mellitus, malignite) ve CAE oluşumu hakkında veriler toplandı. Mikrobiyolojik analizler, 2024 Avrupa Antimikrobiyal Duyarlılık Testi (ADT) yönergelerine uygun olarak patojen tanımlama ve ADT içeriyordu.

Bulgular: Bin yüz yetmiş dokuz hastanın (%51,2'si erkek; ortalama yaş 57,8) %5,4'ünde abdominal cerrahi sonrası CAE gelişti. Enfeksiyon oranları alt gastrointestinal prosedürlerde (%57,8), üst gastrointestinal prosedürlere (%42,2) göre anlamlı derecede daha yüksekti. ≥ 50 yaş hastalarda CAE riski daha yüksekti ($p = 0,01$), eşlik eden hastalıkları ($p = 0,0007$) ve diyabeti ($p = 0,0006$) olanlarda da aynı risk mevcuttu. Enfekte hastalarda ortalama hastanede kalış süresi belirgin şekilde daha uzundu (4,4'e karşı 2,0 gün; $p < 0,0001$). *Escherichia coli* (%39) ve *Enterococcus faecalis* (%22) en sık görülen izolatlar olup, siprofloksasine (%34) ve trimetoprim-sülfametoksazol (%31) karşı belirgin direnç göstermekteydi. Enfeksiyon oranlarındaki azalmalar, gelişmiş ameliyathane ventilasyonu, iyileştirilmiş sterilizasyon uygulamaları ve tek kullanımlık malzemelerin kullanımıyla yakından bağlantılıydı.

Sonuç: Arnavutluk, hedefli altyapı ve prosedür reformları sayesinde CAE önlenmesinde önemli ilerleme kaydetmiştir. Bununla birlikte, özellikle antimikrobiyal direnç ve ulusal bir CAE gözetim sisteminin olmaması gibi devam eden zorluklar, koordineli ve çok disiplinli bir stratejiye olan ihtiyacın altını çizmektedir. Antimikrobiyal yönetimin güçlendirilmesi, perioperatif protokollerin standartlaştırılması ve minimal invaziv cerrahinin kullanımının yaygınlaştırılması, iyileştirmelerin sürdürülmesi için temel önceliklerdir.

Anahtar Kelimeler: Cerrahi alan enfeksiyonu, Arnavutluk, karın cerrahisi, hastane altyapısı, antimikrobiyal direnç, enfeksiyon kontrolü

Introduction

Healthcare-associated infections (HAIs) remain a persistent and critical challenge in modern surgical care. Also known as hospital-acquired or nosocomial infections, HAIs pose a substantial threat to patient safety and quality of care. Although largely preventable through evidence-based infection control strategies, they continue to occur with alarming frequency. HAIs are associated with extended hospital stays, adverse clinical outcomes, long-term disability, rising antimicrobial resistance (AMR), increased healthcare costs, and preventable deaths. Globally, they contribute significantly to both morbidity and mortality, representing a major burden for healthcare systems.

Among HAIs, surgical site infections (SSIs) account for approximately 16%–20% of all cases and exert profound effects on patient outcomes, including delayed wound healing, prolonged hospitalization, increased readmission rates, and elevated mortality[1–4]. The global prevalence of HAIs in acute care hospitals is estimated at 7.1%, but the burden is substantially higher in low- and middle-income countries (LMICs)[5,6]. In these settings, the financial consequences are particularly severe because patients frequently shoulder the majority of healthcare expenses. Developing an SSI can increase household expenditures by more than 10% of annual income, imposing a considerable economic strain[7].

In Albania, earlier reports documented SSI prevalence rates of up to 13.3%, underscoring long-standing infrastructural and procedural gaps in infection control[8,9]. Over the past decade, however, major reforms have been implemented at the University Hospital Center "Mother Teresa," including the renovation of surgical theaters, centralization of sterilization services, introduction of disposable surgical materials, and acquisition of laparoscopic systems. Recent prospective data indicate that these reforms have reduced the SSI incidence following abdominal surgery to 5.4%.

This study aims to evaluate the extent to which infrastructural modernization, improved sterilization standards, and updated clinical practices have contributed to this decline. Unlike previous investigations that primarily addressed clinical or epidemiological aspects, the present analysis highlights hospital modernization as a pivotal determinant in the prevention and control of SSIs in Albania.

Materials and Methods

Inclusion and Exclusion Criteria

All adult patients (≥ 14 years) who underwent abdominal surgery—either elective or emergency—at the Department of General and Digestive Surgery, University Hospital Center "Mother Teresa," Albania, between October 2023 and October 2024 were included. Abdominal procedures were classified as upper gastrointestinal, lower gastrointestinal, hepatobiliary, or hernia-related surgeries. Laparoscopic procedures were analyzed as a separate subgroup to assess their increasing utilization during the study period. Reoperations within the same hospitalization were included when associated with postoperative complications.

Patients admitted solely for diagnostic procedures, minor interventions (e.g., biopsies, wound revisions), or transferred postoperatively from other hospitals were excluded. Pediatric, thoracic, cardiac, trauma, and neurosurgical procedures were also excluded.

Study Design and Context

This research employed a hybrid design combining a narrative review of previously published national data with a prospective observational analysis. The prospective component included all abdominal surgery patients treated between October 2023 and October 2024 at the University Hospital Center "Mother Teresa."

Comparative analyses between pre- and post-reconstruction periods were based on institutional records obtained before (2015–2019) and after (2023–2024) modernization of operating theaters and central sterilization units. Published hospital reports and earlier prevalence surveys represented the pre-reconstruction baseline, whereas current data from the prospective survey characterized the post-reconstruction phase.

Descriptive statistics were used to compare SSI incidence, patient demographics, risk factors, and microbiological patterns across the two periods. The study emphasized systemic infrastructural and procedural improvements rather than individual patient-level matching.

The 2023–2024 prospective survey included 1,179 patients and assessed SSI incidence, associated risk factors, microbiological profiles, and AMR trends. Additionally, literature on SSI epidemiology in Europe and LMICs was reviewed to provide contextual background.

Data Extraction and Thematic Analysis

Data were analyzed under three domains:

• Infrastructure and organization: Upgrades in operating rooms, sterilization processes, use of disposable materials, and patient ward improvements.

• Clinical epidemiology: SSI incidence, patient demographics, comorbidities, and surgical categories.

• Microbiology and AMR: Pathogen distribution and antimicrobial susceptibility profiles.

Microbiological Sampling and Analysis

For patients clinically suspected of SSIs, wound specimens were collected aseptically. In superficial incisional infections, sterile cotton swabs were used after cleaning the wound with saline to eliminate surface contaminants. For deep or purulent infections, aspirates or tissue biopsies were collected to enhance diagnostic accuracy. All specimens were transported promptly to the Microbiology Laboratory of the University Hospital Center "Mother Teresa" and processed within 2 h of collection.

Microbial Identification and Antimicrobial Susceptibility Testing (AST)

Bacterial isolates were cultured on blood and MacConkey agar, followed by biochemical identification using conventional methods and the VITEK® 2 Compact system (bioMérieux, France). AST was performed according to the 2024 European Committee on AST (EUCAST) guidelines, using the automated VITEK® 2 platform. Breakpoints were interpreted per EUCAST standards. Resistance data were analyzed for each organism individually, and antibiotic names are reported as generic compounds.

Infrastructure and Organizational Upgrades

Comparison of conditions before and after departmental renovation revealed substantial improvements:

Before renovation:

• Operating theaters lacked adequate ventilation and temperature regulation.

• Sterilization was decentralized, performed independently by each department with variable standards.

• Reusable cloth drapes and gowns were employed, heightening infection risk.

• Laparoscopic instruments were unavailable.

• Wards were overcrowded (up to four patients per room) and lacked proper ventilation systems.

After renovation:

• Modern operating theaters with biofiltration and climate control systems were constructed.

• Sterilization was centralized under a specialized, trained team.

• Disposable gowns and drapes replaced reusable materials.

• Laparoscopic instruments became standard for minimally invasive surgery.

Table 1. Clinical and demographic characteristics of patients with SSIs.

Patients with SSIs	Number	Percentage
Female	27	42.19%
Male	37	57.81%
Lower gastrointestinal surgery	37	57.81%
Upper gastrointestinal surgery	27	42.19%
Comorbidities	45	70.31%
Arterial hypertension	41	64.06%
Diabetes mellitus	18	28.12%
Cancer	21	32.81%

SSIs, surgical site infections.

• Patient wards were renovated, featuring reduced bed density and air-conditioning systems.

Ethical Approval and Informed Consent

The study complied with the ethical principles of the Declaration of Helsinki (1964) and subsequent amendments. The research protocol was approved by the Ethics Committee of the University of Medicine, Tirana, and the National Agency for Scientific Research and Innovation (approval number: 20, dated: 02.11.2023). Written informed consent was obtained from all participants prior to enrollment. All data were anonymized to ensure confidentiality.

Statistical Analysis

Data analysis was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequencies (n) and percentages (%). Associations between categorical variables were evaluated using the chi-square test, while continuous variables were compared using the Student's t-test. Univariate logistic regression was applied to identify predictors of SSI development, with results reported as odds ratios (ORs) and 95% confidence intervals. Statistical significance was set at $p \leq 0.05$.

Results

A total of 1,179 patients were included, comprising 51.23% men and 48.77% women, with a mean age of 57.8 years (range: 19–92 years). SSIs occurred in 64 patients (5.4%), all of which were classified as superficial. Although infection rates were marginally higher in males, the difference was not statistically significant (OR 1.47, p = 0.12) (Table 1).

Table 2. Microorganisms isolated from SSIs.

Microorganisms isolated from the SSI	Number	Percentage
Escherichia coli	25	39.06%
Enterococcus faecalis	15	23.44%
Klebsiella pneumoniae	6	9.37%
Staphylococcus epidermidis	4	6.25%
Pseudomonas aeruginosa	3	4.69%
Staphylococcus hominis	3	4.69%
Enterobacter cloacae	2	3.13%
Staphylococcus aureus	1	1.56%
Staphylococcus capitis	1	1.56%
Staphylococcus caprae	1	1.56%
Streptococcus agalactiae	1	1.56%
Citrobacter	1	1.56%
Corynebacterium	1	1.56%

SSIs, surgical site infections.

Lower gastrointestinal surgeries carried a greater infection risk, accounting for 58% of SSIs, compared with 42% in upper gastrointestinal procedures. Laparoscopic operations represented 21% of all surgeries and showed a significantly lower SSI rate than open procedures (2.1% vs. 6.3%; $p < 0.05$), confirming the infection-preventive advantage of minimally invasive techniques.

Patients with SSIs were significantly older than those without infection (mean age, 66.2 vs. 57.0 years; $p < 0.0001$). Age ≥ 50 years increased the infection risk nearly threefold (OR: 2.69, p = 0.01). Comorbidities were also strong predictors (OR: 2.71, p = 0.0007), particularly type 2 diabetes mellitus (OR: 2.98, p = 0.0006). Older patients were significantly more likely to have comorbidities (OR 19.59, $p < 0.0001$) (Table 2).

Microbiological cultures confirmed that *Escherichia coli* and *Enterococcus faecalis* were the predominant pathogens, accounting for 39% and 23% of SSIs, respectively.

Resistance patterns are summarized in Table 3. Notably, over one-third of bacterial isolates exhibited resistance to ciprofloxacin, while resistance to trimethoprim–sulfamethoxazole, cefazolin, and levofloxacin exceeded 30%. In contrast, resistance to tetracycline, nitrofurantoin, penicillin, and fosfomycin remained low (< 5%). Patients who developed SSIs experienced hospital stays more than twice as long as those without infection, averaging 4.4 days versus 2.0 days ($p < 0.0001$). No deaths were directly attributable to SSIs during the study period. Overall postoperative mortality among patients undergoing abdominal surgery was 1.1% (13/1,179), and all deaths occurred in individuals with advanced malignancies or severe comorbidities rather than active SSIs. These findings indicate that improvements in infection control contributed not only to reduced morbidity but also to the maintenance of low mortality rates.

Discussion

SSIs are among the most frequent postoperative complications, contributing to increased patient morbidity, prolonged hospital

Table 3. AMR patterns among bacterial isolates from SSIs.

Antibiotic	Resistant isolates (%)
Ciprofloxacin	34%
Trimethoprim–sulfamethoxazole	31%
Cefazolin	30%
Levofloxacin	30%
Amoxicillin-clavulanate	18%
Gentamicin	12%
Tetracycline	3%
Nitrofurantoin	3%
Penicillin	3%
Fosfomycin	3%

AMR, antimicrobial resistance; SSIs, surgical site infections.

stays, and higher healthcare costs. Effective prevention and control of SSIs are widely recognized as key indicators of surgical quality and patient safety[10–12].

In LMICs, SSIs remain a major concern due to limited access to sterile equipment, overcrowded hospitals, and suboptimal infection control practices. The incidence in these settings is often two- to fivefold higher than in high-income countries, resulting in substantial preventable morbidity and mortality[7,13–15].

Prior to this study, national data on SSIs in Albania were scarce[8,16]. The observed decline in SSI rates from 13.3% to 5.4% strongly correlates with recent infrastructural and organizational improvements. These include renovation of operating theaters, centralization of sterilization services, adoption of single-use surgical materials, and the integration of laparoscopic techniques. International evidence supports that ventilated, filtered operating rooms, centralized sterilization units, and disposable materials significantly reduce infection rates. Minimally invasive surgery further lowers SSI risk by reducing wound exposure[17–21]. Our stratified analysis confirmed this protective effect, as laparoscopic procedures demonstrated substantially lower SSI rates, emphasizing the importance of expanding minimally invasive surgery programs in tertiary hospitals.

Despite these advancements, risk factors for SSI in Albania remain consistent with global trends, including advanced age, comorbidities, and diabetes mellitus. The predominance of *Escherichia coli* and *Enterococcus* species among isolates reflects the gastrointestinal origin of contamination[22–24]. Importantly, no SSI-related deaths were observed, and overall postoperative mortality remained low (1.1%), with all deaths occurring in patients with advanced malignancies or severe comorbidities rather than active infections. These findings underscore the clinical significance of modern infection control measures and laparoscopic techniques in reducing morbidity and maintaining low mortality in LMIC surgical settings.

A significant concern is AMR. Resistance rates exceeding 30% for commonly used antibiotics—including ciprofloxacin, trimethoprim–sulfamethoxazole, cefazolin, and levofloxacin—threaten the effectiveness of both prophylactic and therapeutic regimens. In contrast, resistance to tetracycline, nitrofurantoin, penicillin, and fosfomycin remained low (< 5%). This pattern mirrors reports from the European Centre for Disease Prevention and Control documenting rising AMR trends across Europe[25–27]. Accordingly, antimicrobial stewardship programs are as critical as infrastructural and procedural improvements in sustaining progress against SSIs.

Remaining challenges in Albania include:

• Absence of a national SSI surveillance system: Current data are limited to individual hospital reports.

• Unquantified economic burden: Although prolonged hospital stays suggest increased costs, comprehensive analyses are lacking.

• Need for continuous staff training and protocol standardization: Consistent implementation of perioperative infection control measures across hospitals is essential.

Study Limitations

This study has several limitations. First, it was conducted in a single tertiary hospital, which may restrict the generalizability of the findings to other healthcare settings in Albania. Second, only abdominal surgical procedures were evaluated, excluding thoracic, cardiac, traumatic, pediatric, and other surgical categories. Third, the comparison of pre- and post-reconstruction data relied partly on previously published institutional records rather than matched patient cohorts, which may introduce variability in reporting standards. Additionally, the study did not assess long-term postoperative outcomes beyond the hospitalization period, potentially underestimating late-onset SSIs. Finally, although antimicrobial resistance patterns were analyzed, the study did not evaluate the adequacy or adherence to antibiotic prophylaxis protocols, an important determinant of SSI prevention.

Conclusion

This study demonstrates that Albania has achieved substantial reductions in SSIs through hospital modernization, centralized sterilization services, and the adoption of disposable materials and laparoscopic techniques. The SSI incidence decreased from 13.3% to 5.4%, confirming that targeted infrastructural and organizational reforms can significantly enhance patient safety. Despite these advancements, AMR remains a major concern, underscoring the need for a national SSI surveillance system, standardized perioperative protocols, and strengthened antimicrobial stewardship programs. Sustained investment in hospital infrastructure, staff training, and infection prevention initiatives will be essential to maintain these gains and expand their impact across the national healthcare system.

Ethics Committee Approval: The study complied with the ethical principles of the Declaration of Helsinki (1964) and subsequent amendments. The research protocol was approved by the Ethics Committee of the University of Medicine, Tirana, and the National Agency for Scientific Research and Innovation (approval number: 20, dated: 02.11.2023).

Informed Consent: Written informed consent was obtained from all participants prior to enrollment. All data were anonymized to ensure confidentiality.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.K., Concept: A.K., K.L., Design: K.L., E.P., Data Collection or Processing: V.D., E.S., Analysis or Interpretation: A.K., K.L., V.D., E.S., F.M., S.F., I.S., L.Z., E.P., Literature Search: A.K., K.L., V.D., E.P., Writing: E.P.

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A Laboratory Decision-Support System for Reflective Urine Culture Testing: Development of an Interpretable Artificial Intelligence Model

Reflektif İdrar Kültürü Testleri için Bir Laboratuvar Karar Destek Sistemi: Yorumlanabilir Bir Yapay Zekâ Modelinin Geliştirilmesi

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Abstract

Introduction: Urinary tract infections are a common diagnostic challenge. Although urine culture remains the gold standard, it is time-consuming and often ordered reflexively. This study aimed to develop and validate an interpretable machine-learning–based Laboratory Decision-Support System (LDSS) to guide reflective urine culture prioritization using only structured laboratory data.

Materials and Methods: We analyzed a retrospective cohort of 51,923 adult patients. Seven machine learning algorithms were trained, with the Random Forest (RF) model demonstrating the highest accuracy. SHapley Additive exPlanations was employed to ensure model interpretability. A reduced RF model, using the top 10 predictive features, was used to construct three scoring systems: one emphasizing model fidelity, one optimizing diagnostic balance, and one maximizing sensitivity.

Results: The RF model demonstrated excellent performance (external receiver operating characteristic – area under the curve [ROC-AUC]: 0.956). The simplified 10-variable model maintained high accuracy (ROC-AUC: 0.947). Key predictors included bacterial count, leukocyte count, nitrite presence, and patient age. The scoring systems offered flexible options tailored to different diagnostic priorities, with the SAFE-Score achieving 95.3% sensitivity.

Conclusion: The developed LDSS supports rational antibiotic use by reducing unnecessary culture testing. Its explainable structure facilitates collaboration between laboratory professionals and clinicians, contributing to standardized reflective testing workflows and interdisciplinary decision-making and strengthens antimicrobial stewardship, while preserving the central role of urine culture in infection management.

Keywords: Urinary tract infections, machine learning, urine culture

Öz

Giriş: İdrar yolu enfeksiyonları sık karşılaşılan bir tanı sorunudur. Altın standart olan idrar kültürü, hem zaman alıcıdır hem de çoğu zaman gereksiz yere istenir. Bu çalışmada, yalnızca yapılandırılmış laboratuvar verilerini kullanarak reflektif idrar kültürü istemine rehberlik edecek, yorumlanabilir bir makine öğrenimi (ML) tabanlı Laboratuvar Karar Destek Sistemi (LKDS) geliştirilmesi ve doğrulanması amaçlandı.

Gereç ve Yöntem: Retrospektif olarak 51.923 erişkin hastaya ait veriler incelendi. Yedi ML algoritması eğitildi; en yüksek doğruluk Rastgele Orman (Random Forest, RF) modelinde elde edildi. Model şeffaflığı için SHapley Additive exPlanations kullanıldı. En iyi 10 özellikten oluşan sadeleştirilmiş

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Öz

RF modeliyle üç farklı puanlama sistemi geliştirildi: Model doğruluğuna öncelik veren, tanısal dengeyi optimize eden ve hassasiyeti en üst düzeye çıkaran modeller.

Bulgular: RF modeli mükemmel performans gösterdi (harici testler – alıcı işletim karakteristiği eğrisi altında kalan alan [ROC-AUC]: 0,956). Basitleştirilmiş 10 değişkenli model yüksek doğruluğu korumuştur (ROC-AUC: 0,947). Temel öngörücüler arasında bakteri sayısı, lökositler, nitrit ve yaş yer almıştır. Skorlama sistemleri, farklı tanı hedeflerine göre uyarlanmış esnek seçenekler sunmuş ve SAFE-Skoru %95,3 hassasiyete ulaşmıştır.

Sonuç: Geliştirilen LKDS, gereksiz kültür sayısını azaltarak rasyonel antibiyotik kullanımını desteklemektedir. Açıklanabilir yapısı, laboratuvar profesyonelleriyle klinisyenler arasındaki iş birliğini kolaylaştırarak standartlaştırılmış reflektif test süreçlerine ve disiplinler arası karar vermeye katkı sağlar.

Anahtar Kelimeler: İdrar yolu enfeksiyonları, makine öğrenimi, idrar kültürü

Introduction

Urinary tract infections (UTIs) are among the most common infections in clinical practice, with an estimated global incidence exceeding 150 million cases annually[1]. They are associated with substantial healthcare costs, frequent antibiotic prescriptions, and increased diagnostic burden, particularly in outpatient and emergency settings[2,3]. Accurate diagnosis remains challenging due to nonspecific symptoms and reliance on time-consuming laboratory tests[4].

Urine culture is considered the gold standard for UTI diagnosis. However, its 24–48-hour turnaround often necessitates empiric antibiotic treatment before microbiological confirmation[5]. This practice contributes to antimicrobial resistance, now recognized by the World Health Organization as a global health threat[6]. Moreover, up to 60%–70% of urine cultures yield negative or clinically insignificant results, highlighting potential overuse of testing and therapy[7].

Rapid dipstick tests, detecting leukocyte esterase and nitrite, provide immediate screening but show variable performance across populations, with sensitivity and specificity ranging from 68% to 88% and 17% to 98%, respectively[8].

This diagnostic uncertainty has prompted efforts to improve laboratory decision-making, including the use of reflective testing. Reflective testing, increasingly recognized in modern laboratory medicine, involves laboratory physicians adding further analyses or interpretative comments after reviewing initial test results to enhance diagnostic reasoning[9]. In UTIs, this expert-led approach aids accurate interpretation and encourages more judicious use of microbiological testing. Laboratory physicians thus face the dual challenge of minimizing unnecessary culture requests while ensuring patients with a high likelihood of positive cultures are correctly identified.

In most laboratory information systems (LIS), detailed symptom information is not captured; only test orders and preliminary diagnoses, such as International Classification of Diseases (ICD) codes, are typically available. Consequently, the predictive modeling approach in this study relied solely on structured laboratory data. To address this, we developed a standardized, interpretable, and data-driven Laboratory Decision-Support System (LDSS) to optimize urine culture utilization using routine laboratory parameters. The LDSS is not intended to replace clinical diagnoses but to assist laboratory physicians in prioritizing reflex urine culture testing within laboratory workflows. Diagnostic responsibility remains entirely with the treating clinician, while the LDSS provides reproducible, standardized insights derived from LIS data.

Artificial intelligence (AI) and machine learning (ML) have gained increasing attention for developing predictive models in UTI diagnosis. Various algorithms—including Logistic Regression (LR), Random Forests (RFs), Extreme Gradient Boosting (XGBoost), Light Gradient Boosting Machine (LightGBM), and TabNet—have demonstrated robust performance using structured data such as urinalysis results, demographics, and clinical history[10–12]. Reported area under the receiver operating characteristic curve (AUROC) values commonly exceed 0.85, with some studies achieving 0.95 or higher in external validation cohorts[11,13].

Recent studies have highlighted the importance of model interpretability. By employing SHapley Additive exPlanations (SHAP), our LDSS not only ensures transparency but also facilitates clinical integration by illustrating the real-time contribution of each variable. Real-world implementations of ML-based LDSSs have shown reductions in unnecessary culture orders, accelerated treatment decisions, and improved antibiotic stewardship outcomes[12,14].

Despite these advances, challenges remain. Many predictive models are trained on single-center datasets and lack external validation, raising concerns about generalizability across institutions and diverse patient populations[13,15]. Additionally, variability in urinalysis platforms and clinical practice patterns may limit reproducibility and scalability.

Unlike existing tools, the proposed LDSS provides three distinct scoring systems tailored to different clinical priorities, ranging from high-sensitivity triage to specificity-focused decision-making. This flexibility promotes collaboration among biochemists, microbiologists, and clinicians while reducing diagnostic waste by minimizing unnecessary urine culture requests.

The aim of this study was to develop and externally validate a robust, interpretable ML-based LDSS to predict urine culture outcomes in patients with suspected UTIs. By standardizing reflective testing practices, the LDSS supports interdisciplinary decision-making, optimizes resource utilization, and ultimately contributes to rational antibiotic prescribing across healthcare settings.

Materials and Methods

Study Population/Subjects

This study was conducted at İzmir Tepecik Training and Research Hospital. Ethical approval was obtained from the University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital Non-Interventional Research Ethics Committee prior to study initiation (approval number: 2025/02-05, dated: 10.03.2025).

Eligible participants were adults aged ≥18 years who presented as inpatients or outpatients to the main hospital between January 1, 2014, and December 31, 2024, or to its affiliated hospital between January 1 and February 28, 2025. Inclusion criteria required patients to undergo their first urinalysis, complete blood count (CBC), and urine culture, ordered by a specialist physician based on clinical indication.

The study cohort included both culture-positive and culture-negative cases, capturing the full spectrum of patients for whom urine cultures were clinically indicated. Consequently, the dataset reflects real-world test-ordering practices rather than a biased subset of confirmed infections.

Patients were excluded if they had incomplete test results, missing sub-parameters, non-bacterial pathogens in their urine culture, delays exceeding one hour between urine sample collection and laboratory registration, delays exceeding 30 minutes for hemogram samples between phlebotomy and laboratory receipt, or a history of antibiotic treatment prior to testing.

CBC analyses were performed using UniCell DxH 800 analyzers (Beckman Coulter, Miami, FL, USA) from 2014 to 2020 and XN-2000 systems (Sysmex Corporation, Kobe, Japan) from 2020 onward. Urinalysis tests were conducted using fully automated analyzers across three periods: H-800 and FUS-200 systems (Dirui Industrial Co., Changchun, China) from 2014 to 2018; BT Uricell 1280–1600 (Bilimsel Products, İzmir, Türkiye) from 2018 to 2021; and U2610–U1600 (Zybio Corporation, Chongqing, China) from 2021 onward.

Midstream urine samples were collected in sterile containers simultaneously with urinalysis and processed according to standard microbiological procedures. Samples without detectable bacterial growth after 24 hours were incubated for an additional 24 hours; if no growth was observed, the result was reported as “no growth”.

Reagents and calibrators for urinalysis were obtained from authorized manufacturers and were certified and registered products. Quality control materials were sourced from Bio-Rad (California, USA). All results were reviewed and validated for accuracy and reliability by both a clinical biochemistry specialist and a clinical microbiology specialist.

Study Design

Patient identifiers were anonymized, and a dataset comprising age, sex, hemogram, urinalysis, and urine culture results from 55,385 patients (main hospital: 52,854; affiliated hospital: 2,531) was imported into Microsoft Excel 2021 (USA).

Symptom data were not included, as such information is not routinely recorded in LIS. In standard laboratory workflows, test orders are typically accompanied by preliminary diagnoses or ICD codes from the requesting physician, but detailed patient symptoms are not captured. Accordingly, the predictive model in this study was developed exclusively on structured laboratory data, aiming to forecast urine culture outcomes rather than to establish a clinical diagnosis of UTI.

After applying exclusion criteria, the final dataset included 49,720 patients, with an external validation cohort of 2,203 patients. The dataset was subsequently transferred to Python (version 3.13.1, USA) for ML analysis.

Following data cleaning, the main dataset was divided into training, internal test, and external test subsets using a 60:20:20 stratified sampling strategy based on the binary target variable, ensuring preservation of class distribution.

Patient flow throughout the study is depicted in Figure 1, in accordance with the Standards for Reporting Diagnostic Accuracy guidelines.

Data Preprocessing and Training of ML Algorithms

Patient data were initially exported from the LIS into Microsoft Excel. Hemogram values and flow cytometry parameters from urinalysis were used directly due to device standardization. Semi-quantitative dipstick results—reported by urinalysis analyzers as categorical values (e.g., “+,” “++,” “+/-,” “trace”)—were converted into numerical equivalents (e.g., “++” mapped to 2; “trace” standardized to 0.5) to ensure quantitative

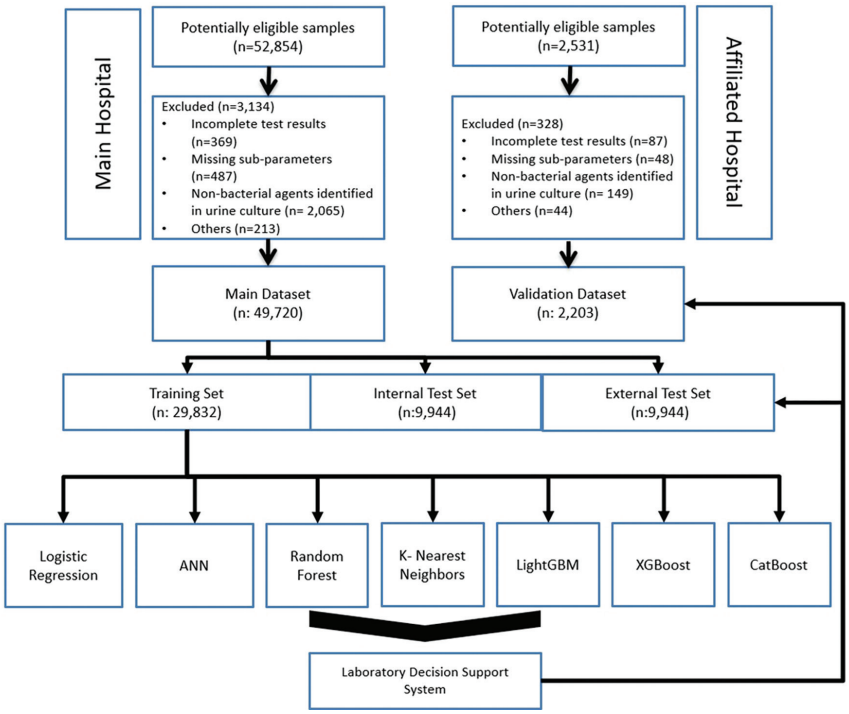

Figure 1. Standards for Reporting Diagnostic Accuracy flow diagram of study participants and urine culture testing.

consistency. Variables describing urine color and appearance were also recategorized by grouping similar classifications (e.g., light yellow to dark red; clear to very cloudy) to standardize the dataset.

Urine culture results were binarized as follows: samples with ≥10,000 colony-forming unit (CFU)/mL bacterial growth were defined as positive (label = 1), while samples with <10,000 CFU/mL, mixed flora, colonization, yeast, or no growth were classified as negative (label = 0).

The 10,000 CFU/mL threshold was selected based on recent evidence and the 2024 European Association of Urology guidelines, which acknowledge that lower colony counts ($\geq 10^3$–10^4 CFU/mL) may be clinically significant in symptomatic or catheterized patients[16]. Nelson et al.[17] demonstrated that these lower thresholds preserve diagnostic accuracy for symptomatic UTIs, supporting their use in reflective testing workflows. Additionally, Werneburg et al.[18] showed that urinalysis parameters reliably predict the absence of infection at this threshold, reinforcing its clinical validity. This definition also aligns with our institutional microbiology reporting standard for significant bacteriuria.

Yeast and colonization findings were labeled as negative (label = 0) based on established microbiological evidence and laboratory reporting standards. In urinary cultures, the presence of Candida species typically reflects colonization or contamination rather than true infection, even at colony counts exceeding 10^4–10^5 CFU/mL, unless accompanied by compatible clinical symptoms[19]. Classifying yeast as negative prevented false-positive propagation in the LDSS and improved the model's clinical specificity.

Similarly, cases labeled as "colonization"—including cultures with mixed flora or non-uropathogenic organisms—were considered negative. This approach aligns with standard microbiology practice, where such findings are reported as clinically non-significant. Although CLSI M100 (2025) does not define colony-count thresholds for colonization or candiduria, its terminology guided our categorization strategy. This interpretation reflects real-world laboratory workflows, ensuring that the LDSS mirrors standardized reporting logic and remains generalizable across institutions[20].

The cleaned dataset was transferred to Python for ML analysis. To enhance model robustness and address class imbalance, a stratified data partitioning scheme was applied, allocating 60% of samples to training and 20% each to internal and external testing. The dataset exhibited natural imbalance, with 22.4% culture-positive and 77.6% culture-negative samples. To mitigate majority-class bias, feature standardization and rebalancing strategies (class_weight='balanced') were applied uniformly across all classifiers.

As a preliminary check, a baseline LR model was trained and evaluated across all data splits. Receiver operating characteristic – area under the curve (ROC-AUC) scores (≈0.74, 0.73, 0.73 for training, internal, and external sets, respectively) and F1 scores (0.55, 0.54, 0.54) demonstrated consistent

generalization without evidence of overfitting or imbalance-driven inflation. The close alignment of these baseline metrics confirmed that stratified sampling preserved class proportions across all subsets (≈22.4% positive vs. 77.6% negative), ensuring reliable model development.

ML Model Selection and Development

The results confirmed that the methodological setup—including stratified sampling and proportional weighting—effectively mitigated class imbalance and provided a reliable foundation for model development. LR was used not as a primary model, but as a diagnostic tool to verify dataset integrity and the fairness of the training process[21].

Model development was performed in Python 3.13.1 using widely adopted libraries and workflows. Seven ML algorithms were evaluated for their suitability with the dataset and their potential effectiveness in predicting urine culture outcomes: RF, XGBoost, LightGBM, CatBoost, LR, Artificial Neural Network (ANN), and K-Nearest Neighbors (KNN).

Variables included in the analysis:

• Demographic: Age, sex

• Hemogram: White blood cell, neutrophil, lymphocyte, monocyte, eosinophil, basophil, hemoglobin (HGB)

• Urine Dipstick: Leukocyte esterase, nitrite, glucose, protein, pH, erythrocyte, bilirubin, urobilinogen, ketone

• Other Urinalysis: Urine color, urine density, appearance

• Flow Cytometry: Bacteria count, cylinder, yeast, urine leukocyte count

Data preprocessing, model training, evaluation, and visualization were conducted using open-source Python libraries:

• Data Processing and Analysis: pandas (v2.2.2), numpy (v2.0.2), optuna (v4.3.0)

• ML Model Development: scikit-learn (v1.6.1), XGBoost (v2.1.4), lightgbm (v4.5.0), catboost (v1.2.8), tensorflow (v2.10), keras (v2.10), torch (v2.6.0 + cu124)

• Model Evaluation and Visualization: matplotlib (v3.10), seaborn (v0.13.2), scipy.stats (v1.9), sklearn.metrics (v1.2), SHAP (v0.47)

Detailed hyperparameter optimization procedures, including search strategies and parameter configurations for each model, are provided in the Supplementary Table 1. Each model was retrained using the optimal hyperparameters identified during tuning. Final model evaluation was based on F1 and ROC-AUC scores derived from the internal test set.

Performance Evaluation

Performance evaluation was conducted using standard Python-based data science libraries. The modeling process was assessed comprehensively through internal cross-validation, hyperparameter tuning, and multiple performance metrics.

Classification Performance Metrics: Model discrimination and predictive capability were evaluated using:

• AUC-ROC

• Area under the precision-recall curve (AUC-PR)

• Sensitivity and Specificity

• Positive predictive value (PPV) and negative predictive value (NPV)

• Positive likelihood ratio (PLR) and negative likelihood ratio (NLR)

• F1 score

Model Interpretability Metrics: To enhance clinical transparency and foster trust in algorithmic decisions, interpretability was assessed using:

• Feature-Importance metrics

• SHAP graphs

This multidimensional evaluation approach balances predictive performance with explainability, providing a robust framework for forecasting urine culture outcomes based solely on laboratory and demographic data.

Development of the LDSS

The LDSS was built using the best-performing ML model identified during model selection. SHAP analysis was employed to select the ten most informative features, and a simplified model was retrained using only these variables. The reduced model maintained performance comparable to the full model, supporting its suitability for practical implementation.

Instead of the default probability threshold of 0.5, an optimized threshold based on Youden's J statistic was applied to improve sensitivity and minimize missed infections. Each selected feature was then converted into a binary indicator using individual cut-points derived from ROC analysis, enabling construction of a straightforward cumulative score.

Feature-importance values were normalized to derive clinically interpretable weights. Highly influential predictors received slightly higher weights, while moderately informative features were scaled conservatively to balance performance with interpretability. The final scoring system was recalibrated using internal data and externally evaluated, demonstrating preserved sensitivity and specificity. This streamlined, transparent design ensures that the LDSS is suitable for routine use within laboratory workflows.

Table 1. Baseline characteristics of the study population, including demographic, clinical, and laboratory variables.

Characteristics[a]	Unit	Main dataset (n = 49,720) mean ± SD	Training set (n = 29,832) mean ± SD	Internal test set (n = 9,944) mean ± SD	External test set (n = 9,944) mean ± SD	Validation set (n = 2,203) mean ± SD	p-value[b] (Main dataset vs. validation set)
Age		38.28 ± 26.85	38.07 ± 26.81	38.89 ± 26.99	38.29 ± 26.83	43.92 ± 28.53	<0.05
Male	Years	39.69 ± 28.20	39.33 ± 28.12	40.09 ± 28.39	40.37 ± 28.26	48.04 ± 28.38	<0.05
Female	Years	37.41 ± 25.96	37.29 ± 25.95	38.17 ± 26.07	37.03 ± 25.85	41.23 ± 28.33	<0.05
Gender							0.152
Male	n (%)	18,871 (38.0%)	11,358 (38.1%)	3,766 (37.9%)	3,747 (37.7%)	870 (39.5%)	
Female	n (%)	30,849 (62.0%)	18,474 (61.9%)	6,178 (62.1%)	6,197 (62.3%)	1,333 (60.5%)	
WBC	$\times 10^9$ cells/L	8.47 ± 4.63	8.5 ± 4.91	8.4 ± 3.86	8.45 ± 4.47	8.45 ± 3.48	0.795
Neutrophil	$\times 10^9$ cells/L	5.1 ± 3.4	5.11 ± 3.34	5.05 ± 3.13	5.11 ± 3.81	5.18 ± 3.14	0.244
Lymphocyte	$\times 10^9$ cells/L	2.45 ± 2.82	2.47 ± 3.27	2.42 ± 1.97	2.43 ± 1.99	2.36 ± 1.26	<0.05
Monocyte	$\times 10^9$ cells/L	0.68 ± 0.85	0.68 ± 1.01	0.68 ± 0.68	0.67 ± 0.37	0.67 ± 0.29	0.168
Eosinophil	$\times 10^9$ cells/L	0.2 ± 0.25	0.2 ± 0.25	0.2 ± 0.25	0.2 ± 0.24	0.19 ± 0.19	<0.05
Basophil	$\times 10^9$ cells/L	0.04 ± 0.06	0.04 ± 0.06	0.03 ± 0.05	0.04 ± 0.07	0.04 ± 0.03	1.000
HGB	g/dL	12.26 ± 1.91	12.26 ± 1.9	12.27 ± 1.92	12.27 ± 1.91	12.56 ± 1.98	<0.05
Bacteria count (urine)	/HPF	33.57 ± 124.45	33.55 ± 127.66	33.93 ± 120.89	33.24 ± 118.07	41.7 ± 157.49	<0.05
LYM (urine)	/HPF	53.46 ± 287.59	53.81 ± 288.38	52.32 ± 279.64	53.53 ± 293.02	64.28 ± 324.2	0.124
Yeast	/HPF	3.85 ± 133.83	5.04 ± 170.15	1.95 ± 36.1	2.2 ± 37.23	3.13 ± 55.43	0.587
Mucus	/HPF	11.32 ± 30.73	11.34 ± 30.69	10.97 ± 28.36	11.62 ± 33.03	22.14 ± 56.43	<0.05
Cylinder	/HPF	0.04 ± 0.22	0.04 ± 0.22	0.04 ± 0.23	0.05 ± 0.23	0 ± 0	<0.05
Density	-	1,016.98 ± 8.17	1,017.02 ± 8.14	1016.95 ± 8.23	1016.9 ± 8.22	1015.86 ± 7.19	<0.05
pH	-	5.9 ± 0.81	5.91 ± 0.82	5.89 ± 0.81	5.9 ± 0.81	6.05 ± 0.52	<0.05
Urine culture							<0.05
Positive	n	11,156 (22.4%)	6,694 (22.4%)	2,231 (22.4%)	2,231 (22.4%)	403 (18.3%)	1.000
Negative	n	38,564 (77.6%)	23,138 (77.6%)	7,713 (77.6%)	7,713 (77.6%)	1,800 (81.7%)	1.000

[a]Categorical variables were not included in this table. [b]Continuous variables were compared using Welch's t-test, and categorical variables were analyzed with Pearson's chi-square test. A p-value <0.05 was considered statistically significant.

Validation of the LDSS

An independent validation dataset, obtained from an affiliated hospital within the same healthcare network, was used to assess the generalizability and robustness of the LDSS through temporal validation. This temporally separated retrospective dataset was entirely independent of all model development phases, including training, feature selection, and score construction.

Performance of the reduced 10-variable RF model and the three derived scoring systems was evaluated within this separate clinical environment. Standard classification metrics were computed and compared with those from the original external test set, providing insight into the system's real-world applicability.

The validation strategy adheres to recommendations from the International Federation of Clinical Chemistry and Laboratory Medicine for evaluating diagnostic tools using independent datasets. This approach strengthens the clinical credibility of the LDSS by demonstrating reproducibility across diverse healthcare settings.

Statistical Analysis

Descriptive statistics are presented as means ± standard deviations (SDs) for continuous variables and as frequencies with percentages for categorical variables. Comparative analyses between the development and validation datasets were conducted using:

- Student's t-test for normally distributed continuous variables
- Welch's t-test for continuous variables with unequal variances or sample sizes
- Pearson's chi-square test for categorical variables
- Z-tests for proportions and McNemar's test for paired categorical outcomes, particularly for comparing model performance metrics across datasets

These statistical comparisons were used to evaluate diagnostic consistency and identify significant differences in classification outcomes, providing insight into the reproducibility and robustness of the LDSS across diverse clinical settings.

All p-values were two-sided, with statistical significance defined as $p < 0.05$. Analyses were conducted using Python 3.13 and its associated statistical packages.

Results

Dataset Description and Data Preprocessing

The analytical cohort comprised 51,923 patient encounters, including 49,720 records from the main institutional database and 2,203 from an affiliated tertiary center. The validation cohort was enriched with inpatients from high-acuity units, such as Palliative Care and Gynecologic Oncology, and was specifically used to assess the external validity of the LDSS.

The validation cohort demonstrated significantly higher age across all demographic strata (total: 43.92 vs. 38.28 years; males: 48.04 vs. 39.69; females: 41.23 vs. 37.41; all $p < 0.05$). Hematologic comparisons revealed statistically significant reductions in lymphocyte count (LYM) and eosinophil count, accompanied by a modest but significant increase in HGB levels ($p < 0.05$).

Among urinalysis variables, the validation group exhibited higher bacterial counts, increased mucus presence, and elevated pH levels, whereas urine specific gravity and cylinder counts were lower ($p < 0.05$ for all). No significant differences were observed in white blood cell (WBC), neutrophil, monocyte, or basophil counts, nor in leukocyte counts, yeast presence, or gender distribution (all $p > 0.05$). Although the proportion of urine culture-positive cases was numerically similar (22.4% vs. 18.3%), this difference reached statistical significance ($p < 0.05$), potentially reflecting distinct microbiologic or clinical characteristics in the validation population.

Overall, these findings indicate that while the two datasets are broadly comparable, the validation cohort exhibits distinct demographic and laboratory profiles, likely due to its inpatient composition. These differences should be considered when interpreting LDSS performance in more complex clinical settings. Detailed summary statistics and p-values for each variable are provided in Table 1.

Hyperparameter Tuning

Each ML model was trained and optimized to achieve optimal performance on our dataset. Final hyperparameter configurations, tailored to the structure of each algorithm, are summarized in the Supplementary Table 2.

Performance Metrics of ML Models

The performance of seven ML models was evaluated using both internal and external test datasets. Ensemble-based methods—RF, CatBoost, and XGBoost—consistently demonstrated high accuracy (≥0.929) and F1 scores (>0.83) across both datasets, highlighting their robustness for clinical prediction tasks.

On the external test set, RF outperformed all other models, achieving the highest ROC-AUC (0.956) and PR-AUC (0.907), indicating superior discrimination and precision-recall trade-off. CatBoost achieved the highest sensitivity (0.771) while maintaining balanced performance across other metrics.

KNN demonstrated exceptional specificity (0.988) and PPV (0.945) in the external set, making it particularly effective for ruling in cases. Conversely, LR, while computationally efficient, showed the lowest sensitivity and F1 scores, limiting its diagnostic utility.

Performance metrics from the external dataset closely mirrored those of the internal test set for all models, reinforcing their generalizability and stability. Comprehensive statistics for both datasets are provided in Table 2 and Figure 2.

Among all evaluated algorithms, RF exhibited the most consistent and highest overall performance, with an internal ROC-AUC of 0.952 (95% confidence interval [CI]: 0.948–0.956) and an external ROC-AUC of 0.956 [95% CI: 0.952–0.960], along with strong PR characteristics.

Given its superior accuracy, consistent generalizability, and interpretability, RF was selected as the core algorithm for integration into the LDSS. SHAP analysis was then performed on the final model to provide insight into the individual contribution of each feature to the predicted outcomes.

SHAP Analysis of the Optimal RF Model

Model interpretability was improved using SHAP, which quantifies the contribution of each feature to the predictions generated by the final RF model. As shown in Figure 3, the most influential features were

- Bacteria_Count (SHAP value: 0.061)
- Urine_Leu_Count (0.055)
- Nitrite (0.052)
- Age and Leukocyte Esterase (both 0.041)

Table 2. Classification performance metrics of the ML models, including accuracy, sensitivity, specificity, and AUC.

Model	Sensitivity	Specificity	PPV	NPV	Accuracy	F1 score	ROC-AUC	PR-AUC
Internal test set								
RF	0.758 (0.741–0.776)	0.985 (0.982–0.987)	0.934 (0.923–0.946)	0.934 (0.929–0.939)	0.934 (0.929–0.938)	0.838 (0.826–0.850)	0.952 (0.948–0.956)	0.897 (0.891–0.903)
XGBoost	0.768 (0.751–0.784)	0.976 (0.973–0.979)	0.902 (0.889–0.916)	0.936 (0.930–0.941)	0.929 (0.925–0.934)	0.830 (0.816–0.842)	0.930 (0.925–0.935)	0.861 (0.854–0.868)
LightGBM	0.681 (0.664–0.699)	0.972 (0.968–0.976)	0.876 (0.862–0.894)	0.913 (0.907–0.919)	0.907 (0.900–0.913)	0.766 (0.751–0.780)	0.916 (0.911–0.921)	0.825 (0.818–0.832)
CatBoost	0.764 (0.747–0.784)	0.980 (0.977–0.983)	0.918 (0.907–0.931)	0.935 (0.930–0.940)	0.932 (0.927–0.937)	0.834 (0.822–0.847)	0.930 (0.925–0.935)	0.861 (0.854–0.868)
LR	0.350 (0.330–0.370)	0.969 (0.965–0.973)	0.765 (0.738–0.791)	0.838 (0.830–0.846)	0.830 (0.823–0.837)	0.480 (0.459–0.501)	0.790 (0.782–0.798)	0.593 (0.583–0.603)
ANN (MLP)	0.561 (0.541–0.582)	0.943 (0.937–0.947)	0.738 (0.717–0.758)	0.881 (0.875–0.888)	0.857 (0.850–0.864)	0.637 (0.621–0.655)	0.844 (0.837–0.851)	0.698 (0.689–0.707)
KNN	0.723 (0.705–0.743)	0.984 (0.981–0.987)	0.929 (0.917–0.940)	0.925 (0.919–0.930)	0.925 (0.920–0.931)	0.813 (0.801–0.827)	0.947 (0.943–0.951)	0.903 (0.897–0.909)
RF (with top 10 variables)*	0.769 (0.761–0.777)	0.981 (0.979–0.984)	0.924 (0.919–0.930)	0.936 (0.931–0.941)	0.934 (0.929–0.939)	0.8397 (0.832–0.847)	0.947 (0.944–0.952)	0.890 (0.884–0.896)
External test set								
RF	0.76 (0.744–0.778)	0.987 (0.984–0.989)	0.943 (0.932–0.953)	0.935 (0.929–0.94)	0.936 (0.931–0.941)	0.842 (0.829–0.854)	0.956 (0.952–0.96)	0.907 (0.901–0.913)
XGBoost	0.767 (0.748–0.784)	0.980 (0.977–0.983)	0.917 (0.906–0.930)	0.936 (0.930–0.942)	0.932 (0.928–0.938)	0.836 (0.824–0.848)	0.932 (0.927–0.937)	0.877 (0.871–0.883)
LightGBM	0.686 (0.666–0.704)	0.976 (0.972–0.979)	0.892 (0.877–0.907)	0.915 (0.909–0.921)	0.911 (0.905–0.916)	0.776 (0.762–0.789)	0.919 (0.914–0.924)	0.840 (0.833–0.847)
CatBoost	0.771 (0.754–0.790)	0.982 (0.979–0.985)	0.924 (0.911–0.936)	0.936 (0.931–0.942)	0.934 (0.929–0.939)	0.840 (0.827–0.852)	0.929 (0.924–0.934)	0.875 (0.868–0.882)
LR	0.339 (0.321–0.358)	0.968 (0.964–0.972)	0.755 (0.725–0.781)	0.835 (0.828–0.842)	0.827 (0.819–0.834)	0.467 (0.445–0.487)	0.793 (0.785–0.801)	0.597 (0.587–0.607)
ANN (MLP)	0.565 (0.544–0.585)	0.937 (0.932–0.943)	0.722 (0.700–0.744)	0.881 (0.874–0.888)	0.854 (0.847–0.861)	0.634 (0.618–0.651)	0.846 (0.839–0.853)	0.707 (0.698–0.716)
KNN	0.719 (0.700–0.738)	0.988 (0.985–0.990)	0.945 (0.933–0.955)	0.924 (0.918–0.929)	0.927 (0.923–0.933)	0.817 (0.803–0.830)	0.947 (0.943–0.951)	0.905 (0.899–0.911)

*Reduced model including only the top 10 predictors selected by SHAP analysis: bacterial count in urine, urinary LYM, urinary nitrite test, patient age, leukocyte esterase activity in urine, HGB concentration, gender, LYM, urine density, and urinary erythrocyte count

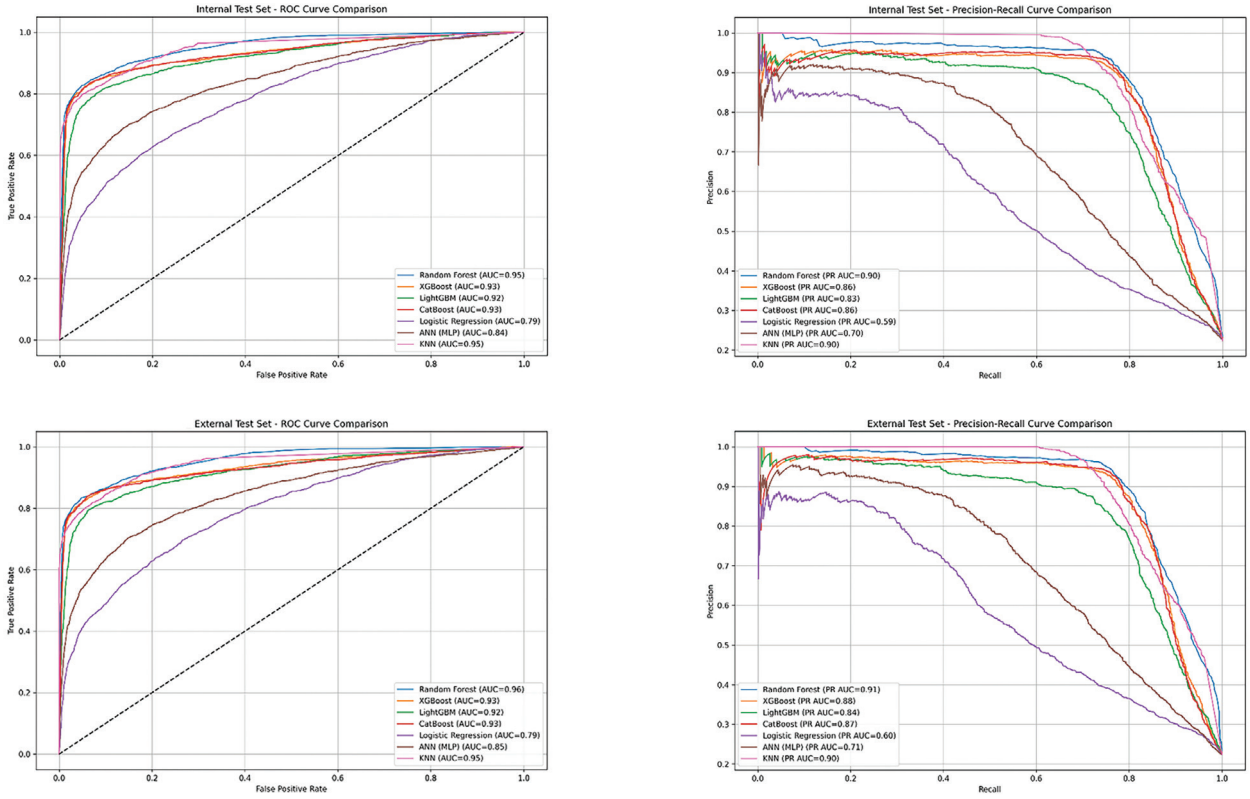

Figure 2. ROC and precision-recall PR curves illustrating the predictive performance of ML models.
PR, precision-recall.

These features correspond with well-established clinical markers of UTI, supporting the biological plausibility of the model.

Features with moderate importance included HGB, Gender, and LYM, with SHAP values ranging from 0.017 to 0.030. Features such as Bilirubin, Urobilinogen, and Ketone contributed minimally, each with SHAP values below 0.003.

Overall, the feature ranking confirms that the model primarily relies on clinically relevant variables, enhancing transparency and supporting its integration into laboratory decision-making.

Performance Metrics of the LDSS

A simplified RF model, built using the top 10 SHAP-derived features, maintained performance comparable to the full-feature model (ROC-AUC: 0.952 vs. 0.947; PR-AUC: 0.897 vs. 0.890), supporting its suitability for clinical implementation (Table 2). Based on these variables, three complementary scoring systems were developed to address distinct operational needs within laboratory workflows (Table 3):

• Model-Prioritized Score: Retains the behavior of the original ML model by assigning weights directly from normalized SHAP values. This version is ideal for institutions seeking high overall discrimination while remaining faithful to the underlying algorithm.

• Dual-Optimization Score: Adjusts feature weights to balance sensitivity and specificity, as reflected in stable metrics across both test datasets (Table 4, Figure 4). This score is intended for laboratories aiming to minimize both missed infections and unnecessary cultures.

• SAFE-Score: Optimized for high sensitivity and NPV, this score is suitable for safety-critical settings where missing true infections is unacceptable—such as high-acuity units, elderly populations, or immunocompromised patients. Its higher sensitivity comes at the expense of specificity, highlighting the trade-off between diagnostic conservatism and resource utilization.

Across all scoring systems, sensitivity remained consistent in external and independent validation cohorts, while specificity varied according to prioritization strategy (Table 4). Together, these tools provide laboratories with flexible options that can be tailored to local clinical priorities, test-ordering practices, and antimicrobial stewardship goals (Figure 4).

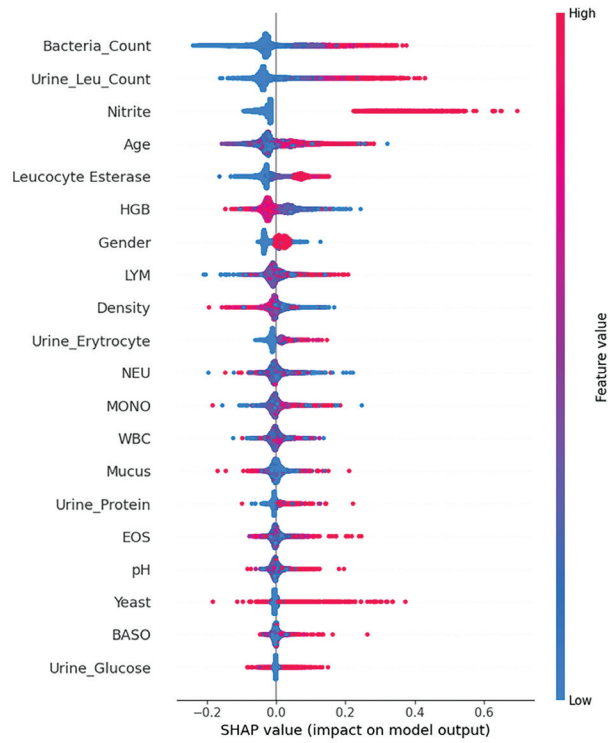

Figure 3. SHAP summary plot showing variable importance in the RF model.

Table 3. Confusion matrix–derived performance metrics of the ML models, including sensitivity, specificity, PPV, and NPV.

Feature	Threshold binarization	Normalized SHAP value	Model-Prioritized Score System[1]	Dual-Optimization Score[2]	SAFE-Score System (sensitive assessment for exclusion)[3]	Scientific justification
Bacteria count	>20, n	0.175	0.20	0.32	0.89	Major diagnostic marker for infection; emphasized clinically.
Urine LYM	>25, n	0.157	0.18	0.22	0.05	Strongly correlates with infection; slightly boosted for sensitivity.
Nitrite	= 1	0.147	0.17	0.15	0.77	Positive nitrite is a direct indicator of gram-negative bacterial activity.
Age	≥65 years	0.118	0.15	0.23	0.42	Increased risk in elderly population (>65 years).
Leucocyte esterase	>0	0.116	0.14	0.13	0.82	Biochemical indicator of leukocytes; moderate importance.
HGB	<12	0.085	0.10	0.12	0.71	Low HGB levels linked to increased infection susceptibility.
Gender	= 1 (Female)	0.062	0.08	0.04	0.06	Higher infection prevalence anatomically in females.
LYM	<1.5	0.051	0.06	0.1	0.65	Low lymphocyte count indicates immunosuppression risk.
Density	>1020	0.048	0.05	0.09	0.03	Higher urine density occasionally correlates with infection.
Urine erythrocyte	>0	0.047	0.05	0.1	0.77	Presence may suggest urinary tract pathology but less specific.

[1]The first system was developed using model-derived, data-driven thresholds and weighting. [2]The second system was designed to optimize both sensitivity and specificity, achieving balanced classification performance. [3]The third system prioritized minimizing false negatives, emphasizing maximum sensitivity and NPV.

Table 4. Performance metrics of the LDSS evaluated using both external test and validation datasets.

A. Results from the external test set.

Method	Sensitivity (%)	Specificity (%)	PPV	NPV	PLR	NLR	Accuracy	F1 score	ROC-AUC	PR-AUC
Model-Prioritized Score System[1]	55.94 (53.87–57.99)	85.83 (85.03–86.59)	53.31 (51.29–55.32)	87.07 (86.30–87.81)	3.95 (3.69–4.22)	0.51 (0.49–0.54)	79.1 (78.31–79.91)	54.59 (52.57–56.60)	70.88 (67.58–74.28)	54.62 (50.71–57.71)
Dual-Optimization Score System[2]	64.77 (62.76–66.72)	76.62% (75.67–77.55)	44.49 (42.79–46.20)	88.26 (87.47–89.01)	2.77 (2.47–3.07)	0.46 (0.44–0.49)	73.96 (73.09–74.82)	52.75 (51.03–54.46)	70.70 (68.70–72.70)	54.63 (52.72–56.72)
SAFE-Score System[3]	95.34 (94.38–96.14)	20.29% (19.41–21.20)	25.70 (24.77–26.66)	93.77 (92.51–94.83)	1.20 (1.03–1.136)	0.23 (0.21–0.25)	37.13 (36.18–38.08)	40.49 (39.44–41.55)	57.81 (57.80–57.83)	60.52 (55.55–65.58)

B. Results from the validation test set.

Method	Sensitivity	Specificity	PPV	NPV	PLR	NLR	Accuracy	F1 score	ROC-AUC	PR-AUC
Model-Prioritized Score System[4]	57.95 (53.00–62.78)	84.78 (83.04–86.41)	46.47 (43.09–49.89)	89.87 (88.74–90.85)	3.81 (3.32–4.37)	0.50 (0.44–0.56)	79.80 (78.06–81.46)	51.51 (47.30–55.37)	71.31 (68.60–73.94)	34.80 (31.05–38.61)
Dual-Optimization Score System[5]	66.50 (61.70–71.07)	76.48 (74.44–78.42)	39.19 (36.65–41.80)	90.92 (89.71–92.00)	2.83 (2.54–3.15)	0.44 (0.38–0.50)	74.63 (72.75–76.43)	49.36 (45.86–53.01)	71.40 (68.82–73.94)	32.35 (29.00–35.66)
SAFE-Score System[6]	94.87 (92.26–96.79)	24.30 (22.33–26.36)	22.22 (21.63–22.83)	95.40 (93.14–96.95)	1.25 (1.21–1.30)	0.21 (0.14–0.32)	37.40 (35.38–39.46)	36.08 (33.46–38.63)	59.58 (58.14–61.03)	22.03 (20.05–24.01)

[1]TP = 1,248; TN = 6,620; FP = 1,093; FN = 983. [2]TP = 1,445; TN = 5,910; FP = 1,803; FN = 786. [3]TP = 2,127; TN = 1,565; FP = 6,148; FN = 104.
[4]TP = 237; TN = 1,521; FP = 273; FN = 172. [5]TP = 272; TN = 1,372; FP = 422; FN = 137. [6]TP = 388; TN = 436; FP = 1,358; FN = 21.

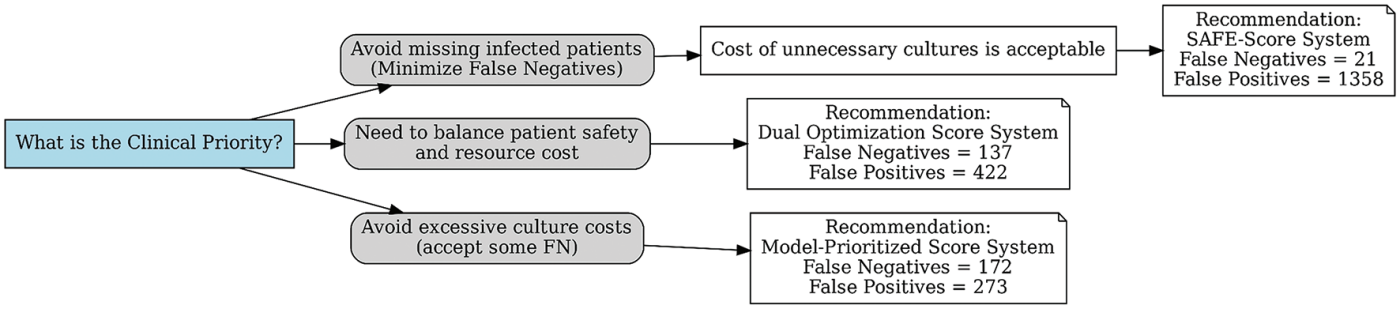

Figure 4. LDSS workflow illustrating selection criteria based on diagnostic accuracy and operational priorities.

Discussion

ML-based approaches offer substantial potential for the early diagnosis of UTIs. With the rising prevalence of antibiotic resistance, reducing unnecessary antibiotic use has become increasingly critical. Recent studies demonstrate that ML models improve diagnostic accuracy by integrating clinical symptoms, medical history, and urinary biomarkers, rather than relying solely on culture results[22].

Moreover, AI–driven decision-support systems can reduce diagnostic workload in hospitals, although their clinical validation remains limited[15]. Urinary biomarkers, such as nitrite and leukocyte esterase, exhibit high sensitivity for UTI diagnosis, yet their integration into ML models is essential to mitigate false-positive results[23]. AI-assisted methodologies are expected to be particularly beneficial for early detection of recurrent UTIs and multidrug-resistant pathogens, potentially improving patient outcomes and guiding more precise therapeutic interventions[23,24].

In this study, we evaluated the performance of multiple ML models in predicting urine culture outcomes and assessed their clinical applicability using explainable AI (XAI) techniques. Validation on a demographically and clinically distinct inpatient cohort further demonstrated the robustness and real-world adaptability of the LDSS. The incorporation of XAI enhanced interpretability, providing insight into the

decision-making process and supporting potential integration in complex healthcare settings.

The LDSS was developed using all physician-ordered urine culture requests, including both culture-positive and culture-negative cases. Consequently, the dataset reflects the complete real-world distribution of suspected UTIs encountered in laboratory practice, enabling the model to learn discriminative patterns for both infection and non-infection samples. Importantly, the LDSS functions solely as a laboratory-level decision-support tool rather than a diagnostic system. Its predictions are limited to variables available in the LIS and are intended to complement, not replace, physicians' diagnostic judgment.

Gender and Age-Related UTI Incidence

In our study, UTIs were significantly more common in female patients than in males. This finding aligns with existing literature and reinforces the well-established notion that women are more susceptible to UTIs due to urogenital anatomy, hormonal fluctuations, and lifestyle factors. Schmiemann et al.[1] reported that UTI incidence in women is four to five times higher than in men. Similarly, Hooton et al.[25] identified a higher risk in women attributable to a shorter urethra and variability in periurethral microbial flora. Additional risk factors include age, postmenopausal hormonal changes, and a history of recurrent infections.

Age also emerged as a critical determinant, with UTI incidence progressively increasing—particularly among women aged 65 years and older. While Foxman et al.[26] reported peak incidence in women aged 15–29, with a secondary rise in postmenopausal groups, and Møller et al.[11] linked estrogen depletion after age 50 to heightened susceptibility, our study identified older age (≥65 years) as an independent risk factor for positive urine culture in the LDSS model. This finding underscores the importance of incorporating age as a predictive variable and reflects the growing burden of UTIs in elderly populations.

Performance of ML Models

The predictive performance of the models developed in this study is consistent with, and in several cases surpasses, previously reported ML approaches for UTI prediction. Among the algorithms tested, ensemble-based models—particularly RF and CatBoost—demonstrated consistently high accuracy, balanced sensitivity and specificity, and favorable F1 scores. Compared to prior models reported by de Vries et al.[27] and Flores et al.[2], our RF model showed superior performance across multiple evaluation metrics. Likewise, our CatBoost implementation outperformed the model described by Mancini et al.[13], which exhibited lower AUC and F1 values in a comparable clinical context.

Tree-based gradient boosting methods, such as XGBoost and LightGBM, also performed robustly and yielded results similar to high-performing models developed by Choi et al.[5] and Lin et al.[28], indicating strong generalizability across diverse patient populations. In studies by Dhanda et al.[29] and Taylor et al.[30], RF and XGBoost models similarly demonstrated superior discriminatory capacity, achieving AUC-ROC values of 0.85 and 0.90, respectively.

The KNN model achieved precision metrics comparable to prior studies; however, its limited interpretability may constrain clinical adoption[7]. Conversely, LR, while highly interpretable, exhibited lower sensitivity and F1 scores—consistent with Ramgopal et al.[10], where the model tended to overpredict positive cases, reducing precision. ANN (MLP) models, though commonly employed in UTI prediction studies, demonstrated moderate performance in our dataset, slightly below previously reported benchmarks[2].

Overall, these results reinforce the value of ensemble ML methods in the context of a LDSS for UTI prediction. They offer high predictive accuracy and consistent performance across internal and external validation cohorts, supporting their applicability in real-world clinical settings.

Several studies have investigated ML–based urine culture prediction, varying in complexity and generalizability. Seheult et al.[31] developed a decision-tree algorithm across multiple institutions to identify urinalysis predictors of culture positivity, reporting ROC-AUC values of approximately 0.78–0.79; however, their study lacked external validation and interpretability assessment. By comparison, our model achieved higher discrimination during development (ROC-AUC = 0.94–0.96) under cross-validation. Following conversion into a simplified score-based LDSS, real-world performance remained consistent (ROC-AUC ≈ 0.70–0.72; F1 ≈ 0.50–0.55). This decline reflects the expected trade-off between model complexity and clinical interpretability, as the LDSS was designed for practical integration into LIS rather than maximizing algorithmic precision[31].

Sergounioti et al.[32] applied ensemble classifiers, including RF and XGBoost, to real-world laboratory data, achieving AUROC values of 0.79–0.82. However, their models combined clinical and laboratory parameters and lacked transparent feature-importance analysis. In contrast, our LDSS relied solely on structured laboratory data, achieved comparable discrimination (0.70–0.72), and preserved interpretability and reproducibility through rule-based score calibration via the Model-Prioritized and Dual-Optimization systems.

Sheele et al.[33] investigated bacteriuria prediction in an emergency-department cohort using mixed clinical–laboratory features, yielding AUC-ROC values of 0.86–0.93 depending

on the CFU/mL threshold. While their results were strong in a high-acuity population, our laboratory-only LDSS achieved comparable sensitivity (up to 95%) in routine diagnostic settings, highlighting its potential as a front-end decision-support tool for reflex culture testing.

Collectively, previous studies demonstrated the feasibility of ML-assisted urine culture prediction but often emphasized algorithmic performance over interpretability and clinical applicability. The present study addresses this gap by establishing a transparent, externally validated, and operational LDSS framework that maintains clinically acceptable performance while remaining fully interpretable and implementable within routine laboratory workflows.

Explainability and Feature Importance

SHAP-based feature-importance analysis in our study revealed a variable ranking that aligns with and extends existing literature. The most influential predictors were bacterial count, urine leukocyte count, nitrite, age, and leukocyte esterase. These findings are consistent with the meta-analysis by Devillé et al.[8], which reported that combining nitrite and leukocyte esterase yielded a sensitivity of 88% and specificity of 98% for UTI diagnosis. Similarly, Lachs et al.[34] demonstrated that integrating these parameters with clinical symptoms significantly improves diagnostic accuracy.

Notably, our model also identified HGB levels, sex, and LYMs as important features with relatively high SHAP values, suggesting sensitivity to broader systemic or demographic factors that may influence infection risk. This aligns with Zhao et al.[35], who reported age and sex among the top predictors in a SHAP-based post-urostomy UTI risk model, and Wang et al.[36], who found that systemic inflammatory markers and age were highly important in predicting post-surgical UTIs.

The predominance of microscopic urinalysis variables—particularly bacterial and leukocyte counts—over clinical or demographic features underscores the model's responsiveness to diagnostic biomarkers. This differentiates our approach from models such as Lee et al.[37], which focused on predicting antimicrobial resistance patterns but also leveraged SHAP analysis for interpretability.

Recent literature highlights the limitations of reflexive urine culture testing in the absence of clinical context. Munigala et al.[38] and others have shown that reflex algorithms triggered by markers like leukocyte esterase or nitrite may reduce test volume but compromise diagnostic precision when symptom data are unavailable. Fakih et al.[39] similarly argue that urinalysis alone is insufficient for accurate UTI diagnosis in asymptomatic patients, risking overdiagnosis and overtreatment.

Our study addresses the diagnostic gap through a reflective developed solely using structured laboratory data. Because symptom data are typically absent from LIS, the LDSS optimizes culture utilization within real-world laboratory constraints. Rather than functioning as an autonomous decision-maker or reflex trigger, the system serves as a reflective tool, providing SHAP-based analytical insights to support laboratory physicians' expert interpretation.

This reflective framework promotes standardized testing and interdisciplinary consultation. In equivocal cases, LDSS outputs can facilitate dialogue between laboratory and clinical teams, helping reconcile test reduction with diagnostic safety. Such an approach advances rational microbiological testing and provides a scalable model for clinician-laboratory collaboration[40].

The LDSS demonstrated robust predictive performance across internal and external datasets, supporting its seamless integration into routine laboratory workflows and reflective testing processes. The system is designed not to replace culture testing but to prioritize it based on evidence-driven probability, maintaining diagnostic stewardship.

To enhance accessibility for readers from diverse clinical and laboratory backgrounds, this study emphasizes the translational relevance of the LDSS over computational complexity. Its explainable design—supported by SHAP analysis and simplified scoring systems—enables non-technical users to interpret outputs transparently. While technical details were included to ensure methodological transparency and reproducibility, the interpretability of the system fosters trust, usability, and interdisciplinary communication between laboratory specialists and treating physicians. By promoting shared understanding of data-driven reasoning, the LDSS supports faster decision-making, improved test stewardship, and enhanced integration of laboratory insights into clinical workflows.

LDSS

Although symptom data were unavailable in the laboratory dataset, the LDSS was intentionally designed to function within the routine workflow of laboratory medicine, where test requests are frequently submitted without accompanying clinical narratives. By aligning the model with real-world laboratory constraints, the LDSS remains applicable and scalable across diverse clinical settings.

To improve interpretability and minimize unnecessary complexity, feature selection was applied to reduce the number of input variables. Prior studies have consistently demonstrated that parsimonious models are better suited for clinical implementation, as they are easier to interpret and maintain,

while preserving acceptable predictive performance[41,42]. Accordingly, subsequent model development was restricted to ten key parameters that did not result in a statistically or clinically meaningful decline in performance. This strategy ensured an optimal balance between model simplicity and predictive accuracy.

Several published studies have similarly developed LDSS frameworks based on urine culture data, including those reported by de Vries et al.[27], Dhanda et al.[29], Del Ben et al.[43], and Flores et al.[2] Among these, Del Ben et al.[43] employed a decision-tree-based approach, whereas the remaining studies selected RF as the primary algorithm. The LDSS developed by de Vries and colleagues demonstrated performance metrics comparable to those observed in the present study, with AUC-ROC values ranging from 0.70 to 0.80. Although their model achieved a higher PPV, its NPV was lower than that of our model, highlighting differences in clinical trade-offs between false-positive and false-negative predictions.

Notably, Dhanda et al.[29] and Flores et al.[2] implemented scoring systems that stratified patients into high- and low-risk groups, an approach that is conceptually aligned with the strategy adopted in the present study. Across key performance metrics, the predictive accuracy of their models was broadly comparable to that of our system.

What distinguishes our LDSS is the integration of three distinct predictive models within a unified decision-making framework. To our knowledge, this is the first study to report the implementation of such a multi-model structure for UTI prediction. This design enables clinicians and laboratory physicians to select among alternative strategies according to specific clinical priorities, such as maximizing case detection or minimizing unnecessary diagnostic testing.

Although the SAFE-Score achieved excellent sensitivity, its specificity was limited (approximately 20%), a trade-off that may raise concerns regarding potential overtesting. Importantly, the LDSS was intentionally designed to accommodate this limitation by offering three complementary scoring strategies, each reflecting a distinct clinical philosophy. These include prioritization of patient safety (SAFE-Score), balanced diagnostic performance (Dual Optimization), and strict adherence to model-derived predictions (Model-Prioritized). Rather than enforcing a one-size-fits-all solution, the LDSS functions as a flexible framework that facilitates consensus-based decision-making, allowing institutions to align model selection with local clinical expectations and operational priorities.

Crucially, the proposed system is not static. By continuously incorporating real-world data—particularly cases in which algorithmic recommendations are compared with expert laboratory physician judgments—the LDSS can be iteratively retrained and refined. As additional large-scale datasets are accumulated over time, improvements in specificity and overall diagnostic balance are anticipated, reflecting the inherent capacity of ML models to evolve with expanding data inputs. In this respect, the LDSS serves not only as an immediate decision-support tool but also as a scalable platform for continuous learning and performance optimization.

Within the Turkish healthcare context, reflective testing has not yet been systematically implemented. Nevertheless, the LDSS offers a structured and standardized framework that may facilitate its adoption, reduce inappropriate urine culture requests, and support antimicrobial stewardship initiatives. Moreover, the Ministry of Health of Türkiye has recently introduced a “Rational Laboratory Utilization” directive that explicitly promotes reflex and reflective testing practices [44]. This regulatory emphasis is expected to accelerate the integration of reflective testing into routine laboratory workflows, highlighting the timeliness and practical relevance of the proposed system.

Finally, the LDSS was designed for seamless integration into routine clinical practice through Microsoft Excel, a widely available and familiar platform in most healthcare settings. All three predictive models are embedded within a single interface and generate concurrent outputs, enabling direct comparison and transparent interpretation at the point of use.

Due to time constraints, the validation cohort was relatively small. Nevertheless, implementation of the LDSS within our hospital’s central laboratory is planned, where it will be deployed to support real-time microbiological decision-making. This implementation will allow prospective validation of the system within routine laboratory workflows, evaluation of its diagnostic impact, and quantification of downstream outcomes, including reductions in unnecessary urine cultures, shorter turnaround times, and improved antibiotic stewardship. In addition, future multicenter studies across diverse healthcare systems are planned, incorporating structured clinical variables such as symptomatology, comorbidities, and medication history to further enhance the model’s generalizability and clinical relevance.

Study Limitations

Although this study leveraged a large dataset and included external validation, several limitations should be acknowledged. First, all data were derived from a single healthcare network, which may limit generalizability to institutions with different patient populations, laboratory infrastructures, or clinical workflows. Second, the retrospective study design precluded assessment of the LDSS in real-time clinical decision-making; prospective implementation studies are therefore required to determine its effects on clinical practice and patient outcomes.

Third, the model relied exclusively on structured laboratory data and did not incorporate patient symptoms, comorbidities, medication history, or clinical notes—factors known to influence UTI risk assessment and antibiotic prescribing. In routine clinical care, integration of such information is primarily the responsibility of the treating physician, who orders diagnostic tests based on patient history, clinical presentation, and prevailing guidelines. In contrast, laboratory physicians are tasked with processing submitted specimens according to standardized pre-analytical and analytical protocols. Although pre-preanalytical factors, such as appropriate test selection, are important, these data are rarely available to LIS in a structured, analyzable format. Consequently, most LIS environments contain only coded test orders and limited demographic information, without access to patient symptomatology or detailed clinical context.

Within these real-world constraints, the LDSS was designed not as a replacement for clinical judgment but as a complementary, interpretable decision-support tool that standardizes reflective testing and promotes communication between laboratory and clinical teams. Accordingly, the system functions as a laboratory-based reflex testing prioritization tool rather than as a diagnostic or therapeutic decision-making platform.

Fourth, despite robust performance in both internal and external test sets, the relatively small independent validation cohort—enriched for high-acuity inpatients—may introduce spectrum bias and lead to overestimation of sensitivity in complex clinical populations. Fifth, although the conventional definition of significant bacteriuria is $\geq 10^5$ CFU/mL, this study adopted a $\geq 10^4$ CFU/mL threshold based on emerging clinical evidence and institutional practice. Future investigations should evaluate the effects of alternative thresholds on model calibration and performance across different clinical settings.

Sixth, scoring weights and feature thresholds were calibrated using a fixed probability cutoff and Youden's index derived from the present dataset. Optimal thresholds may vary across institutions and will require local adjustment to maintain the desired balance between sensitivity and specificity. Finally, while SHAP values were employed to enhance model interpretability, clinician acceptance, usability, and integration into routine workflows were not formally assessed. Future implementation studies are therefore essential to evaluate user engagement, potential alert fatigue, and cost-effectiveness prior to widespread clinical deployment.

Conclusion

We developed and preliminarily validated an interpretable, multi-model LDSS designed to improve the efficiency of urine culture utilization. By integrating ensemble ML approaches with SHAP-based interpretability, the system demonstrated strong discriminatory performance while offering flexible scoring strategies that prioritize sensitivity, specificity, or an optimized balance between the two. The LDSS has the potential to reduce unnecessary urine cultures, support antimicrobial stewardship efforts, and promote standardized, evidence-based laboratory decision-making.

Future work will focus on prospective, real-world implementation across diverse clinical settings. Planned enhancements include integration with electronic health record–derived clinical data, local calibration of decision thresholds, and systematic evaluation of clinical impact, user adoption, and cost-effectiveness. These steps are critical for translating this early-stage model into a scalable and clinically actionable decision-support tool.

Ethics Committee Approval: Ethical approval was obtained from the University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital Non-Interventional Research Ethics Committee prior to study initiation (approval number: 2025/02-05, dated: 10.03.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: F.D., İ.A., Concept: F.D., İ.A., Design: F.D., M.A., İ.A., Data Collection or Processing: F.D., M.A., İ.A., Analysis or Interpretation: F.D., A.D., Literature Search: F.D., M.A., A.D., Writing: F.D., A.D.

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Supplementary Table 1. https://d2v96fxpocvxx.cloudfront.net/d363ec1e-9e5e-4591-a00a-d656bfcabb80/content-images/218f51a5-ad5b-4d21-939d-cba6f579c419.pdf

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Case of Periprosthetic Joint Infection Associated with *Streptococcus dysgalactiae* subsp. *dysgalactiae*

Streptococcus dysgalactiae subsp. *dysgalactiae* ile İlişkili Periprostetik Eklem Enfeksiyonu Olgusu

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Abstract

Periprosthetic joint infection (PJI) is a serious complication following joint prosthesis implantation. Although *Streptococcus dysgalactiae* subsp. *equisimilis* is increasingly recognized as an emerging human pathogen, *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD) is traditionally considered an animal-associated organism. However, recent reports indicate that SDSD can infrequently cause invasive human infections. SDSD has been linked to PJIs, endocarditis, and cellulitis of the upper and lower extremities. To date, only two cases of SDSD-related PJI have been described in the literature. Here, we present a rare case of late-onset PJI caused by SDSD following knee arthroplasty.

Keywords: Periprosthetic joint infection, *Streptococcus dysgalactiae* subsp. *dysgalactiae*, streptococcal infection

Öz

Periprostetik eklem enfeksiyonu (PJI), eklem protezinin implante edilmesi sonrası ciddi bir komplikasyondur. *Streptococcus dysgalactiae* subsp. *equisimilis*, giderek daha fazla önemli bir insan patojeni olarak tanınırken, *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD) genellikle bir hayvan patojeni olarak kabul edilmektedir. Son yıllarda, SDSD'nin nadiren insanlarda enfeksiyonlara neden olabileceği bildirilmiştir. SDSD, periprostetik eklem enfeksiyonları, endokardit ve üst ve alt ekstremite selüliti ile ilişkilendirilmiştir. Bugüne kadar SDSD ile ilişkili iki periprostetik eklem enfeksiyonu vakası literatürde belgelenmiştir. Bu çalışma, diz protezi cerrahisi sonrasındaki geç dönemde meydana gelen bir SDSD kaynaklı periprostetik eklem enfeksiyonunu sunmayı amaçlamaktadır.

Anahtar Kelimeler: Protez eklem enfeksiyonu, *Streptococcus dysgalactiae* subsp. *dysgalactiae*, *streptokokal enfeksiyon*

Introduction

Periprosthetic joint infection (PJI) is one of the most serious complications of joint prosthesis implantation. Reported incidence rates range from 0.5% to 2% for knee prostheses and from 0.5% to 1.0% for hip prostheses[1]. PJIs are typically classified into three categories: early onset (<3 months postoperatively), delayed onset (3–12 months), and late onset (>12 months)[2]. Early-onset infections are usually linked to perioperative contamination, whereas late-onset infections often present acutely and arise from pathogens originating at sites of compromised skin integrity, following dental or gingival procedures, or through hematogenous spread from the respiratory or urinary tract[3].

The microbiological profile also differs by onset category. Early-onset PJIs are commonly caused by *Staphylococcus aureus*, Gram-negative bacilli, anaerobes, or polymicrobial flora. Delayed-onset infections are frequently associated with coagulase-negative staphylococci, *Cutibacterium* (formerly *Propionibacterium*) spp., or enterococci. Late-onset PJIs are

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most often due to *Staphylococcus aureus*, Gram-negative bacilli, or beta-hemolytic streptococci[4]. Although streptococci are less common than staphylococci, they account for a notable proportion of PJIs (8%–11%)[5].

Streptococcus dysgalactiae causes more than 80% of invasive infections attributed to beta-hemolytic streptococci outside groups A and B[6]. This organism, a Lancefield group C streptococcus, is divided into two subspecies: *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) and *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD)[7]. SDSD is a well-recognized cause of bovine mastitis and is traditionally regarded as an animal-associated pathogen. However, rare human infections have been documented, including PJI, septic shock, endocarditis, and cellulitis of the upper and lower extremities[8].

This case report describes a rare late-onset PJI caused by SDSD following knee arthroplasty.

Case Report

A 68-year-old man presented to the emergency department with a two-day history of swelling, left knee pain, and inability to bear weight on the affected limb. He reported no recent respiratory or urinary tract infections, dental procedures, or other events that might predispose to bacteremia. His medical history was notable for total knee arthroplasty performed two years earlier for gonarthrosis.

On examination, the patient was alert, oriented, cooperative, and in good general condition. Vital signs were within normal limits: temperature, 36.8 °C; blood pressure, 130/80 mmHg; and heart rate, 80 beats/min. The left knee demonstrated mild erythema, swelling, marked tenderness, and increased warmth. Range of motion was painful, and no sinus tract was observed.

Laboratory investigations revealed a hemoglobin level of 12.1 g/dL, white blood cell count of 13,890/mm³, erythrocyte sedimentation rate of 30 mm/hour, C-reactive protein level of 23.4 mg/dL, and procalcitonin level of 0.9 ng/mL. Anteroposterior radiographs of the knee showed no osseous abnormalities. Superficial ultrasonography demonstrated a loculated joint effusion measuring approximately 1 cm in maximum diameter.

Given the patient's symptoms, elevated inflammatory markers, and ultrasonographic findings, arthrocentesis of the left knee was performed, yielding 50 mL of purulent fluid. Synovial fluid analysis showed a leukocyte count >125,000/mm³ with 80% polymorphonuclear leukocytes. Gram staining revealed Gram-positive cocci and numerous leukocytes. The synovial glucose level was undetectable, while the simultaneous blood glucose level was 155 mg/dL.

According to the 2021 European Bone and Joint Infection Society criteria, the patient was diagnosed with prosthetic joint infection. Empirical therapy with levofloxacin 750 mg/day and teicoplanin 400 mg/day was initiated after synovial fluid samples were sent for culture. On the second day of treatment, the erythema around the knee progressed, raising concerns regarding possible levofloxacin resistance; therefore, ceftriaxone 1 g twice daily was added.

Culture of the synovial fluid identified SDSD using the Vitek® MS MALDI-TOF (BioMérieux) system. Antibiotic susceptibility testing demonstrated sensitivity to vancomycin, teicoplanin, trimethoprim–sulfamethoxazole, clindamycin, and penicillin; resistance to tetracycline; and intermediate susceptibility to levofloxacin. Based on these results, empirical therapy was revised: levofloxacin and teicoplanin were discontinued, and ceftriaxone 1 g twice daily was continued.

The orthopedic team confirmed the diagnosis of prosthetic joint infection and recommended a two-stage revision procedure. During the first stage, the prosthesis was removed and extensive debridement of soft tissue and bone was performed. Antibiotic-loaded bone cement was then implanted. At least three periprosthetic tissue specimens were obtained intraoperatively, although culture remained negative, likely due to the prior initiation of antibiotics.

By the third week of ceftriaxone therapy, the patient demonstrated marked clinical and laboratory improvement. Before discharge, the antimicrobial regimen was transitioned to oral amoxicillin–clavulanic acid (1 g twice daily) to complete a six-week treatment course.

DISCUSSION

Streptococcus dysgalactiae is a virulent organism capable of causing severe infections such as cellulitis, necrotizing soft tissue infections, and streptococcal toxic shock syndrome, often presenting with clinical features similar to those of Streptococcus pyogenes[9]. In 1996, Vandamme et al.[10] reclassified *Streptococcus dysgalactiae* into two subspecies: SDSE, predominantly a human pathogen, and SDSD, traditionally regarded as an animal pathogen[10]. SDSE is increasingly recognized as a significant pathogen in humans and is associated with pharyngitis, skin and soft tissue infections, bacteremia, endocarditis, and septic arthritis[11,12]. Although SDSD is primarily linked to bovine mastitis, sporadic human infections have been described in recent years[12].

Reports in the literature have documented SDSD-associated prosthetic joint infections, endocarditis, and both upper and lower extremity cellulitis[10]. Zoonotic transmission is believed

to play a key role, particularly among individuals with occupational or environmental exposure to fish, livestock, or other animals, as well as among immunocompromised patients[13]. Koh et al.[12] described three cases of SDSD-related soft tissue infection following injuries sustained while handling raw seafood. Nathan et al.[10] reported a case of septic shock secondary to SDSD-related upper extremity cellulitis in 2021. The first published case of SDSD-related prosthetic joint infection was reported by Park et al.[13] in 2012, followed by a second case reported by Afacan et al.[14] in 2024, in which animal contact and chronic comorbidities were identified as potential risk factors.

In the present case, the patient had no history of direct animal exposure or contact with seafood; however, he resided in a rural area and regularly consumed unpasteurized milk. Additionally, the patient was receiving disease-modifying antirheumatic drugs for rheumatoid arthritis, which may have contributed to immune compromise. Given these factors, we suspect that consumption of unpasteurized milk could have been a plausible route of exposure, supporting the view that SDSD may act as an opportunistic pathogen in susceptible hosts.

The optimal management of human infections caused by *Streptococcus dysgalactiae* has not been fully established[15]. Given the phenotypic similarity between SDSE and SDSD strains, it is hypothesized that treatment strategies effective for SDSE may also be applicable to SDSD-related infections[15]. *Streptococcus dysgalactiae* is generally susceptible to penicillin, and standard therapy often involves penicillin or third-generation cephalosporins. In cases of bacteremia, ceftriaxone is recommended, whereas vancomycin is preferred in patients with severe beta-lactam allergies[16]. Although *Streptococcus dysgalactiae* rarely develops resistance to penicillin or cephalosporins, resistance to quinolones and macrolides has been reported[10].

Previous reports illustrate diverse treatment approaches. Afacan et al.[14] described a prosthetic joint infection treated with surgical debridement, followed by intravenous teicoplanin (400 mg daily for six weeks) and sequential oral therapy with fusidic acid (500 mg three times daily) and levofloxacin (500 mg daily); the patient remained asymptomatic during one-year follow-up. In Park et al.[13] case of SDSD-related prosthetic joint infection, vancomycin (2 g/day) was administered for seven weeks due to oxacillin-resistant SDSD, followed by a two-stage reimplantation. Postoperative reassessment revealed increased warmth, pain, and elevated acute-phase reactants, prompting an additional six-week course of secapene pivoxil hydrochloride (300 mg/day), after which no clinical deterioration was observed over six months[13]. In the cellulitis cases reported by Koh et al.[12], ciprofloxacin and clindamycin were successfully used in patients with documented cephalosporin allergy.

Conclusion

In summary, the optimal duration, selection, and combination of antibiotics for SDSD-related prosthetic joint infections remain controversial. In the present case, levofloxacin demonstrated intermediate susceptibility; therefore, therapy was continued with ceftriaxone (1 g twice daily). After three weeks, marked clinical improvement allowed transition to oral amoxicillin–clavulanic acid (1 g twice daily) to complete a six-week course. The patient was subsequently discharged in stable condition.

Ethics

Informed Consent: Written informed consent was obtained.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.S., S.A.T., Z.B.Y., Concept: E.S., S.A.T., Z.B.Y., Design: E.S., S.A.T., Z.B.Y., Data Collection or Processing: E.S., S.A.T., Z.B.Y., Analysis or Interpretation: E.S., S.A.T., Z.B.Y., Literature Search: E.S., S.A.T., Z.B.Y., Writing: E.S., S.A.T., Z.B.Y.

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Generalized Tetanus in an Unvaccinated Adult Following Foot Injury: A Case Report

Ayak Yaralanmasını Takiben Aşısız Bir Erişkinde Gelişen Jeneralize Tetanos: Olgu Sunumu

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Abstract

Tetanus is an acute and potentially fatal infection caused by *Clostridium tetani*, characterized by profound neuromuscular involvement. Although effective treatment options remain limited, tetanus remains a significant public health concern because it is entirely preventable through vaccination. Here, we describe a case of generalized tetanus in a 21-year-old unvaccinated man who developed the disease after a nail puncture wound sustained at a construction site. The patient presented with classic manifestations, including trismus, risus sardonicus, generalized muscle rigidity, and autonomic instability. Despite prompt administration of tetanus vaccine, human tetanus immunoglobulin, and metronidazole, he experienced worsening muscle spasms and respiratory compromise that necessitated mechanical ventilation and deep sedation in the intensive care unit. His clinical course was further complicated by ventilator-associated pneumonia and subglottic stenosis related to prolonged intubation, the latter of which was successfully treated with bronchoscopic dilation. Following 70 days of intensive care and inpatient management, the patient recovered and was discharged. This case underscores the essential role of vaccination in preventing tetanus and highlights the complexities involved in the multidisciplinary management of severe tetanus.

Keywords: *Clostridium tetani*, tetanus, tetanus prophylaxis

Öz

Tetanos, *Clostridium tetani* adlı anaerobik bakterinin ürettiği nörotoksin tetanospazminin etkisiyle nöromüsküler sistemi tutan, tonik kas spazmları ile karakterize ve mortalitesi yüksek potansiyel olarak ölümcül bir enfeksiyon hastalığıdır. Bu çalışmada, inşaat alanında ayağına çivi batması sonrası tetanos aşısı olmayan 21 yaşındaki erkek hastada gelişen genelleşmiş tetanos olgusu sunulmaktadır. Hastada trismus, risus sardonicus, yaygın kas rijiditesi ve otonomik disfonksiyon gibi tipik klinik bulgular gözlenmiştir. Erken dönemde tetanos aşısı, insan tetanos immünoglobulini ve metronidazol tedavisi başlanmasına rağmen hastada ilerleyici kas spazmları ve solunum yetmezliği gelişmiş, yoğun bakım koşullarında mekanik ventilasyon ve sedasyon uygulanmıştır. Klinik seyir sırasında ventilatör ilişkili pnömoni ve uzun süreli entübasyona bağlı subglottik stenoz gibi komplikasyonlar ortaya çıkmış, bronkoskopik dilatasyon ile başarılı şekilde tedavi edilmiştir. Hasta 70 günlük yoğun bakım ve servis takibi sonrasında klinik iyileşme göstererek taburcu edilmiştir. Bu olgu, tetanosun önlenmesinde aşılamanın kritik önemini ve ağır tetanos vakalarının multidisipliner yönetimindeki zorlukları vurgulamaktadır.

Anahtar Kelimeler: *Clostridium tetani*, tetanos, tetanus profilaksisi

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Introduction

Tetanus is an acute, potentially fatal infectious disease caused by the anaerobic bacterium *Clostridium tetani*, which affects the neuromuscular system through a potent neurotoxin and is characterized by tonic muscle contractions[1]. *Clostridium tetani* exists in soil and the gastrointestinal tract of mammals in the form of resilient spores. These spores can enter the body through skin injuries, where they transform into a vegetative form and produce the neurotoxin tetanospasmin[2]. Tetanospasmin induces widespread, simultaneous contraction of agonist and antagonist muscle groups, a phenomenon known as tetanic spasm. The toxin primarily targets motor endplates at the neuromuscular junction, resulting in rigidity, muscle spasms, and autonomic dysfunction[3].

Tetanus manifests in several clinical forms, including generalized, localized, cephalic, and neonatal tetanus, with generalized tetanus being the most common and severe[4]. Diagnosis is primarily clinical, based on characteristic muscle rigidity and spasms, combined with a history of insufficient immunization and recent trauma[5]. Although vaccination programs have largely reduced its incidence, tetanus remains a public health concern in developing countries due to incomplete immunization coverage[6]. This case report aims to contribute to the literature by presenting a patient who developed generalized tetanus following a nail puncture injury in the absence of prior vaccination.

Case Report

A 21-year-old man with no known chronic illnesses presented to an external healthcare center with difficulty swallowing, sore throat, and abdominal and back pain. He was subsequently referred to our hospital's emergency department with a preliminary diagnosis of tetanus. Detailed history revealed that approximately five days earlier, he had stepped on a nail at a construction site and had not sought medical care for wound management or tetanus vaccination.

Five days after the injury, he developed pain in the nape, back, and abdomen, accompanied by muscle spasms and dysphagia. On admission, his vital signs, including temperature, were within normal limits. Physical examination revealed a conscious, oriented, and cooperative patient with the characteristic "risus sardonicus" (sardonic smile) (Figure 1). Additional findings included neck stiffness, trismus, and generalized hypertonia. Abdominal palpation demonstrated a "board-like" rigidity. Examination of the right foot revealed a 0.5 × 1 cm non-necrotic puncture wound. No surrounding lesions required debridement, so the wound was left intact.

Laboratory investigations showed leukocytosis (14,000/mm³), markedly elevated creatine kinase (CK, 2,522 U/L; reference range 30–200 U/L), normal C-reactive protein (0.41 mg/dL), and creatinine (1 mg/dL). Given the patient's unknown immunization status, history of nail puncture, and classic clinical features, a preliminary diagnosis of tetanus was made. Treatment was initiated with intravenous (IV) metronidazole 500 mg four times daily.

The patient was admitted to the intensive care unit for close monitoring in a quiet, dark environment to minimize muscle spasms. Oral baclofen (20 mg twice daily) was initiated to control spasms, and tramadol (50 mg three times daily) was administered for severe muscle pain. On the second day of hospitalization, worsening muscle spasms and increased respiratory difficulty necessitated elective intubation, and sedation was initiated with IV midazolam (5 mL/hour) and fentanyl (1 mL/hour).

As spasms persisted, baclofen was increased to 20 mg three times daily, and continuous rocuronium infusion (2 mL/hour) was added to achieve neuromuscular blockade. On the fifth day, the patient developed fever and elevated acute-phase reactants; thoracic computed tomography revealed pneumonic infiltration, raising suspicion for ventilator-associated pneumonia. Empirical antimicrobial therapy was initiated with meropenem (1 g three times daily) and tigecycline (100 mg loading dose followed by 50 mg twice daily intravenously). Upon detection of Acinetobacter baumannii in aspirate culture, meropenem was discontinued, and colistin (300 mg loading dose followed by 150 mg twice daily IV) was administered. Antibacterial therapy was completed over a total of 14 days.

On the eighth day, persistent muscle spasms despite baclofen and rocuronium prompted initiation of dexmedetomidine infusion (5 mL/hour) for additional analgesic and anxiolytic support. By the 25th day, clinical stabilization allowed sequential discontinuation of rocuronium, dexmedetomidine, and midazolam. During follow-up, the patient experienced prolonged fever and tachycardia; with no other infection focus identified, these findings were attributed to autonomic dysfunction.

Following clinical stabilization, the tracheostomy was closed on day 40, and the patient was transferred to the Infectious Diseases Service. On day 60, he developed acute stridor and dyspnea, raising suspicion of tracheostomy-related complications. Thoracic surgery consultation and bronchoscopic evaluation revealed subglottic stenosis secondary to prolonged intubation, which was successfully treated with rigid bronchoscopy and dilation. The patient's condition gradually improved, and he was discharged fully recovered on day 70 of hospitalization.



Figure 1. Risus sardonicus.

Discussion

In resource-rich countries, the incidence of tetanus has declined dramatically since the 1940s due to universal tetanus toxoid vaccination programs[5]. In contrast, tetanus remains endemic in resource-limited settings[7]. Between 2013 and 2022, a total of 267 tetanus cases and 13 deaths were reported in the United States[8]. According to World Health Organization (WHO)/United Nations Children's Fund Estimates of National Immunization Coverage, DTP1 (the first dose of the diphtheria, tetanus, and pertussis vaccine) coverage in Türkiye has remained consistently high, ranging from 94% to 98% between 2020 and 2024, indicating that childhood tetanus immunization is well maintained nationally[9].

However, surveillance data from the WHO indicate that tetanus has not been completely eliminated in Türkiye. Reported cases numbered 9 in 2020, none in 2021, 10 in 2022, 12 in 2023, and increased markedly to 34 in 2024. These data suggest that tetanus remains a rare but persistent public health concern with fluctuations in incidence over time[9]. Vaccination significantly reduces the risk of tetanus, as demonstrated in a study of 53 cases by Saltoglu et al.[10], in which only 28.3% of patients had received a single tetanus vaccine dose prior to hospital admission, while 71.7% had not received primary immunization.

The incubation period of tetanus averages 8 days, typically ranging from 3 to 21 days[8]. In a Turkish study by Saltoglu et al.[10], the mean incubation period was 11.5 days (range: 2–40 days), with 22.6% of patients presenting within 7 days and 77.3% after 7 days[10]. Case reports demonstrate considerable variability: Kaya et al.[11] reported an incubation period of 2 days, Kuzucuoğlu et al.[12] observed symptom onset at 15 days post-exposure, and Sah et al.[5] reported onset at 7 days. In the present case, symptoms appeared on day 5 post-exposure, consistent with the reported range.

Tetanus is primarily a clinical diagnosis, based on characteristic findings in the absence of alternative explanations, as no laboratory test can definitively confirm or exclude the disease[8]. Generalized tetanus, observed in approximately 80% of cases, is the most common form[3]. Patients typically present with tonic contractions and intermittent severe spasms of skeletal muscles. Trismus is the most frequent early sign, followed by dysphagia and neck stiffness[13]. As the disease progresses, continuous and widespread muscle spasms may occur, leading to rigidity of the back muscles. Spasms can be severe enough to cause opisthotonus or fractures of long bones and vertebrae[14].

In tetanus, consciousness and awareness are typically preserved, so muscle spasms are associated with severe pain. Involvement of the facial muscles may produce the characteristic sardonic smile, or risus sardonicus. When autonomic dysfunction occurs, patients may exhibit blood pressure fluctuations, tachycardia, and fever[15]. In a retrospective analysis of 29 tetanus cases, Pérez-González et al.[16] reported trismus, or "lockjaw," as the most common clinical finding.

Our patient presented to the emergency department approximately five days after stepping on a nail, with complaints of dysphagia, headache, and pain in the back, neck, and abdomen. During hospitalization, signs of autonomic dysfunction—including fever, tachycardia, and hypertension—were noted in addition to generalized muscle spasms. Given the history of nail puncture and widespread hypertonia, tetanus was strongly suspected.

Comprehensive, universally accepted clinical guidelines for tetanus management remain limited, largely because tetanus predominantly occurs in resource-limited settings, which hinders the conduct of randomized controlled trials[17]. Evidence supporting the effectiveness of tetanus treatments is scarce, with only six randomized controlled trials published over the past 20 years[18].

The primary principles of tetanus management include neutralization of tetanus toxin, antibiotic therapy, wound debridement to prevent further toxin production, and mitigation of toxin effects on the central nervous system[19]. Early removal of infected tissue and foreign bodies is critical to preventing toxin production[20]. However, inadequate wound debridement can reduce the efficacy of antimicrobial therapy against *Clostridium tetani*, as demonstrated in a

study of 84 Vietnamese patients with severe tetanus, in which 45 *Clostridium tetani* isolates were recovered from wound samples[21]. Preferred antibiotics include metronidazole and penicillin, typically administered for 7–10 days[20].

In our patient, a 0.5 × 1 cm non-necrotic puncture wound was present on the right foot. As no surrounding tissue required debridement, the wound was left intact. Given the high clinical suspicion for tetanus, IV metronidazole 500 mg four times daily was initiated.

Tetanus toxin binds irreversibly to neural tissues, so only circulating free toxin can be neutralized. Consequently, passive immunization through administration of tetanus antitoxin is associated with reduced mortality[22]. Tetanus immunoglobulin (TIG) should be administered intramuscularly as soon as possible after diagnosis. Because TIG neutralizes only free toxin, early administration can prevent progression by blocking toxin entry into the nervous system. Previously, doses of 3,000–6,000 units were suggested; however, current Centers for Disease Control and Prevention guidelines recommend a single intramuscular dose of 500 units[8].

As natural immunity is not conferred following tetanus infection, all patients require a full active immunization series. Vaccination should be initiated at the time of diagnosis[23]. Accordingly, in our patient, who presented with a provisional diagnosis of tetanus and unknown vaccination status, a single intramuscular dose of 500 units TIG was administered at multiple anatomical sites, along with one dose of tetanus vaccine. A schedule for primary immunization was planned, with two additional tetanus vaccine doses at 1 and 6 months.

The principal determinant of tetanus-related mortality is generalized muscle spasms, which may lead to life-threatening complications, including respiratory failure, aspiration, and multisystem dysfunction. Although high-quality evidence is limited, sedation and muscle relaxants, combined with mechanical ventilation when indicated, have been shown to improve survival. Benzodiazepines, such as diazepam and midazolam, act by enhancing gamma-aminobutyric acid receptor activity and are commonly used for muscle relaxation[24]. Neuromuscular blocking agents are indicated in severe cases when sedation alone is insufficient.

In the present case, oral baclofen (20 mg twice daily) was initially administered for pre-intubation muscle spasms, along with tramadol (50 mg three times daily) for severe pain. On the second day of hospitalization, worsening spasms prompted elective intubation and transition to IV midazolam and fentanyl for sedation. Persistent spasms necessitated escalation of baclofen and initiation of continuous rocuronium (esmeron) infusion for neuromuscular blockade. With the onset of autonomic dysfunction, including fever and tachycardia, dexmedetomidine—an alpha-2 adrenergic agonist with sedative, analgesic, and sympatholytic properties—was added. Following clinical stabilization, doses of sedatives and spasmolytics were gradually reduced, and by day 25 all such therapies were discontinued. The patient's condition stabilized, and he was transferred to the Infectious Diseases Service on day 40 of hospitalization.

Although tetanus is rare in the modern era, case reports continue, particularly among unvaccinated individuals in developing countries. In cases of penetrating trauma, absent or incomplete vaccination, or the presence of suspicious clinical signs, tetanus should always be considered. For patients with uncertain immunization status, the need for both tetanus vaccine and TIG should be evaluated regardless of wound characteristics. Early recognition, prompt administration of TIG and antibiotics, and appropriate supportive care remain crucial in reducing morbidity and mortality associated with tetanus.

Ethics

Informed Consent: Written informed consent was obtained.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.S., F.M., F.Ö., Concept: E.S., F.M., F.Ö., Design: E.S., F.M., F.Ö., Data Collection or Processing: E.S., F.M., F.Ö., Analysis or Interpretation: E.S., F.M., F.Ö., Literature Search: E.S., F.M., Writing: E.S.

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CASE REPORT / OLGU SUNUMU

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Unveiling the Unexpected: Two Rare Cases of Human *Fasciola hepatica* Infection

Beklenmeyeni Ortaya Çıkarma: İnsanlarda *Fasciola hepatica* Enfeksiyonunun İki Nadir Vakası

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Abstract

Fascioliasis is a parasitic zoonotic infection caused by the liver flukes *Fasciola hepatica* and *Fasciola gigantica*. Although herbivorous mammals such as cattle, sheep, horses, and buffalo serve as primary hosts, humans can act as accidental hosts. Human infection typically occurs through ingestion of watercress or other aquatic plants contaminated with larvae from water sources in areas where these animals graze. Clinical manifestations include fever, right upper-quadrant abdominal pain, nausea, fatigue, and eosinophilia. Diagnosis relies on a combination of parasitological, laboratory, and imaging investigations, which can be challenging. Nevertheless, treatment with the antihelminthic drug triclabendazole is effective and straightforward. Here, we report two cases of human fascioliasis identified during the evaluation of abdominal pain, highlighting the importance of considering parasitic infections in the differential diagnosis of liver lesions accompanied by eosinophilia in endemic regions.

Keywords: *Fasciola hepatica*, fascioliasis, antihelminthic, triclabendazole

Öz

Fasiyoliyaz, *Fasciola hepatica* ve *Fasciola gigantica*'nın neden olduğu asıl konağı sığır, koyun, at, manda gibi otçul memeliler olmasına rağmen insanların da rastlantısal bir konak olduğu zoonotik paraziter bir enfeksiyondur. İnsanlara bulaş daha çok bu tür hayvanların beslendiği yerlerde kontamine sularla temas etmiş su teresi bitkilerinin tüketilmesiyle meydana gelmektedir. Ateş, karın ağrısı, bulantı, halsizlik, sağ üst kadran ağrısı gibi semptomlarla seyreden bu zoonotik enfeksiyonun tanısında parazitolojik, laboratuvar ve görüntüleme yöntemleri kullanılmaktadır. Tanının konulmasında güçlüklerle karşılaşılmasına rağmen tedavisinde antihelmintik ilaç olan triclabendazol'un kullanıldığı bu enfeksiyonun tedavisi kolaydır. Karın ağrısı etiyolojisi araştırılırken fasciolasis tanısı konulan iki olgunun sunulduğu bu çalışmada, endemik bölgelerde eozinofilinin eşlik ettiği karaciğer lezyonları olan hastaların ayırıcı tanısında, paraziter enfeksiyonların da göz önünde bulundurulması gerektiği anlaşılmaktadır.

Anahtar Kelimeler: *Fasciola hepatica*, fascioliyaz, antihelmintik, triclabendazol

Introduction

Fascioliasis is a parasitic infection of the hepatobiliary system caused by the trematodes *Fasciola hepatica* and *Fasciola gigantica*, which may occasionally infect human[1]. This disease is most prevalent in cattle- and sheep-raising regions in temperate climates and affects an estimated 0.4–5.6% of the population in our country [2]. Infection begins when eggs excreted in the feces of animals carrying adult parasites in their bile ducts contaminate freshwater sources. Humans

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acquire the infection by consuming aquatic plants harboring the encysted larval stage (metacercariae) or by drinking contaminated water[3].

Fascioliasis presents in two distinct clinical stages. During the hepatic migratory phase, patients typically exhibit fever, abdominal pain, elevated liver enzyme levels, and marked eosinophilia. In the biliary phase, when adult parasites localize in the bile ducts, patients often experience right upper-quadrant pain, which may be accompanied by obstructive complications such as acute pancreatitis or cholestasis[4–6]. The diagnosis of fascioliasis is challenging because the infection presents with a broad spectrum of clinical manifestations. Accurate identification requires stage-specific clinical findings supported by microscopic stool examination and serological assays such as the Indirect Hemagglutination Assay (IHA), Immunofluorescence Antibody Test, Complement Fixation Test, and Enzyme-Linked Immunosorbent Assay (ELISA). Molecular diagnostic techniques, in combination with imaging modalities such as abdominal ultrasonography or contrast-enhanced computed tomography (CT), may also be necessary.

During the acute phase, stool microscopy may yield negative results; therefore, serological tests that detect specific serum antibodies are essential. Owing to the parasite's migration through hepatic parenchyma, imaging findings during the hepatic phase commonly include serpiginous, hypoattenuated subcapsular tracts. These tracts are visualized on contrast-enhanced CT, whereas magnetic resonance imaging (MRI) typically shows hypointense signals on T1-weighted and hyperintense signals on T2-weighted sequences[4]. However, imaging findings may vary and may also include hepatomegaly, nodular lesions, splenomegaly, and lymphadenopathy[6,7].

Triclabendazole, an anthelmintic agent, is the treatment of choice for fascioliasis, and the diagnosis relies on a combination of these clinical, serological, and imaging findings[5,8].

Case Report 1

A 62-year-old woman presented with a four-month history of fatigue, weakness, and prominent right upper-quadrant abdominal pain. An abdominal ultrasound performed at an external center showed mild parenchymal echogenicity extending to the peripheral subcapsular region of the right hepatic lobe. She was referred to our clinic for further evaluation, including assessment for parasitic infections. The patient resided in the Mutki district of Bitlis Province, worked in agriculture, and reported consuming watercress. She had no significant family history, and her physical examination findings were unremarkable.

Laboratory tests revealed an absolute eosinophil count of 5,100/μL (reference range: 0.05–0.5 × 10^3/μL) and an eosinophil percentage of 23.5% (reference range: 0.5%–6%). Liver function tests were within normal limits, and serology for hepatitis A, B, and C viruses and brucellosis was negative. The IHA result for hydatid cyst was negative, whereas the ELISA titer for fascioliasis was 1:1280. No parasite eggs were detected in direct or concentrated stool examinations.

Abdominal MRI showed patchy, heterogeneous, and poorly demarcated hyperintense areas on T2-weighted sequences involving hepatic segments VI and VII, with occasional extension into the subcapsular region (Figure 1). Based on the clinical, laboratory, and imaging findings, fascioliasis was strongly suspected.

The patient was treated with triclabendazole (2 × 10 mg/kg) administered orally in a single day. Marked improvement in clinical symptoms was noted within one month. Follow-up laboratory testing at three months demonstrated normalization of the eosinophil count. A repeat abdominal MRI revealed peripheral contrast-enhancing hypodense lesions extending to hepatic segments VI–VII, with a significant reduction in size compared with the initial scan.

Case Report 2

A 42-year-old woman presented to the internal medicine clinic with a one-month history of fatigue, exhaustion, and right upper-quadrant abdominal pain. Initial imaging suggested a possible parasitic infection, prompting referral to our infectious diseases clinic. Further history revealed that she resided in Bitlis city, was a housewife engaged in agricultural work, and regularly consumed watercress. Her brother had previously experienced similar symptoms and had been treated for fascioliasis at another center. Physical examination findings were unremarkable, with normal vital signs.

Laboratory investigations demonstrated an absolute eosinophil count of 7,170/μL (reference range: 0.05–0.5 × 10^3/μL) and

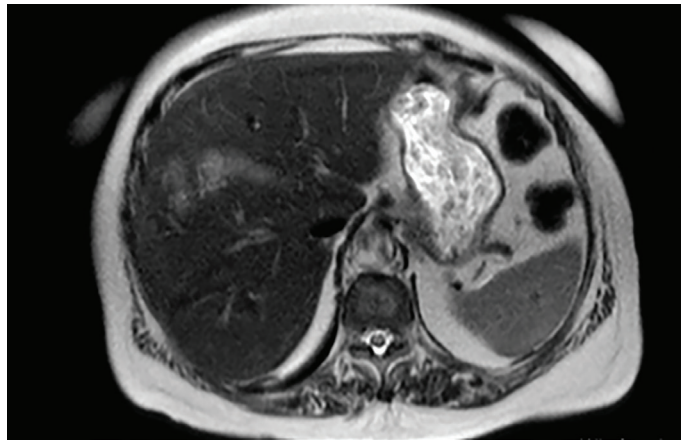

Figure 1. Irregular, patchy, heterogeneous lesions in hepatic segments VI–VII.

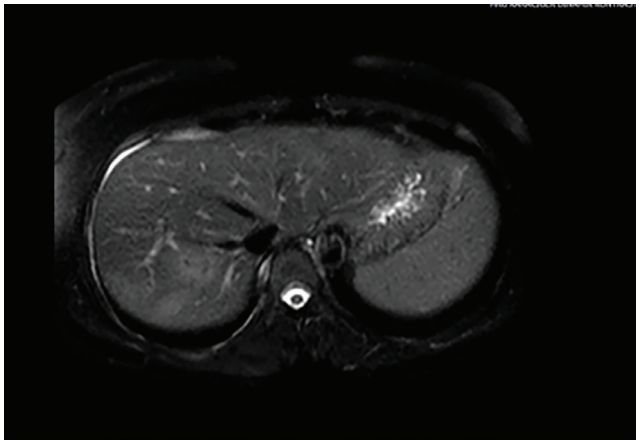

Figure 2. Irregular lesions in hepatic segments III and VII–VIII without obvious contrast enhancement.

an eosinophil percentage of 60.1% (reference range: 0.5%–6%). Serologic testing for viral hepatitis and brucellosis was negative, as were the hydatid cyst IHA and fascioliasis ELISA. Stool microscopy and parasitological examinations revealed no parasite eggs.

Abdominal MRI showed distinct, irregular, non-enhancing lesions with diffusion restriction, extending to the subcapsular region, and demonstrating T2-weighted hyperintensity in hepatic segments III and VII–VIII (Figure 2). Considering the patient's family history, laboratory findings, and imaging results, a diagnosis of fascioliasis was established.

The patient received oral triclabendazole (2 × 10 mg/kg) as a single-day treatment. At three months, follow-up laboratory tests showed normalization of the eosinophil count, and MRI revealed significant regression of the hepatic lesions.

Discussion

Fascioliasis progresses through two clinical stages: the hepatic phase and the biliary phase. Transmission occurs primarily through ingestion of raw aquatic vegetables, particularly watercress, which harbor the parasite's encysted larval stage, the metacercariae[9]. After oral ingestion, metacercariae traverse the intestinal wall, penetrate the hepatic capsule, and reach the liver, resulting in abdominal pain and other manifestations of the acute hepatic phase, including capsular irritation and subcapsular abscess formation[10]. Adult parasites that establish themselves in the bile ducts subsequently begin producing eggs. Consequently, a history of consuming watercress may raise suspicion for fascioliasis in patients presenting with hepatobiliary symptoms.

Kaya et al.[5], in a prospective study of 30 patients with fascioliasis, reported that abdominal pain was present in all cases, with eosinophilia being the most prominent laboratory finding in both the hepatic and biliary phases. Similarly, in a report of two cases of hepatic fascioliasis originating outside the United States, both patients presented with abdominal pain and marked peripheral eosinophilia[11]. A study from Taiwan involving two patients with a history of consuming unpurified water and freshwater plants, who were diagnosed with hepatic fascioliasis through surgical intervention, identified acute-phase findings including absolute eosinophilia, intrahepatic cystic lesions, and right upper-quadrant pain in both cases[12].

Gündağ et al. [13] conducted a retrospective evaluation of 26 cases in our country, finding that 16 patients (61.5%) resided in rural areas and peripheral eosinophilia was documented in all patients . Both cases in the present report share a history of rural residence and peripheral eosinophilia, consistent with previous literature.

During the acute (hepatic) phase of fascioliasis, patients may present with fever, abdominal pain, arthralgia, and elevated eosinophil counts. In contrast, the chronic biliary phase may manifest as biliary obstruction, cholangitis, pancreatitis, diarrhea, vomiting, and anorexia[14]. In the current cases, clinical findings were indicative of the acute phase, including abdominal pain and fatigue, whereas biliary involvement was not observed.

Definitive diagnosis is established by identifying adult parasites or eggs, typically via stool examination; however, eggs may be absent during the hepatic phase. Consequently, serological tests and imaging modalities play a critical role in diagnosis[15,16]. In our cases, stool examinations were negative, consistent with acute-phase infection, and serological tests were positive in one patient and negative in the other.

A review by Kabaalioğlu et al.[17] of 23 radiologically confirmed cases of fascioliasis reported multiple, confluent, linear, tract-like hypodense hepatic lesions without contrast enhancement on CT during the hepatic phase. Ultrasonography demonstrated adult parasites as echogenic particles in the gallbladder or common bile duct without posterior shadowing. MRI findings were described as hypo- or isointense on T1-weighted sequences and hyperintense on T2-weighted sequences[17].

In the first case, both ultrasound and MRI were utilized for evaluation, whereas in the second case, MRI alone was performed, guided by high eosinophil counts, MRI lesions consistent with fascioliasis, and a relevant family history. Imaging findings from both cases, including the ultrasound of the first patient, demonstrated lesions consistent with those described in the literature[15].

Triclabendazole, a narrow-spectrum anthelmintic belonging to the class of ampholytic benzimidazole derivatives, is active against *Fasciola hepatica*, *Fasciola gigantica*, and *Paragonimus* species. It is effective against *Fasciola* infections at all stages[18,19]. Both patients received a single-day oral dose of

20 mg/kg triclabendazole, obtained from abroad, and were closely monitored. Common indicators of treatment response include regression of clinical symptoms, normalization of eosinophil counts, reduction in *Fasciola* serology titers, and resolution of imaging abnormalities[20]. Complete treatment response is typically observed at three months[21]. At the three-month follow-up, both patients exhibited near-complete resolution of symptoms, normalization of eosinophil counts and percentages, and significant regression of lesions on imaging. However, repeat ELISA titers could not be determined due to insufficient testing.

These two cases contribute to the existing literature by highlighting the clinical characteristics of fascioliasis in our region. The patients' rural living conditions, engagement in agricultural activities, and consumption of watercress provide evidence of region-specific risk factors and transmission routes. Our findings are consistent with previous case reports, while also emphasizing regional characteristics and the importance for clinicians to consider fascioliasis in the differential diagnosis of patients presenting with hepatobiliary symptoms.

Conclusion

In regions where fascioliasis is endemic, clinicians should consider this infection in the differential diagnosis of patients presenting with prolonged fever, fatigue, abdominal pain, elevated liver enzymes, and eosinophilia. A thorough medical history—including consumption of green aquatic plants, particularly watercress, and involvement in animal husbandry—can provide valuable diagnostic clues. These cases also highlight the importance of integrating clinical, laboratory, and imaging findings for accurate diagnosis. Moreover, they demonstrate that appropriate dosing of triclabendazole can achieve successful clinical and radiological improvement.

Ethics

Informed Consent: Informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: T.T., M.K., Concept: T.T., İ.H.Ş., H.T.M., M.K., Design: T.T., İ.H.Ş., H.T.M., M.K., Data Collection or Processing: T.T., İ.H.Ş., H.T.M., M.K., Analysis or Interpretation: T.T., İ.H.Ş., H.T.M., M.K., Literature Search: T.T., İ.H.Ş., H.T.M., M.K., Writing: T.T., İ.H.Ş., H.T.M., M.K.

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RESEARCH ARTICLE / ARAŞTIRMA

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A Five-Year Single-Center Experience with *Fasciola hepatica* Infection

Fasciola hepatica Enfeksiyonu ile İlgili Beş Yıllık Tek Merkez Deneyimi

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Abstract

Introduction: *Fasciola hepatica* infection (fascioliasis) is an uncommon zoonotic parasitic disease in humans that primarily involves the hepatobiliary system. It may present with a broad spectrum of clinical manifestations, ranging from obstructive jaundice and cholangitis to chronic biliary inflammation and cirrhosis. This study aimed to describe the clinical, laboratory, and radiological characteristics of fascioliasis cases diagnosed over a five-year period between 01.01.2020 and 01.01.2025 in a tertiary care center and to highlight its relevance in differential diagnosis.

Materials and Methods: We conducted a retrospective review of adult patients diagnosed with *Fasciola hepatica* infection between 2020 and 2025 at Elazığ Fethi Sekin City Hospital. Hospital records, electronic files, and discharge summaries were examined. Clinical presentations, laboratory parameters, and radiological findings at initial admission and at one-month follow-up were evaluated. Analyses were performed using SPSS version 20.0, and appropriate statistical tests were applied according to data distribution.

Results: Twenty-six patients met the inclusion criteria (mean age: 51 ± 10.93 years; 73% female). Most patients lived in rural areas (61.5%). Abdominal pain was reported in 50% of cases, and peripheral eosinophilia was documented in all patients. Ultrasonography, computed tomography, and magnetic resonance imaging frequently suggested malignancy (69.2%); however, these lesions regressed after treatment with triclabendazole. Significant reductions were observed in alkaline phosphatase (ALP), lactate dehydrogenase (LDH), eosinophil count, and leukocyte count, while serum albumin levels increased after treatment ($p < 0.001$). Stool microscopy was performed in two patients but failed to detect parasitic eggs.

Conclusion: Fascioliasis should be considered in the differential diagnosis of hepatic lesions, particularly in patients from rural settings who present with eosinophilia and abdominal pain. Early diagnosis can be achieved through a combination of serology, imaging modalities, and strong clinical suspicion. ALP, LDH, and eosinophil levels are valuable, non-invasive indicators for monitoring treatment response.

Keywords: Eosinophilia, *Fasciola hepatica*, hepatic lesions, parasitic infection, triclabendazole

Öz

Giriş: Fasiyoliyazis, insanlarda nadir görülen bir paraziter enfeksiyon olup, safra yollarını etkileyebilir ve obstrüktif sarılık ile kolanjitten siroza kadar geniş bir klinik yelpazede bulgu verebilir. Bu çalışmanın amacı, üçüncü basamak bir sağlık merkezinde beş yıl içinde tanı konulan fasiyoliyazis vakalarının klinik, laboratuvar ve radyolojik bulgularını tanımlamak ve ayırıcı tanıdaki yerine dikkat çekmektir.

Gereç ve Yöntem: Elazığ Fethi Sekin Şehir Hastanesi'nde 2020–2025 yılları arasında *Fasciola hepatica* tanısı alan erişkin hastaların dahil edildiği retrospektif bir çalışma yürütüldü. Hastane kayıtları, dosyalar ve taburcu özetleri incelendi. Başvuru anındaki ve birinci ay takiplerindeki klinik, laboratuvar ve radyolojik bulgular SPSS versiyon 20.0 programı ile değerlendirildi. Verilerin dağılımına göre uygun istatistiksel testler uygulandı.

Bulgular: Çalışmaya 26 hasta dahil edildi (ortalama yaş: 51 ± 10,93 yıl; %73'ü kadın). Hastaların çoğu (%61,5) kırsal bölgelerde yaşamaktaydı. %50'sinde karın ağrısı mevcutken, tüm vakalarda eozinofili saptandı. Görüntülemelerde hastaların %69,2'sinde malignite şüphesi oluşmuş, ancak triklabendazol tedavisini takiben lezyonlarda gerileme gözlenmiştir. Tedavi sonrası alkalen fosfataz (ALP), laktat dehidrogenaz (LDH), eozinofil ve

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Öz

lökosit düzeylerinde anlamlı azalma, albümin seviyelerinde ise artış saptandı (p<0,001). Dışkı mikroskopisinde yumurta iki hastada araştırılmış, ancak saptanamamıştır.

Sonuç: Fasiyoliyazis, özellikle eozinofili ve karın ağrısı olan ve kırsal kökenli hastalarda hepatik lezyonların ayırıcı tanısında dikkate alınmalıdır. Klinik şüpheyle birlikte serolojik testler ve görüntüleme yöntemleri erken tanıyı kolaylaştırır. ALP, LDH ve eozinofil düzeyleri tedavi yanıtının izlenmesinde faydalı, invaziv olmayan parametrelerdir.

Anahtar Kelimeler: Eozinofili, *Fasciola hepatica*, hepatik lezyon, paraziter enfeksiyon, triklabendazol

Introduction

Fascioliasis is a neglected tropical disease caused by *Fasciola hepatica* and *Fasciola gigantica*, and it poses a significant global public health concern, particularly in endemic regions. Approximately 50% of infected individuals remain asymptomatic[1]. The prevalence of foodborne zoonotic trematode infections may reach up to 21% in tropical and subtropical areas, and fascioliasis disproportionately affects rural communities. An estimated 180 million people worldwide are at risk of infection. The parasite primarily invades the hepatic and biliary systems of mammals—including humans and livestock—resulting in substantial pathological damage[2]. Although ruminants such as sheep, goats, and cattle serve as the main hosts, humans become infected as accidental hosts. *Fasciola hepatica* is a leaf-shaped trematode measuring approximately 2–4 cm in length[1].

Human infection occurs through ingestion of water or aquatic plants contaminated with metacercariae, the infective larval stage[3]. After passing through the stomach, the larvae penetrate the duodenal wall, migrate into the peritoneal cavity, and then traverse the liver capsule to reach the biliary tree[4]. Human fascioliasis progresses through two distinct clinical stages: the hepatic and biliary phases. The hepatic phase typically begins 1–3 months after ingestion of metacercariae and involves larval migration from the liver parenchyma into the bile ducts[1,4,5]. Common manifestations during this stage include abdominal pain, fever, eosinophilia, and elevated liver function test results[4,6–11]. The biliary phase commonly presents with intermittent right upper quadrant pain, with or without features of cholangitis or cholestasis[12–14].

Extrahepatic manifestations may also occur during the acute phase, likely mediated by immunologic or allergic mechanisms. Reported findings include Löffler syndrome, right-sided pleural effusion with eosinophilia, urticaria associated with pruritus and dermatographism, and, rarely, pericarditis, cardiac conduction abnormalities, meningeal symptoms, focal neurological deficits, or seizures[12–14].

In non-endemic settings, diagnosis is frequently delayed because of the disease's rarity and its overlapping clinical features with other hepatobiliary conditions[1,4]. Serological testing is particularly valuable for early diagnosis because assays typically yield positive results during the early migration phase—before eggs are detectable in stool samples. These tests include indirect hemagglutination, complement fixation, counterimmunoelectrophoresis, immunofluorescence, and enzyme-linked immunosorbent assay. Although serological tests demonstrate high sensitivity, their specificity remains suboptimal. Imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) aid diagnosis in the hepatic phase, whereas ultrasonography (USG) is more informative during the biliary phase[8,9]. Nevertheless, definitive diagnosis should be supported by both serological and parasitological evidence[15].

Triclabendazole is the treatment of choice for fascioliasis. When triclabendazole is unavailable or when patients fail to respond to therapy, alternative agents—such as nitazoxanide, albendazole, or bithionol—may be considered[2].

Although fascioliasis is considered uncommon, it is encountered more frequently in rural regions of our country where pastoral lifestyles and livestock exposure are prevalent. In clinical practice, the disease may be radiologically misinterpreted as a hepatic tumor, metastatic lesion, or cholecystitis/cholestasis. In this study, we aimed to increase clinical awareness of fascioliasis and to highlight that patients from rural areas who present with eosinophilia and are initially evaluated for malignancy or biliary pathology may, in fact, have underlying Fasciola infection.

Materials and Methods

This retrospective study included adult patients diagnosed with *Fasciola hepatica* infection between 2020 and 2025 at Elazığ Fethi Sekin City Hospital. Patient information was collected from the hospital information system, medical records, and discharge summaries.

Laboratory investigations comprised complete blood count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), total and direct bilirubin, serum albumin,

coagulation parameters, and *Fasciola hepatica* indirect hemagglutination assay (IHA) tests. Stool examinations were not performed in all patients due to the limited sensitivity of egg detection in chronic infection and because some patients presented during the acute phase when eggs are not yet detectable.

Abdominal USG was performed in all patients as the primary imaging modality. Patients with suspicious lesions on USG underwent further evaluation with MRI or CT. One patient presenting with clinical features of cholestasis underwent diagnostic endoscopic retrograde cholangiopancreatography (ERCP). All patients were followed from initial presentation up to six months after treatment.

The study was approved by the Elazığ Fethi Sekin City Hospital Non-Interventional Research Ethics Committee (approval number: 268525027, dated: 06.02.2025).

Statistical Analysis

Statistical analyses were performed using IBM SPSS statistics version 20.0 (IBM Corp., Armonk, NY, USA). Normality of variable distributions was assessed using visual inspection and analytical tests (Kolmogorov–Smirnov and Shapiro–Wilk tests). Normally distributed continuous variables were analyzed using the paired-samples t-test, whereas non-normally distributed variables were analyzed using the Wilcoxon signed-rank test. Categorical variables were evaluated using the McNemar test. Non-parametric variables are reported as median and interquartile range. A p-value < 0.05 was considered statistically significant.

Results

In this study, 26 patients were diagnosed with *Fasciola hepatica* infection. The mean age was 51 ± 10.9 years, and the female-to-male ratio was 19:7, indicating a higher prevalence among females (73%). Most patients resided in rural areas (61.5%, $n = 16$), whereas 38.5% ($n = 10$) were from urban regions (Table 1).

Abdominal pain was reported in 13 patients (50%), while nausea and weight loss were each observed in 4 patients (15.3%). Jaundice was present in only one patient, and another experienced intermittent diarrhea for one month. Physical examination revealed mild right upper quadrant tenderness in 12 patients (46.2%) and hepatomegaly in 3 patients (11.5%).

All patients had a positive IHA test. Radiological imaging (MRI, CT, or ultrasound) suggested a "tumor/metastasis?" in 69.2% of cases ($n = 18$), whereas only 28.7% ($n = 7$) were reported as "suspected *Fasciola hepatica*" (Figure 1). Lesions initially interpreted as tumors or malignancies resolved after treatment, a difference that was statistically significant ($p < 0.001$).

Imaging studies were normal in 8 patients. One patient was diagnosed during ERCP, where the parasite was directly visualized; this patient also tested positive by IHA and had

Table 1. Demographic datas.

Parameter	Value
Age (mean ± SD)	51 ± 10.94
Sex (F/M)	19 (73%)/7 (27%)
Rural/urban	16 (61.5%)/10 (38.5%)

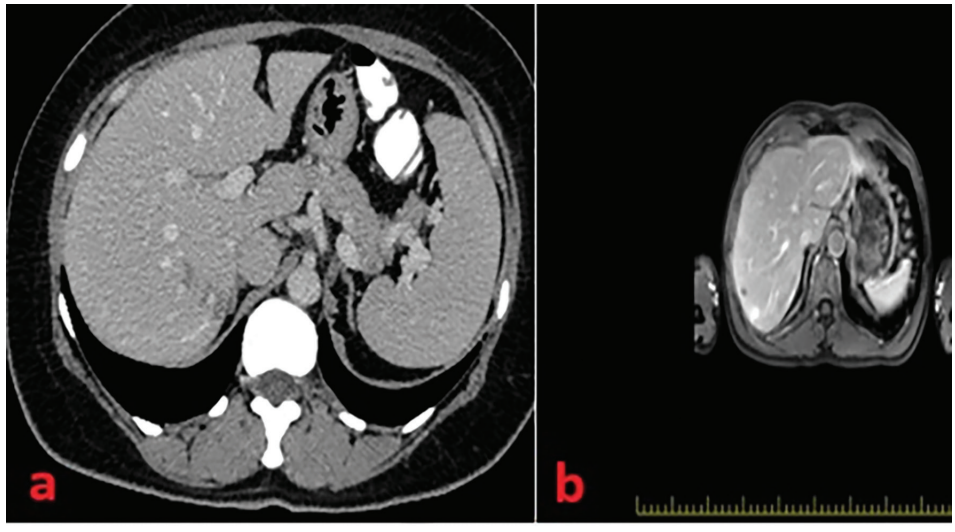

Figure 1. (a) Upper abdominal CT scan of a patient with fascioliasis showing multiple hypodense nodules in the liver with a heterogeneous appearance, diffusely distributed but predominantly central. (b) Upper abdominal CT scan of a patient with fascioliasis demonstrating a heterogeneously hypodense nodule located peripherally in the liver.

been initially hospitalized with a preliminary diagnosis of cholecystitis.

Eosinophilia (>500/µL) was present in all patients upon admission. Pre- and post-treatment laboratory values during the first month are presented in Table 2. Alkaline phosphatase (ALP), LDH, AST, eosinophil count, and leukocyte count significantly decreased after treatment, whereas albumin levels increased (all $p < 0.001$). No significant changes were observed in ALT, total or direct bilirubin, coagulation parameters, or platelet count between admission and post-treatment assessments.

Microscopic stool examination was performed in only two patients; *Fasciola hepatica* eggs were not detected in either case.

All patients received a single oral dose of triclabendazole (10–12 mg/kg), repeated after two weeks. No adverse effects were reported. Patients in the hepatic phase did not receive antibiotics, whereas one patient in the biliary phase was treated with ceftriaxone for seven days due to an initial diagnosis of cholecystitis.

Clinical improvement, resolution of eosinophilia, and reduction in hepatic lesion size were observed in all patients at the one-month follow-up. Complete clinical and laboratory recovery was achieved in all patients by six months.

Discussion

Fascioliasis is an infectious disease caused by the liver flukes *Fasciola hepatica* and *Fasciola gigantica*, primarily affecting ruminants such as sheep, goats, and cattle, with humans serving as incidental hosts[4]. In most human cases, the clinical course is mild. The incidence of *Fasciola hepatica* infection has increased since the 1980s, and several geographic regions have been identified as endemic for human fascioliasis[5,6]. In our country, research has predominantly focused on animals, with limited studies involving humans; consequently, the true prevalence of human fascioliasis remains unknown[16,17]. A separate study in our province reported a seroprevalence of *Fasciola hepatica* immünoglobulin G of 2.78%[18].

Diagnosis of fascioliasis can be challenging in non-endemic areas due to its rarity and potential confusion with other hepatic or biliary diseases, often resulting in delayed identification.

Table 2. Laboratory parameters before and after treatment.

n = 26 patients	Pre-treatment (mean)	Post-treatment (mean)	p-value
ALP, U/L	167	110	<0.001
LDH, U/L	238	206	<0.001
Eosinophil, 10_9/L	5896	1168	<0.001
Leukocyte, 10_9/L	12746	8656	<0.001
Albumin, g/L	40	42	<0.001

Traditionally, diagnosis relies on detection of parasite eggs in stool samples; however, this method is unreliable because of typically low egg output[4,7]. Serological tests, which generally become positive during the early hepatic migratory phase before eggs are detectable in stool, are valuable for early diagnosis. Although these tests demonstrate good sensitivity, their specificity is limited due to potential cross-reactivity with other parasitic infections[4,7,18,19]. In our study, all patients tested positive for *Fasciola hepatica* using the IHA test, while stool examinations conducted in two patients did not detect parasite eggs.

In endemic regions, very young children and women are more frequently affected by fascioliasis. Studies from Türkiye have also reported a female predominance[20,21], which is consistent with our findings.

Eosinophilia was present in all patients (n = 26) at initial presentation. The mean eosinophil count decreased from 5,896.9/µL at admission to 1,168.9/µL post-treatment, a statistically significant reduction. These results support the use of eosinophil count as a reliable laboratory parameter for both suspecting and monitoring fascioliasis.

The mean leukocyte count at presentation was 12,746.2/µL, which decreased to 8,656.2/µL after treatment. At admission, 61.5% of patients (n = 16) had leukocyte counts above 11,000/µL (reference range in adults according to Tietz: 4.5–11.0 × 10^9/L), which declined to 15.4% (n = 4) following treatment. This difference was statistically significant ($p < 0.001$). The elevated leukocyte counts at baseline were primarily due to eosinophilia, and levels decreased in parallel with eosinophil counts after treatment.

The mean albumin level increased from 40.1 g/L at admission to 42.6 mg/dL at follow-up. Although baseline albumin levels were within the normal range in all patients, the increase was statistically significant ($p < 0.001$), suggesting that fascioliasis may transiently impair hepatic albumin synthesis due to hepatocellular involvement.

In fascioliasis, elevated ALP is thought to reflect hepatocellular and biliary injury[19,22]. In our study, the mean ALP level at presentation was 167.96 mg/dL (range: 69–296), which decreased to 110.6 mg/dL (range: 50–206) post-treatment. This reduction was statistically significant ($p < 0.001$), supporting the use of ALP as a marker for evaluating treatment response.

Elevated LDH in fascioliasis likely reflects hepatocellular damage. The mean LDH level at presentation was 238.85 mg/dL (range: 16–383), decreasing to 206.96 mg/dL (range: 144–291) after treatment, with a statistically significant difference ($p < 0.001$). No significant changes were observed in GGT, bilirubin, or coagulation parameters before and after treatment.

Triclabendazole, an imidazole derivative, is effective against all developmental stages of *Fasciola* spp. It is typically administered as a single dose of 10 mg/kg, repeated after 2–4 weeks; in severe cases, a dose of 20 mg/kg may be used. Treatment efficacy exceeds 90% in most cases[17]. Response is monitored by reductions in eosinophil counts, disappearance of eggs in stool, decreased antibody titers, and regression in radiological findings[23–25]. Stool examinations were not performed in all patients due to the low sensitivity of egg detection in chronic fascioliasis.

Conventional imaging modalities such as MRI and CT are commonly used for diagnosis[20]. ERCP has recently gained prominence, particularly in diagnosing biliary-phase fascioliasis. In our country, Tuna et al.[24] reported a 44-year-old patient diagnosed via ERCP who presented with abdominal pain, nausea, and vomiting. In our cohort, 18 patients had liver lesions suspicious for tumors or metastases on abdominal CT or MRI. One patient was diagnosed via ERCP, highlighting the utility of this technique in selected cases. In contrast, only seven patients had imaging findings suggestive of *Fasciola hepatica*. These observations emphasize that fascioliasis should be included in the differential diagnosis of hepatic masses or suspected metastases, particularly in patients presenting with abdominal pain and eosinophilia.

Conclusion

Fascioliasis is an underdiagnosed yet treatable parasitic infection. In regions with rural populations, it should be considered in patients presenting with eosinophilia, hepatic lesions, or abdominal pain. Non-invasive biomarkers, including ALP, LDH, and eosinophil counts, are useful for monitoring treatment response. Our findings contribute to the growing body of literature on fascioliasis and underscore the value of serological and imaging tools for early diagnosis.

Ethics

Ethics Committee Approval: The study was approved by the Elazığ Fethi Sekin City Hospital Non-Interventional Research Ethics Committee (approval number: 268525027, dated: 06.02.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.G., Z.Ç., Concept: Ö.G., Y.K., Design: Ö.G., Y.K., Data Collection or Processing: Ö.G., Y.K., Z.Ç., Analysis or Interpretation: Ö.G., Y.K., Literature Search: Ö.G., Z.Ç., Writing: Ö.G., Y.K., Z.Ç.

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Herpes Simplex Virus Encephalitis–Associated Hyponatremia and Anti-NMDA Receptor Encephalitis

Herpes Simplex Virüs Ensefaliti İlişkili Hiponatremi ve Anti-NMDA Reseptör Ensefaliti

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Abstract

A 78-year-old male presented to the emergency department with a two-day history of high-grade fever and confusion. Cerebrospinal fluid analysis was positive for *Herpes simplex* virus type 1 (HSV-1) via polymerase chain reaction, and intravenous acyclovir therapy was initiated. During hospitalization, the patient developed hyponatremia, which was attributed to cerebral salt wasting syndrome based on elevated urinary sodium levels and clinical hypovolemia. Hyponatremia and consciousness improved following acyclovir therapy and aggressive isotonic fluid replacement. After being discharged, the patient experienced recurrent cognitive decline, leading to a second hospitalization. Further evaluation resulted in a diagnosis of anti–N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis. Treatment with intravenous immunoglobulin resulted in gradual improvement in consciousness. This case highlights the coexistence of HSV encephalitis–associated cerebral salt wasting syndrome and subsequent anti-NMDAR encephalitis.

Keywords: Encephalitis, *Herpes simplex* virus, hyponatremia, cerebral salt wasting syndrome, anti-NMDAR encephalitis

Öz

Yetmiş sekiz yaşında erkek hasta, iki gündür devam eden yüksek ateş ve bilinç değişikliği şikayetiyle acil servise başvurdu. Beyin omurilik sıvısı incelemesinde polimeraz zincir reaksiyon yolu ile *Herpes simplex* virüsü tip 1 (HSV-1) için pozitif saptandı ve asiklovir 30 mg/kg/günden IV tedavi başlandı. Takiplerinde hiponateremi gelişen hastanın idrar sodyum atılımının yüksek olması ve hipovolemisi serebral tuz kaybı sendromunu düşündürdü. Asiklovir tedavisinin yanı sıra agresif izotonik tedavi ile hiponatremisi ve bilinci düzeldi. Taburculuğu sonrası yeniden bilinç değişikliği gelişmesi üzerine ikinci kez hastaneye yatırıldı. Yapılan incelemeler sonucunda anti- N-metil- D-aspartat reseptör (Anti-NMDAR) ensefaliti tanısı konuldu. İntravenöz immunoglobulin tedavisi sonrası hastanın bilinci kademeli olarak düzeldi. Bu olgu HSV ensefalit ilişkili serebral tuz kaybı sendromuna ve sonrasında gelişebilecek anti-NMDAR ensefalitine karşı farkındalığı artırmak amacı ile sunuldu.

Anahtar Kelimeler: Ensefalit, *Herpes simplex*, hiponatremi, serebral tuz kaybı sendromu, anti-NMDAR ensefalit

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Introduction

Herpes simplex virus (HSV) is the most common cause of acute sporadic viral encephalitis in adults. Hyponatremia frequently occurs in central nervous system disorders, including HSV encephalitis[1]. Although the syndrome of inappropriate antidiuretic hormone secretion (SIADH) is a well-recognized cause of renal sodium loss, a similar electrolyte disturbance may also arise from cerebral salt wasting syndrome (CSWS). CSWS is characterized by excessive renal sodium excretion in the setting of intracranial pathology, resulting in hyponatremia accompanied by a reduction in extracellular fluid volume[2,3]. Differentiating CSWS from SIADH is essential because management strategies differ substantially: SIADH typically requires fluid restriction, whereas CSWS necessitates aggressive isotonic fluid replacement.

In addition, patients recovering from HSV encephalitis may develop post-infectious autoimmune encephalitis, most commonly anti–N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis. This risk underscores the importance of continued clinical vigilance after hospital discharge. In a prospective cohort of 51 patients with HSV encephalitis, autoimmune encephalitis developed in 14 (27%), and neuronal antibodies were identified in all affected individuals; among these cases, anti-NMDAR antibodies were detected in 9 (64%) patients[4].

Here, we report a case of HSV encephalitis–associated hyponatremia followed by anti-NMDAR encephalitis and review the relevant literature.

Case Presentation

A 78-year-old man presented to the emergency department with a two-day history of high fever and altered consciousness. His medical history was notable only for benign prostatic hyperplasia managed with tamsulosin; no other comorbidities or medications were reported. On admission, his temperature was 37.8 °C, blood pressure 110/70 mmHg, and respiratory rate 16 breaths/min. He was unresponsive, non-cooperative, disoriented, and exhibited no meningeal signs.

Initial laboratory evaluation showed serum creatinine 1.28 mg/dL, sodium 140 mmol/L, and C-reactive protein 1.3 mg/L. Brain magnetic resonance imaging (MRI) revealed T2-FLAIR hyperintense lesions in the left temporal lobe and hippocampus (Figure 1). Lumbar puncture demonstrated cerebrospinal fluid (CSF) with 210 leukocytes/mm³ (predominantly lymphocytes), 50 erythrocytes/mm³, glucose 49 mg/dL (serum glucose 98 mg/dL), and protein 69 mg/dL. Intravenous empirical therapy with ceftriaxone 4 g/day, ampicillin 12 g/day, and acyclovir 30 mg/kg/day was initiated. CSF polymerase chain reaction (PCR) subsequently returned positive for HSV-1; antibiotic therapy was discontinued, and acyclovir was continued.

On the 7th day of his hospitalization, serum sodium had decreased to 134 mmol/L, and spot urine sodium was elevated at 99 mmol/L. Physical examination revealed dry mucous membranes and reduced skin turgor, consistent with hypovolemia. A diagnosis of CSWS was made, and aggressive isotonic saline administration (4–5 L/day) was initiated. Urine sodium levels remained ≥110 mmol/L, while serum sodium stabilized between 133 and 135 mmol/L.

During the first 2 weeks, the patient remained markedly lethargic, responding only with facial expressions. Following a generalized tonic–clonic seizure, levetiracetam therapy was started. Blood and urine cultures were obtained after inflammatory markers increased (CRP 60 mg/L; procalcitonin 0.21 ng/mL). Meropenem 3 g/day was added for suspected nosocomial infection, and *Pseudomonas aeruginosa* was isolated from the urine culture. By the end of the second week, the patient's consciousness improved and oral intake resumed. Meropenem was administered for a total of 7 days, and acyclovir was discontinued after 21 days. The patient subsequently underwent physiotherapy, showed clinical and laboratory improvement, and was discharged in stable condition.

One week after discharge, he again developed altered consciousness and experienced a focal seizure involving the right upper extremity. Upon readmission to another hospital,

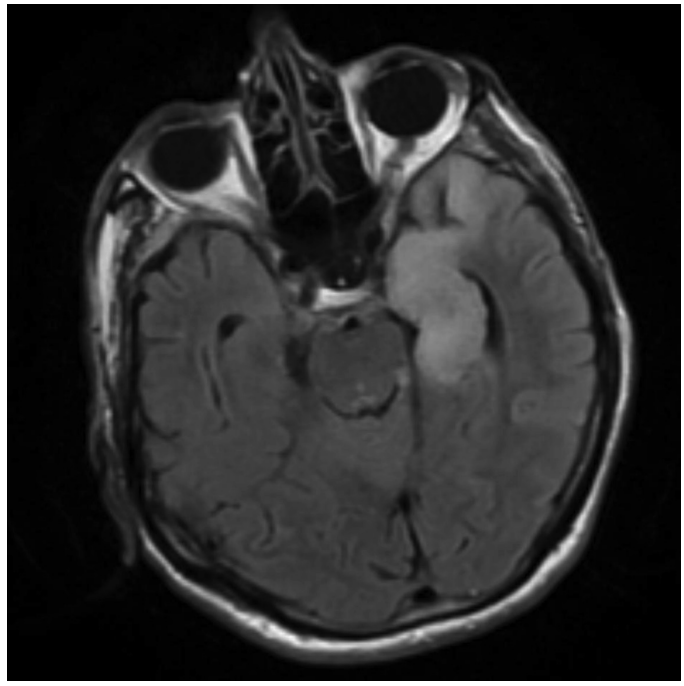

Figure 1. Brain MRI demonstrating T2-FLAIR hyperintense lesions in the left temporal lobe and hippocampus.

CSF testing was positive for anti-NMDAR antibodies, confirming anti-NMDAR encephalitis. After transfer to our facility, the patient received intravenous immunoglobulin therapy, resulting in gradual restoration of consciousness. He was eventually discharged with significant clinical improvement.

Discussion

HSV encephalitis is the most common cause of sporadic encephalitis in adults and carries significant mortality despite timely antiviral treatment[5]. MRI often reveals temporal lobe involvement. CSF may show lymphocytic pleocytosis, elevated protein levels, and erythrocytes. HSV PCR in CSF has >95% sensitivity and specificity for diagnosis. Hyponatremia has been reported in 33–83% of patients with HSV encephalitis cases. Başaran et al.[1], evaluated 67 patients with infectious encephalitis. Hyponatremia was detected in 56% of those with HSV encephalitis and in 19% of patients with other infectious encephalitis forms without HSV; this difference was shown to be statistically significant. Hyponatremia, disorientation, and the presence of lesions on initial brain computed tomography are poor prognostic indicators in HSV encephalitis, as demonstrated by Riancho et al.[5] in a Spanish study.

Hyponatremia may have several underlying mechanisms and may present with hypovolemia due to high fever, inadequate fluid intake, or vomiting, or euvolemia due to SIADH or CSWS. Characterizing the specific etiology underlying hyponatremia in these patients is often challenging. In hyponatremic presentations associated with central nervous system pathology and increased urinary sodium excretion, laboratory and clinical data must be interpreted together[6]. In another series of 79 encephalitis cases, hyponatremia occurred in 22 patients (27.8%): 12 were classified as SIADH, 2 as CSWS, and 8 remained unclassified[7]. SIADH and CSWS are the most frequent etiologies, and their treatments differ completely; SIADH requires fluid restriction, whereas CSWS mandates aggressive volume repletion. Unlike SIADH, CSWS is accompanied by extracellular volume depletion, although quantifying such losses is difficult. In our patient, diminished skin turgor, dry mucous membranes, and elevated urinary sodium excretion were observed. He received aggressive isotonic fluid resuscitation, after which serum sodium normalized. These findings were consistent with CSWS in the context of HSV encephalitis.

Anti-NMDAR encephalitis is an autoimmune encephalitis that predominantly affects the limbic system. It is mediated by immunoglobulin G antibodies targeting the NR1 subunit of the NMDA receptor in the central nervous system. The disease typically follows an acute or subacute course and presents with a combination of seizures, psychiatric disturbances, cognitive impairment, and movement disorders[8]. Titulaer et al.[9] reported that among patients aged ≥45 years, anti-NMDAR encephalitis occurs with similar frequency in males and females, and its heterogeneous clinical manifestations often result in a broad initial differential diagnosis. Although older age is generally associated with a milder disease course, delayed diagnosis and treatment can lead to poorer outcomes.

Anti-NMDAR encephalitis may develop during or after HSV encephalitis. The underlying mechanism involves HSV-induced neuronal injury, which exposes neuronal antigens and subsequently triggers autoantibody formation against NMDA receptors. Clinically, the two disease phases are distinct. The acute HSV encephalitis phase is characterized by fever, headache, seizures, and altered mental status, reflecting viral infection and inflammation localized to the temporal lobes. In contrast, the secondary autoimmune phase is dominated by psychiatric symptoms, memory impairment, speech dysfunction, dyskinesias, and behavioral abnormalities, often mimicking primary psychiatric disorders[10–12].

In a cohort of 58 patients with autoimmune encephalitis following HSV encephalitis, antibody positivity confirmed the diagnosis, and 74% had anti-NMDAR antibodies. The most common clinical features included behavioral changes (93%), decreased level of consciousness (57%), choreoathetosis (47%; observed in all children aged ≤4 years), seizures (38%), and dysautonomia (27%)[9].

First-line immunotherapy for anti-NMDAR encephalitis includes intravenous methylprednisolone and/or intravenous immunoglobulin (IVIG). In the present case, administration of IVIG resulted in marked clinical improvement and eventual full recovery.

Conclusion

Clinicians should be aware of the importance of recognizing hyponatremia due to CSWS during HSV encephalitis. Early differentiation of hyponatremia subtypes and timely initiation of appropriate antiviral therapy and fluid management are critical for optimizing patient outcomes. HSV encephalitis may also trigger an autoimmune encephalitis. After the diagnosis of HSV encephalitis, it is necessary to remain vigilant for persistent or newly developing symptoms, especially those related to the central nervous system or those that may present with similar psychiatric complaints. Early diagnosis and appropriate treatment significantly reduce mortality and morbidity, as patients who received early diagnosis and treatment had better outcomes at the end of therapy.

Ethics

Informed Consent: Written informed consent was obtained.

Footnotes

Authorship Contributions

Surgical and Medical Practices: D.U., S.ZU., S.D.Ç., Z.K., B.D., H.E., Concept: D.U., S.ZU., S.D.Ç., A.E., B.D., H.E., Design: D.U., S.ZU., S.D.Ç., Z.K., A.E., B.D., H.E., Data Collection or Processing: D.U., S.ZU., S.D.Ç., Z.K., A.E., B.D., H.E., Analysis or Interpretation: D.U., S.ZU., Z.K., A.E., B.D., H.E., Literature Search: D.U., S.ZU., H.E., Writing: D.U., S.ZU., A.E., H.E.

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A Case of Chronic Hepatitis C with Treatment Failure of Glecaprevir/Pibrentasvir

Glekaprevir/Pibrentasvir Tedavi Başarısızlığı Gelişen Kronik Hepatit C Olgusu

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Abstract

Direct-acting antivirals have achieved high sustained virologic response (SVR) rates in the treatment of chronic hepatitis C virus (HCV) infection. However, data on retreatment strategies following failure of glecaprevir/pibrentasvir (G/P) therapy remain limited. We report the case of a 48-year-old man with genotype 3 chronic HCV infection who experienced virologic failure despite full adherence to an 8-week G/P regimen. The patient was subsequently treated with sofosbuvir/velpatasvir/voxilaprevir (SOF/VEL/VOX) for 12 weeks, resulting in normalization of liver enzyme levels and undetectable HCV ribonucleic acid at the end of treatment and 12 weeks after treatment, confirming achievement of SVR12. This case demonstrates that SOF/VEL/VOX may represent an effective and safe salvage therapy for patients with genotype 3 HCV infection who fail prior G/P treatment and provides clinical insight to guide retreatment decisions.

Keywords: Chronic viral hepatitis C, direct-acting antivirals, hepatitis C

Öz

Doğrudan etkili antiviraller, kronik hepatit C virüsü (HCV) enfeksiyonunun tedavisinde yüksek kalıcı virolojik yanıt (SVR) oranlarına ulaşılmasını sağlamıştır. Ancak glekaprevir/pibrentasvir (G/P) tedavisinin başarısızlığı sonrasında yeniden tedavi stratejilerine ilişkin veriler sınırlı kalmaktadır. Bu yazıda, genotip 3 kronik HCV enfeksiyonu olan ve 8 haftalık G/P rejimine tam uyum göstermesine rağmen virolojik başarısızlık gelişen 48 yaşındaki bir erkek olgu sunulmaktadır. Hasta daha sonra 12 hafta süreyle sofosbuvir/velpatasvir/voxilaprevir (SOF/VEL/VOX) ile tedavi edilmiş olup, tedavi sonunda ve tedavi bitiminden 12 hafta sonra karaciğer enzim düzeylerinin normalleştiği ve HCV ribonükleik asidinin saptanamaz olduğu gösterilerek SVR12 elde edildiği doğrulanmıştır. Bu olgu, önceki G/P tedavisinde başarısızlık gelişen genotip 3 HCV enfeksiyonlu hastalarda SOF/VEL/VOX'un etkili ve güvenli bir kurtarma tedavisi seçeneği olabileceğini göstermekte ve yeniden tedavi kararlarına klinik açıdan yol gösterici nitelik taşımaktadır.

Anahtar Kelimeler: Kronik viral hepatit C, doğrudan etkili antiviraller, hepatit C

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide. More than half of infected individuals progress to chronic infection, which may persist lifelong if left untreated[1,2]. The management of hepatitis C has evolved rapidly with the introduction of direct-acting antivirals (DAAs), leading to highly effective and well-tolerated treatment

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regimens. Current guidelines recommend treatment for all patients without contraindications[3].

Despite the high success rates of DAA-based therapies, a small proportion of patients experience virologic failure. Evidence regarding optimal retreatment strategies for patients who fail glecaprevir/pibrentasvir (G/P) therapy remains limited and has not been sufficiently evaluated[3,4]. Here, we present a case of chronic HCV infection in which virologic suppression was not achieved following G/P therapy, and successful retreatment was subsequently accomplished using an alternative DAA regimen. Written informed consent was obtained from the patient for publication of this case.

Case Report

A 48-year-old man presented after testing positive for anti-HCV antibodies during routine laboratory screening. He was asymptomatic at presentation. Although he was aware of his hepatitis C diagnosis, he had not previously sought medical follow-up or treatment. His medical history was notable for intravenous drug use approximately 20 years earlier. He had no known chronic illnesses, was not taking regular medications, and had no family history of hepatitis.

On physical examination, vital signs were within normal limits, and no abnormal findings were observed. Laboratory evaluation revealed alanine aminotransferase (ALT) of 155 U/L (reference <45 U/L), aspartate aminotransferase (AST) of 142 U/L (reference <45 U/L), platelet count of 87,000/µL (reference >100,000/µL), and HCV–ribonucleic acid (RNA) level of 16,000,000 IU/mL. Serologic testing showed negative hepatitis B surface antigen, negative antibody to hepatitis B surface antigen, negative antibody to hepatitis B core antigen immunoglobulin G (IgG), positive antibody to hepatitis A virus IgG, and negative antibody to human immunodeficiency virus antibodies. Other laboratory parameters were within normal limits, including a white blood cell count of 5,000/µL, hemoglobin level of 14.8 g/dL, hematocrit of 44.4%, and serum creatinine of 1.04 mg/dL.

The fibrosis-4 (FIB-4) score was calculated as 6.29, and the AST-to-platelet ratio index (APRI) score was 3.98. Hepatobiliary ultrasonography demonstrated a liver craniocaudal length of 156 mm, which was at the upper limit of normal, with regular contours and no evidence of cirrhosis or splenomegaly.

The patient was initiated on the pangenotypic regimen G/P (100 mg/40 mg). At week 8 of treatment, laboratory testing showed ALT of 136 U/L, AST of 95 U/L, HCV-RNA of 2,000,000 IU/mL, and a platelet count of 81,000/µL, indicating virologic failure. Changes in laboratory parameters during treatment are summarized in Table 1.

Although the HCV-RNA level decreased during the fourth week of G/P therapy, a high viral load was detected at week 8. Repeat testing confirmed persistently elevated HCV-RNA levels. Consequently, the patient's adherence to therapy was carefully evaluated and confirmed to be appropriate, with regular and correct medication intake. The patient denied the use of concomitant medications, alcohol, or other substances.

HCV genotyping revealed genotype 3 infection. An antiviral resistance analysis was requested from the Public Health Microbiology Reference Laboratory; however, the test was not processed. Based on current clinical guidelines, a review of the available literature, and the antiviral agents accessible in our country, retreatment with sofosbuvir/velpatasvir/voxilaprevir (SOF/VEL/VOX) (400 mg/100 mg/100 mg) was initiated and continued for 12 weeks.

Table 1. Laboratory results during G/P treatment.

Parameter	Before treatment	Week 4	Week 8
ALT	155 U/L	59 U/L	136 U/L
AST	142 U/L	50 U/L	95 U/L
HCV-RNA	16,000,000 IU/mL	15,000 IU/mL	2,000,000 IU/mL
Platelet	87,000/µL	82,000/µL	81,000/µL

ALT, alanine aminotransferase; AST, aspartate aminotransferase; G/P, glecaprevir/pibrentasvir; HCV-RNA, hepatitis C virus/ribonucleic acid.

Table 2. Laboratory results during SOF/VEL/VOX treatment.

Parameter	Before treatment	Week 4	Week 8	Week 12
ALT	145 U/L	56 U/L	50 U/L	53 U/L
AST	102 U/L	40 U/L	40 U/L	39 U/L
HCV-RNA	3,900,000 IU/mL	62 IU/mL	30 IU/mL	Negative
Platelet	81,000/µL	89,000/µL	91,000/µL	100,000/µL

ALT, alanine aminotransferase; AST, aspartate aminotransferase; G/P, glecaprevir/pibrentasvir; HCV-RNA, hepatitis C virus/ribonucleic acid; SOF/VEL/VOX, sofosbuvir/velpatasvir/voxilaprevir.

Throughout the treatment period, ALT and AST levels were closely monitored and demonstrated a gradual decline. At the end of treatment, ALT was 53 U/L, AST was 39 U/L, and the platelet count was 100,000/µL. HCV-RNA testing performed at the end of therapy showed undetectable viral levels (Table 2).

At 12 weeks post-treatment, the patient's ALT was 31 U/L, AST was 37 U/L, platelet count was 104,000/µL, and HCV-RNA was not detected. Liver enzymes had returned to normal, and thrombocytopenia had resolved. Sustained virologic response (SVR) was confirmed at the end of treatment and 12 weeks post-treatment.

Discussion

The efficacy of DAA therapies for HCV infection is well established. G/P therapy achieves SVR at 12 weeks post-treatment (SVR12) in over 95% of patients, including those with prior DAA exposure, with virologic failure rates ranging from 0% to 1%[5–10]. However, evidence regarding retreatment strategies for patients who fail G/P therapy remains limited.

In the EXPEDITION-8 trial, 343 treatment-naive cirrhotic patients with genotypes 1–6 received G/P for 8 weeks, with 18% infected with genotype 3. The overall SVR12 rate was 97.7%, while patients with genotype 3 achieved 95.2%[11]. This study highlighted the relatively lower success rate in genotype 3 infections, consistent with our case of a patient with genotype 3 who failed G/P therapy.

Treatment options for chronic hepatitis C patients with prior NS5A inhibitor exposure are limited. Pibrentasvir exhibits a higher in vitro resistance barrier than other NS5A inhibitors, making combination therapy with sofosbuvir a promising alternative[3,12]. The MAGELLAN-3 study reported a 96% SVR12 rate with 12–16 weeks of G/P plus sofosbuvir and ribavirin in patients with previous G/P treatment failure[13].

According to European Association for the Study of the Liver (EASL) guidelines, genotype 3 patients with prior treatment experience and no cirrhosis may be treated with SOF/VEL or G/P. For patients with cirrhosis, the triple regimen SOF/VEL/VOX is recommended[3]. In our patient, these standard regimens could not be implemented because sofosbuvir-containing drugs were unavailable locally. Although cirrhosis was not assessed via biopsy or noninvasive methods, elevated fibrosis scores (APRI and FIB-4) suggested advanced liver disease. Based on available evidence and treatment options, SOF/VEL/VOX therapy was initiated.

The efficacy of SOF/VEL/VOX in patients who fail G/P therapy remains unclear, as most studies predate widespread G/P use[14]. While EASL guidelines recommend 12 weeks of SOF/VEL/VOX for all DAA-experienced patients, data specific to G/P failures are limited. SOF/VEL/VOX serves as a salvage regimen for patients with prior NS5A inhibitor exposure[3,11]. In a study of 31 patients who failed G/P therapy and received SOF/VEL/VOX, 94% achieved SVR12, supporting its efficacy[4]. Consistent with these findings, our patient achieved SVR12 following SOF/VEL/VOX therapy. A limitation of this case was the absence of resistance testing following G/P failure.

This case demonstrates several distinctive features that add real-world evidence to the management of G/P failure. Despite the high efficacy of G/P across genotypes, including genotype 3, our patient exhibited an unusual virologic pattern: an initial on-treatment response followed by viral rebound at week 8, a rare occurrence in the literature. Although resistance testing and standard retreatment algorithms could not be applied, SOF/VEL/VOX led to complete virologic suppression. The regimen was well tolerated, even in the context of laboratory indicators suggestive of advanced fibrosis, underscoring its therapeutic potential in challenging scenarios.

Conclusion

In conclusion, a 12-week course of SOF/VEL/VOX can achieve SVR12 in genotype 3 chronic hepatitis C patients with G/P treatment failure. This regimen should be considered a safe and effective salvage option in similar clinical contexts.

Ethics

Informed Consent: Written informed consent was obtained from the patient for publication of this case.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.A., Y.Ç., Concept: S.A., Design: S.A., Y.Ç., Data Collection or Processing: S.A., Analysis or Interpretation: S.A., Literature Search: S.A., Y.Ç., Writing: S.A., Y.Ç.

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Knowledge Level, Vaccination Rates, and Determinants of HPV Vaccination among People Living with HIV in Türkiye: A Cross-sectional Study

Türkiye'de HIV ile Yaşayan Kişilerde HPV Aşısına İlişkin Bilgi Düzeyi, Aşılama Oranları ve HPV Aşılamasını Etkileyen Faktörler: Kesitsel Bir Çalışma

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Abstract

Introduction: Human papillomavirus (HPV) vaccination has not yet been included in national immunization programs in many low- and middle-income countries, including Türkiye. People living with human immunodeficiency virus (PLWH) are a priority group for vaccination due to their heightened susceptibility to persistent HPV infection and HPV-related cancers. Identifying barriers to vaccination is essential for developing effective prevention strategies.

Materials and Methods: We conducted a cross-sectional study between April and July 2024 among PLWH receiving care at a tertiary training and research hospital. Data were collected using a structured questionnaire covering sociodemographic characteristics, HPV knowledge, vaccination history, and attitudes. Statistical analyses were performed to identify factors associated with HPV knowledge and vaccine uptake.

Results: A total of 120 individuals participated: 92.5% (n = 111) were male, 6.7% (n = 8) female, and 0.8% (n = 1) transgender. The mean age was 34.6 years. Overall, 60.0% (n = 72) reported awareness of HPV, but only 12.5% (n = 15) had received at least one vaccine dose. Among those aware of HPV, uptake was 18.1%. Vaccination was significantly higher among participants with a previous HPV infection (25.0%, $p < 0.05$) and those informed by physicians (45.8%, $p < 0.001$). Among unvaccinated participants, 48.8% (n = 51) indicated they would accept the vaccine only if it were provided free of charge. Higher education level, younger age, and non-heterosexual orientation were significantly associated with greater HPV knowledge.

Conclusion: Despite moderate awareness, HPV vaccination coverage among PLWH in Türkiye remains unacceptably low, primarily due to economic and informational barriers. Expanding cost-free vaccination programs and reinforcing physician recommendations are critical to improving vaccine uptake and reducing the burden of HPV-related diseases in this high-risk population.

Keywords: Barriers, cost, health care, HIV, HPV, knowledge, vaccination

Öz

Giriş: Türkiye dâhil olmak üzere birçok düşük ve orta gelirli ülkede insan papilloma virüsü (HPV) aşısı henüz ulusal bağışıklama programına dâhil edilmemiştir. İnsan bağışıklık yetmezliği virüsü (HIV) ile yaşayan kişiler, HPV enfeksiyonu ve buna bağlı kanserlere karşı artmış duyarlılıkları nedeniyle aşılama açısından öncelikli bir risk grubudur. Aşılama önündeki engellerin belirlenmesi, etkili korunma stratejilerinin geliştirilmesi için önem taşımaktadır.

Gereç ve Yöntem: Çalışma Nisan–Temmuz 2024 tarihleri arasında, üçüncü basamak bir eğitim ve araştırma hastanesinde HIV ile yaşayan kişiler arasında yürütüldü. Yapılandırılmış bir anket aracılığıyla katılımcıların sosyodemografik özellikleri, HPV hakkındaki bilgi düzeyleri, aşı öyküleri ve aşıya ilişkin tutumları değerlendirildi. HPV bilgisi ve aşılama oranlarıyla ilişkili faktörler istatistiksel olarak analiz edildi.

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Bulgular: Çalışmaya toplam 120 kişi katıldı; katılımcıların %92,5'i (n = 111) erkek, %6,7'si (n = 8) kadın ve %0,8'i (n = 1) transgender olarak tanımlandı. Katılımcıların ortalama yaşı 34,6 idi. Katılımcıların %60,0'ı HPV hakkında bilgi sahibi olduğunu bildirmiş, ancak yalnızca %12,5'i en az bir doz HPV aşısı yaptırmıştı. HPV konusunda bilgi sahibi olanlar arasında aşılama oranı %18,1 olarak bulundu. Aşılanma oranı, önceden HPV enfeksiyonu geçirenlerde (%25,0; $p < 0.05$) ve hekimden bilgi alanlarda (%45,8; $p < 0,001$) anlamlı olarak daha yüksek saptandı. Aşılanmamış bireylerin %48.8'i (n = 51) aşının yalnızca ücretsiz olması durumunda aşı yaptıracağını belirtti. Daha yüksek eğitim düzeyi, genç yaş ve heteroseksüel olmayan yönelim, HPV konusunda daha yüksek bilgi düzeyi ile anlamlı olarak ilişkili bulundu.

Sonuç: Türkiye'de HIV ile yaşayan bireylerde HPV farkındalığı orta düzeyde olmasına rağmen aşılama oranı belirgin biçimde düşüktür. Ekonomik ve bilgiye erişimle ilgili engeller bu durumu belirleyen temel faktörlerdir. Ücretsiz aşılama programlarının yaygınlaştırılması ve hekimlerin aşı önerilerinin güçlendirilmesi, bu yüksek riskli grupta HPV ilişkili hastalık yükünü azaltmak açısından kritik öneme sahiptir.

Anahtar Kelimeler: Engeller, maliyet, sağlık hizmetleri, HIV, HPV, bilgi düzeyi, aşılama

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection (STI) worldwide and is associated with a broad spectrum of diseases, including genital warts and cancers of the cervix, anus, and oropharynx[1,2]. People living with human immunodeficiency virus (HIV; PLWH) are particularly susceptible to persistent HPV infection and HPV-related malignancies due to HIV-induced immunodeficiency, which impairs viral clearance and increases oncogenic potential[3–6]. Among PLWH, men who have sex with men (MSM) are especially vulnerable to anal HPV infection and the subsequent development of anal cancer[7].

HPV vaccination is highly effective in preventing infections caused by high-risk HPV types. Leading health authorities, including the World Health Organization, the European AIDS Clinical Society, and the Centers for Disease Control and Prevention, recommend HPV vaccination for all eligible individuals, including PLWH[8,9]. Nevertheless, in many low- and middle-income countries (LMICs), such as Türkiye, HPV vaccines are not universally incorporated into national immunization programs. This, combined with out-of-pocket payment requirements, significantly restricts access, particularly for economically disadvantaged PLWH[10].

Limited knowledge about HPV and its associated risks further impedes vaccine uptake. Studies in LMICs have shown that PLWH often possess inadequate awareness of HPV-related diseases and the benefits of vaccination, contributing to low vaccination coverage[11,12]. Despite the high prevalence of HIV–HPV coinfection in LMICs, data from Türkiye regarding HPV knowledge, attitudes, and vaccine access among PLWH remain scarce. This study aimed to assess the knowledge and attitudes of PLWH toward HPV and HPV vaccination in Türkiye and to identify sociodemographic and clinical factors influencing vaccination uptake. To the best of our knowledge, this is the first study from Türkiye to report HPV knowledge and vaccination data among PLWH. By situating the findings within the broader LMIC context, the results may inform targeted strategies to enhance HPV prevention among high-risk populations.

Materials and Methods

This cross-sectional observational study was conducted between April 1 and July 1, 2024, among PLWH aged 18 years or older who was receiving care at a training and research hospital. Data were collected using a structured questionnaire administered through face-to-face interviews. The questionnaire included demographic variables (age, gender, education level, disease history, and number of sexual partners in the past year) as well as items assessing knowledge of HPV and attitudes toward HPV vaccination.

Ethical approval was obtained from the Clinical Research Ethics Committee of University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital (approval number: 4,338, dated: 12.03.2024). Written informed consent was obtained from all participants prior to enrollment. Data collection was performed anonymously to ensure participant confidentiality.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 21.0 (Armonk, NY, USA). Descriptive statistics were used to summarize participants' sociodemographic and behavioral characteristics. Categorical variables were presented as frequencies and percentages, and associations between categorical variables were assessed using the chi-square test or Fisher's exact test, as appropriate. Variables with a p-value <0.10 in bivariate analysis were included in a multivariate logistic regression model to identify independent predictors of HPV vaccination. Statistical significance was set at $p < 0.05$.

Results

Sociodemographic and Behavioral Characteristics

A total of 120 participants were enrolled in the study. The majority were male (92.5%, n = 111), followed by female (6.7%, n = 8) and

transgender individuals (0.8%, n = 1). Regarding age distribution, 16.7% (n = 20) were younger than 25 years, 37.5% (n = 45) were 25–35 years, 32.5% (n = 39) were 35–45 years, and 13.3% (n = 16) were older than 45 years.

All participants had completed at least primary education. Specifically, 5.0% (n = 6) had primary education, 9.2% (n = 11) middle school, 25.8% (n = 31) high school, 43.3% (n = 52) university, and 16.7% (n = 20) postgraduate education.

Regarding sexual orientation, 42.5% (n = 51) identified as homosexual, 32.5% (n = 39) as heterosexual, and 16.7% (n = 20) as bisexual. In the past year, 12.5% (n = 15) reported no sexual partners, 30.0% (n = 36) had one partner, 33.3% (n = 40) had 2–5 partners, and 19.2% (n = 23) had more than five partners.

Overall, 26.7% (n = 32) reported a history of HPV infection, 32.5% (n = 39) had undergone HPV/Pap smear testing, and 52.5% (n = 63) reported previous STIs other than HIV and HPV.

HPV Knowledge Level

Among participants, 60.0% (n = 72) reported awareness of HPV. To verify self-reported knowledge, participants were asked five specific HPV-related questions. Of these, 55.8% correctly identified that "HPV causes genital warts," and 48.3% correctly recognized that "HPV can cause cancer." The statements "HPV only causes disease in women" and "HPV only causes disease in homosexual men" were correctly rejected by 95.8% and 98.3% of participants, respectively. The statement "Condoms completely prevent HPV transmission" had the lowest correct response rate (28.3%).

HPV knowledge was significantly associated with education, age, and sexual orientation. Knowledge rates were highest among university or postgraduate graduates (69.6%) and lowest among participants with primary or middle school education (5.0%, p = 0.015).

When analyzed by age, 70.0% of participants under 25 years, 70.5% of those aged 25–35 years, and 64.0% of those aged 35–45 years reported awareness of HPV, compared to only 26.9% of participants over 45 years, indicating a significant decline in knowledge with increasing age (p = 0.002).

By sexual orientation, 68.6% of homosexual participants reported awareness of HPV, compared with 60.0% of bisexual and 41.0% of heterosexual participants. Knowledge among homosexual and bisexual participants was significantly higher than among heterosexual participants (p = 0.012).

No significant associations were observed between HPV knowledge and gender or number of sexual partners. However, participants with a history of HPV infection were more likely to report awareness than those without (90.6% vs. 48.9%, p < 0.001). Similarly, individuals with a history of other STIs had higher HPV knowledge than those without (68.3% vs. 50.9%, p = 0.045) (Table 1).

Knowledge and Attitudes Regarding HPV Vaccines

Overall, 40.8% of participants reported awareness of HPV vaccines, while 59.2% were uninformed. Among participants with knowledge of the vaccine, 6.1% believed no effective vaccine existed, 22.4% thought the vaccine protects only against warts, and 65.3% correctly recognized that it protects against both warts and cancer. Additionally, 42.9% believed the vaccine would be ineffective if not administered early, and 36.7% thought it would be ineffective after wart development.

Table 1. Association between HPV knowledge level and sociodemographic and clinical variables.

Variable	Reported HPV knowledge: Yes, n (%)	Reported HPV knowledge: No, n (%)	p-value
Education level			**0.015**
Primary/middle school	1 (5.0)	16 (95.0)	
High school	12 (37.5)	20 (62.5)	
University/ postgraduate	59 (69.6)	26 (30.4)	
Age group			**0.002**
<25	14 (70.0)	6 (30.0)	
25–35	31 (70.5)	13 (29.5)	
35–45	25 (64.0)	14 (36.0)	
>45	4 (26.9)	12 (73.1)	
Sexual orientation			**0.012**
Homosexual	35 (68.6)	16 (31.4)	
Bisexual	12 (60.0)	8 (40.0)	
Heterosexual	16 (41.0)	23 (59.0)	
History of HPV infection			**<0.001**
Yes	29 (90.6)	3 (9.4)	
No	41 (48.9)	43 (51.1)	
History of STIs			**0.045**
Yes	43 (68.3)	20 (31.7)	
No	29 (50.9)	28 (49.1)	
Gender			**0.538**
Male	67 (60.4)	44 (39.6)	
Female/transgender	5 (55.6)	4 (44.4)	
Number of sexual partners (last 1 year)			**0.619**
0	8 (53.3)	7 (46.7)	
1	22 (61.1)	14 (38.9)	
2–5	24 (60.0)	16 (40.0)	
>5	18 (64.3)	10 (35.7)	

Statistical analysis was performed using the chi-square test and Fisher's exact test where appropriate.

The main sources of information were physicians (33.3%), friends (27.8%), the internet (16.7%), social media (13.9%), and non-governmental organizations (NGOs) (8.3%).

Vaccination Status

Among all participants, 12.5% (n = 15) had received at least one dose of the HPV vaccine. Among the unvaccinated, the most common reasons for not receiving the vaccine were lack of knowledge (48.6%), high cost (21.0%), perception that vaccination was unnecessary (18.1%), prior HPV infection leading to perceived ineffectiveness (2.9%), and older age (4.8%). No participants cited fear of side effects or distrust in vaccine efficacy.

Of participants reporting HPV knowledge (n = 72), only 18.1% (n = 13) had been vaccinated. Vaccination rates were significantly higher among individuals with a history of HPV infection compared to those without (25.0% vs. 8.0%, p = 0.02).

Vaccination rates did not differ significantly by number of sexual partners in the past year (range: 9.8%–15.8%) or by sexual orientation, although rates were slightly higher among homosexual participants (14.5%) than among heterosexual (9.5%) or bisexual (13.0%) participants (Table 2).

Receiving information from a physician was strongly associated with vaccination: 45.8% of participants informed by a doctor were vaccinated, compared with 4.2% of others (p < 0.001; odds ratio = 19.46), highlighting the key role of healthcare providers in promoting vaccination (Table 3). Vaccination rates were 25.0% among participants who received information from friends or the internet but 0% among those relying on social media or NGOs.

Of the vaccinated participants, 20.0% received one dose, 33.3% received two doses, and 46.7% completed the three-dose series. Vaccine types included bivalent (20.0%), quadrivalent (33.3%), and nonavalent (40.0%), with 6.7% unable to recall the type.

Access to the HPV Vaccine and Perception of Cost

Among unvaccinated participants, 10.8% believed the HPV vaccine was free, 19.2% knew it was paid and were aware of the price, 25.0% knew it was paid but were unaware of the price, and 45.0% had no opinion regarding the cost.

Nearly half of participants (48.6%) indicated they would only consider vaccination if it were free. Among those willing to pay, 31.4% could allocate less than 3,000 TRY, 15.2% could allocate between 3,000 and 6,000 TRY, and 4.8% between 6,000 and 10,000 TRY. The difference in willingness to pay among these budget groups was statistically significant (chi-square = 46.28; p < 0.001), confirming cost as a major barrier to HPV vaccination.

Table 2. Association between HPV vaccination status and sociodemographic and clinical variables.

Variable	Vaccinated, n (%)	Not vaccinated, n (%)	p-value
Age group			**0.237**
<25	4 (20.0)	16 (80.0)	
25–34	4 (8.9)	41 (91.1)	
≥35	7 (12.7)	48 (87.3)	
Education level			**0.974**
High school or below	6 (13.0)	40 (87.0)	
University	7 (11.9)	52 (88.1)	
Postgraduate	2 (13.3)	13 (86.7)	
Gender			**0.471**
Female	0 (0.0)	8 (100.0)	
Male	15 (13.5)	96 (86.5)	
Other	0 (0.0)	1 (100.0)	
Sexual orientation			**0.791**
Heterosexual	4 (9.5)	38 (90.5)	
Homosexual	8 (14.5)	47 (85.5)	
Bisexual	3 (13.0)	20 (87.0)	
Number of sexual partners (last year)			**0.847**
0–1	8 (14.5)	47 (85.5)	
2–4	4 (9.8)	37 (90.2)	
≥5	3 (12.5)	21 (87.5)	
HPV/pap smear test			**0.030**
Yes	9 (23.1)	30 (76.9)	
No	6 (7.4)	75 (92.6)	
History of HPV infection			**0.020**
Yes	8 (25.0)	24 (75.0)	
No	7 (8.0)	81 (92.0)	
History of STIs			**0.951**
Yes	8 (12.7)	55 (87.3)	
No	7 (12.3)	50 (87.7)	
Knowledge about HPV			**<0.001**
Yes	13 (18.1)	59 (81.9)	
No	2 (4.3)	45 (95.7)	

Chi-square and Fisher's exact test were used to evaluate associations between categorical variables.

Table 3. Association between HPV vaccination status and source of information.

Source of information	Vaccinated, n (%)	Not vaccinated, n (%)	Total, n	Vaccination rate (%)
Doctor	11	13	24	45.8
Friends	5	15	20	25.0
Internet	3	9	12	25.0
NGOs	0	6	6	0.0
Social media	0	10	10	0.0

Vaccination rates according to the source of information were compared using Fisher's exact test. The rate of vaccination was significantly higher among participants who received information from a physician ($p < 0.001$, Fisher's exact test; OR = 19.46).

NGOs, non-governmental organizations.

Discussion

This study demonstrates that although most PLWH in Türkiye were aware of HPV, only a small proportion had received the vaccine, highlighting a notable knowledge–behavior gap. The relatively high knowledge level observed—higher than previously reported in the literature[13,14]—may be attributed to the generally high educational attainment of the sample[15,16] and greater awareness among younger participants, who likely have easier access to information, higher digital health literacy, and are more frequently targeted by vaccination campaigns[17].

The finding that homosexual and bisexual individuals had higher HPV knowledge aligns with prior studies[18,19]. This may reflect their more frequent participation in screening programs and greater engagement with health education services. Additionally, the broader availability of HPV-related counseling for MSM living with HIV may further reinforce this disparity.

Higher awareness among participants who had previously experienced HPV or other STIs may indicate an accelerated learning effect associated with personal illness experience[20].

In our study, most participants reported obtaining information about HPV primarily through social networks and other digital sources. This is consistent with previous research, which identifies digital media, social networks, and the internet as the main sources of HPV-related information, even among PLWH[21,22]. While these platforms can facilitate information dissemination, they also carry a risk of misinformation. This underscores the need for healthcare professionals to provide accurate counseling and direct individuals to reliable sources via formal health services.

Being informed does not always translate into behavioral change. In the case of HPV, knowledge does not necessarily lead to the adoption of preventive behaviors such as vaccination. Indeed, only 12.5% of participants reported having been vaccinated, and just 46.7% had completed the three-dose series. Similar gaps between awareness and vaccine uptake have been documented among PLWH in other contexts[23,24]. The low vaccination rates despite relatively high knowledge suggest that general education does not directly reflect health literacy, highlighting the importance of targeted public health education and the need for health systems to bridge the gap between knowledge and action.

A recent randomized controlled trial from Brazil demonstrated that a personalized, web-based intervention significantly increased both HPV vaccine initiation and completion rates among PLWH compared with standard educational approaches[25]. Similarly, a systematic meta-analysis found that digital reminders and provider-based interventions, including SMS reminders and electronic health record prompts, were more effective in promoting HPV vaccination uptake than client education alone[26]. In Türkiye, where HPV vaccines are not included in the national immunization program and economically disadvantaged groups face additional access barriers, such low-cost and scalable digital interventions could play a critical role in improving vaccination coverage among PLWH.

The unexpectedly low vaccination rates among individuals engaging in high-risk sexual behaviors suggest that these groups are not adequately targeted in vaccination programs, revealing potential strategic gaps. Previous research has similarly documented suboptimal HPV vaccine uptake among individuals with histories of risky sexual behavior, including heterosexually active adults at increased risk for HIV and transgender or gender-diverse populations, who often experience overlapping behavioral and structural risk factors[27,28]. In our study, participants reporting risky sexual behaviors, such as having multiple sexual partners, did not show a statistically significant association with vaccination status. This finding suggests that, even within high-risk groups, awareness may not readily translate into preventive action, and access-related or systemic barriers may be more decisive.

Conversely, some studies have reported that MSM living with HIV are more likely to accept HPV vaccination[29]. A systematic review indicated that HPV vaccine uptake among HIV-negative MSM ranged from 2.7% to 91.5%, whereas among MSM

living with HIV, it ranged from 5.6% to 90.0%, highlighting both heterogeneity and generally higher uptake in the latter group[30]. A recent meta-analysis further reported HPV vaccine acceptance among men with same-sex orientation at approximately 62.2%, compared with 47.0% among men in general[31]. In our study, although homosexual participants exhibited higher vaccination rates than heterosexual participants, the difference was not statistically significant, likely due to limited sample size. Greater engagement of MSM with healthcare services, coupled with exposure to comprehensive sexual health education and counseling, may explain the relatively higher vaccine acceptance observed in this group.

Among individuals with a history of HPV infection, the knowledge rate was 90.6%, compared with 48.9% among those without such a history. Although this knowledge increase was positively reflected in vaccination behavior, only 25.0% of individuals with prior HPV infection were vaccinated, compared with 8.0% without such history. Thus, while increased knowledge may enhance vaccination behavior, the uptake remains suboptimal. The low vaccination rates among individuals with HPV-related disease history suggest that opportunities for secondary prevention are underutilized. Failure to provide vaccination counseling to individuals who have undergone HPV testing or have a history of warts may result in missed vaccination opportunities. Similar patterns have been reported in the general population, where women with high-grade cervical lesions remain unvaccinated despite clear eligibility, often due to misconceptions about age limits, prior HPV infection, or lack of provider recommendation[32]. Additionally, catch-up vaccination in older age groups remains underused[33].

Increasing HPV vaccination among (PLWH) requires more than simply providing information. Our analysis revealed that the source of information critically influences the decision to vaccinate. Physician-delivered information has consistently been identified as the strongest predictor of vaccination[34–36]. Healthcare providers who actively recommend the HPV vaccine significantly increase uptake, whereas the absence of such recommendations remains a major barrier[37]. However, even when providers recognize the importance of vaccination, practical, evidentiary, and ethical challenges may hinder recommendations, particularly for older or high-risk populations, including gay, bisexual, and other MSM[38]. These findings highlight the pivotal role of healthcare professionals in initiating vaccination discussions, correcting misconceptions, and facilitating access for eligible individuals.

Conversely, in our study, vaccination rates were significantly lower among participants who relied on social media or internet sources for information. Although the internet can increase awareness of HPV vaccination, it may also disseminate misinformation, contributing to vaccine hesitancy[39].

When participants were asked why they had not been vaccinated, lack of knowledge and cost were the most frequently cited reasons. Notably, 48.6% of unvaccinated participants stated that they would only receive the vaccine if it were free. This aligns with evidence from the Eastern Mediterranean Region, where the high cost of the HPV vaccine—especially in non-Gavi-eligible middle- and high-income countries—was identified as a major barrier to immunization. In contexts without vaccine subsidies, financial burden substantially reduces vaccination rates, even among high-risk populations[40]. Considering the burden of HPV-related infections and cancers in PLWH, providing free vaccination to this group is critically important.

Evidence from multiple settings indicates that HPV vaccination can be cost-effective for PLWH by preventing HPV-related diseases and reducing healthcare expenditures. In China, a Markov model analysis demonstrated that vaccinating women living with HIV aged 18–45 years, particularly with the quadrivalent vaccine, was cost effective and substantially reduced genital warts, cervical cancer incidence, and related mortality compared with no vaccination[41]. In Germany, dynamic transmission modeling suggested that extending HPV vaccination to MSM up to ages 26 or 45 could avert thousands of anogenital cancers and warts, with incremental cost-effectiveness ratios within acceptable thresholds[42]. Similarly, in the United States, modeling studies in HIV-positive MSM indicated that treating high-grade squamous intraepithelial lesions combined with adjuvant HPV vaccination in individuals aged 38 years or older was cost effective compared with no intervention[43]. Collectively, these findings suggest that HPV vaccination offers favorable economic value for both women and men living with HIV. Ensuring insurance coverage, implementing health policies to reduce out-of-pocket costs, expanding reimbursement mechanisms, and integrating HPV vaccination into publicly funded programs may substantially improve access for PLWH, particularly in countries such as Türkiye, where financial barriers remain significant.

Given that lack of knowledge and high vaccine cost remain major barriers to HPV vaccination among PLWH, interventions should also address social and psychological factors influencing uptake. In particular, stigma associated with HIV and other STIs may discourage individuals from seeking vaccination or discussing it with partners. Culturally sensitive education, community engagement, and the creation of a supportive, non-judgmental healthcare environment may facilitate vaccine access, empower informed decision-making, and encourage open communication about HPV prevention[11].

Although this study identifies important trends, several methodological limitations must be acknowledged. First, the sample size was limited in certain subgroups, such as women and heterosexual individuals, which may have reduced the ability to detect statistically significant differences. For variables showing no significant differences, this may reflect insufficient statistical power rather than a true absence of effect. The cross-sectional design precludes causal inferences; therefore, relationships should be interpreted as associations rather than causation. Additionally, reliance on self-reported data may introduce response bias. Multicenter studies with larger samples are needed to confirm these findings and enable broader generalization.

This research did not receive specific funding, and the authors declare no conflicts of interest.

Conclusion

In this study, 60.0% of participants reported awareness of HPV, yet only 12.5% had received at least one dose of the HPV vaccine. Among participants aware of HPV, the vaccination rate was 18.1%. Higher vaccination rates were observed among individuals with a history of HPV infection and those who received information from a physician. Greater HPV knowledge scores were associated with higher education, younger age, and non-heterosexual orientation. Notably, 48.8% of unvaccinated participants indicated they would accept the vaccine only if it were free. These findings suggest that, in this study population, vaccination coverage among PLWH remains substantially lower than awareness levels. Cost-related barriers and limited physician-delivered information were identified as key factors influencing uptake. Incorporating HPV vaccination into publicly funded programs and providing structured counseling within HIV care settings may enhance vaccine access and uptake among PLWH in Türkiye.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Clinical Research Ethics Committee of University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital (approval number: 4,338, dated: 12.03.2024).

Informed Consent: Written informed consent was obtained from all participants prior to enrollment.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.G., Concept: Ö.G., N.D.D., D.Y.S., Design: Ö.G., D.Y.S., Data Collection or Processing: Ö.G., N.D.D., C.A.T., O.D., A.Ö., D.Y.S., Analysis or Interpretation: Ö.G., O.D., Literature Search: Ö.G., N.D.D., C.A.T., A.Ö., Writing: Ö.G., N.D.D., D.Y.S.

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Prevalence and Antimicrobial Resistance Trends of *Staphylococcus aureus* in a University Hospital: A 6.5-Year Retrospective Analysis

Bir Üniversite Hastanesinde *Staphylococcus aureus* Prevelansı ve Antimikrobiyal Direncin Değişimi: 6.5 Yıllık Retrospektif Bir Analiz

Elif Seren Tanrıverdi*, Yusuf Yakupoğulları, Hasine Ak, Deste Ceylan, Barış Otlu

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Abstract

Introduction: *Staphylococcus aureus* is a major pathogen causing infections in both community and hospital settings. Surveillance of this organism is essential for effective planning of preventive strategies. This study aimed to evaluate changes in the isolation frequency and antimicrobial resistance patterns of *Staphylococcus aureus* strains in a university hospital over recent years.

Materials and Methods: In this retrospective cross-sectional study, data on *Staphylococcus aureus* strains isolated at İnönü University Faculty of Medicine Hospital between January 2019 and June 2025 were collected. Demographic information of patients and antimicrobial resistance profiles of the isolates were analyzed. Changes in strain prevalence and resistance rates over the years were assessed using linear regression analysis.

Results: A total of 3,514 *Staphylococcus aureus* strains were examined, including 861 (24.5%) community-acquired and 2653 (75.5%) hospital-acquired. The median prevalence of *Staphylococcus aureus* among all cultures was 9.96%, showing a nonsignificant variation from 9.2% to 11.57% over the study period. Comparison of pre- and post-pandemic periods revealed a significant increase in hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) ($p < 0.05$), whereas changes in community-acquired MRSA were not significant. Among hospital isolates, methicillin resistance approximately doubled, whereas no significant change was observed in community-acquired strains. Methicillin-resistant strains exhibited two- to seven-fold higher resistance to several antimicrobials, including quinolones, aminoglycosides, macrolides, and co-trimoxazole, compared to methicillin-susceptible strains. During the study period, resistance to amikacin, gentamicin, and erythromycin significantly decreased, while resistance to tetracycline and clindamycin increased.

Conclusion: Although the overall prevalence of *Staphylococcus aureus* remained stable, methicillin resistance increased steadily, particularly in hospital-acquired strains. Targeted strategies to control these highly resistant strains should be a priority.

Keywords: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, methicillin-susceptible *Staphylococcus aureus*, antimicrobial resistance, trend analysis

Öz

Giriş: *Staphylococcus aureus*, hem toplum hem de hastanede enfeksiyona neden olan başlıca patojenlerden biridir. Bu etkene ait sürveyans verilerinin analizi, önleyici girişimlerin doğru planlanması adına önemlidir. Bu çalışmada bir üniversite hastanesinde *Staphylococcus aureus* suşlarının izolasyon sıklığının ve antimikrobiyal direnç özelliğinin son yıllarda bir değişim gösterip-göstermediğinin saptanması amaçlanmıştır.

Gereç ve Yöntem: Bu kesitsel çalışmada, İnönü Üniversitesi Tıp Fakültesi Hastanesi'nde, Ocak 2019 ile Haziran 2025 tarihleri arasında izole edilen *Staphylococcus aureus* suşlarına ait veriler geriye dönük olarak toplandı. Suşların saptandığı hastaların demografik bilgileri ve izolatların antimikrobiyal direnç özellikleri çalışma kapsamında değerlendirildi. Yıllara göre suş ve direnç frekansındaki değişimler doğrusal regresyon ve eğilim analizi ile araştırıldı.

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Öz

Bulgular: Çalışmada, 861'i (%24,5) toplum kaynaklı ve 2653'ü (%75,5) hastane kaynaklı enfeksiyon etkeni olarak değerlendirilen toplam 3514 *Staphylococcus aureus* suşu incelendi. Tüm üremeler arasında *Staphylococcus aureus*'un ortanca prevalansı %9,96 idi ve yıllar içinde %9,2–11,57 arasında istatistiksel olarak anlamlı olmayan bir değişim gösterdi. Pandemi öncesi ve sonrası dönemler karşılaştırıldığında; hastane kaynaklı metisilin dirençli *Staphylococcus aureus* (MRSA) oranlarında anlamlı bir artış saptanırken ($p < 0,05$), toplum kaynaklı MRSA oranlarında anlamlı bir değişiklik gözlenmedi. Hastane izolatları arasında metisilin direnç oranı, çalışma süresince yaklaşık iki kat olmak üzere anlamlı artış gösterirken toplum kökenli suşlardaki artış anlamlı bulunmadı. Metisilin dirençli suşlarda, metisilin-duyarlılara göre kinolonlar, aminoglikozitler, makrolidler ve ko-trimoksazol başta olmak üzere birçok antimikrobiyale karşı anlamlı olarak iki ila yedi kat daha fazla direnç saptandı. Çalışma süresince amikasin, gentamisin ve eritromisin direnci anlamlı azalış gösterirken tetrasiklin ve klindamisin direncinde anlamlı artış bulundu.

Sonuç: Saptanan veriler, *Staphylococcus aureus*'un prevalansında önemli bir değişim olmadığını ancak metisilin direncinin özellikle hastane kökenli suşlarda düzenli bir artış trendi göstererek yükseldiğini ortaya koymuştur. Birçok antibiyotiğe karşı daha yüksek direnç taşıyan bu suşların kontrol altına alınması öncelikli hedeflerden biri olmalıdır.

Anahtar Kelimeler: *Staphylococcus aureus*, metisiline dirençli *Staphylococcus aureus*, metisiline duyarlı *Staphylococcus aureus*, antimikrobiyal direnç, trend analizi.

Introduction

Staphylococcus aureus is an aerobic, gram-positive coccus, testing positive for catalase and coagulase. In humans, it colonizes many body sites, primarily the nose and skin. Through its capsule, cell wall components, and numerous lytic enzymes and toxins, it can cause rapidly developing invasive infections with significant tissue damage under appropriate conditions. Therefore, it can infect multiple parts of the human body in all age groups and both sexes, and unlike many opportunistic hospital pathogens, it is also an important infectious agent in healthy individuals[1].

Staphylococcus aureus is one of the pathogens that can cause infections both in the community and in hospitals. Studies report that it accounts for 7.7%–17.8% of community-acquired bloodstream infections[2] and approximately three-quarters of skin and soft tissue infections[3]. A multicenter prospective study indicated that *Staphylococcus aureus* infections occur more frequently in patients, especially in surgical departments, wound care units, burn and trauma units, and intensive care units; it is responsible for over 10% of hospital-acquired sepsis cases and is the most frequently isolated pathogen in bone and soft tissue infections[4].

Methicillin-resistant *Staphylococcus aureus* (MRSA) significantly limits treatment options and increases morbidity and mortality in life-threatening infections. MRSA bacteremia has been found to result in approximately twice the mortality of methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteremia[5]. MRSA infections are also associated with a 6–14 day longer hospital stay and an additional cost of USD 3,200–9,600 per patient compared with MSSA infections[6]. To control MRSA and other *Staphylococcus aureus* strains in hospitals, interventions such as hand hygiene, wound and catheter care bundles, antimicrobial stewardship, and the use of rapid laboratory diagnostics have been implemented[7]. Following these measures, a considerable decrease in *Staphylococcus aureus* infections has been observed. For instance, an analysis of 130 hospitals in the United States reported a 43% decrease in all *Staphylococcus aureus* infections between 2005 and 2017, more pronounced for MRSA than MSSA[8]. Later studies reported that hospital-acquired MRSA bloodstream infections decreased by 17% annually, especially between 2005 and 2012; however, this rate slowed during 2013–2016, and a slight increase was observed in MSSA infections[9].

However, in recent years, especially in our country, limited data have been published on changes in the frequency of *Staphylococcus aureus* isolation and resistance rates. Moreover, the Coronavirus Disease 2019 (COVID-19) pandemic, which began in March 2020 and lasted at least two years, caused significant changes in community health practices and hospital services, but there is little information on its impact on *Staphylococcus aureus*. Recent reports suggest that the pandemic influenced both the incidence and resistance patterns of *Staphylococcus aureus*, with some studies reporting shifts in detection rates and MRSA proportions[10,11].

This study aimed to investigate the isolation and antimicrobial resistance frequencies of *Staphylococcus aureus* strains in a university hospital over the past 6.5 years, interpret the findings in the context of current scientific evidence, and discuss possible contributing factors.

Materials and Methods

Study Design and Data Collection

This study was retrospectively planned and conducted at İnönü University Turgut Özal Medical Center, a tertiary care hospital with 1,585 beds. In this cross-sectional study, *Staphylococcus aureus* strains isolated from various clinical samples between

January 1, 2019, and June 1, 2025, were analyzed. Demographic characteristics of the patients, including age and gender, type of clinical sample, and isolate-related data, were collected from the hospital information system.

Considering the patient's hospital admission date, isolates from samples collected ≥48 hours after admission were classified as hospital-acquired, while strains isolated from outpatient samples or from clinical samples collected within <48 hours of admission were classified as community-acquired. For repeated growth from the same clinical sample of a patient, only one isolate was included. If *Staphylococcus aureus* growth occurred in different clinical samples from the same patient, one isolate per sample was included. For each study year, the total number of microorganisms grown in laboratory cultures was determined, and the proportional frequency of *Staphylococcus aureus* strains among total isolates was calculated.

Laboratory Tests: Culture, Identification, and Susceptibility

Clinical samples were inoculated onto appropriate culture media, primarily 5% sheep blood agar and eosin methylene blue agar, and incubated aerobically at 35 °C. Colonies suspected to be *Staphylococcus aureus* were identified based on colony morphology, Gram staining, catalase, and coagulase tests, and confirmed using the Vitek MS (bioMérieux, France) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system.

Susceptibility of the isolates to penicillin, cefoxitin, erythromycin, clindamycin, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, tetracycline, amikacin, gentamicin, rifampin, vancomycin, and linezolid was tested using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Condalab, Spain). When necessary, minimal inhibitory concentrations were determined using the VITEK 2 automated identification and susceptibility system with GP-664 antimicrobial susceptibility cards (bioMérieux, France), the broth microdilution method, or E-test strips (Bioanalyse, Türkiye). In total, 1210 isolates (mainly from intensive care unit patients) were tested using VITEK 2 cards, 314 isolates using E-tests, and 72 isolates using broth microdilution, primarily for vancomycin and linezolid. All susceptibility tests were performed and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria[12]. During the study period, the *Staphylococcus aureus* ATCC 29213 strain was used as a control for susceptibility testing.

Methicillin resistance of *Staphylococcus aureus* isolates was determined using a 30 µg cefoxitin disk (Bioanalyse, Türkiye) according to EUCAST guidelines. Isolates with a cefoxitin inhibition zone diameter <22 mm were classified as MRSA, and those with ≥22 mm were classified as MSSA[12].

Statistical Analysis

Categorical data are presented as numbers and percentages. Data analysis was performed using SPSS Statistics (version 20.0, IBM, Chicago, IL, USA). Descriptive statistics were calculated for demographic and clinical variables. The Pearson chi-square test was used to compare categorical variables. Temporal trends in MRSA prevalence and antimicrobial resistance rates were assessed using simple linear regression models. A p-value <0.05 was considered statistically significant.

Results

A total of 3,514 *Staphylococcus aureus* isolates obtained from various clinical samples between January 1, 2019, and June 1, 2025, were examined. Of these, 861 (24.5%) were classified as community-acquired and 2,653 (75.5%) as hospital-acquired. Community-acquired isolates were most frequent in 2024 (160 strains), while hospital-acquired isolates peaked in 2021 (521 strains).

Community-acquired strains were most frequently isolated from wound samples (394 isolates, 45.8%), whereas hospital-acquired strains were most commonly isolated from sputum and lower respiratory tract samples (830 isolates). The numerical distribution of *Staphylococcus aureus* strains by year and clinical sample type is presented in Table 1.

According to susceptibility data, 1,072 (30.5%) of the studied strains were identified as MRSA and 2442 (69.5%) as MSSA. Among community-acquired *Staphylococcus aureus* strains, 234 (27.2%) were MRSA, whereas among hospital-acquired strains, 838 (31.6%) were MRSA; the difference was not statistically significant. Comparison of pre- and post-pandemic periods showed a statistically significant increase in hospital-acquired MRSA strains ($p < 0.05$), whereas no significant change was observed in community-acquired MRSA strains.

As illustrated in Figures 1A and 1B, yearly fluctuations in MRSA and MSSA proportions were observed. Statistical analysis revealed a significant increasing trend for hospital-acquired MRSA ($p < 0.05$), while yearly changes in community-acquired MRSA were not statistically significant. Linear regression analysis confirmed a significant annual increase in hospital-acquired MRSA rates ($p < 0.05$).

Among all *Staphylococcus aureus* isolates (excluding vancomycin and linezolid), the highest susceptibility was observed for amikacin (96.6%), while the highest resistance was seen for penicillin (90.1%). Resistance to all tested antibiotics was significantly higher in MRSA strains compared to MSSA. Specifically, rifampin resistance was higher in community-acquired MRSA strains, levofloxacin resistance was higher in

hospital-acquired MSSA strains, and gentamicin resistance was higher in hospital-acquired MRSA strains.

The comparison of resistance characteristics of *Staphylococcus aureus* strains according to hospital- and community-acquired MRSA and MSSA status is presented in Table 2. Trend analysis (Table 3) showed significant decreases in resistance to amikacin (MRSA, p = 0.02), gentamicin (MRSA, p = 0.02; MSSA, p = 0.04), and erythromycin (MRSA, p = 0.01; MSSA, p = 0.03), while significant increases were observed for tetracycline (MSSA, p = 0.01) and clindamycin (MSSA, p = 0.01). No significant yearly changes were detected for ciprofloxacin, levofloxacin, rifampin, trimethoprim-sulfamethoxazole, or penicillin (all p > 0.05). When pre- and post-pandemic periods were compared, hospital-acquired MRSA strains showed a significant increase (p < 0.05), whereas antibiotic susceptibilities for most agents did not change significantly (p > 0.05).

Table 1. Distribution of *Staphylococcus aureus* strains by year and clinical sample type.

Year		Sample type, n (%)						Total
		Abscess	Urine	Blood	Wound	Sputum	Other	
2019	CA	17 (12.3%)	23 (16.7%)	21 (15.2%)	61 (44.2%)	7 (5%)	9	138
	HA	44 (13.8%)	17 (5.3%)	70 (22%)	64 (20%)	102 (32%)	22	319
2020	CA	16 (20.5%)	14 (18%)	6 (7.7%)	39 (50%)	1 (1.3%)	2	78
	HA	43 (12.2%)	13 (3.7%)	92 (26.1%)	64 (18.2%)	107 (30.4%)	33	352
2021	CA	9 (6%)	51 (34.2%)	13 (8.7%)	62 (41.6%)	4 (2.7%)	10	149
	HA	42 (8%)	36 (6.9%)	112 (21.5%)	117 (22.5%)	176 (33.8%)	38	521
2022	CA	8 (6.3%)	40 (31.7%)	12 (9.5%)	58 (46%)	1 (0.8%)	7	126
	HA	33 (7.4%)	31 (6.9%)	89 (19.9%)	129 (28.9%)	123 (27.5%)	42	447
2023	CA	7 (5.8%)	38 (31.4%)	10 (8.3%)	57 (47.1%)	6 (5%)	3	121
	HA	35 (8.8%)	29 (7.3%)	90 (22.5%)	81 (20.3%)	134 (33.5%)	31	400
2024	CA	11 (6.9%)	42 (26.3%)	18 (11.3%)	85 (53%)	1 (0.6%)	3	160
	HA	30 (6.7%)	34 (7.6%)	107 (23.9%)	117 (26.1%)	130 (29%)	30	448
2025	CA	4 (4.5%)	25 (28.1%)	10 (11.3%)	32 (36%)	1 (1.1%)	17	89
	HA	10 (6%)	9 (5.4%)	35 (21.1%)	46 (27.7%)	58 (35%)	8	166

CA, community-acquired; HA, hospital-acquired.

Table 2. Comparison of resistance characteristics of *Staphylococcus aureus* strains to tested antibiotics according to hospital- or community-acquired status and MRSA/MSSA classification.

Antibiotic	Hospital-acquired		Community-acquired		p	Total MRSA	Total MSSA	p
	MRSA	MSSA	MRSA	MSSA				
AK	7.3	1.7	8.5	1.8	>0.05	7.5	1.7	**<0.001**
GN	15.2	4.5	9.2	6	**0.038**	14	4.8	**0.002**
CIP	26.5	8.4	27.3	10.4	>0.05	26.7	8.9	**<0.001**
LEV	4.9	1.7	7.7	1.3	>0.05	5.6	1.6	**<0.001**
TE	41	6	38.7	7.3	>0.05	40.6	6.4	**<0.001**
RIF	14.3	5.3	22.6	7.2	**0.033**	16	6	**0.009**
SXT	10.5	1.5	14.5	2.1	>0.05	10.9	1.6	**0.001**
DA	43.5	13	40	14.4	>0.05	42.7	13.4	**0.022**
E	50.1	14.7	43.2	14.9	>0.05	48.6	14.6	**0.014**
P	100	83.8	100	81.3		100	83.2	

In the table, resistance rates of hospital-acquired MRSA strains were compared with those of community-acquired MRSA strains, and resistance rates of hospital-acquired MSSA strains were compared with those of community-acquired MSSA strains. On the right side of the table, total resistance rates for MRSA and MSSA strains are shown. Statistically significant differences are indicated in bold. Since all MRSA strains were resistant to penicillin, a statistical comparison between MRSA and MSSA for penicillin was not performed. Statistical analysis was performed using the Pearson chi-square test; p < 0.05 was considered significant. AK, amikacin; CIP, ciprofloxacin; DA, clindamycin; E, erythromycin; GN, gentamicin; LEV, levofloxacin; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible; P, penicillin; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline.

Trend analysis of the isolation and resistance patterns of *Staphylococcus aureus* over the study period showed that its frequency among all culture isolates ranged from 9.2% to 11.57%, without a statistically significant change. Although methicillin resistance increased among community-acquired isolates, this trend was not statistically significant. In contrast, the increase in hospital-acquired MRSA frequency was statistically significant. Yearly changes in community- and hospital-acquired MRSA and MSSA strains during the study period are shown in Figure 1.

In community-acquired isolates, methicillin resistance decreased in 2021 and 2022 compared with the previous two years, but increased approximately twofold over the following three years. However, this increase was not statistically significant when the entire study period was considered ($p > 0.05$). In hospital-acquired isolates, the MRSA rate showed a significant increasing trend with an almost linear yearly increase.

Trend analysis of susceptibility to the tested antimicrobials by year revealed that resistance to amikacin in MRSA strains, and to gentamicin and erythromycin in both MRSA and MSSA strains, significantly decreased. In contrast, resistance to tetracycline and clindamycin significantly increased in MSSA strains. Detailed trend analysis data for changes in antibiotic resistance during the study period are presented in Table 3.

Table 3. Annual trends of antibiotic resistance rates in MRSA and MSSA isolates (2019–2025).

Antibiotic	Strain	Slope (trend)	R- squared	p
AK	MRSA	−1.733	0.684	0.02
	MSSA	−0.245	0.21	0.30
GN	MRSA	−3.284	0.663	0.02
	MSSA	−1.196	0.592	0.04
CIP	MRSA	−0.074	0.001	0.95
	MSSA	0.055	0.003	0.91
The LEV	MRSA	−0.944	0.4	0.3
	MSSA	−0.873	0.326	0.18
TE	MRSA	0.243	0.003	0.91
	MSSA	0.6	0.762	0.01
RIF	MRSA	−3.008	0.467	0.09
	MSSA	−0.652	0.552	0.06
STX	MRSA	−0.481	0.058	0.60
	MSSA	0.247	0.279	0.22
DA	MRSA	−1.588	0.213	0.30
	MSSA	1.174	0.764	0.01
E	MRSA	−7.83	0.738	0.01
	MSSA	−1.618	0.607	0.03
P	MRSA	−6.487	0.474	0.09
	MSSA	−5.574	0.525	0.07

AK, amikacin; GN, gentamicin; CIP, ciprofloxacin; LEV, levofloxacin; TE, tetracycline; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; DA, clindamycin; E, erythromycin; P, penicillin; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

Discussion

Until the early 2000s, *Staphylococcus aureus* was considered the second most common pathogen in hospital infections after *Escherichia coli* and the most frequently identified pathogen in community-acquired sepsis and soft tissue infections[13]. In a 2004 study conducted at a university hospital in our country, *Staphylococcus aureus* was reported as the most common pathogen among both community- and hospital-acquired bloodstream infections[14]. Similarly, in a 2003 study in the intensive care units of a training and research hospital, it was identified as the most frequently isolated pathogen in all clinical samples[15].

However, since the mid-2000s, significant declines in the incidence of this pathogen have been recorded, particularly in hospitals in developed countries, due to the implementation of targeted infection control measures. Following the enactment of the "Infection Control Regulation for Inpatient Treatment Institutions" in 2005 in Türkiye, measures such as hand hygiene, patient, wound, and catheter care practices, prospective active surveillance, pathogen screening, isolation of carriers, standardization of disinfection and sterilization procedures, measures to prevent microbial spread, and appropriate antimicrobial use have led to a several-fold reduction in hospital infection rates[16]. These measures have been particularly effective against *Staphylococcus aureus*. In a nine-year surveillance analysis conducted in the intensive care units of a university hospital in Türkiye, the rate of *Staphylococcus aureus* decreased from 11% in 2007 to 3.2% in 2015[17].

Conversely, recent studies from some countries have shown that *Staphylococcus aureus* has exhibited an increasing trend, particularly after 2015. This rise has been associated with an increasing number of patients with multiple risk factors and comorbidities[10,18].

The findings of our study revealed that the incidence of *Staphylococcus aureus* remained stable at approximately 9%–11.5% over the last 6.5 years, showing no significant change in overall isolation. Although this study does not cover as long a period as the studies mentioned above, it provides important data showing that the isolation frequency of *Staphylococcus aureus* in a tertiary regional hospital has fluctuated within a narrow range since 2019, without a clear trend of increase or decrease.

In our study, more than half (54%) of community-acquired *Staphylococcus aureus* strains were isolated from wound and



Figure 1. Changes in methicillin resistance over the years in community-acquired (a) and hospital-acquired (b) *Staphylococcus aureus* strains. MRSA, methicillin-resistant *Staphylococcus* aureus; MSSA, methicillin-susceptible *Staphylococcus aureus*.

abscess cultures, and 10.5% were isolated from blood cultures. Among hospital-acquired isolates, approximately 55% were from these three clinical sample types. Additionally, about 27% of community-acquired isolates were obtained from urine, while more than 30% of hospital-acquired strains were isolated from sputum and lower respiratory tract samples. These findings indicate that at least 90% of community-acquired *Staphylococcus aureus* isolates originated from wound, abscess, blood, and urine, whereas more than 85% of hospital-acquired isolates were from wound, abscess, blood, and respiratory tract samples.

These data highlight unique features of *Staphylococcus aureus* in infection pathogenesis, such as its presence in multiple body sites, primarily the hands, skin, and nose, facilitating access to sterile sites when tissue integrity is compromised. Through its capsule, polysaccharide A, lipoteichoic acid, coagulase, staphylokinase, hyaluronidase, hemolysins, leukocidins, and other virulence factors, *Staphylococcus aureus* can rapidly accomplish colonization, infection, and invasion processes[19].

Although *Staphylococcus aureus* shares important similarities with *Escherichia coli*, one of the most common human pathogens, in terms of adaptation to commensal life in the human body and its broad repertoire of virulence and pathogenicity factors, it remains a unique pathogen with a much wider spectrum of infections. This bacterium, an important outbreak pathogen in hospitals, can also spread efficiently among individuals in the community. In a study conducted in the United States, prior antibiotic treatment was identified as the only independent risk factor associated with *Staphylococcus aureus* colonization of household members and surfaces. The study reported that if a household member carried *Staphylococcus aureus*, the same strain was found in nearly half of the other household members[20]. Therefore, for measures aiming to control this pathogen in both the community and hospitals to be successful, such characteristics must be taken into consideration.

The second key aspect investigated in this study was the level of resistance of *Staphylococcus aureus*, particularly to methicillin and other antimicrobial agents, and whether these resistance characteristics changed over the study period. Our data showed that the frequency of MRSA among the isolates was approximately 30%. Although methicillin resistance in community-acquired isolates increased in a fluctuating pattern from 2019 to 2025, this increase was not statistically significant. In contrast, methicillin resistance in hospital-acquired isolates showed a statistically significant increase of more than twofold, following an almost linear trend.

This steady increase over the 6.5-year study period is a concerning finding, indicating the need to review antibiotic usage practices that may influence the selection of MRSA strains in our hospital as well as to implement strategies for monitoring resistant strains and preventing their spread. To determine whether this increase is specific to our hospital or reflects a national-level trend, further studies are required in our country. Future investigations should be conducted comprehensively to assess the impact of MRSA-related infections on patient survival, morbidity, and hospital costs. Such studies will provide an accurate estimate of the burden imposed by these strains on the healthcare system and guide preventive efforts and collaborations on a national scale.

In our study, gentamicin resistance was significantly higher in hospital-acquired MRSA strains compared with community-acquired MRSA strains, whereas rifampin resistance was significantly higher in community-acquired MRSA strains than in hospital-acquired strains. This pattern is most likely related to differences in the intensity and type of antibiotic use in the community versus hospitals. Overall susceptibility data showed that resistance rates of *Staphylococcus aureus* strains were more strongly associated with methicillin resistance than with whether the strain was hospital- or community-acquired. Indeed, methicillin-resistant strains exhibited approximately two- to seven-fold higher resistance than methicillin-susceptible strains across all tested antibiotics. These findings indicate that methicillin resistance in *Staphylococcus aureus*

is not only a marker for beta-lactam resistance but also an important indicator of multidrug resistance.

Quinolones are indispensable drugs because of their broad gram-positive and gram-negative coverage, activity against aerobic and anaerobic bacteria, effective distribution to many body sites—particularly the respiratory and urinary tracts—availability for oral and parenteral administration, and low incidence of side effects. The quinolone resistance observed in our study appears relatively high compared with 2023 European surveillance data, which also follow EUCAST criteria[21]. In a four-year analysis of intensive care isolates from a university hospital near our region, the greatest increases in resistance were reported for ciprofloxacin and levofloxacin[22]. These findings underscore the need for more selective use of quinolones in both hospitals and the community as well as careful attention to susceptibility results.

In our study, amikacin and co-trimoxazole were identified as the two most effective antimicrobials against all isolates, regardless of whether they were hospital- or community-acquired, or methicillin-resistant or susceptible. The preserved efficacy of amikacin may be related to its parenteral administration and its use typically in combination therapy, which likely limited overuse. However, the high effectiveness of co-trimoxazole, despite the absence of such limiting factors, is notable. It is likely that relatively high resistance to this drug among gram-negative pathogens has led to its reduced overall use, thereby preserving its activity against species such as *Staphylococcus aureus*.

Our country has long faced a growing problem of antimicrobial resistance to last-resort drugs in gram-negative pathogens[23–25]. One major contributing factor is the higher overall use of antimicrobial drugs compared with many other countries worldwide[26]. When the susceptibility data for *Staphylococcus aureus* from our study are compared with resistance patterns in gram-negative pathogens, they appear relatively more favorable. Although the dynamics of antimicrobial resistance differ substantially between gram-negative and gram-positive bacteria, analyzing the relatively low (<20%) resistance observed for many antibiotics in *Staphylococcus aureus* and understanding its components may provide useful insights for addressing the longstanding issue of excessive resistance in gram-negative pathogens.

Our study also includes data from the period affected by the COVID-19 pandemic, which lasted for two years starting in March 2020 in our country. During this time, measures aimed at reducing pathogen transmission in the community—such as social distancing, curfews, mask use, and hand hygiene—were widely implemented. Concurrently, hospitals experienced an increased population of severely ill patients requiring intensive care, broad-spectrum antimicrobials, immunosuppressive therapy, and life support. Studies from our country reported a decreasing trend in many community pathogens during this period[27,28], whereas increases were observed in certain hospital pathogens and resistance patterns[29].

In our study, hospital-acquired MRSA increased significantly in the post-pandemic period compared with pre-pandemic years ($p < 0.05$), whereas community-acquired MRSA showed no significant change. These findings are consistent with recent international reports describing a rise in hospital-onset MRSA bacteremia during the COVID-19 pandemic[10]. This trend has been attributed to prolonged intensive care admissions, increased use of invasive devices, and greater empirical use of broad-spectrum antibiotics during the pandemic[11]. Conversely, the absence of a significant change in community-acquired MRSA may reflect reduced interpersonal contact and transmission in the community due to lockdowns and social restrictions. Taken together, our results underscore the divergent impact of the pandemic on hospital versus community *Staphylococcus aureus* epidemiology and emphasize the importance of continuous surveillance in both settings.

Recent national data support our findings. Yılmaz et al.[30] analyzed resistance trends in 17 hospitals across Türkiye and reported that methicillin resistance in *Staphylococcus aureus* decreased significantly between 2019 and 2020 ($p = 0.03$), followed by an increase in 2021 ($p = 0.04$), returning to pre-pandemic levels. In the same study, susceptibility to fluoroquinolones and gentamicin improved during the pandemic. Furthermore, Hamidi and Yılmaz.[31] demonstrated a significant increase in the consumption of broad-spectrum antibiotics, including piperacillin-tazobactam, meropenem, fluoroquinolones, and teicoplanin, in a tertiary hospital during the pandemic. These changes in antimicrobial prescribing may have contributed to the post-pandemic rise in hospital-acquired MRSA observed in our study. Together, these national findings highlight that pandemic-related changes in both resistance patterns and antibiotic use had a measurable impact on *Staphylococcus aureus* epidemiology in Türkiye, reinforcing the importance of sustained surveillance and antimicrobial stewardship.

Additionally, the major earthquakes that struck our region in February 2023 introduced multiple risk factors potentially threatening public health. When evaluated in the context of these adverse conditions, our data showed two noticeable peaks in MRSA strains in 2020 and 2023 compared with previous years; however, there was no significant difference in the overall incidence of *Staphylococcus aureus* over the entire study period.

Study Limitations

This study has several limitations. Its retrospective, single-center design may limit generalizability. Despite the exclusion of duplicate isolates, misclassification could have occurred if a patient initially had MSSA and later developed MRSA during the same episode. As a laboratory-based study, clinical data were not analyzed. Finally, regression analyses based on annual aggregated data over 6.5 years should be interpreted as descriptive rather than predictive.

Conclusion

In this study, the trends in *Staphylococcus aureus* isolation frequency and antimicrobial resistance were examined in a tertiary hospital serving a city representing approximately 1% of the national population. MRSA strains showed a significant increase during the study period. It is important to determine whether a similar increase is occurring at the national level and, if so, to promptly plan and implement appropriate preventive measures.

Ethics

Ethics Committee Approval: This study was conducted with the ethical approval of İnönü University Scientific Research and Publication Ethics Committee (approval number: 2025/7409, dated: 25.03.2025).

Informed Consent: This study was conducted retrospectively using anonymized laboratory and archival data. No direct patient contact or intervention was involved. All data were de-identified prior to analysis, ensuring that no personally identifiable information was accessible to the researchers. Therefore, individual informed consent was not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.S.T., Concept: E.S.T., Y.Y., B.O., Design: E.S.T., Y.Y., D.C., B.O., Data Collection or Processing: E.S.T., H.A., D.C., Analysis or Interpretation: E.S.T., H.A., D.C., Literature Search: E.S.T., Y.Y., H.A., B.O., Writing: E.S.T., Y.Y., B.O.

Conflict of Interest: Barış Otlu, the author of this article, is a member of the editorial board of the Mediterranean Journal of Infection, Microbes and Antimicrobials. However, he was not involved in any stage of the editorial review or decision making process for this manuscript.

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Corynebacterium striatum in the Respiratory Tract of Intensive Care Unit Patients: Pathogen or Colonizer? A Decade-Long Retrospective Study

Yoğun Bakım Ünitesinde *Corynebacterium striatum*: Solunum Yolu Enfeksiyonunda Etken mi, Kolonizan mı? 10 Yıllık Retrospektif Çalışma

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Abstract

Introduction: *Corynebacterium striatum* has emerged as a significant nosocomial pathogen, particularly among intensive care unit (ICU) patients. This study aimed to determine whether *Corynebacterium striatum* isolated from respiratory samples represents true infection or colonization and to identify risk factors distinguishing these conditions.

Materials and Methods: We conducted a retrospective cohort study from June 2015 to June 2025, including adult ICU patients with *Corynebacterium striatum* isolated from respiratory specimens. Patients were classified into pneumonia or colonization/contamination (CC) groups. Risk factors were analyzed using descriptive statistics, univariate comparisons, and multivariate binary logistic regression to identify independent predictors of pneumonia.

Results: Among 396 patients, 126 (31.8%) were classified in the pneumonia group and 270 (68.2%) in the CC group. Chronic obstructive pulmonary disease (COPD; 31% vs. 2.2%, $p < 0.001$) and cardiovascular disease (22.2% vs. 11.5%, $p = 0.005$) were more prevalent in the pneumonia group. Multivariate analysis identified COPD (adjusted odds ratio [OR] = 26.67; 95% confidence interval [CI] = 5.05–140.91; $p < 0.001$) and Bartlett score (adjusted OR = 20.61; 95% CI = 10.99–38.61; $p < 0.001$) as independent predictors of pneumonia. The area under the Receiver Operating Characteristic curve for the Bartlett score was 0.941 (95% CI = 0.914–0.968); a score ≥1 had 92% sensitivity and 93% specificity for predicting pneumonia.

Conclusion: Isolation of *Corynebacterium striatum* from respiratory samples in ICU patients should not be automatically dismissed as contamination, particularly in patients with COPD or specimens with higher Bartlett scores. Assessment of specimen quality provides valuable diagnostic insight, and ongoing surveillance of antimicrobial resistance remains essential.

Keywords: Colonization, *Corynebacterium striatum*, intensive care unit, pneumonia

Öz

Giriş: *Corynebacterium striatum*, son yıllarda özellikle yoğun bakım ünitesinde (YBÜ) takip edilen hastalarda önemli bir nozokomiyal patojen olarak öne çıkmaktadır. Bu çalışmanın amacı, solunum örneklerinden izole edilen *Corynebacterium striatum*'un gerçek enfeksiyonu mu yoksa kolonizasyonu mu temsil ettiğini belirlemek ve bu durumları ayırt etmeye yardımcı olabilecek risk faktörlerini saptamaktır.

Gereç ve Yöntem: Bu retrospektif kohort çalışma, Haziran 2015–Haziran 2025 tarihleri arasında hastanemiz YBÜ'de takip edilen ve solunum örneklerinde *Corynebacterium striatum* izole edilen hastalar çalışmaya dahil edildi. Hastalar pnömoni ve kolonizasyon/kontaminasyon (KK) grupları

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Öz

olarak ikiye ayrıldı. Risk faktörleri; tanımlayıcı istatistikler, tek değişkenli karşılaştırmalar ve bağımsız pnömoni belirleyicilerini saptamak amacıyla çok değişkenli ikili lojistik regresyon analizi ile değerlendirildi.

Bulgular: Toplam 396 hastanın 126'sı (%31,8) pnömoni, 270'i (%68,2) KK grubunda yer aldı. Kronik obstrüktif akciğer hastalığı (KOAH) (sırasıyla %31'e karşı %2,2; p < 0,001) ve kardiyovasküler hastalık (sırasıyla %22,2'ye karşı %11,5; p = 0,005) pnömoni grubunda daha yaygındı. Çok değişkenli analizde KOAH (düzeltilmiş olasılık oranı [OR] = 26,67; %95 güven aralığı [GA] = 5,05–140,91; p < 0,001) ve Bartlett skoru (düzeltilmiş OR = 20,61; %95 GA = 10,99–38,61; p < 0,001) pnömoni için bağımsız risk faktörleri olarak saptandı. Bartlett skorunun Alıcı İşletim Karakteristiği eğrisi altındaki alanı 0,941 (%95 GA = 0,914–0,968) idi. ≥1 Bartlett skoru, pnömoniyi öngörmede %92 duyarlılık ve %93 özgüllük sağladı.

Sonuç: YBÜ'de takip edilen hastalarda solunum örneklerinden *Corynebacterium striatum* izolasyonu, özellikle KOAH'ı olan veya örnekte yüksek Bartlett skoru saptanan hastalarda, otomatik olarak kontaminasyon olarak değerlendirilmemelidir. Çalışmamız, *Corynebacterium striatum*'un YBÜ hastalarında alt solunum yolu enfeksiyonları için önemli bir nozokomiyal patojen olabileceğini göstermektedir.

Anahtar Kelimeler: Kolonizasyon, *Corynebacterium striatum*, yoğun bakım ünitesi, pnömoni

Introduction

Corynebacterium striatum, formerly considered a harmless skin commensal, has recently emerged as a significant opportunistic pathogen, particularly in healthcare settings. It is increasingly isolated from clinical specimens, especially in patients with prolonged hospital stays, invasive devices, or prior antibiotic exposure, and has been implicated in various nosocomial infections, including respiratory tract infections (RTIs), bloodstream infections, and surgical site infections[1–3].

In hospitals, particularly among immunocompromised patients, those with chronic pulmonary disease or malignancy, or those undergoing hemodialysis, *Corynebacterium striatum* infections are associated with high morbidity and mortality. Lee et al.[3] reported that *Corynebacterium striatum* isolated from blood cultures was predominantly healthcare-associated and linked to 30-day mortality. In a retrospective cohort of intensive care unit (ICU) patients, *Corynebacterium striatum* was identified as the etiologic agent in a substantial proportion of ventilator-associated pneumonia cases, with outcomes comparable to those caused by *Staphylococcus aureus*[3,4].

Although traditionally underrecognized, the rising frequency and clinical severity of *Corynebacterium striatum* infections underscore the need for heightened awareness and accurate microbiological identification to differentiate colonization from true infection. Modern diagnostic tools, including matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and genome sequencing, have enhanced detection and epidemiological surveillance of this pathogen[1,5].

Recent studies indicate that *Corynebacterium striatum* frequently exhibits resistance to β-lactams, macrolides, aminoglycosides, and fluoroquinolones, while retaining susceptibility primarily to glycopeptides (vancomycin, teicoplanin) and oxazolidinones (linezolid)[6,7].

This study aims to evaluate the clinical significance and risk factors of RTIs caused by *Corynebacterium striatum* in ICU patients and to provide guidance for distinguishing true infections from colonization or contamination.

Materials and Methods

Study Design

This retrospective cohort study was conducted at a tertiary research hospital in İzmir, Türkiye, from June 2015 to June 2025. Eligible participants were adult ICU inpatients who met all of the following criteria: (a) ICU admission; (b) isolation of *Corynebacterium striatum* from a respiratory tract culture obtained ≥48 hours after ICU admission; and (c) age ≥18 years. Patients were classified into two groups for analysis: colonization/contamination (CC; n = 270) and pneumonia (n = 126).

Sample size calculations were performed using OpenEpi Version 3.01, estimating that 264 patients were required to achieve 80% power with a type I error rate of 5%.

The study was approved by the University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital Ethics Committee for Non-Interventional Research (approval number: 2025/05-24, dated: 12.06.2025). As this was a retrospective observational study using de-identified patient data, written informed consent was waived by the ethics committee.

Definition of CC and Infection

RTIs were defined according to the Centers for Disease Control and Prevention and European Centre for Disease Prevention and Control Criteria for hospital-acquired pneumonia and ventilator-associated pneumonia[8,9]. A positive culture from respiratory specimens (tracheal aspirate, bronchoalveolar lavage, or sputum) was considered indicative of infection only if accompanied by compatible clinical findings, including new

or progressive pulmonary infiltrates on imaging, fever (>38 °C), leukocytosis or leukopenia, and purulent tracheal secretions.

Colonization or contamination was defined as the isolation of microorganisms from respiratory samples in the absence of clinical, radiological, or laboratory evidence of infection. Repeated isolation of the same organism without clinical deterioration was also considered colonization. Classification of patients as pneumonia or colonization was performed by an infectious diseases specialist during consultation, based solely on CDC criteria and without reference to the Bartlett score during the initial clinical assessment.

Data Collection

The primary outcome was 30-day mortality. Secondary variables included the antimicrobial resistance profile of *Corynebacterium striatum*, Bartlett scores of respiratory specimens, and concomitant infections. Patient demographics, comorbidities, and other clinical variables were extracted from electronic medical records.

Microbiological Analysis

Respiratory specimens were evaluated using Gram staining and direct microscopic examination. The quality of all specimens was routinely assessed using the Bartlett scoring system. For this study, Bartlett scores were calculated retrospectively for analytical purposes only and were not used to guide diagnostic classification, minimizing the risk of classification bias. The Bartlett score quantifies polymorphonuclear leukocytes, squamous epithelial cells, and mucus under low-power fields (LPF) microscopy (×100)[10].

Scores were assigned as follows:

- Neutrophils per 10 LPFs: <10 = 0; 10–25 = +1; >25 = +2
- Presence of mucus: +1
- Epithelial cells per 10 LPFs: 10–25 = −1; >25 = −2

The total Bartlett score was obtained by summing these parameters

Following microscopic evaluation, specimens were inoculated onto 5% sheep blood agar, eosin methylene blue (EMB) agar and chocolate agar (bioMérieux®, France). Blood and EMB agar plates were incubated aerobically at 35 °C for 24–48 hours, while chocolate agar plates were incubated under 5% CO_2 at 35 °C. Colonies with morphology consistent with Corynebacterium species were identified using MALDI-TOF MS (Bruker Daltonics®, Germany). Antimicrobial susceptibility testing was performed using the disk diffusion method on Mueller–Hinton Fastidious agar (bioMérieux®, France) according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Susceptibility results were interpreted based on the EUCAST breakpoints valid at the time of testing[11]. Cases in which additional microorganisms were detected alongside *Corynebacterium striatum* in the same specimen were recorded as concomitant microorganisms.

Statistical Analysis

Normality of continuous variables was assessed using the Kolmogorov–Smirnov test. Non-normally distributed variables were summarized as median and interquartile range (IQR; Q1–Q3). Categorical variables were presented as counts (n) and percentages (%).

Variables with $p < 0.10$ in univariate analyses—including age, sex, comorbidities, intubation status, Coronavirus Disease 2019 (COVID-19) status, concomitant microorganisms, and Bartlett score—were included in a multivariate binary logistic regression model to identify independent predictors of pneumonia caused by *Corynebacterium striatum*. Multicollinearity was assessed using variance inflation factors and was not observed. Associations were expressed as adjusted odds ratios (ORs) with 95% confidence intervals (CIs).

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the Bartlett score in distinguishing true *Corynebacterium striatum* pneumonia from colonization. The area under the curve (AUC) with 95% CI was calculated, and the optimal cut-off value was determined using the Youden index. Statistical significance was defined as $p < 0.05$. Analyses were performed using IBM SPSS Statistics, version 26.0.

Results

A total of 396 patients were included, of whom 126 (31.8%) were classified in the pneumonia group and 270 (68.2%) in the CC group. In the pneumonia group, 120 lower respiratory tract samples (95.2%) and six sputum samples (4.8%) were evaluated, whereas 246 lower respiratory tract samples (85.9%) and 24 sputum samples (14.1%) were observed in the CC group. The proportions of specimen types were similar between the two groups ($p = 0.214$), indicating that subsequent analyses were balanced with respect to specimen type.

Patient characteristics are summarized in Table 1. The median age of patients with pneumonia was 76.5 years (IQR, 64.8–85.3), comparable to the CC group (75 [63.5–83] years; $p = 0.350$). Gender distribution was similar, with males comprising 57.9% of the pneumonia group and 60.7% of the CC group ($p = 0.596$). Among comorbidities, chronic obstructive pulmonary disease (COPD; 31% vs. 2.2%, $p < 0.001$) and cardiovascular disease (22.2% vs. 11.5%, $p = 0.005$) were significantly more frequent in patients with pneumonia. No significant differences were observed for diabetes mellitus, hypertension, or chronic kidney disease. Thirty-day all-cause mortality was significantly higher in the pneumonia group compared with the CC group (43.7% vs. 11.1%; $p < 0.001$).

Among the 396 patients, 328 (82.8%) were intubated at the time of respiratory culture sampling, whereas 68 (17.2%) were not. The rate of tracheal intubation did not differ significantly between the pneumonia and CC groups.

Analysis of the antimicrobial resistance profile of *Corynebacterium striatum* isolates showed the highest resistance to penicillin (96,1%), followed by gentamicin (27.8%), ciprofloxacin (19.2%), and clindamycin (13.4%). Resistance to erythromycin was low (3.3%), and no resistance was detected to vancomycin or linezolid. The resistance patterns were similar between the pneumonia and CC groups, with no statistically significant differences.

The presence of concomitant bacterial microorganisms and the coexistence of COVID-19 infection were evaluated. Concomitant bacterial microorganisms was similarly distributed between the pneumonia and CC groups, with no statistically significant differences. Although COVID-19 infection was more frequently observed in the pneumonia group. Similarly, immunosuppressive conditions, including cancer, did not differ significantly between groups.

Table 1. Characteristics of patients in the pneumonia and CC groups.

	Pneumonia group (n = 126)	CC group (n = 270)	p-value
Age, years	76.5 (64.8–85.3)	75 (63.5–83)	0.350
Gender, male	73 (57.9)	164 (60.7)	0.596
Comorbidities			
Diabetes mellitus	50 (39.7)	129 (47.8)	0.133
Hypertension	21 (16.7)	52 (19.3)	0.535
COPD	39 (31)	6 (2.2)	<0.001
Cardiovascular disease	28 (22.2)	31 (11.5)	0.005
Cerebrovascular disease	6 (4.8)	16 (5.9)	0.638
Chronic kidney disease	9 (7.1)	12 (4.4)	0.264
Cancer/ immunosuppression	20 (15.9)	24 (8.9)	0.039
Tracheal intubation	110 (87.3)	218 (80.7)	0.107
COVID-19 infection	22 (17.5)	23 (8.5)	0.021
Concomitant microorganisms*	32 (25.4)	82 (30.4)	0.309
Bartlett score	2 (2–2)	0 (0–0)	<0.001
Penicillin resistance	(41.4%)	258 (95.6)	0.316
30-day mortality	55 (43.7)	30 (11.1)	<0.001

Data are presented as median (IQR) or n (%).*Concomitant microorganisms: Cases in which additional microorganisms were detected alongside *Corynebacterium striatum* in the same respiratory sample. CC, colonization/contamination; COPD, chronic obstructive pulmonary disease; COVID-19, Coronavirus Disease 2019; IQR, interquartile range.

The Bartlett score, reflecting the quality of sputum specimens, was markedly higher in the pneumonia group compared with the CC group. In the binary logistic regression analysis (Table 2), COPD and Bartlett score were identified as independent risk factors for pneumonia caused by *Corynebacterium striatum*. Patients with COPD had a 26.7-fold higher likelihood of developing pneumonia (adjusted OR = 26.67; 95% CI = 5.05–140.91; $p < 0.001$), whereas each one-point increase in Bartlett score was associated with a 20.6-fold increase in the odds of pneumonia (adjusted OR = 20.61; 95% CI = 10.99–38.61; $p < 0.001$). ROC analysis of the Bartlett score yielded an AUC of 0.941 (95% CI = 0.914–0.968; Figure 1). A Bartlett score of ≥1 demonstrated 92% sensitivity and 93% specificity for predicting pneumonia.

Cancer showed a borderline association with pneumonia (adjusted OR = 3.33; 95% CI = 0.99–11.17; $p = 0.052$), whereas cerebrovascular disease was not significantly associated. The overall model was statistically significant (Omnibus test, $p < 0.001$; -2 Log Likelihood = 165.59) and demonstrated good calibration (Hosmer–Lemeshow test, $p = 0.135$).

Discussion

Corynebacterium striatum is increasingly recognized as a significant cause of nosocomial infections and outbreaks worldwide, affecting both immunocompromised and immunocompetent patients and occasionally leading to severe or fatal invasive disease[12]. Experimental studies have shown that *Corynebacterium striatum* induces marked lung inflammation and pathological changes, with a more pronounced inflammatory response observed in immunocompromised mice[13].

The primary objective of this study was to determine whether *Corynebacterium striatum* isolates from respiratory samples of ICU patients represent true infection or colonization and to identify risk factors that differentiate these conditions. By clarifying this distinction, our findings aim to support more accurate clinical decision-making and optimize management strategies for critically ill patients with *Corynebacterium striatum* isolation.

Table 2. Risk factors for pneumonia based on binary logistic regression analysis (enter method).

Covariate	Adjusted OR (95% CI)	p-value
COPD	26.67 (5.05–140.91)	<0.001
Cerebrovascular disease	1.98 (0.66–5.95)	0.223
Cancer	3.33 (0.99–11.17)	0.052
Bartlett score	20.61 (10.99–38.61)	<0.001

Omnibus test: $p < 0.001$. -2 Log Likelihood: 165.59. Hosmer–Lemeshow test: $p = 0.135$. CI, confidence interval; COPD, chronic obstructive pulmonary disease; OR, odds ratio.

We evaluated 396 ICU patients with respiratory specimens positive for *Corynebacterium striatum*. Age and sex distributions were similar between the pneumonia and CC groups; however, significant differences were observed in comorbidity profiles. COPD and cardiovascular disease were more prevalent in the pneumonia group, with COPD emerging as a strong independent risk factor in multivariate analysis (adjusted OR = 26.67; 95% CI = 5.05–140.91; $p < 0.001$). These findings suggest that *Corynebacterium striatum* may act as a true respiratory pathogen, particularly in patients with underlying structural lung disease. Previous studies similarly reported that *Corynebacterium striatum* infections predominantly occur in individuals with severe COPD or other chronic pulmonary conditions[13]. To our knowledge, this study is the first to demonstrate that COPD is not only a predisposing condition but also an independent risk factor for invasive infection caused by *Corynebacterium striatum*.

In our cohort, 30-day all-cause mortality was significantly higher in the pneumonia group than in the CC group (43.7% vs. 11.1%; $p < 0.001$), emphasizing the clinical relevance of *Corynebacterium striatum* pneumonia. This observation aligns with previous reports; a study from South Korea comparing pneumonia caused by *Corynebacterium striatum* and methicillin-resistant *Staphylococcus aureus* found similarly high mortality rates in both groups[14]. These findings highlight *Corynebacterium striatum* as an emerging cause of severe pneumonia, particularly among critically ill patients.

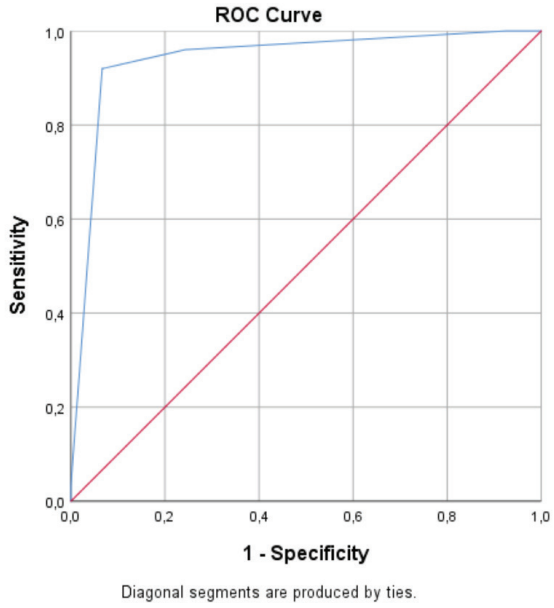

Figure 1. ROC curve of the Bartlett score for the diagnosis of pneumonia.

ROC, receiver operating characteristic.

In contrast to prior studies, the distribution of immunosuppressed patients was similar between the pneumonia and CC groups in our cohort. This may reflect the high prevalence of underlying comorbidities and overall critical illness among ICU patients, potentially masking the effect of immunosuppression as a differentiating factor. Similarly, endotracheal intubation rates did not differ significantly between groups, likely because the uniformly severe clinical condition of ICU patients limited the discriminatory value of this variable.

In this study, *Corynebacterium striatum* isolates exhibited high resistance rates to penicillin (41.4), gentamicin (27.8), ciprofloxacin (19.2), and clindamycin (13.4), whereas resistance to erythromycin was low (3.3), and no resistance was observed for vancomycin or linezolid. These findings are consistent with previous reports indicating that *Corynebacterium striatum* frequently exhibits multidrug resistance while remaining generally susceptible to glycopeptides and oxazolidinones[15,16]. The observation that both colonizing and pathogenic isolates shared similar resistance profiles suggests that antimicrobial resistance in *Corynebacterium striatum* is largely driven by environmental selection pressure from sustained antibiotic exposure in hospital settings rather than by intrinsic infection-related properties of the strain. This underscores *Corynebacterium striatum* as a highly adapted nosocomial colonizer capable of persisting in ICU environments and indicates that antimicrobial resistance alone cannot distinguish true infection from colonization.

Recent studies have reported increased *Corynebacterium striatum* isolation during the COVID-19 pandemic[17]. In our cohort, COVID-19 infection was more frequently observed in the pneumonia group, reflecting the known susceptibility of patients with viral pneumonia to secondary bacterial infections and impaired airway defense mechanisms. However, as COVID-19 status did not remain an independent predictor in multivariate analysis, this association should be interpreted cautiously and may serve as a marker of overall disease severity rather than a direct causal factor.

The Bartlett score, reflecting specimen quality, was significantly higher in patients with pneumonia. Each one-point increase was associated with a 20.6-fold increase in the odds of pneumonia. ROC analysis demonstrated excellent discriminative performance of the Bartlett score in distinguishing true *Corynebacterium striatum* pneumonia from colonization or contamination, with an AUC of 0.941 (95% CI = 0.914–0.968). A Bartlett score ≥1 yielded 92% sensitivity and 93% specificity for predicting pneumonia, suggesting that this threshold may serve as a practical cut-off in clinical settings. These results emphasize that sputum quality assessment is not merely a laboratory procedure but a

clinically meaningful parameter that can guide interpretation of *Corynebacterium striatum* isolates.

Study Limitations

This study has several limitations. First, it was a retrospective, single-center study, which may limit generalizability. Second, detailed data on prior antibiotic use, duration of mechanical ventilation, severity of illness, and other invasive procedures were incomplete, potentially introducing confounding variables. Third, although the Bartlett score was calculated retrospectively and not used for clinical adjudication, the strong association observed may partly reflect conceptual overlap between specimen quality and the clinical definition of pneumonia, potentially inflating its effect size. The Bartlett score, validated for sputum samples, was retrospectively applied to lower respiratory tract specimens from intubated patients, which may limit generalizability.

Conclusion

This study underlines that *Corynebacterium striatum* isolation from respiratory specimens in ICU patients should not be automatically dismissed as contamination, particularly in patients with COPD. The study also highlights the diagnostic value of specimen quality, suggesting that isolates with higher Bartlett scores are more likely to represent true infection rather than colonization.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital Ethics Committee for Non-Interventional Research (approval number: 2025/05-24, dated: 12.06.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: Y.N., Design: Y.N., P.K., S.A., Data Collection or Processing: Y.N., M.A.Ö., Analysis or Interpretation: Y.N., P.K., M.A.Ö., S.A., Literature Search: Y.N., S.A., Writing: Y.N.

Conflict of Interest: No conflict of interest was declared by the author(s).

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Global Bibliometric Analysis of Research on Perinatal Transmission and Prevalence in Maternal HIV, Hepatitis B, and Hepatitis C Infections

Maternal HIV, Hepatit B ve Hepatit C Enfeksiyonlarında Perinatal Bulaş ve Prevalansa İlişkin Araştırmaların Küresel Bibliyometrik Analizi

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Abstract

Introduction: This study aimed to perform a bibliometric analysis of research addressing the risk and prevalence of perinatal transmission in maternal human immunodeficiency virus (HIV), Hepatitis B, and Hepatitis C infections.

Materials and Methods: Using predefined keywords, we retrieved all articles published between 1982 and 2025. Only original research articles written in English and published after 1982 were included. A total of 1.058 records were identified, of which 1.016 were published in English. Data were exported, and duplicate records were removed using Mendeley. Quantitative analyses and visualizations were conducted using Microsoft Excel, VOSviewer, and the Bibliometrix package in R Studio. After duplicate removal, 1.015 articles were included in the final analysis.

Results: The United States was the most productive country, contributing 457 articles (44.98%). Newell was the most prolific author, with 32 publications. The most highly cited article was "Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission," which received 738 citations. Overall, research output addressing perinatal transmission risk and prevalence in maternal HIV, Hepatitis B, and Hepatitis C infections has increased markedly over the past three decades.

Conclusion: The increasing volume of publications reflects growing international interest and an expanding research scope in this field. Over the past three decades, a substantial rise has been observed in studies examining perinatal transmission risk and prevalence in maternal HIV, Hepatitis B virus, and Hepatit C infections.

Keywords: Maternal HIV, maternal Hepatitis B, maternal Hepatitis C, perinatal transmission, vertical transmission

Öz

Giriş: Yapılan çalışmanın amacı, maternal insan immün yetmezlik virüsü (HIV), Hepatit B ve Hepatit C enfeksiyonlarında perinatal bulaş riski ve prevalansını bibliyometrik analiz yöntemiyle incelemektir.

Gereç ve Yöntem: Çalışmada, anahtar kelimeler kullanılarak 1982–2025 yılları arasında yayımlanan tüm makaleler taranmıştır. Yalnızca 1982 sonrası yayımlanmış, İngilizce dilinde yazılmış orijinal araştırmalar dahil edilmiştir. Toplam 1,058 kayıt saptanmış, bunların 1,016'sı İngilizce yayınlardan oluşmuştur. Veriler dışa aktarılmış ve Mendeley programı kullanılarak tekrarlayan kayıtlar çıkarılmıştır. Nicel analizler ve görselleştirmeler Microsoft Excel, VOSviewer ve Bibliometrix (R Studio) yazılımları kullanılarak gerçekleştirilmiştir. Nihai analiz 1,015 makale üzerinden yapılmıştır.

Bulgular: Amerika Birleşik Devletleri 457 makale (%44,98) ile en üretken ülke olarak belirlenmiştir. En üretken yazar 32 makale ile Newell'dır. En çok atıf alan çalışma ise "Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1

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Öz

transmission" başlıklı makale olup 738 atıf almıştır. Ayrıca, son otuz yılda maternal HIV, Hepatit B ve Hepatit C enfeksiyonlarında perinatal bulaş riski ve prevalansını ele alan araştırma eğilimlerinde belirgin bir artış gözlenmiştir.

Sonuç: Bu alandaki çalışmaların sayısındaki artış, konunun uluslararası ölçekte daha fazla ilgi gördüğünü ve araştırma kapsamının genişlediğini göstermektedir. Ayrıca, son otuz yılda maternal HIV, Hepatit B ve Hepatit C enfeksiyonlarında perinatal bulaş riski ve prevalansını inceleyen çalışmalarda belirgin bir artış gözlenmiştir.

Anahtar Kelimeler: Maternal HIV, maternal Hepatit B, maternal Hepatit C, perinatal bulaş, vertikal bulaş

Introduction

Maternal viral infections, particularly human immunodeficiency virus (HIV), Hepatitis B virus (HBV), and Hepatitis C virus (HCV), represent a major global concern for maternal and neonatal health[1–3]. These infections share common perinatal transmission pathways and have significant implications for mother-to-child transmission, neonatal morbidity, and long-term health outcomes. Preventive strategies, including antenatal screening, antiviral therapy, and neonatal immunoprophylaxis, constitute overlapping public health interventions across these conditions, providing a strong rationale for their combined evaluation within a single bibliometric framework[4].

Although numerous studies have investigated the perinatal transmission and prevalence of maternal HIV, HBV, and HCV infections, their global publication trends, collaborative networks, and primary research foci have not yet been systematically examined[5]. Bibliometric analyses offer quantitative and visual assessments of key research indicators, such as publication output, citation patterns, author and country collaborations, and major thematic domains within a given field[6,7]. These approaches have become increasingly valuable for identifying research gaps and emerging areas of scientific interest.

Among the tools commonly used in bibliometric research, software such as VOSviewer and Bibliometrix enables the visualization of co-authorship networks and thematic clusters, thereby facilitating more nuanced interpretations of bibliographic data[6,7]. In recent years, these methods have been increasingly applied, particularly in maternal and child health research, to evaluate global research trends and evolving thematic priorities[5].

The present study aims to conduct a global bibliometric analysis of research addressing perinatal transmission and prevalence in maternal HIV, HBV, and HCV infections. By systematically examining publication output, citation impact, author and country collaborations, key conceptual themes, and their temporal evolution, this study seeks to elucidate the scientific production dynamics within this field and identify existing research gaps[5–7].

Materials and Methods

Search Strategy

The bibliographic data for this study were obtained from the Web of Science database. The search was conducted on November 2, 2025, and included articles published between 1982 and 2025. Searches were limited to English-language publications and were performed within the title, abstract, and keyword fields using the following terms: "maternal HIV," "maternal Hepatitis B," "maternal Hepatitis C," "perinatal transmission," "vertical transmission," "prevalence," "epidemiology," and "risk."

In the initial screening phase, 1.356 records were identified. As the earliest relevant publication dated back to 1982, all records published between 1982 and 2025 were considered. After applying inclusion criteria, only original research articles (n = 1.058) written in English (n = 1.016) and published after 1982 were retained for further analysis. Bibliographic data were exported, and duplicate records were screened using Mendeley. Following duplicate removal (n = 1), a total of 1.015 articles were included in the final analysis. Quantitative analyses and visualizations were performed using Microsoft Excel, VOSviewer, and the Bibliometrix package in R Studio.

Data Processing and Analysis

The bibliographic data retrieved from the Web of Science database were exported for further processing, and duplication control was conducted using the Mendeley software. Following this procedure, no additional duplicate records were identified. Bibliometric analyses and visualizations were carried out using Microsoft Excel, VOSviewer, and Bibliometrix.

Key bibliometric indicators, including publication year, authorship, country of origin, journal distribution, and citation counts, were analyzed. In addition, collaboration networks and keyword co-occurrence analyses were conducted to identify research collaborations and thematic patterns. The main analytical steps applied in this study are summarized in Table 1.

Table 1. Search strategy.

Type	Criteria
Database	Web of Science
	(maternal HIV OR maternal "Hepatitis B" OR maternal "Hepatitis C*") AND ("perinatal transmission" OR "vertical transmission*") AND ("prevalence" OR "epidemiology" OR "risk*")
Subject area	Bibliometric analysis of perinatal transmission risk and prevalence in maternal HIV, Hepatitis B, and Hepatitis C infections
Time period	1982–2025
Document type	Article
Publication status	Final
Publication type	Journal
Language	English
Duplication control	November 2, 2025 (Mendeley); duplicates = 1
Included in the analysis	1.015

Data Processing and Analysis

Data Processing

The data required for the bibliometric analysis were obtained from the Web of Science database. Relevant records were exported and transferred to Microsoft Excel for initial organization. The data were categorized by article, country, author, and journal. Duplication control was subsequently performed using the Mendeley software, and one duplicate record was identified and removed.

In the next step, a word preprocessing procedure was conducted. Terms were extracted from author keywords, titles, and abstracts and processed using VOSviewer (Leiden University, Leiden, the Netherlands) and the Bibliometrix software package.

Statistical Analysis

Citation reports, publication trends, and the most productive countries, authors, and journals were analyzed using the R-based Bibliometrix software. Word frequency analyses and visualizations were performed using VOSviewer, which is freely accessible at www.vosviewer.com. Bibliometric network visualizations were generated using nodes and links. Nodes represent words or concepts included in the analysis, and node size indicates the frequency of the corresponding item within the field. Links represent co-occurrence relationships between nodes, with line thickness and proximity reflecting the strength of the association between items[7,8].

Results

Summary of the Data

Between 1982 and 2025, a total of 1.015 articles were identified, originating from 342 sources and collectively containing 21.739 references. On average, each document received 33.10 citations. The bibliographic dataset comprised 6.968 authors, who collectively used 1.609 keywords. A total of 35 documents were single-authored. The proportion of publications involving international collaboration was 34.29% (Figure 1 and Table 2). A steady and marked increase in annual scientific production was observed over time (Figure 2).

Countries

The screening identified publications from 92 countries addressing the risk and prevalence of perinatal transmission in maternal HIV, HBV, and HCV infections. The United States ranked first, contributing 457 publications (44.98%), followed by England (n = 107), South Africa (n = 100), China (n = 73), Italy (n = 70), France (n = 61), Brazil (n = 55), Canada (n = 50), Kenya (n = 42), and India (n = 30). The United States also exhibited the highest level of international collaboration (Figures 3 and 4).

Authors

The analysis included a total of 6,968 authors. The author with the highest number of publications was Newell, with 32 articles. Additionally, Newell had the highest h-index and the greatest total number of citations (Table 3, Figure 5)[9].

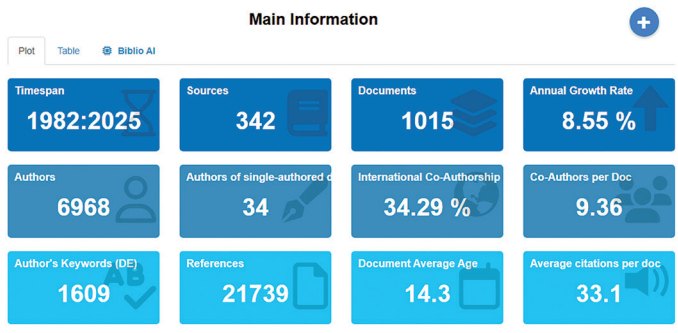

Figure 1. Summary of main bibliometric indicators.

The journals AIDS, JAIDS: Journal of Acquired Immune Deficiency Syndromes, PLOS One, Journal of Medical Virology, and Journal of Infectious Diseases were among the leading publications reporting studies on global research trends in perinatal transmission risk and prevalence in maternal HIV, Hepatitis B, and Hepatitis C infections. Analysis of 2025 metrics showed that AIDS had the highest CiteScore and was also the most cited journal that year (Figure 6, Table 4).

The authors' keywords and their frequencies are shown in the visualization generated using VOSviewer. Circle sizes correspond to the frequency of keyword usage, while colored clusters indicate groups of keywords that frequently occur together. Connecting lines represent the relationships and co-occurrence frequencies between keywords. The visualization displays ten distinct color clusters. The largest cluster is associated with the keyword "vertical transmission," followed by "HBV" and "HIV-1" (Figure 7).

Table 2. Summary of bibliographic information.

Description	Conclusion
Basic data information	
Time span	1982–2025
Sources (journals)	342
Number of documents	1.015
Annual growth rate (%)	8.55
Average age of documents	14.3
Average citations per document	33.1
References	21.739
Document contents	
Keywords (ID)	1.597
Author keywords (Deutsch)	1.609
Authors	
Authors	6.968
Authors of single-authored documents	34
Author collaborations	
Single-authored documents	35
Co-authors per document	9.36
International co-authorship (%)	34.29
Document type	
Articles	1.015

Citation Topic (Meso Level)

Meso-level citation analysis reveals the thematic structure and interdisciplinary distribution of literature on perinatal viral transmission. As shown in Table 5, publications in this field are predominantly focused on HIV (n = 532) and hepatitis (n = 366). In contrast, other infectious diseases and related disciplines have a relatively small number of publications.

Table 3. Five most productive authors publishing on this topic.

Author	The number of publications	h-index	Total citations
Newell[9]	32	25	2.537
Fowler[10]	20	14	1.129
Myer et al.[11]	15	8	299
Abrams et al.[12]	14	12	834
Resti et al.[13]	14	12	619

Table 4. Top five journals publishing the most on this topic and their citation counts (based on 2025 data).

Journals[a]	Numbe of publications[b]	h-index	Citations per article	Annual citations
	Total			
1	73	41	65.82	4.805
2	40	23	33.53	1.341
3	28	12	14.57	408
4	23	15	31.22	718
5	22	17	74.36	1.636

[a]Journals: 1) AIDS, 2) JAIDS: Journal of Acquired Immune Deficiency Syndromes, 3) PLOS One, 4) Journal of Medical Virology, 5) Journal of Infectious Diseases. [b]Bibliometric analysis of perinatal transmission risk and prevalence in maternal HIV, Hepatitis B, and Hepatitis C infections. HIV: human immunodeficiency virus.

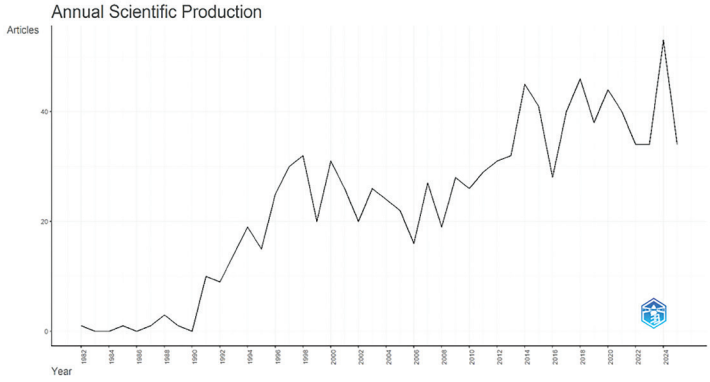

Figure 2. Annual scientific production.

Most Cited Articles

The most cited articles are summarized in Table 6. The first column lists the first author and publication year, the second column provides the journal name and DOI, and the third column shows the total number of citations. The study by Cooper et al.[14] ranks first, with 738 citations (Table 6).

Discussion

Table 5. Distribution of publications on perinatal viral transmission by meso-level citation topics.

Citation topic (meso level)	Number of publications (n)
HIV	532
Hepatitis	366
Sexually transmitted infections	19
Virology: Identification & Sequencing	17
Parasitology: Malaria, toxoplasmosis, & coccidiosis	11
Healthcare policy	8
Obstetrics & gynecology	8
Reproductive biology	6
Immunology	5
Virology–general	4
Antibiotics & antimicrobials	4
Photoproductivity	4
Tuberculosis & leprosy	3
Virology: Tropical diseases	3
Other fields*	26

This table presents the distribution of publications on perinatal viral transmission according to meso-level citation topics. Other fields include nursing, psychiatry, nutrition, oncology, and various basic science disciplines. *Other fields include examples such as pediatrics, public health, epidemiology, and molecular or interdisciplinary studies not represented as separate categories in the table. HIV, human immunodeficiency virus.

This bibliometric analysis provides a comprehensive overview of global research on perinatal transmission risk and prevalence in maternal HIV, HBV, and HCV infections between 1982 and 2025. By evaluating publication output, citation impact, and thematic patterns, the study highlights both the evolution of scientific interest and existing gaps in the literature. The annual growth rate of 8.55% indicates that this topic has gained substantial global significance in public health and clinical practice[6].

Overall, the results show a steady and marked increase in research output over time, reflecting growing international awareness of the public health importance of preventing mother-to-child transmission of viral infections. The dominance of high-income countries, particularly the United States and several European nations, underscores the role of strong research infrastructure and funding mechanisms in shaping the global scientific landscape. At the same time, the increasing contributions from regions with a high disease burden suggest a gradual diversification of research activity.

This analysis reveals a predominantly HIV-centered structure in the literature on perinatal viral transmission, with HBV and, particularly, HCV being comparatively underrepresented. The predominance of HIV-related publications is evident in publication volume, citation counts, and the prominence of HIV-focused journals and authors. While this pattern reflects the historical prioritization of HIV in global health research and the early development of effective preventive interventions, it should not be interpreted as indicating lower clinical or public health importance of HBV or HCV[24,25]. The epidemiology, natural history, and prevention strategies of HBV and HCV differ substantially from those of HIV; therefore, findings derived from HIV-related data should not be directly extrapolated to hepatitis infections without careful consideration[26,27].

Table 6. Top 10 most cited articles.

Authors	Years	Journal	DOI	Total citations
Cooper et al.[14]	2002	Journal of Acquired Immune Deficiency Syndromes	10.1097/00126334-200204150-00009	738
Blanche et al.[15]	1989	New England Journal of Medicine	10.1056/NEJM198906223202502	504
Townsend et al.[16]	2008	AIDS	10.1097/QAD.0b013e3282f9b67a	427
Giaquinto et al.[17]	2005	Clinical Infectious Diseases	10.1086/427287	420
Coutsoudis et al.[18]	2004	Journal of Infectious Diseases	10.1086/420834	324
Landesman et al.[19]	1996	New England Journal of Medicine	10.1056/NEJM199606203342501	300
Fideli, et al.[20]	2001	Aids Research and Human Retroviruses	10.1089/088922201750290023	299
Wiseman et al.[21]	2009	Medical Journal of Australia	10.5694/j.1326-5377.2009.tb02524.x	287
Zou et al.[22]	2012	Journal of Viral Hepatitis	10.1111/j.1365-2893.2011.01492.x	279
Tuomala et al.[23]	2002	New England Journal of Medicine	10.1056/NEJMoa991159	278

The relatively small number of studies investigating the perinatal transmission of HBV and HCV underscores a notable gap in the literature. Considering the global burden of chronic hepatitis and its long-term consequences for maternal and child health, additional epidemiological, clinical, and implementation-focused research is warranted[26]. Furthermore, the distribution of meso-level citation topics reveals limited representation of obstetrics and gynecology, reproductive biology, and

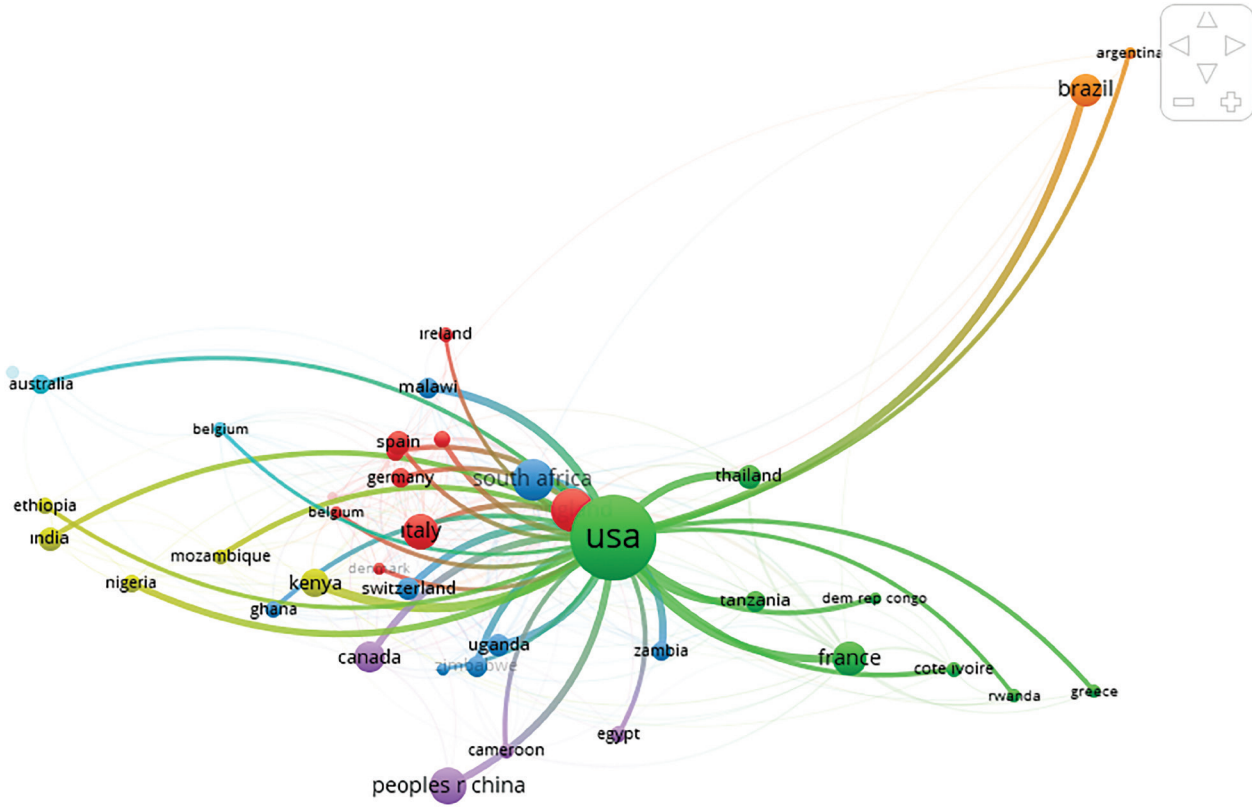

Figure 3. Country collaboration network.

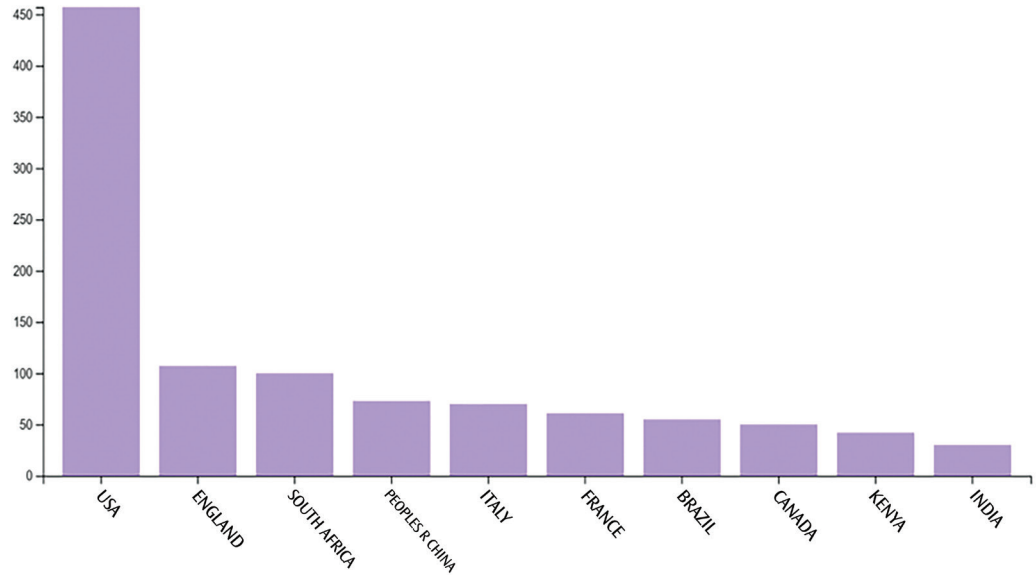

Figure 4. Ranking of countries publishing on the risk and prevalence of perinatal transmission in maternal HIV, Hepatitis B, and Hepatitis C infections.

HIV, human immunodeficiency virus.

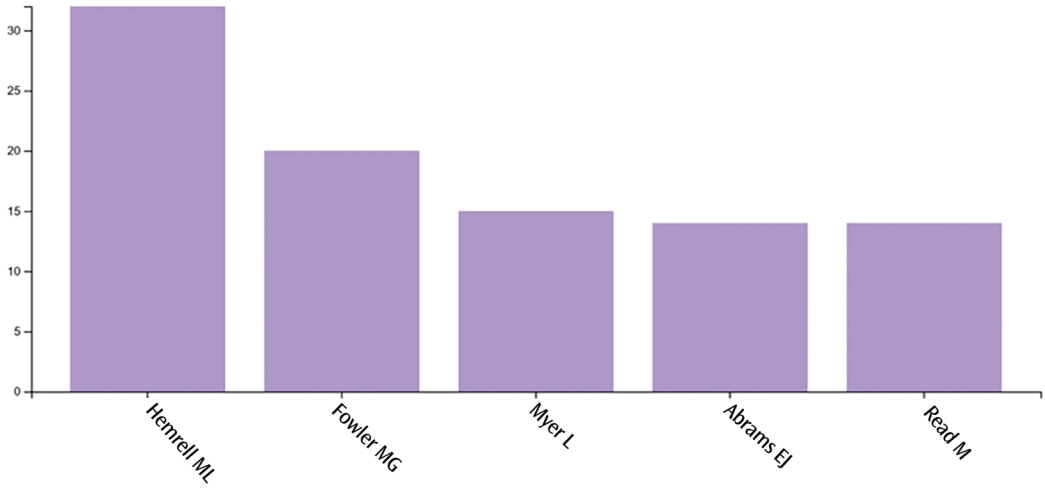

Figure 5. Most prolific authors.

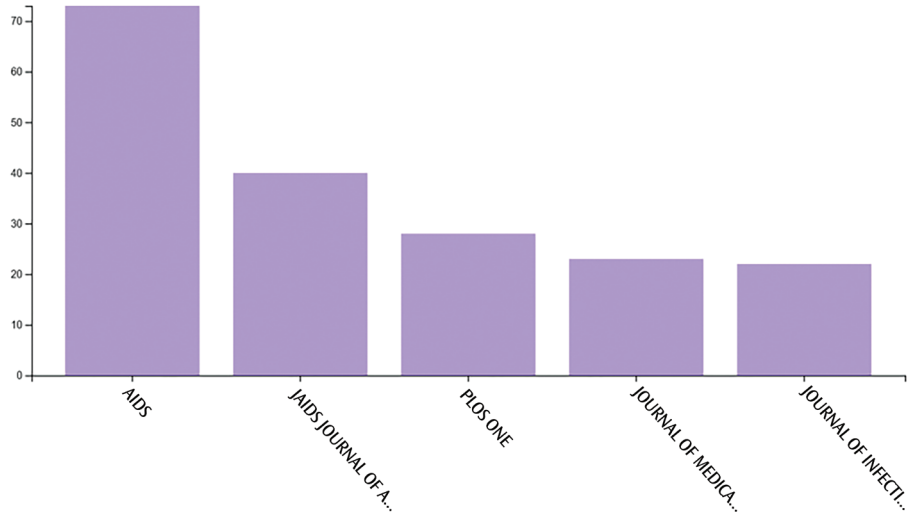

Figure 6. Most productive journals.

health policy, suggesting that perinatal viral transmission has been primarily studied from a biomedical perspective[27]. Context-specific studies from low- and middle-income countries—where access to screening and preventive interventions may be inconsistent—are particularly essential to inform effective and sustainable prevention strategies[25].

Moreover, the high level of multiauthorship and international collaboration observed in this field reflects the complex and multidisciplinary nature of research on perinatal infections. The concentration of scientific output within specific research groups indicates that long-standing expertise has significantly influenced the development of this research area, as demonstrated by the sustained contributions of leading investigators[9–12].

Keyword analyses indicate that research on perinatal transmission has adopted a multidisciplinary approach, encompassing prevention strategies, clinical outcomes, and maternal-to-child transmission mechanisms.

In summary, this bibliometric analysis demonstrates substantial growth and diversification in research on the perinatal transmission of maternal viral infections while highlighting the persistent dominance of HIV-focused literature. Addressing the relative underrepresentation of HBV and HCV in future studies will be critical for developing equitable and effective global strategies to prevent perinatal transmission and improve maternal and neonatal health outcomes.

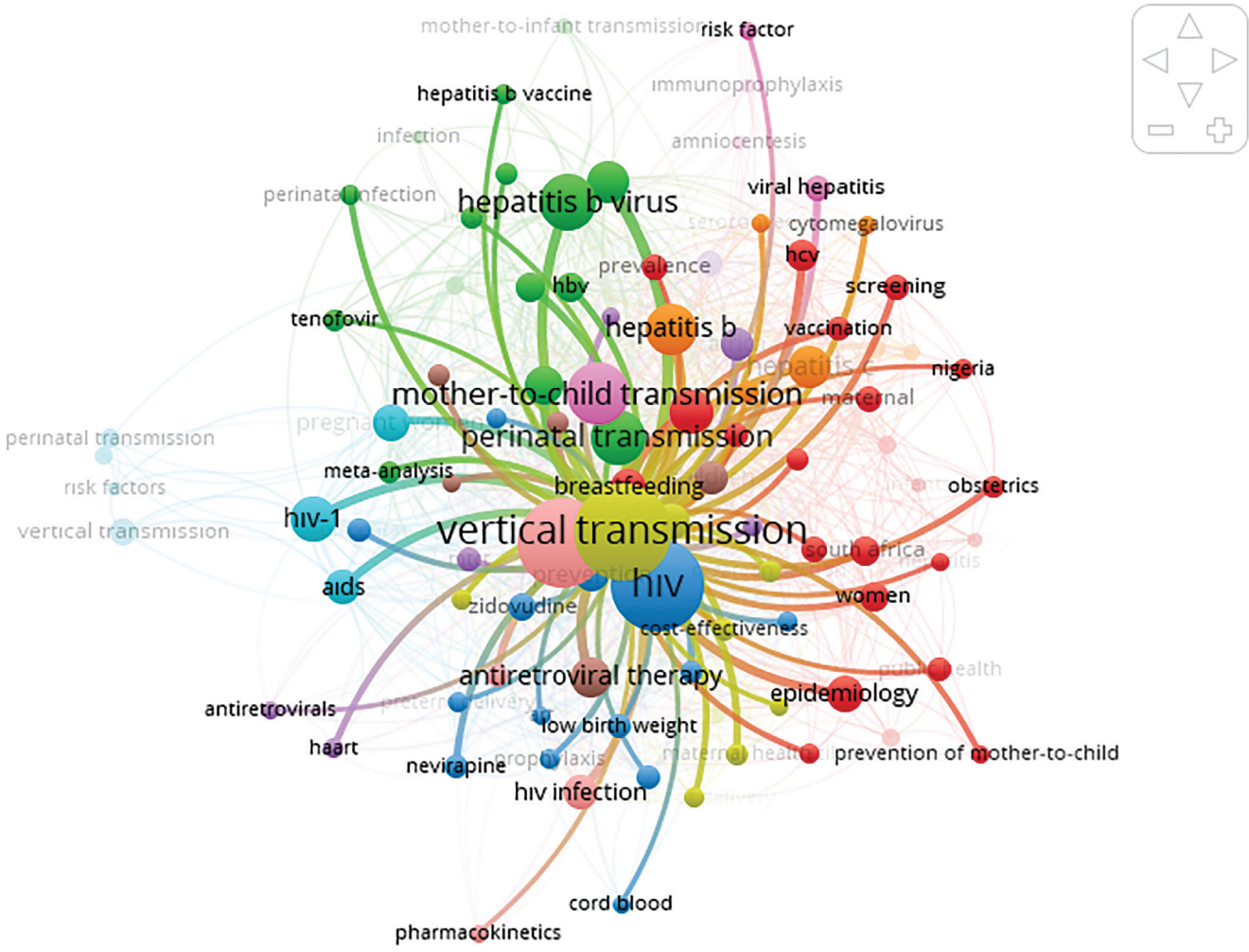

Figure 7. Visualization of authors' keywords and their frequencies.

Study Limitations

This study has several limitations. First, the analysis was limited to publications indexed in the Web of Science database; consequently, relevant studies available in other databases may not have been captured. Second, restricting inclusion to English-language articles may have reduced the representation of research conducted in non-English-speaking regions. Additionally, bibliometric analyses rely on citation-based indicators, which can be influenced by factors unrelated to scientific quality, including journal visibility, publication practices, and regional citation behaviors. Furthermore, recently published articles may exhibit lower citation counts due to time-lag effects. Despite these limitations, this study provides a comprehensive and informative overview of global research trends in perinatal transmission of maternal HIV, Hepatitis B, and Hepatitis C infections.

Conclusion

This bibliometric analysis demonstrates a sustained and growing global scientific interest in preventing the perinatal transmission of maternal HIV, Hepatitis B, and Hepatitis C infections. Despite this overall growth, research output remains disproportionately concentrated in specific countries and established research groups, highlighting persistent inequalities in global research capacity. Addressing these disparities will require targeted capacity-building initiatives, improved data-sharing mechanisms, and the expansion of collaborative research networks that actively involve low- and middle-income countries.

Although HIV-related research dominates the field, evidence regarding the perinatal transmission of Hepatitis B and C remains relatively limited. Future epidemiological and clinical studies focusing on HBV and HCV are therefore essential to inform comprehensive and equitable prevention strategies. Strengthening international collaborations, sustaining multidisciplinary research approaches,

and aligning scientific outputs with global wmaternal and child health policies may substantially contribute to reducing perinatal transmission and improving maternal and neonatal outcomes worldwide.

Ethics

Ethics Committee Approval: As this study is a bibliometric analysis, ethics committee approval was not required. Nevertheless, the study was conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Not required.

Footnotes

Authorship Contributions

Concept: T.D., İ.Ö.D., Design: T.D., İ.Ö.D., Data Collection or Processing: Z.B., Analysis or Interpretation: Z.B., Literature Search: T.D., İ.Ö.D., Z.B., Z.O., Writing: T.D., İ.Ö.D., Z.B., Z.O.

Conflict of Interest: No conflict of interest was declared by the author(s).

Financial Disclosure: The author(s) declared that this study received no financial support.

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CASE REPORT / OLGU SUNUMU

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Prolonged Antifungal Therapy and Survival in a Liver Transplant Recipient with Rhino-Orbitocerebral Mucormycosis: A Case Report

Rino-Orbito-Serebral Mukormikozlu Bir Karaciğer Nakli Alıcısında Uzun Süreli Antifungal Tedavi ve Sağkalım: Olgu Sunumu

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Abstract

Mucormycosis is a rare and rapidly progressive fungal infection associated with high mortality, particularly among immunocompromised individuals. Solid organ transplant recipients remain at risk not only during the early posttransplant period but also during episodes of intensified immunosuppression for rejection. We report the case of a 14-year-old female liver transplant recipient who developed rhino-orbitocerebral mucormycosis that was initially misdiagnosed as a dental abscess and orbital cellulitis. Early clinical suspicion, prompt histopathological confirmation, aggressive surgical management—including enucleation and partial maxillectomy—and prolonged antifungal therapy (liposomal amphotericin B followed by posaconazole) resulted in survival despite cerebral involvement. At 18 months posttransplantation, the patient remains alive and continues antifungal therapy, with radiologically stable residual lesions. This case highlights the importance of maintaining a high index of suspicion, ensuring early diagnostic confirmation, and implementing prolonged, individualized antifungal therapy to achieve favorable outcomes in liver transplant recipients with cerebral mucormycosis.

Keywords: Mucormycosis, liver transplantation, solid organ transplantation, antifungal therapy, cerebral involvement

Öz

Mukormikoz, özellikle immün yetmezliği olan hastalarda görülen, yüksek mortalite ile seyreden nadir ve fulminan bir fungal enfeksiyondur. Solid organ nakli alıcıları, yalnızca erken nakil sonrası dönemde değil, aynı zamanda rejeksiyon tedavisi sırasında uygulanan yoğun immünosupresyon dönemlerinde de risk altındadır. Bu yazıda, başlangıçta dental apse ve orbital selülit olarak yanlış değerlendirilen, rino-orbito-serebral mukormikoz gelişen 14 yaşında bir kadın karaciğer nakli alıcısının olgusu sunulmaktadır. Erken klinik şüphe, hızlı histopatolojik doğrulama, enükleasyon ve parsiyel maksillektomiyi içeren cerrahi debridman ile liposomal amfoterisin B'yi takiben posakonazol ile uygulanan uzun süreli antifungal tedavi, serebral tutulum varlığına rağmen sağkalım ile sonuçlanmıştır. Nakilden 18 ay sonra hasta, radyolojik olarak stabil rezidüel lezyonlar eşliğinde antifungal tedavi altında yaşamını sürdürmektedir. Bu olgu, serebral mukormikoz gelişen karaciğer nakli alıcılarında sağkalımın sağlanmasında yüksek klinik şüphe ve bireyselleştirilmiş, uzun süreli tedavinin önemini vurgulamaktadır.

Anahtar Kelimeler: Mukormikoz, karaciğer nakli, solid organ nakli, antifungal tedavi, serebral tutulum

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Introduction

Infections remain among the most frequent complications after liver transplantation (LT) and continue to be a leading cause of posttransplant mortality. Opportunistic infections, particularly invasive fungal diseases, pose a significant threat to transplant recipients who require lifelong immunosuppressive therapy. Although these infections most commonly occur within the first few months after LT—when the intensity of immunosuppression is greatest—they may also develop later during periods of augmented immunosuppressive therapy for acute rejection. Therefore, transplant recipients remain vulnerable to opportunistic infections during both the early and late posttransplant periods.

Invasive mucormycosis is a rare but highly aggressive fungal infection associated with a reported mortality rate of 40%–50%[1,2]. The principal pathogenic genera include Rhizopus, Mucor, Lichtheimia, Syncephalastrum, Saksenaea, Cunninghamella, Rhizomucor, and Apophysomyces[3]. The disease is characterized by angioinvasion leading to vascular thrombosis, hemorrhagic necrosis, and tissue infarction. It may spread contiguously to adjacent anatomical structures or disseminate hematogenously to distant organs[4]. Established risk factors include hematologic malignancies, prolonged neutropenia, uncontrolled diabetes mellitus, solid organ transplantation, extended corticosteroid use, and human immunodeficiency virus infection[5]. Although most cases occur within 3–6 months after transplantation, mucormycosis in liver transplant recipients has frequently been reported as early as the first posttransplant month[5].

Prompt diagnosis and early intervention are critical determinants of outcome. The cornerstone of management consists of reversal of predisposing factors whenever feasible, aggressive surgical debridement, and early initiation of appropriate antifungal therapy. Rhino-orbitocerebral mucormycosis (ROCM) represents a rapidly progressive and life-threatening manifestation, particularly in immunocompromised individuals such as solid organ transplant (SOT) recipients. The present case is notable for the unusually early onset of ROCM following LT. This report emphasizes the need for heightened clinical vigilance for atypical presentations in immunosuppressed patients, as early recognition and coordinated multidisciplinary management are essential to improving survival.

Case Report

A 14-year-old girl with intrahepatic cholestasis underwent living-donor orthotopic LT in October 2023. The procedure was uneventful, and she had no significant comorbidities. Postoperatively, she was monitored in the intensive care unit, where her clinical course remained stable. Standard immunosuppressive therapy consisting of tacrolimus, mycophenolate mofetil, and prednisolone was administered.

On postoperative day 33, she developed a dental abscess and was referred to our clinic one week later. At admission, she was febrile (38 °C) and in moderate clinical condition. Her heart rate was 89 beats/min, and blood pressure was 122/70 mmHg. Physical examination revealed marked swelling, erythema, and warmth in the right periorbital region. The right eyelid was severely edematous, completely closed, and tender on palpation. A hemorrhagic superficial erosion was also observed on the right nasal ala, raising suspicion for mucormycosis. The remainder of the systemic examination was unremarkable. Laboratory investigations showed leukocytosis with a white blood cell count of 21,850/mm^3 (89% neutrophils), elevated C-reactive protein (16.2 mg/dL; reference range: 0–0.5 mg/dL), and procalcitonin of 0.53 ng/mL. Based on the presumptive diagnoses of orbital cellulitis and mucormycosis, empiric intravenous therapy with piperacillin–tazobactam (4.5 g four times daily), teicoplanin (400 mg twice as a loading dose, followed by 400 mg once daily), and liposomal amphotericin B (5 mg/kg/day) was initiated.

For further evaluation of orbital involvement, the ophthalmology team performed cranial and orbital magnetic resonance imaging, and the otolaryngology team obtained paranasal computed tomography. On postoperative day 41, imaging demonstrated right orbital proptosis; soft tissue densities extending into the periorbital region and temporal fossa; thickening of the extraocular muscles; heterogeneity of the intraconal fat; and a focal lesion in the right lateral pons. A biopsy of the nasal mucosa revealed gray–brown necrotic tissue fragments measuring 0.2–1.0 cm. Histopathological examination using periodic acid–Schiff and Grocott Methenamine Silver staining demonstrated broad, aseptate, ribbon-like hyphae, consistent with mucormycosis (Figure 1).

During the fifth postoperative week, the patient developed right periorbital swelling accompanied by altered mental status. Magnetic resonance imaging (MRI) demonstrated restricted diffusion in the right lateral pons, suggestive of a mycotic embolism. Imaging also revealed involvement of the right periorbital, maxillary, and temporal soft tissues, along with signal abnormalities in the optic nerve and extraocular muscles.

By the sixth postoperative week, follow-up MRI showed abscess formation within the pontine lesion, bilateral dural enhancement, and progressive involvement of the optic nerve and rectus muscles (Figure 2).

At postoperative week 9, partial regression of the pontine lesion was observed; however, new extension into the

cavernous sinus, optic canal, and cavernous segment of the internal carotid artery was noted. By the seventh postoperative month, MRI demonstrated further progression along the temporal muscle and trigeminal nerve. The pontine lesion measured approximately 8 × 25 mm, and the right globe was absent following surgical intervention (Figure 3). At 16 months, follow-up MRI revealed radiologically stable residual lesions without evidence of further progression.

On postoperative day 55, the patient underwent endoscopic sinus surgery, right eye enucleation, and partial maxillectomy performed by the ophthalmology and otolaryngology teams. Liposomal amphotericin B therapy was continued for approximately 20 days, along with antibacterial agents, until clinical stabilization was achieved; antibacterial therapy was subsequently discontinued. Due to persistent cranial involvement, liposomal amphotericin B was maintained until discharge. On postoperative day 140, the patient was discharged on oral posaconazole (300 mg/day). Because radiological evidence of residual cranial infection persisted, antifungal therapy was continued. At 18 months posttransplantation, she remains under regular outpatient follow-up and continues long-term antifungal treatment.

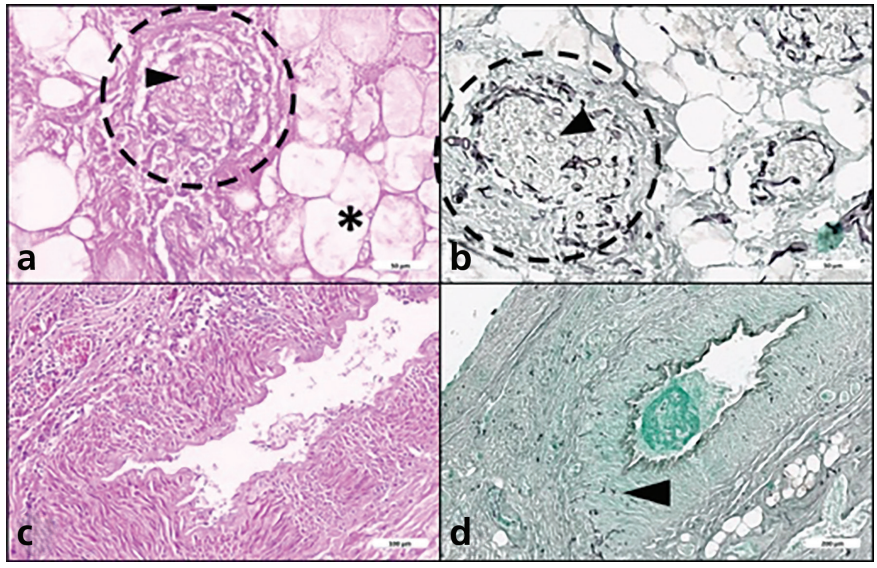

Figure 1. Histopathological findings of nasal mucormycosis. (a) Ischemic mucosa and submucosal tissue demonstrating broad, ribbon-like fungal hyphae (arrowhead), also observed within an intravascular space (circled) (H&E stain; ×200). (b) GMS stain of the region shown in panel a highlighting fungal hyphae in both intravascular (circled) and perivascular areas (×200). (c) A large submucosal vascular structure exhibiting perivascular ischemic changes without clearly identifiable fungal elements (H&E stain; ×100). (d) GMS staining of the region shown in panel c revealing distinctly visible fungal hyphae (arrowhead) (×50).

GMS, Grocott Methenamine Silver; H&E, hematoxylin and eosin.

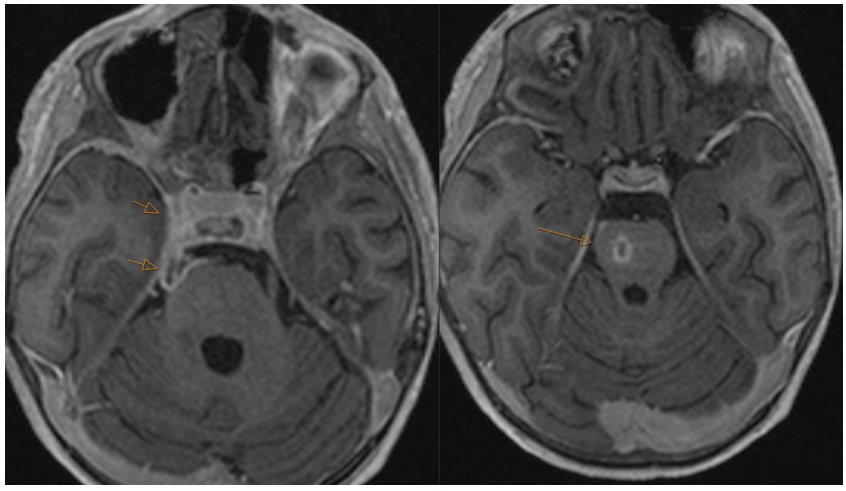

Figure 2. Contrast-enhanced T1-weighted fat-saturated magnetic resonance images obtained two days apart demonstrating progressive right orbital proptosis and extension of the mucormycosis lesion into the cavernous sinus (arrow).

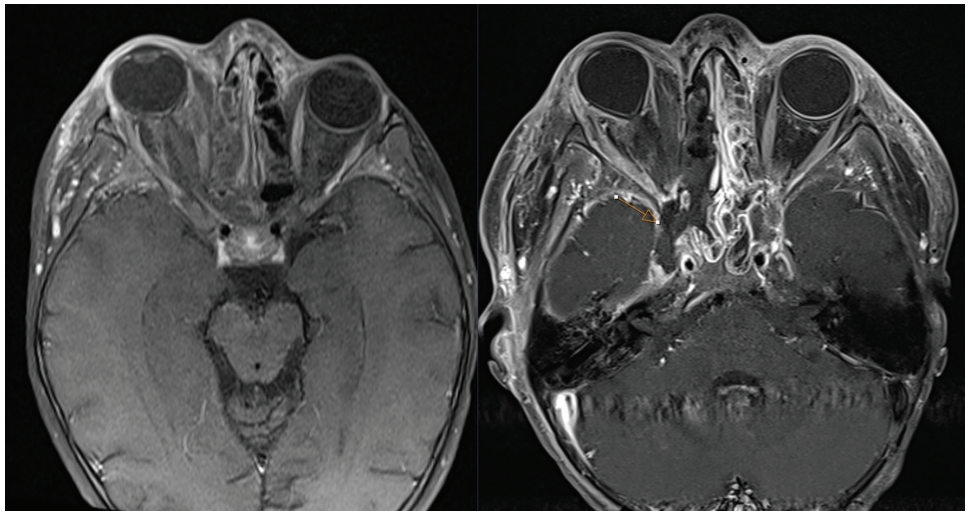

Figure 3. Follow-up contrast-enhanced T1-weighted fat-saturated magnetic resonance image obtained after right eye enucleation demonstrating linear enhancement along the trigeminal nerve (left image) and a peripherally enhancing abscess within the pons, consistent with disseminated mucormycosis.

Discussion

Mucormycosis is a rare opportunistic fungal infection most commonly caused by *Rhizopus*, *Rhizomucor*, and *Cunninghamella* species. In SOT recipients, the reported incidence ranges from 0.4% to 16%, whereas in liver transplant recipients, the incidence is generally lower (0%–2%)[6,7]. Although invasive mold infections typically occur later after transplantation, mucormycosis can also develop during the early posttransplant period[8]. The mean time to onset has been reported as approximately 2.7 months after transplantation. However, a 2024 tertiary-center case series of invasive fungal infections in SOT recipients documented mucormycosis in a kidney transplant recipient as early as postoperative day 61[8,9].

Several risk factors predispose liver transplant recipients to mucormycosis, including high-dose corticosteroid therapy, neutropenia, renal failure, prior antifungal prophylaxis with voriconazole or caspofungin, cholestasis, multiple blood transfusions, bacterial coinfections, and retransplantation[10,11]. In SOT recipients, the most frequently involved sites are the paranasal sinuses (39%), lungs (24%), skin (19%), central nervous system (9%), and gastrointestinal tract (7%)[12]. Rhino-orbital or rhinomaxillary mucormycosis typically originates in the nasal cavity and extends to the paranasal sinuses, ethmoid air cells, and sphenoid sinuses. Therefore, in transplant recipients presenting with fever, maxillary swelling, or radiologic evidence of sinus opacification, mucormycosis should be strongly considered.

The present case is notable for the unusually early development of ROCM following LT. Although mucormycosis has traditionally been described in the late posttransplant period, this case highlights the need for heightened clinical suspicion even during the early phase, particularly when patients present with atypical features such as dental abscess or orbital cellulitis.

Cerebral mucormycosis usually occurs via contiguous spread from the paranasal sinuses to the orbit and brain and is associated with high mortality. Retroorbital fungal foci causing pain have rarely been reported[13], and such extension frequently results in cavernous sinus thrombosis and death. Notably, our patient survived despite cerebral involvement, which is typically associated with a poor prognosis. Mortality rates remain substantial, with 90-day survival reported at only 50%–60% despite aggressive surgical and antifungal therapy[14]. The favorable outcome in our patient may be attributed to early clinical suspicion, prompt diagnostic confirmation, timely initiation of appropriate antifungal therapy, and aggressive surgical intervention.

The optimal duration of antifungal therapy for mucormycosis remains undefined. Current guidelines recommend continuation of treatment until complete resolution of clinical symptoms and radiologic abnormalities, with regular reassessment[14]. Decisions regarding treatment discontinuation should be individualized and generally require evidence of complete surgical debridement, adequate clinical response, and absence of fungal elements on follow-up evaluation. In our patient, antifungal therapy was ongoing at 18 months posttransplantation. To our knowledge, this represents only the second reported case of ROCM in a liver transplant recipient successfully managed with combined antifungal therapy and radical surgical intervention[14]. Recent studies continue to demonstrate that although mucormycosis in SOT recipients is uncommon, it is associated with considerable mortality, underscoring the critical importance of early recognition and combined surgical–antifungal management strategies[15,16].

Conclusion

ROCM is a rare, acute, and aggressive infection that predominantly affects immunocompromised patients, including those with diabetes, malignancy, or solid organ transplantation. Its angioinvasive nature is associated with high morbidity and mortality. Optimal management is based on three key principles: early and radical surgical debridement, control of predisposing factors, and prompt initiation of effective antifungal therapy.

Invasive fungal infections following LT require a high index of suspicion, early imaging, rapid mycological confirmation, and timely multidisciplinary intervention. This case underscores that even in immunosuppressed patients presenting with nonspecific symptoms, early recognition and aggressive management are critical for improving survival. Furthermore, it highlights the importance of individualized, prolonged antifungal therapy in achieving favorable outcomes in liver transplant recipients with cerebral mucormycosis.

Ethics

Informed Consent: Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.K., Concept: S.D., Design: M.Ö., Data Collection or Processing: S.A.T., Analysis or Interpretation: S.Y., Literature Search: E.E.K., Writing: E.E.K.

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Physicians' Attitudes and Early Uptake Tendencies Toward the Newly Introduced Adult RSV Vaccine in Türkiye: A Nationwide Survey Study

Türkiye'de Yeni Kullanıma Giren Erişkin RSV Aşısına Hekimlerin Tutumları ve Erken Dönem Uygulama Eğilimleri: Ulusal Bir Anket Çalışması

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Abstract

Introduction: Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infections, particularly among older adults. The recent introduction of adult RSV vaccines in Türkiye necessitates an assessment of physicians' knowledge and recommendation behaviors regarding this new vaccine. This study aimed to evaluate physicians' knowledge, attitudes, and early recommendation practices toward the RSV vaccine and to identify factors associated with vaccine recommendation.

Materials and Methods: This cross-sectional study was conducted via an online survey among 223 physicians across Türkiye using voluntary participation. Demographic characteristics, professional experience, and vaccination practices were analyzed using the Mann–Whitney U test and logistic regression analysis.

Results: The mean knowledge score was 7.6 ± 2.1 (on an 11-point scale), indicating moderate awareness. Only 10.3% of participants had recommended the RSV vaccine in the past 3 months, reflecting limited early uptake, while 42.6% were aware of its licensure for adults aged ≥60 years. Knowledge scores were significantly higher among faculty members and infectious diseases specialists compared with general practitioners and other specialties ($p = 0.019$ and $p = 0.011$, respectively). In multivariable analysis, higher knowledge score remained an independent predictor of vaccine recommendation (adjusted odds ratio = 1.54, 95% confidence interval: 1.12–2.11; $p = 0.007$). The most frequently reported barriers included lack of knowledge (76.2%), limited accessibility (44.4%), and reimbursement concerns (40.8%).

Conclusion: Despite generally positive attitudes toward RSV vaccination, early recommendation of the newly introduced adult RSV vaccine by physicians was limited. Clearer clinical guidance and educational support may be necessary to improve its adoption in clinical practice in Türkiye.

Keywords: Respiratory syncytial virus, adult vaccination, physician awareness, knowledge and attitudes

Öz

Giriş: Respiratuar sinsityal virüs (RSV), özellikle ileri yaş yetişkinlerde alt solunum yolu enfeksiyonlarının önemli bir nedenidir. Yetişkin RSV aşılarının Türkiye'de yakın dönemde kullanıma sunulması, hekimlerin bu yeni aşıya yönelik bilgi düzeyi ve öneri davranışlarının değerlendirilmesini gerekli kılmaktadır. Bu çalışmada, Türkiye'de hekimlerin RSV aşısına yönelik bilgi düzeyleri, tutumları ve erken öneri davranışları değerlendirilmiş ve aşı önerisi ile ilişkili faktörler araştırılmıştır.

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Öz

Gereç ve Yöntem: Bu kesitsel çalışma, Türkiye genelinde farklı branşlardan 223 hekimin gönüllü katılımıyla çevrim içi anket yöntemi kullanılarak yürütülmüştür. Demografik özellikler, mesleki deneyim ve aşılama uygulamaları Mann–Whitney U testi ve lojistik regresyon analizi ile değerlendirilmiştir.

Bulgular: Ortalama bilgi puanı 11 üzerinden 7,6 ± 2,1 olup hekimlerin orta düzeyde bir bilgiye sahip olduğunu göstermiştir. Katılımcıların sadece %10,3'ü son üç ay içinde RSV aşısını önermiştir. Aşının ≥60 yaş yetişkinlerde ruhsatlandırıldığının farkında olanların oranı %42,6'dır. Bilgi puanları, öğretim üyeleri ve enfeksiyon hastalıkları uzmanlarında pratisyen hekimler ve diğer branşlara göre anlamlı olarak daha yüksektir (p = 0,019 ve p = 0,011). Çok değişkenli analizde daha yüksek bilgi puanı, aşı önerisinde bulunmanın bağımsız bir belirleyicisi olarak saptanmıştır (düzeltilmiş olasılık oranı = 1,54; %95 güven aralığı: 1,12–2,11; p = 0,007). En sık bildirilen engeller bilgi eksikliği (%76,2), sınırlı erişilebilirlik (%44,4) ve geri ödeme sorunları (%40,8) olarak belirlenmiştir.

Sonuç: RSV aşısına yönelik genel olarak olumlu tutumlara rağmen, yeni uygulamaya konulan yetişkin RSV aşısının hekimler tarafından önerilmesi, erken uygulama döneminde sınırlı kalmıştır. Türkiye'de klinik uygulamada benimsenmesini iyileştirmek için daha net klinik kılavuzlara ve eğitim desteğine ihtiyaç duyulabilir.

Anahtar Kelimeler: Respiratuar sinsityal virüs, yetişkin aşılaması, hekim farkındalığı, bilgi ve tutumlar

Introduction

Respiratory syncytial virus (RSV) is a well-established cause of lower respiratory tract infections. Although historically associated with severe disease in infants, RSV also contributes substantially to morbidity, hospitalization, and mortality among adults aged ≥60 years and individuals with comorbidities[1-5]. Recent estimates indicate that RSV accounts for over 300,000 deaths annually in this age group worldwide[6,7].

In Türkiye, national respiratory virus surveillance data show that RSV is consistently detected during seasonal viral circulation and may represent approximately 9%–10% of hospitalized cases of severe acute respiratory infection[8]. Hospital-based studies from Türkiye similarly suggest that RSV is a significant cause of respiratory morbidity among adults requiring hospitalization[9,10]. These findings highlight that RSV constitutes a clinically relevant and potentially underrecognized burden in Türkiye, underscoring the need for effective preventive strategies, including adult vaccination[11].

RSV vaccines for adults have recently been licensed in multiple countries and became available for clinical use in Türkiye in May 2025. As healthcare professionals play a central role in vaccination programs, their awareness, knowledge, and willingness to recommend newly introduced vaccines are critical to real-world implementation[3,12]. However, systematic data on physicians' awareness of RSV vaccination in Türkiye remain limited.

This study aimed to evaluate physicians' knowledge, attitudes, and perceived barriers regarding RSV vaccination in Türkiye and to examine variations across specialties and levels of professional experience during the early implementation phase following vaccine introduction.

Materials and Methods

Study Design and Participants

This cross-sectional study was conducted using an online survey between July 18 and August 10, 2025. Physicians involved in adult patient care across Türkiye were invited to participate. The target population included specialists in infectious diseases, internal medicine, pulmonology, family medicine, and related disciplines, as well as residents, general practitioners, and faculty members. The survey was developed using Google Forms and distributed through professional networks and social media platforms. A total of 223 physicians voluntarily completed the questionnaire.

Sample Size Estimation

Sample size estimation was performed prior to data collection using the standard formula for estimating a single population proportion in cross-sectional studies. As the true prevalence of adequate RSV knowledge and vaccine recommendation among physicians in Türkiye was unknown, a conservative prevalence estimate of 50% was used to ensure the maximum required sample size. Assuming a 95% confidence level and a 5% margin of error, the minimum required sample size was calculated to be 384 participants. To account for potential incomplete responses, the target sample size was increased to at least 400 physicians. However, a total of 223 physicians ultimately completed the survey, representing a broad range of specialties, professional levels, and healthcare settings. Although the final sample size was lower than the initial target, it is comparable to those reported in previous international physician surveys on adult vaccination and is considered adequate for descriptive reporting and exploratory analyses[13].

Questionnaire Development

The questionnaire was developed based on a comprehensive review of the literature on RSV and adult vaccination

practices[5,12,14-17]. The research team aimed to design a concise, practical instrument that could be completed within a limited time while capturing essential information on physicians' knowledge, attitudes, and perceived barriers. The draft version was evaluated by infectious disease specialists, and minor revisions were implemented to enhance clarity and relevance.

Each knowledge item was scored as 1 for a correct response and 0 for an incorrect response, yielding a maximum possible score of 11. The item assessing awareness of vaccine licensure was excluded from the knowledge score exclusively in the linear regression analysis, in which the knowledge score was treated as the dependent variable, to minimize the risk of collinearity. In contrast, in the logistic regression model, the total knowledge score (including this item) was retained as an independent variable.

The final questionnaire comprised five main sections: (1) demographic and professional characteristics, (2) knowledge regarding RSV and its vaccination, (3) attitudes toward RSV vaccination, (4) perceived barriers to vaccine implementation, and (5) interest in further education on RSV and vaccination practices. The questionnaire was structured to ensure scientific rigor while maintaining practical feasibility, allowing busy physicians to complete it efficiently without compromising the reliability of the data obtained. The full questionnaire is provided in the Supplementary Materials.

Several items, including those assessing vaccine recommendation practices, perceived barriers, clinical risk groups, and RSV-related complications, were designed as multiple-response questions, permitting participants to select more than one applicable option. Consequently, the percentages reported for these items may exceed 100%. Knowledge scores were analyzed as a continuous variable. The descriptive term "moderate," as used in the Results section, reflects the mean score expressed as a percentage of the maximum possible score (approximately 69%), rather than a predefined categorical classification.

Data Collection

The survey was disseminated through professional networks. Participation was voluntary and anonymous, and no identifying information was collected. Accordingly, a nonprobability convenience sampling approach was employed. Electronic informed consent was obtained from all participants prior to survey completion.

Ethics Approval

This study was approved by the Ankara Bilkent City Hospital Medical Research Scientific and Ethical Review Board (TABED) (approval number: TABED 1-25-1519, approval date: 16.07.2025).

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics version 28.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as mean ± standard deviation or median with interquartile range, as appropriate, while categorical variables were presented as frequencies and percentages. Normality of data distribution was assessed visually and using the Kolmogorov–Smirnov test.

Comparisons of continuous variables between two groups were performed using the independent samples T-test or the Mann–Whitney U test, as appropriate. For comparisons involving more than two groups, one-way analysis of variance with Tukey's HSD post hoc test was applied for normally distributed data, whereas the Kruskal–Wallis test with Dunn–Bonferroni post hoc correction was used for non-normally distributed data. Categorical variables were compared using the chi-square test or Fisher's exact test when expected cell counts were less than 5.

Knowledge scores were compared across subgroups defined by professional status, specialty, and years of experience. A multivariable logistic regression model was constructed to identify independent predictors of RSV vaccine recommendation, including knowledge score, professional status, specialty, age, gender, and years of experience. The results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). In addition, a multiple linear regression model was used to evaluate factors associated with higher knowledge scores.

All statistical tests were two-sided, and a p-value of <0.05 was considered statistically significant.

Results

Participant Characteristics

A total of 223 physicians routinely managing adult patients completed the survey. The mean age was 36.1 ± 8.8 years, and the majority were female (70.4%). Participants represented a range of professional positions, specialties, and workplace settings, with nearly half specializing in infectious diseases. Detailed demographic and professional characteristics are presented in Table 1.

In terms of clinical practice, the median number of adult patients evaluated per week was 100 (interquartile range: 50–200), and most participants reported caring for patients aged ≥60 years.

Knowledge Scores

Overall, participants demonstrated a moderate level of knowledge, with a mean score of 7.6 ± 2.1. Knowledge scores

differed significantly by professional status and specialty, with general practitioners scoring lower than specialists and faculty members (p = 0.019). No significant differences were observed according to years of professional experience (p = 0.114).

Knowledge scores also varied across specialties (p = 0.011). Post hoc analyses revealed that infectious diseases physicians had significantly higher scores than physicians from other specialties (p = 0.046), whereas no other between-group differences were significant. A summary of knowledge scores across professional status, specialty, and experience subgroups is presented in Table 2.

In an exploratory linear regression model, awareness of RSV vaccine licensure was independently associated with higher knowledge scores (β = 1.00, 95% CI: 0.50–1.50; p < 0.001), whereas professional position and years of experience were not significant predictors (Supplementary Table 1).

Table 1. Demographic and professional characteristics of participants (n = 223).

Characteristic	n (%) or mean ± SD
Age (years)	36.1 ± 8.8
Sex	
– Female	157 (70.4)
– Male	66 (29.6)
Professional status	
– Specialist	106 (47.5)
– Resident	72 (32.3)
– Faculty member	23 (10.3)
– General practitioner	22 (9.9)
Specialty field	
– Infectious diseases	108 (48.4)
– Family medicine	35 (15.7)
– Internal medicine	28 (12.6)
– Pulmonary diseases	15 (6.7)
– Others (including emergency medicine and smaller specialties)	37 (16.5)
Years of professional experience	
– 0–5 years	80 (35.9)
– 6–10 years	68 (30.5)
– 11–15 years	38 (17.0)
– ≥16 years	37 (16.6)
Workplace	
– City hospital	73 (32.7)
– University hospital	21 (9.4)
– Training and research hospital	91 (40.8)
– Primary care center	29 (13.0)
– Private practice	9 (4.0)

SD, standard deviation.

Attitudes and Recent Practice

Pneumococcal, influenza, and hepatitis B vaccines were the most frequently recommended vaccines for adults aged ≥60 years over the past 3 months. In this period, 29 participants (13.0%) reported not recommending any vaccine to adults in this age group. Here, "recommendation" refers to physician advice rather than confirmed prescription or administration.

When asked about reasons for not recommending vaccines, 71.3% indicated that the item was not applicable, as they routinely recommend vaccines. Other reported barriers included not systematically reviewing patients' vaccination records, lack of institutional protocols, and reimbursement or coverage issues. Detailed information on recommended vaccines and reasons for non-recommendation is provided in Supplementary Tables 2 and 3.

Regarding RSV vaccination, 43.9% of participants stated that it should definitely be administered to adults aged ≥60 years; however, only a minority had recommended it in the past 3 months. Physicians who recommended the vaccine had significantly higher knowledge scores than those who did not (p = 0.004).

Awareness of RSV vaccine licensure was limited, with fewer than half of participants correctly identifying approval for adults aged ≥60 years (Supplementary Table 4). As this item contributed to the overall knowledge score, physicians who answered correctly had higher total scores (p <0.001); therefore, licensure awareness was excluded as a separate variable in multivariable analyses to avoid collinearity.

Table 2. Knowledge scores of participants according to subgroups (n = 223).

Subgroup	Mean ± SD	p-value
Professional status		0.019
– Faculty member	8.22 ± 1.51	
– Specialist	7.80 ± 1.83	
– Resident	7.50 ± 2.27	
– General practitioner	6.36 ± 2.36	
Specialty		0.011
– Infectious diseases	7.99 ± 2.03	
– Internal medicine	7.57 ± 2.10	
– Pulmonary diseases	7.67 ± 1.68	
– Family medicine	7.14 ± 2.44	
– Others	6.92 ± 1.59	
Professional experience		0.114
– 0–5 years	7.18 ± 2.12	
– 6–10 years	7.87 ± 2.01	
– 11–15 years	7.95 ± 1.86	
– ≥16 years	7.70 ± 2.09	

SD, standard deviation.

Awareness was slightly higher among infectious diseases specialists and faculty members, but overall familiarity with vaccine availability remained low, with only 31.4% knowing that the RSV vaccine was currently available in Türkiye. Subgroup distributions of physicians' attitudes and practices regarding RSV vaccination are presented in Supplementary Table 4.

Predictors of RSV Vaccine Recommendation

In multivariable logistic regression (Table 3), higher knowledge scores were independently associated with RSV vaccine recommendation within the past 3 months (adjusted OR = 1.54, 95% CI: 1.12–2.11; p = 0.007). None of the other factors—including professional status, specialty, age, gender, or years of experience—were significantly associated. The model demonstrated good overall fit (Nagelkerke R^2 = 0.19; Hosmer–Lemeshow p = 0.680).

Barriers to RSV Vaccination

Lack of knowledge was the most commonly reported barrier to recommending RSV vaccination across professional groups. Accessibility and reimbursement concerns were also frequently cited (Supplementary Table 5). Comparisons by professional status and specialty revealed significant differences in reported cost/reimbursement and accessibility concerns (p = 0.012 and p = 0.023, respectively) (Supplementary Tables 5 and 6). Most participants expressed interest in receiving further education or updates regarding RSV and its vaccines.

Table 3. Multivariable logistic regression for predictors of RSV vaccine recommendation (n = 223).

Variable	Adjusted OR	95% CI	p-value
Knowledge score (per 1-point increase)	1.54	1.12–2.11	0.007
Professional status			
Resident (ref)	1.00	—	—
Specialist	1.28	0.61–2.69	0.510
Faculty member	1.46	0.57–3.78	0.432
General practitioner	0.79	0.23–2.68	0.713
Specialty			
Infectious diseases (ref)	1.00	—	—
Internal medicine	0.84	0.36–1.95	0.680
Pulmonary diseases	1.09	0.41–2.89	0.860
Family medicine	0.93	0.39–2.19	0.870
Other specialties	0.77	0.33–1.79	0.540
Gender (female vs. male)	1.17	0.61–2.22	0.630
Age (per year)	0.98	0.95–1.02	0.340
Years of experience (per year)	1.02	0.95–1.09	0.620

Model summary: Nagelkerke R2 = 0.19. Hosmer–Lemeshow p = 0.680. Overall model p <0.001.

RSV, respiratory syncytial virus; OR, odds ratio; CI, confidence interval.

Discussion

This study evaluated physicians' knowledge, attitudes, and recommendation practices regarding adult RSV vaccination in Türkiye during the early period following vaccine introduction. Conducted within the first three months of clinical availability, our findings provide an early snapshot of physician behavior toward RSV vaccination in adults aged ≥60 years. Although participants generally expressed favorable attitudes toward RSV vaccination and recognized RSV as a clinically important respiratory pathogen, actual recommendation rates remained limited.

Overall, physicians demonstrated a moderate level of knowledge regarding RSV infection and its prevention. Despite this, only a minority actively recommended the vaccine to eligible patients. Similar gaps between awareness of disease severity and real-world vaccination practices have been reported in previous studies. A recent study from Türkiye highlighted ongoing knowledge gaps related to RSV prevention among healthcare professionals[18], while studies from Greece and the United States indicate that physicians may acknowledge the clinical importance of RSV yet remain hesitant to recommend vaccination during the early period following licensure[12,13]. Early logistical and implementation challenges have also been documented across European healthcare systems[19]. Collectively, these findings suggest that awareness of disease severity alone may be insufficient to support early clinical adoption of newly introduced adult vaccines.

In our multivariable analysis, higher knowledge scores were independently associated with a greater likelihood of RSV vaccine recommendation. Similarly, greater familiarity with RSV has been linked to higher recommendation rates in multivariable analyses conducted during the first season of RSV vaccine availability in other settings[12]. However, this relationship should be interpreted within the broader implementation context. The knowledge construct in this study encompassed not only general vaccine-related information but also awareness of local availability and clinical use. Accordingly, the frequently reported barrier of "lack of knowledge" may reflect uncertainty related to real-world implementation such as vaccine accessibility, procurement pathways, or institutional availability—rather than an absolute deficit in clinical knowledge about RSV.

Within the Turkish healthcare system, structural factors including limited accessibility, absence of routine institutional delivery pathways, and lack of reimbursement may have contributed to the observed gap between awareness and recommendation behavior. At the time of the survey, RSV

vaccines had recently been introduced for adult use but were not yet included in the national immunization program and were not reimbursed by the social security system. Similar practical challenges during early vaccine deployment have been reported in other healthcare systems[11,19], and awareness of such factors may influence physicians' likelihood of recommending vaccination in clinical practice.

The observed gap between knowledge and recommendation behavior may therefore reflect early-stage adoption challenges rather than persistent reluctance among physicians. The absence of clearly defined national guidance regarding target populations and implementation pathways may have further contributed to hesitancy in active recommendations during this initial period. Nevertheless, this early assessment provides a valuable baseline for monitoring future changes as national communication strategies, reimbursement policies, and clinical guidance become more clearly established. Experiences from other adult immunization programs, including influenza and pneumococcal vaccination, suggest that structured education, institutional support, and accessible implementation pathways can enhance physician engagement and recommendation practices over time[3]. Future studies are warranted to evaluate how physician recommendation behavior evolves as RSV vaccination strategies and national implementation frameworks are more clearly defined.

Study Limitations

This study has several limitations. Its cross-sectional design precludes causal inferences, and the reliance on self-reported responses may introduce recall bias. Additionally, the relatively limited sample size and voluntary participation may restrict the generalizability of the findings. Because the questionnaire included heterogeneous items assessing different constructs such as knowledge, attitudes, and clinical practices internal consistency analysis for the overall instrument was not performed, which may be considered a methodological limitation. The study was conducted during the initial months following the introduction of adult RSV vaccines in Türkiye; thus, observed awareness levels likely reflect an early, rather than steady-state, snapshot of physician knowledge and behavior. Future multicenter studies with larger and more diverse samples could provide a more comprehensive understanding of physicians' evolving knowledge and practices. Moreover, qualitative research may help elucidate the cognitive, emotional, and organizational factors influencing vaccine recommendation behaviors.

Conclusion

This study provides early insight into physicians' perceptions and practices regarding adult RSV vaccination in Türkiye following vaccine availability. Although physicians demonstrated general awareness of RSV as a clinically relevant pathogen, actual recommendation rates remained low, highlighting a gap between knowledge and recommendation behavior. These findings suggest that enhancing physicians' practical familiarity with vaccine access pathways and institutional implementation processes may be crucial for supporting integration of RSV vaccination into routine adult care. Further studies are warranted to evaluate how physician recommendation behaviors evolve as national implementation frameworks and vaccination strategies become more clearly established.

Ethics

Ethics Committee Approval: This study was approved by the Ankara Bilkent City Hospital Medical Research Scientific and Ethical Review Board (TABED) (approval number: TABED 1-25-1519, approval date: 16.07.2025).

Informed Consent: Electronic informed consent was obtained from all participants prior to survey completion.

Footnotes

Authorship Contributions

Concept: Z.O., R.G., Design: Z.O., H.A., R.G., Data Collection or Processing: Z.O., H.A., Analysis or Interpretation: Z.O., Literature Search: Z.O., H.A., R.G., Writing: Z.O.

Conflict of Interest: No conflict of interest was declared by the authors.

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Supplementary Table Links: https://d2v96fxpocvxx.cloudfront.net/beb8919b-f013-4ea1-b1c8-40332e840fe1/content-images/44b7758b-2bcf-45c2-b01e-9c540c1a3d86.pdf

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Clinical Predictors of Mortality in *Achromobacter* spp. Infections: A 10-Year Retrospective Analysis

Achromobacter spp. Enfeksiyonlarında Mortalitenin Klinik Belirleyicileri: 10 Yıllık Retrospektif Bir Analiz

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Abstract

Introduction: *Achromobacter* infections are increasingly recognized among high-risk patient populations; however, data regarding factors associated with mortality remain limited. This study aimed to evaluate the clinical, microbiological, and treatment-related predictors of 30-day mortality in adult patients with *Achromobacter* spp. infections.

Materials and Methods: This retrospective, single-center study included adult patients (≥18 years) diagnosed with *Achromobacter* species infections between 2016 and 2025. Demographic characteristics, comorbidities, clinical severity parameters, microbiological findings, and treatment-related variables were analyzed. Univariable and multivariable logistic regression analyses were performed to identify independent predictors of 30-day all-cause mortality.

Results: A total of 218 patients were included in the analysis, and the 30-day mortality rate was 18.3%. In the multivariable analysis, a higher Charlson comorbidity index score, the presence of septic shock, and delayed initiation of targeted antimicrobial therapy (≥72 hours) were independently associated with increased mortality. Age and the use of immunosuppressive therapy were excluded from the multivariable model because of collinearity with comorbidity burden. Carbapenem resistance was not associated with mortality in either the univariable or multivariable analyses.

Conclusion: Mortality in *Achromobacter* spp. infections appears to be primarily driven by host vulnerability, disease severity, and the timing of targeted antimicrobial therapy rather than antimicrobial resistance alone. Early risk stratification and prompt initiation of appropriate targeted therapy are therefore critical for improving clinical outcomes in this high-risk population.

Keywords: *Achromobacter*, mortality, comorbidity, septic shock, delayed antimicrobial therapy

Öz

Giriş: *Achromobacter* enfeksiyonları yüksek riskli hasta popülasyonlarında giderek daha sık tanımlanmaktadır; ancak mortalite ile ilişkili faktörlere dair veriler sınırlıdır. Bu çalışmada, erişkin hastalarda *Achromobacter* spp. enfeksiyonlarında 30 günlük mortalitenin klinik, mikrobiyolojik ve tedaviye ilişkin belirleyicilerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Bu retrospektif, tek merkezli çalışmaya 2016–2025 yılları arasında *Achromobacter* spp. enfeksiyonu tanısı alan ≥18 yaş erişkin hastalar dahil edilmiştir. Demografik özellikler, komorbiditeler, klinik şiddet parametreleri, mikrobiyolojik bulgular ve tedaviye ilişkin değişkenler analiz edilmiştir. Otuz günlük tüm nedenlere bağlı mortalitenin bağımsız belirleyicilerini saptamak amacıyla tek değişkenli ve çok değişkenli lojistik regresyon analizleri yapılmıştır.

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Öz

Bulgular: Toplam 218 hasta çalışmaya dahil edilmiş olup 30 günlük mortalite oranı %18,3 olarak saptanmıştır. Çok değişkenli analizde, daha yüksek Charlson Komorbidite İndeksi skoru, septik şok varlığı ve etkene yönelik antimikrobiyal tedavinin gecikmiş başlanması (≥72 saat) mortalite ile bağımsız olarak ilişkili bulunmuştur. Yaş ve immünsüpresif tedavi kullanımı, komorbidite yükü ile kolinearite göstermesi nedeniyle çok değişkenli modele dahil edilmemiştir. Karbapenem direnci, ne tek değişkenli ne de çok değişkenli analizlerde mortalite ile ilişkili bulunmamıştır.

Sonuç: *Achromobacter* spp. enfeksiyonlarında mortalite, antimikrobiyal dirençten ziyade konak kırılganlığı, enfeksiyonun klinik şiddeti ve etkene yönelik tedavinin zamanlaması ile belirlenmektedir. Yüksek riskli bu hasta grubunda erken risk sınıflaması ve uygun hedefe yönelik tedavinin zamanında başlanması klinik sonuçların iyileştirilmesinde kritik öneme sahiptir.

Anahtar Kelimeler: *Achromobacter*, mortalite, komorbidite, septik şok, gecikmiş antimikrobiyal tedavi

Introduction

Achromobacter species (spp.), particularly *Achromobacter xylosoxidans*, are environmental, nonfermenting Gram-negative bacilli that have been increasingly isolated in recent years, especially among high-risk populations such as patients with cystic fibrosis and immunocompromised hosts[1-3]. Although initially regarded as opportunistic pathogens, *Achromobacter* spp. are now recognized as clinically significant causes of both nosocomial and community-acquired infections. This shift in recognition has been facilitated by improved diagnostic capabilities and increasing clinical awareness[1,4,5].

These organisms are most frequently associated with respiratory tract infections, bloodstream infections, and catheter-related infections[6]. Their pathogenic potential is supported by biofilm formation and multiple antimicrobial resistance mechanisms, including metallo-β-lactamase production, porin loss, and efflux pump activation[3,7,8]. Collectively, these resistance determinants substantially limit therapeutic options and complicate clinical management[8].

Despite their increasing clinical relevance, studies systematically evaluating prognostic determinants in *Achromobacter* infections remain limited. Most available publications consist of descriptive case series that focus primarily on clinical and microbiological characteristics rather than on the independent analysis of mortality predictors[4,6]. However, in Gram-negative bloodstream infections, identifying epidemiological patterns and prognostic determinants is essential for optimizing clinical management and improving patient outcomes[9]. In Türkiye, the epidemiological characteristics of *Achromobacter* infections and the factors independently associated with mortality have not yet been adequately characterized.

Therefore, this study aimed to evaluate the clinical, microbiological, and treatment-related factors associated with 30-day all-cause mortality among adult patients (≥18 years) diagnosed with *Achromobacter* spp. infections at a tertiary care center. In particular, the independent effects of host vulnerability, infection severity, antimicrobial resistance profiles, and the timing of targeted antimicrobial therapy on mortality were systematically assessed. We hypothesized that these factors would contribute differentially and independently to mortality risk.

Materials and Methods

This study was designed as a retrospective, single-center observational investigation. Adult patients (≥18 years) who were followed at a tertiary care university hospital between January 1, 2016, and December 31, 2025, and who had *Achromobacter* spp. isolated from microbiological cultures in the presence of clinical findings consistent with infection were included. Study data were retrospectively retrieved from the hospital information management system and microbiology laboratory records.

Ethics committee approval for this study was obtained from the İstanbul Medipol University Clinical Research Ethics Committee (decision number: 82, date: 08.01.2026). The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. Because of the retrospective and observational design of the study, the requirement for informed consent from patients or their legal representatives was waived by the ethics committee. No intervention affecting patient safety was performed during the study period. All data were anonymized and analyzed in accordance with confidentiality principles.

Only the first episode of *Achromobacter* spp. infection was included for each patient, and recurrent infections in the same patient were excluded from the analysis. Cases classified as polymicrobial infections or colonization were also excluded. This approach was adopted to ensure a homogeneous study population and to better reflect the true clinical course of infection and its impact on mortality.

Collected data included demographic characteristics (age, sex, and body mass index) as well as underlying clinical conditions. Clinical variables included heart failure, chronic

obstructive pulmonary disease, diabetes mellitus, chronic kidney disease, neurological and rheumatological disorders, solid and hematological malignancies, a history of solid organ or hematopoietic stem cell transplantation, use of immunosuppressive therapy, neutropenia, and the presence of cystic fibrosis.

Immunosuppressive therapy was defined as the use of immunosuppressive medications following solid organ or hematopoietic stem cell transplantation; receipt of chemotherapy, targeted therapy, or biological agents for active malignancy; or systemic corticosteroid therapy administered for ≥14 days at a dose of ≥20 mg/day of prednisone or its equivalent[10]. Neutropenia was defined as an absolute neutrophil count of <500 cells/mm³ or a count between 500 and 1,000 cells/mm³ with a declining trend[11]. Comorbidity burden was assessed using the Charlson comorbidity index (CCI) and was included in the analyses as a continuous variable[12].

The clinical severity of infection was determined by the patient's admission setting (intensive care unit or ward) and the presence of sepsis or septic shock, as defined by Sepsis-3 criteria[13]. To quantitatively assess infection severity, the previously described Pitt bacteremia score (PBS) was calculated[14]. The use of invasive devices, including central venous catheters, urinary catheters, and other devices, was also recorded.

Sources and foci of infection were classified according to standard diagnostic criteria established by the Centers for Disease Control and Prevention[15]. Analyses also included the presence of bacteremia and the need for source control.

Identification of isolates from clinical specimens was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility testing was conducted using the BD Phoenix™ automated microbiology system (BD Diagnostics, Sparks, MD, USA), and results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria applicable during the study period[16]. Based on EUCAST-recommended clinically interpretable breakpoints for *Achromobacter* spp., susceptibility analyses were limited to trimethoprim–sulfamethoxazole, piperacillin–tazobactam, and meropenem. Carbapenem resistance was defined according to meropenem resistance per EUCAST interpretive criteria valid during the study period. Multidrug resistance was defined as resistance to at least three antimicrobial classes, following the criteria of Magiorakos et al.[17].

Empirical therapy was defined as antibiotic treatment initiated before microbiological results were available, and its appropriateness was assessed based on the *in vitro* susceptibility of the isolate. Targeted therapy was defined as treatment adjusted according to antimicrobial susceptibility results. Time to initiation of targeted therapy was calculated from the collection of the first positive culture to the start of an appropriate antibiotic. A duration of ≥72 hours was classified as delayed targeted therapy. The ≥72-h cut-off was determined in accordance with routine microbiological processing times, reflecting the expected interval required to obtain definitive susceptibility results and allowing differentiation between early and delayed therapy initiation.

The primary outcome was defined as 30-day all-cause mortality following *Achromobacter* spp. isolation. For patients who died, the day of death was recorded; survivors were followed for 30 days.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 29.0 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Kolmogorov–Smirnov test. Continuous variables with a nonnormal distribution were expressed as median and interquartile range, whereas categorical variables were presented as counts and percentages (%).

Comparisons between groups were performed using the Mann–Whitney U test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. To identify factors associated with 30-day mortality, univariable logistic regression analyses were initially conducted.

The multivariable logistic regression model was constructed based on clinical relevance and assessment of collinearity rather than solely on univariable p-value thresholds. Variables considered clinically meaningful and not exhibiting significant collinearity were included in the multivariable analysis. Collinearity was evaluated using variance inflation factor analysis and correlation matrix assessment. Variables demonstrating clinically meaningful overlap and statistical collinearity were not entered simultaneously into the final model. Specifically, age and use of immunosuppressive therapy were excluded due to collinearity with the CCI. The final multivariable model comprised four variables. The events-per-variable ratio was 10 (40 events for 4 covariates), consistent with conventional recommendations for logistic regression model stability. Model calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test.

Results are reported as odds ratios (ORs), adjusted ORs, 95% confidence intervals (CIs), and p values. A two-sided p-value <0.05 was considered statistically significant.

Results

A total of 218 adult patients diagnosed with *Achromobacter* spp. infection were included in the study. Thirty-day mortality occurred in 18.3% of patients (n = 40), whereas 81.7% (n = 178) survived during the follow-up period. Patients with and without 30-day mortality were compared with respect to demographic characteristics, comorbidity burden, clinical severity of infection, microbiological findings, and treatment-related variables.

In descriptive analyses, patients who died within 30 days were older and had a significantly higher median CCI score than survivors. Hematological malignancy, a history of hematopoietic stem cell transplantation, use of immunosuppressive therapy, and neutropenia were more frequently observed among patients who died (Table 1).

Admission to the intensive care unit, presence of sepsis or septic shock, requirement for mechanical ventilation, and higher PBS were significantly more frequent in patients who died. Similarly, bacteremia was more common in the mortality group. The distribution of infection sources and foci was comparable between patients with fatal and nonfatal outcomes. The majority of isolates were *A. xylosoxidans*, and antimicrobial resistance patterns were similar across groups.

Empirical antibiotic regimens included fourth-generation cephalosporins (cefepime) in 23.4% (n = 51), third-generation cephalosporins in 21.6% (n = 47), carbapenems in 21.1% (n = 46), piperacillin–tazobactam plus an aminoglycoside in 20.6% (n = 45), and piperacillin–tazobactam monotherapy in 13.3% (n = 29). After antimicrobial susceptibility results became available, all patients were switched to targeted therapy. The most frequently used agents for targeted therapy were piperacillin–tazobactam (35.3%, n = 77), trimethoprim–sulfamethoxazole (33.0%, n = 72), and meropenem (31.7%, n = 69). Mortality rates did not differ significantly across empirical or targeted therapy subgroups.

In contrast, time to initiation of targeted therapy was significantly longer in patients who died, and delayed targeted therapy (≥72 hours) was more frequently observed in this group. Furthermore, the proportion of patients receiving empirical therapy concordant with in vitro susceptibility results was significantly lower among those who died (Table 2).

In univariable logistic regression analysis, age, CCI, presence of septic shock, use of immunosuppressive therapy, and delayed

Table 1. Demographic characteristics and comorbidities according to 30-day mortality.

Variable	Overall, n = 218 (%)[1]	Death n = 40 (18.3%)[1]	Survived n = 178 (81.7%)[1]	p-value[2]
Patient-related factors				
Age (years) (median, IQR)	61 (58–63)	74 (71–76)	60 (57–62)	<0.001
Male sex	130 (59.6)	23 (57.5)	107 (60.1)	0.90
BMI (kg/m²) (median, IQR)	25.3 (23.9–26.3)	25.3 (23.8–26.3)	25.2 (24.0–26.3)	0.65
Comorbidities				
CCI (median, IQR)	4.5 (3.8–5.1)	4.9 (4.2–5.4)	4.4 (3.6–4.9)	0.003
Heart failure	36 (16.5)	8 (20.0)	28 (15.7)	0.67
COPD	42 (19.3)	4 (10.0)	38 (21.3)	0.15
DM	65 (29.8)	12 (30.0)	53 (29.8)	0.90
CKD	48 (22.0)	8 (20.0)	40 (22.5)	0.90
Neurological disease	31 (14.2)	7 (17.5)	24 (13.5)	0.57
Rheumatologic disease	18 (8.3)	4 (10.0)	14 (7.9)	0.74
Solid organ malignancy	54 (24.8)	8 (20.0)	46 (25.8)	0.57
SOT	22 (10.1)	4 (10.0)	18 (10.1)	0.90
Hematologic malignancy	58 (26.6)	26 (65.0)	32 (18.0)	<0.001
HSCT	26 (11.9)	16 (40.0)	10 (5.6)	<0.001
Immunosuppressive therapy	72 (33.0)	26 (65.0)	46 (25.8)	<0.001
Neutropenia	57 (26.1)	18 (45.0)	39 (21.9)	0.005
Cystic fibrosis	9 (4.1)	1 (2.5)	8 (4.5)	0.69

[1]n (%) and median (interquartile range [IQR] 25–75) values. [2]Pearson's chi-squared test, Wilcoxon rank-sum test, or Fisher's exact test. BMI, body mass index; CCI, Charlson comorbidity index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HSCT, hematopoietic stem cell transplant; SOT, solid organ transplantation.

Table 2. Clinical severity and microbiological characteristics according to 30-day mortality.

Variable	Overall n = 218 (%)[1]	Death n = 40 (18.3%)[1]	Survived n = 178 (81.7%)[1]	p-value[2]
Clinical characteristics and severity				
ICU admission	101 (46.3)	32 (80.0)	69 (38.8)	<0.001
Presence of sepsis	98 (45.0)	26 (65.0)	72 (40.4)	0.01
Presence of septic shock	52 (23.9)	18 (45.0)	34 (19.1)	<0.001
MV	66 (30.3)	25 (62.5)	41 (23.0)	<0.001
PBS (median, IQR)	3 (2–4)	6 (5–7)	2 (2–3)	<0.001
Invasive device use*	186 (85.3)	36 (90.0)	150 (84.3)	0.42
Presence of Bacteremia	117 (53.7)	30 (75.0)	87 (48.9)	0.005
Origin of infection				
Community-acquired infection	62 (28.4)	9 (22.5)	53 (29.8)	0.41
Healthcare-associated infection	156 (71.6)	31 (77.5)	125 (70.2)	0.28
Source of infection				
Primary BSI	52 (23.9)	14 (46.7)	38 (43.7)	0.78
Pneumonia	26 (11.9)	6 (20.0)	20 (23.0)	0.74
CR-BSI	20 (9.2)	5 (16.7)	15 (17.2)	0.95
Intra-abdominal infection	12 (5.5)	3 (10.0)	9 (10.3)	0.96
SSTI	4 (1.8)	1 (3.3)	3 (3.4)	1.00
UTI	3 (1.4)	1 (3.3)	2 (2.3)	0.75
Microbiological characteristics				
Achromobacter xylosoxidans	168 (77.1)	31 (77.5)	137 (77.0)	0.95
Non-xylosoxidans spp.	50 (22.9)	9 (22.5)	41 (23.0)	0.95
Meropenem resistance	69 (31.7)	14 (35.0)	55 (30.9)	0.42
Piperacillin–tazobactam resistance	61 (28.0)	13 (32.5)	48 (27.0)	0.49
Trimethoprim–sulfamethoxazole resistance	58 (26.6)	11 (27.5)	47 (26.4)	0.88
Treatment-related variables				
Source control was performed	25 (11.5)	4 (10.0)	21 (11.8)	0.89
Appropriate empirical therapy	62 (28.4)	5 (12.5)	57 (32.0)	0.01
Targeted therapy delay (hours) (median, IQR)	44 (36–52)	96 (84–110)	38 (32–44)	<0.001
Targeted therapy delay ≥72 hours	62 (28.4)	21 (52.5)	41 (23.0)	<0.001

[1]n (%) and median (IQR 25–75) values. [2]Pearson's chi-squared test, Wilcoxon rank-sum test, or Fisher's exact test. *Central venous catheter, urinary catheter, and/or percutaneous endoscopic gastrostomy. ICU, intensive care unit; MV, mechanical ventilation; PBS, Pitt bacteremia score (range 0–14); CR-BSI, catheter-related bloodstream infection; SSTI, skin and soft tissue infection; UTI, urinary tract infection; IQR, interquartile range.

initiation of targeted therapy (≥72 hours) were associated with 30-day mortality. Carbapenem resistance was not significantly associated with mortality in univariable analysis but was further evaluated in the multivariable model based on clinical relevance.

Following collinearity assessment, age and immunosuppressive therapy were excluded from the multivariable model due to collinearity with CCI. In the final multivariable model, higher CCI score, presence of septic shock, and delayed initiation of targeted therapy (≥72 hours) remained independently associated with 30-day mortality, whereas carbapenem resistance was not (Table 3). The Hosmer–Lemeshow goodness-of-fit test demonstrated satisfactory model calibration ($\chi^2 = 3.57$, df = 8, $p = 0.894$).

Discussion

In this 10-year single-center cohort, 30-day mortality among adult patients with *Achromobacter* spp. infections was independently associated with high comorbidity burden, presence of septic shock, and delayed initiation of targeted antimicrobial therapy. In contrast, carbapenem resistance was not independently associated with mortality. These findings

Table 3. Univariable and multivariable logistic regression analyses of factors associated with 30-day mortality.

Variable	Univariable OR (95% CI)[1]	p-value[2]	Multivariable aOR (95% CI)[1]	p-value[2]
Age (per year)	1.08 (1.04–1.12)	<0.001	—	—
CCI (per point)	1.74 (1.21–2.51)	0.003	1.75 (1.16–2.66)	0.008
Septic shock	5.01 (2.43–10.32)	<0.001	5.10 (2.32–11.24)	<0.001
Immunosuppressive therapy	5.10 (2.40–10.80)	<0.001	—	—
≥72 h delay in targeted therapy	3.69 (1.81–7.53)	<0.001	4.63 (2.07–10.32)	<0.001
Carbapenem resistance	1.21 (0.59–2.46)	0.32	1.18 (0.51–2.71)	0.46

[1]ORs and aORs with 95% CIs. [2]Univariable and multivariable logistic regression analyses. Variables with clinical relevance in univariable analysis were included in the multivariable model. Collinearity was assessed prior to model construction. aOR, adjusted odds ratio; CI, confidence interval; CCI, Charlson comorbidity index; OR, odds ratio.

extend beyond the predominantly descriptive nature of the existing literature on *Achromobacter* and address an important gap regarding prognostic determinants.

Recent studies have characterized the clinical and microbiological spectrum of *Achromobacter* infections, demonstrating that these infections predominantly occur in patients with significant underlying comorbidities[18-20]. Although mortality rates have been reported, independent predictors of mortality have not been systematically evaluated using multivariable models. Consequently, prognostic determinants in *Achromobacter* infections remain insufficiently defined. Our study addresses this limitation by identifying independent variables associated with mortality.

The independent association between higher CCI and mortality is consistent with previous observations that *Achromobacter* infections are more frequent in patients with substantial comorbid conditions[18-20]. However, prior reports have not systematically quantified the independent impact of cumulative comorbidity burden on mortality. In the broader literature on Gram-negative bloodstream infections, elevated CCI scores have been shown to independently increase mortality risk[21]. Our findings suggest that, in *Achromobacter* infections, prognosis is influenced less by isolated risk factors and more by cumulative host vulnerability and reduced physiological reserve. These data support the concept that mortality is shaped by overall host frailty rather than a single underlying disease.

Septic shock also emerged as a strong independent predictor of mortality. Large multicenter intensive care studies have consistently reported significantly higher mortality among patients with septic shock compared to those without shock[22]. Septic shock represents the clinical manifestation of severe systemic inflammatory dysregulation and organ dysfunction and is a universal driver of adverse outcomes, irrespective of the infecting pathogen. Our results indicate that, once *Achromobacter* infection progresses to hemodynamic instability and organ failure, its mortality dynamics parallel those observed in severe sepsis in general populations.

Delay in targeted antimicrobial therapy of ≥72 hours was independently associated with mortality, representing the most clinically actionable finding of this study. The importance of early and appropriate antimicrobial therapy in sepsis is well established. Delayed initiation of effective therapy in septic shock significantly increases mortality[23], prolonged time to first antimicrobial administration is linked to clinical deterioration and progression to shock[24], and early antibiotic administration reduces hospital mortality in large sepsis cohorts[25]. Our findings indicate that *Achromobacter* infections follow this broader Gram-negative sepsis paradigm, in which timely administration of effective antimicrobial therapy critically influences outcomes.

The absence of an independent association between carbapenem resistance and mortality is noteworthy. Although resistance patterns complicate antimicrobial selection and therapeutic strategy, the resistance phenotype alone does not appear to determine prognosis. This observation suggests that mortality in *Achromobacter* infections is primarily driven by host vulnerability and infection severity rather than resistance classification per se. When effective alternative therapeutic options are available and administered promptly, carbapenem resistance does not independently translate into increased mortality. These findings highlight the importance of clinical risk stratification and timely targeted therapy over reliance on isolated resistance metrics.

This study represents one of the largest single-center cohorts to evaluate independent mortality predictors in *Achromobacter* spp. infections using multivariable analysis. While prior reports have largely been descriptive, our analysis integrates host-related variables, clinical severity parameters, and treatment timing within a structured prognostic framework. The 10-year study period reflects sustained clinical experience and enhances the consistency of findings. The use of validated instruments, such as the CCI and PBS, strengthens methodological rigor. Inclusion of both bloodstream and nonbloodstream infections improves clinical representativeness.

Study Limitations

Several limitations of this study should be acknowledged. The retrospective, single-center design restricts causal inference and limits generalizability. Molecular characterization of resistance mechanisms was not performed, preventing genotype–phenotype correlation analyses. Antimicrobial management was not protocolized and may have been influenced by clinician-dependent variability. Despite multivariable adjustment, residual confounding cannot be entirely excluded. Because delayed targeted therapy is inherently a time-dependent variable, the potential for immortal-time bias or indication bias cannot be fully ruled out. Although time to targeted therapy was calculated from culture collection to initiation of appropriate therapy, early deaths occurring before susceptibility results were available may have affected the observed association. Therefore, residual time-related bias should be considered when interpreting this finding. Further multicenter studies with larger independent cohorts are needed to better define prognostic determinants in *Achromobacter* infections.

Conclusion

This study demonstrates that 30-day mortality in *Achromobacter* spp. infections is primarily influenced by host vulnerability, infection severity, and the timing of targeted antimicrobial therapy, rather than by antimicrobial resistance. The CCI, presence of septic shock, and a ≥72-h delay in targeted therapy emerged as independent predictors of mortality, whereas carbapenem resistance was not associated with mortality. These findings underscore the critical importance of early risk stratification and timely initiation of appropriate targeted therapy in the management of *Achromobacter* spp. infections.

Ethics

Ethics Committee Approval: Ethics committee approval for this study was obtained from the İstanbul Medipol University Clinical Research Ethics Committee (decision number: 82, date: 08.01.2026). The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.

Informed Consent: Because of the retrospective and observational design of the study, the requirement for informed consent from patients or their legal representatives was waived by the ethics committee.

Footnotes

Authorship Contributions

Surgical and Medical Practices: R.D., A.M., Concept: A.M., Design: R.D., A.M., Data Collection or Processing: R.D., Analysis or Interpretation: R.D., Literature Search: R.D., Writing: R.D., A.M.

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Brucella-Associated Subacute Thyroiditis with Positive Blood Culture: A Case Report and Literature Review

Brucella İlişkili Subakut Tiroidit ve Pozitif Kan Kültürü: Bir Olgu Sunumu ve Literatür İncelemesi

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Abstract

Brucellosis is a zoonotic infection with diverse clinical manifestations; however, thyroid involvement is exceedingly rare. Non-suppurative *Brucella* thyroiditis may clinically mimic subacute (De Quervain) thyroiditis, potentially leading to diagnostic delay. We report the case of a 50-year-old female livestock worker who presented with fatigue, sore throat, and right-sided cervical swelling. Laboratory evaluation revealed suppressed thyroid-stimulating hormone levels. Neck ultrasonography demonstrated thyroid nodules without evidence of lymphadenopathy. *Brucella* serology was positive, and *Brucella* spp. was isolated from blood culture, confirming microbiologically proven bacteremia. The patient was treated with streptomycin and doxycycline, followed by rifampicin and doxycycline, for a total treatment duration of 45 days, resulting in complete clinical recovery. In addition, a literature review identified six previously reported cases of non-suppurative *Brucella* thyroiditis. Most patients presented with painful thyroid swelling and transient thyrotoxicosis. Blood culture positivity was uncommon, whereas serological testing remained the primary diagnostic tool. All reported cases responded favorably to doxycycline-based combination therapy. In endemic regions, brucellosis should be considered in patients presenting with thyroid inflammation and biochemical thyrotoxicosis, particularly among individuals with occupational exposure to livestock. Early recognition and appropriate antimicrobial therapy can prevent misdiagnosis, unnecessary corticosteroid use, and invasive procedures.

Keywords: Brucellosis, subacute thyroiditis, thyroid involvement, bacteremia, zoonotic infection

Öz

Bruselloz, geniş klinik spektruma sahip zoonotik bir enfeksiyondur; ancak tiroid tutulumu son derece nadirdir. Non-süpüratif *Brucella* tiroiditi klinik olarak subakut (De Quervain) tiroiditi taklit edebilir ve tanıda gecikmeye yol açabilir.

Bu yazıda, halsizlik, boğaz ağrısı ve sağ servikal şişlik şikâyeti ile başvuran 50 yaşında, hayvancılıkla uğraşan kadın hasta sunulmuştur. Laboratuvar incelemesinde tiroid stimülan hormon (TSH) düzeyi baskılı saptanmıştır. Boyun ultrasonografisinde lenfadenopati olmaksızın tiroid nodülleri izlenmiştir. *Brucella* aglütinasyon testi pozitif bulunmuş ve kan kültüründe *Brucella* spp. üremesi saptanarak bakteriyemi mikrobiyolojik olarak doğrulanmıştır. Hasta streptomisin ve doksisiklin kombinasyonu ile tedavi edilmiş, ardından rifampisin ve doksisiklin ile toplam 45 günlük tedavi tamamlanmış ve klinik olarak tam iyileşme sağlanmıştır.

Ayrıca literatür taramasında non-süpüratif *Brucella* tiroiditi tanısı alan altı olguya ulaşılmıştır. Bildirilen olguların çoğunda ağrılı tiroid şişliği ve geçici tirotoksikoz saptanmış, kan kültürü pozitifliği nadir görülmüş ve tanı çoğunlukla serolojik yöntemlerle konulmuştur. Tüm olgular doksisiklin temelli kombinasyon tedavisine iyi yanıt vermiştir.

Endemik bölgelerde ve hayvancılık ile uğraşan hastalarda tiroid inflamasyonu ve biyokimyasal tirotoksikoz varlığında bruselloz ayırıcı tanıda düşünülmelidir. Erken tanı ve uygun antimikrobiyal tedavi gereksiz kortikosteroid kullanımı ve invaziv girişimleri önleyebilir.

Anahtar Kelimeler: Bruselloz, subakut tiroidit, tiroid tutulumu, bakteriyemi, zoonotik enfeksiyon

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Introduction

Brucellosis is a globally prevalent zoonotic infection, particularly endemic in Mediterranean and Middle Eastern countries[1]. The diagnosis is established in patients with compatible clinical findings using standard tube agglutination (STA) testing and/or isolation of *Brucella* spp. from blood or other body fluids[2]. Although brucellosis can involve multiple organ systems, involvement of the thyroid gland is extremely rare[3]. To date, fewer than a dozen cases of non-suppurative *Brucella* thyroiditis have been reported in the literature. Clinically, this condition may mimic subacute, autoimmune, or suppurative thyroiditis, thereby leading to diagnostic confusion[4].

Because of its rarity and non-specific clinical presentation, *Brucella* thyroiditis may be overlooked, particularly in patients presenting with thyrotoxicosis and cervical pain. In endemic regions, failure to consider brucellosis in the differential diagnosis of subacute thyroiditis may result in delayed diagnosis and treatment.

Herein, we report the case of a livestock worker who initially presented with cervical swelling and suppressed thyroid-stimulating hormone (TSH) levels and was subsequently diagnosed with *Brucella*-associated subacute thyroiditis. In addition, we reviewed previously reported cases to better characterize the clinical features, diagnostic approaches, and treatment outcomes associated with this rare entity.

Case Report

A 50-year-old female farmer presented with fatigue, sore throat, and right-sided neck swelling that had persisted for one week. She reported working with livestock and noted a history of abortions among her animals. She denied recent tick bites, hunting exposure, or travel history. In addition, she reported joint pain and a sensation of chest pressure, but no fever, night sweats, or weight loss.

On physical examination, a 4-cm firm, mobile, and painless swelling resembling a lymph node was palpated in the right cervical region (Figure 1). The thyroid gland was not enlarged in the midline and was non-tender on palpation. Hepatomegaly was not detected, whereas splenomegaly was present. Cardiac auscultation revealed no murmurs.

Laboratory investigations revealed the following thyroid function results: TSH, 0.073 mIU/L (reference range: 0.55–4.78); free T3, 4.2 ng/L (2.3–4.2); and free T4, 2.13 ng/L (0.89–1.76). Anti-thyroid peroxidase and anti-thyroglobulin antibodies were negative. Other laboratory parameters were within normal limits, except for an elevated erythrocyte sedimentation rate (32 mm/h), alanine aminotransferase level (50 U/L; reference range: 0–45), and C-reactive protein level (26 mg/L; reference range: 0–5). The complete blood count demonstrated a predominance of monocytes.

Neck ultrasonography revealed thyroid nodules but no evidence of pathological lymphadenopathy, and the patient was subsequently referred to the endocrinology department. Additional differential diagnostic tests, including anti- human immunodeficiency virus antibodies, hepatitis markers, Venereal Disease Research Laboratory test, and tularemia microagglutination test, were negative (samples were sent to the public health laboratory for analysis).

The Brucella Rose Bengal test was positive, and the STA-Coombs test showed a titer of 1:1280. Subsequent blood cultures grew *Brucella* spp., thereby confirming the diagnosis of *Brucella* thyroiditis.

The patient initially received streptomycin and doxycycline for 10 days, followed by rifampicin and doxycycline, completing a total treatment duration of 45 days. During follow-up, her systemic symptoms and neck swelling resolved completely. Thyroid scintigraphy demonstrated heterogeneous and hyperplastic uptake with both hypoactive and hyperactive nodules. Fine-needle aspiration biopsy revealed benign thyroid cytology. The patient recovered fully and continues to attend regular follow-up visits.



Figure 1. Cervical swelling observed in the patient at presentation.

Literature Search Strategy

A systematic literature search was conducted to identify all previously reported cases of brucellosis-associated thyroiditis. The PubMed, Scopus, and Web of Science databases were searched from their inception to January 2026. The search strategy included combinations of the following keywords: *"brucellosis," "Brucella," "subacute thyroiditis," "thyroiditis,"* and *"thyroid involvement."*

Case reports or case series describing subacute or acute non-suppurative thyroiditis associated with *Brucella* infection were included in the review. Only articles published in English or Turkish with full-text availability were considered eligible. Cases of suppurative thyroiditis or thyroid abscess were excluded, as were reports in which thyroid involvement was incidental in the context of predominant multiorgan disease.

In addition, the reference lists of the retrieved articles were manually screened to identify additional relevant publications. Six individual cases met the inclusion criteria and were therefore included in the present review.

Discussion

Brucellosis is a systemic granulomatous infection that can involve nearly every organ; however, thyroid involvement remains exceptionally rare, even in endemic regions. In a large Turkish cohort of 1028 patients with brucellosis, only one case of thyroid involvement was reported, highlighting the rarity of this manifestation[5]. The clinical and laboratory characteristics of previously reported non-suppurative cases are summarized in Tables 1 and 2.

The thyroid gland is relatively resistant to bacterial infection due to its encapsulated structure, high iodine content,

Table 1. Clinical characteristics of previously reported *Brucella* thyroiditis cases.

Author	Year	Country	Age/sex	Type of thyroiditis	Main clinical features	Diagnostic methods
Sırmatel and Akarsu[6]	2004	Türkiye	34/M	Subacute	Fever, throat pain, arthralgia, and thyrotoxicosis	Serology, TFT
Küçükbayrak et al.[4]	2007	Türkiye	19/F	Subacute	Neck pain, fever, weight loss, night sweats, palpitations	IgM, scintigraphy
Alinaghian and Avijgan[10]	2016	Iran	62/F	Acute non-suppurative	Odynophagia and high fever	Serology, USG, and scintigraphy
Fatani et al.[11]	2018	Egypt	53/F	Subacute	Fever and sore throat	Serology, TFT
Cvetkova et al.[8]	2019	North Macedonia	55/M	Acute non-suppurative	Fever, thyroid pain, and arthralgia	USG, serology
Lahoud et al.[12]	2024	Lebanon	48/F	Subacute	Neck pain, thyrotoxicosis, and night sweats	Serology, scintigraphy
Present case	2025	Türkiye	50/F	Subacute	Cervical swelling, fatigue, and sore throat	Serology, scintigraphy, and USG

TFT, thyroid function tests; USG, ultrasonography; IgM, immunoglobulin M; M, male; F, female.

Table 2. Laboratory findings and treatment outcomes of reported *Brucella* thyroiditis cases.

Author	Blood culture	Thyroid function	Serology	Treatment	Outcome
Sırmatel and Akarsu[6]	Negative	Hyperthyroidism (high fT4, fT3)	Positive	Doxycycline ± Rifampicin (8 weeks)	Recovery
Küçükbayrak et al.[4]	Negative	Hyperthyroidism	IgM positive	Doxycycline + Streptomycin (6 Weeks)	Recovery
Alinaghian and Avijgan[10]	**Positive**	Low TSH, high fT4	Wright 1:1280	Doxycycline + rifampicin (3 months) + Strep + Steroid (14 Days)	Recovery
Fatani et al.[11]	Negative	Hyperthyroidism	Positive	Doxycycline + Rifampicin + Ciprofloxacin (6 Weeks)	Recovery
Cvetkova et al.[8]	Not reported	Mild thyrotoxicosis (only high fT4)	Positive	Gentamicin for 10 days, doxycycline, and ciprofloxacin for 45 days	Recovery
Lahoud et al.[12]	Not reported	Suppressed TSH, high T3/T4	Positive	Doxycycline + 900-mg Rifampicin (6 weeks)	Recovery
Present case	**Positive**	Suppressed TSH	Positive	Doxycycline + Rifampicin (6 weeks) + Streptomycin (10 days)	Recovery

TSH, thyroid-stimulating hormone; IgM, immunoglobulin.

rich vascular supply, and extensive lymphatic drainage. Nevertheless, infection can occur via hematogenous spread or direct invasion. Clinically, *Brucella* thyroiditis may closely mimic classical subacute (De Quervain) thyroiditis, suppurative thyroiditis, or autoimmune thyroid disease, which can lead to diagnostic delays[4,6-9].

As shown in Table 1, most reported patients were middle-aged women presenting with fever, anterior neck pain, and painful thyroid enlargement. Transient thyrotoxicosis was observed in the majority of cases, characterized by suppressed TSH and elevated thyroid hormone levels, whereas thyroid autoantibodies were generally negative. Imaging findings, including ultrasonography and scintigraphy, were consistent with subacute thyroiditis[6-12].

Importantly, Table 2 demonstrates that blood culture positivity was uncommon in previously reported cases, with serological testing remaining the primary diagnostic method. In contrast, our patient had microbiologically confirmed *Brucella* bacteremia, strengthening the etiological association and underscoring the value of obtaining blood cultures in febrile patients from endemic regions[10].

Subacute thyroiditis is most commonly associated with viral infections[9]; however, not all cases are viral, particularly in brucellosis-endemic areas. Our patient initially presented with cervical swelling and suppressed TSH levels, consistent with classical subacute thyroiditis. Occupational exposure and positive blood cultures confirmed the infectious etiology. This case emphasizes that brucellosis should be considered in the differential diagnosis of thyroiditis, especially in individuals with livestock exposure in endemic regions.

As shown in Table 2, all reported patients responded favorably to doxycycline-based combination therapy, most commonly doxycycline plus rifampicin, with or without an aminoglycoside. No mortality, recurrence, or permanent thyroid dysfunction has been documented[6-12]. These findings suggest that early recognition and prompt initiation of appropriate antimicrobial therapy result in excellent outcomes and can prevent unnecessary corticosteroid use or surgical intervention.

Conclusion

Brucella thyroiditis is a rare but clinically significant manifestation of brucellosis. In endemic regions, brucellosis should be considered in patients presenting with painful thyroid swelling and biochemical thyrotoxicosis, particularly among those with occupational exposure. Early microbiological confirmation and prompt initiation of appropriate antimicrobial therapy can ensure complete recovery and prevent misdiagnosis or unnecessary corticosteroid use.

Ethics

Informed Consent: Written informed consent was obtained from the patient for publication of this case report.

Footnotes

Conflict of Interest: The author declares no conflict of interest.

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OLGU SUNUMU / CASE REPORT

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Management of BK Virus Nephropathy with IVIG in a Kidney Transplant Recipient Unresponsive to Immunosuppression Reduction

BK Virüs Nefropatisinin İmmünsüpresyon Azaltımına Yanıtsız Bir Böbrek Nakli Alıcısında IVIG ile Tedavisi

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Abstract

BK virus (BKV)-associated nephropathy (BKVN) is a complication that can lead to graft loss after kidney transplantation. The primary approach to this disease is reduction of immunosuppressive therapy; however, some cases are non-responsive to this strategy. This case report presents a patient with BKVN that was non-responsive to immunosuppressive reduction and was treated with intravenous immunoglobulin (IVIG). A 62-year-old male patient with a history of kidney transplantation from a living donor 2 years earlier presented with deterioration of renal function. High BKV loads were detected, and the diagnosis of BKVN was confirmed by renal biopsy. Despite discontinuation of mycophenolate mofetil, the viral load continued to increase, and graft function continued to deteriorate. Subsequently, the patient was treated with IVIG. BKV levels in plasma and urine decreased, but renal function showed only minimal improvement. This case highlights the effectiveness of IVIG in resistant BKVN and underscores the role of proactive screening methods in preventing graft rejection.

Keywords: Nephropathy, renal transplantation, BK virus, IVIG

Öz

BK virüsü ile ilişkili nefropati (BKVN), böbrek nakli sonrası greft kaybına yol açabilen bir komplikasyondur. Bu hastalığa yönelik önceki yaklaşım, immünosüpresif tedavinin azaltılmasıdır, ancak bazı vakalar bu yaklaşıma yanıt vermemektedir. Bu vaka sunumu, immünosüpresif tedavinin azaltılmasına yanıt vermeyen ve intravenöz immünoglobulin (IVIG) ile tedavi edilen BKVN'li bir hastayı sunmaktadır. İki yıl önce canlı donörden böbrek nakli öyküsü olan 62 yaşındaki erkek hasta, böbrek fonksiyonlarında bozulma ile başvurdu. Yüksek BK virüsü (BKV) yükü tespit edildi ve böbrek biyopsisi ile BKVN tanısı doğrulandı. Mikofenolat mofetil tedavisinin kesilmesine rağmen viral yük artmaya ve greft fonksiyonu bozulmaya devam etti. Bunun üzerine hasta IVIG ile tedavi edildi. Plazma ve idrardaki BKV düzeyleri azaldı, ancak böbrek fonksiyonlarında minimal bir iyileşme görüldü. Bu vaka, dirençli BKVN'de IVIG'nin etkinliğini ve greft reddini önlemede proaktif tarama yöntemlerinin rolünü göstermektedir.

Anahtar Kelimeler: Nefropati, böbrek nakli, BK virüsü, IVIG

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Introduction

Chronic kidney disease is a global health issue that causes significant morbidity and mortality worldwide[1]. Kidney transplantation is considered the most effective treatment method in terms of survival and quality of life for patients with end-stage renal disease[2]. Kidney transplant recipients must receive regular immunosuppressive therapy to prevent graft rejection. These treatment regimens typically include a combination of calcineurin inhibitors, such as tacrolimus, antiproliferative agents such as mycophenolate mofetil (MMF), and steroids. However, this potent immunosuppression renders patients vulnerable to opportunistic infections, particularly viral infections[3].

BK virus (BKV) is a polyomavirus that is common in the human population and usually remains latent. Reactivation of BKV in immunosuppressed kidney transplant recipients can lead to viral replication in renal tubular epithelial cells and potentially result in BKV-associated nephropathy (BKVN). BKVN is a complication that can lead to progressive graft dysfunction, interstitial nephritis, and graft loss[4].

The primary approach to managing BKVN is the reduction of immunosuppressive therapy. The main goal of this strategy is to enable the immune system to control viral replication. However, this approach requires a balance between BKV clearance and the risk of graft rejection. Treatment options are limited in refractory cases because there is no specific antiviral agent with proven efficacy. In such cases, the role of intravenous immunoglobulin (IVIG) is being investigated. The immunomodulatory effects of IVIG and its potential to contain neutralizing antibodies against BKV make it a possible treatment option for refractory BKVN[5]. In this case, we aimed to discuss the efficacy and outcomes of IVIG treatment in a patient with BKVN who did not respond to standard immunosuppression reduction therapy.

Case Report

A 62-year-old male patient was admitted to the nephrology clinic with a history of kidney transplantation from a living donor two years earlier. The patient had diabetes mellitus, hypertension, and gout. He had not attended routine follow-up appointments for approximately one year. Laboratory tests performed at presentation showed an increase in baseline creatinine from 1.0 to 1.95 mg/dL.

Because of this deterioration, the patient was evaluated for possible causes of kidney dysfunction. He did not report any recent oral intake problems or diarrhea. There was no history of contrast imaging within the previous month. No new medications or herbal products were reported. Blood glucose and blood pressure measurements were within normal limits before and during hospitalization. The results for other viral infectious agents that could cause kidney dysfunction were negative. The patient's tacrolimus level was low (3.42 µg/L), and the dose was adjusted accordingly. The patient was initially evaluated with a preliminary diagnosis of graft rejection. BKV polymerase chain reaction (PCR) positivity was detected at a level of 12,747,732,633 copies/mL in urine and 987,870 copies/mL in plasma. The pathological examination of the renal allograft biopsy performed to confirm the diagnosis was reported as consistent with BKVN. Figures 1 and 2 show the examination of slides stained with hematoxylin and eosin, whereas Figure 3 shows the examination performed using immunohistochemical markers.

The patient was receiving standard triple immunosuppressive therapy, including tacrolimus, MMF, and prednisolone, after kidney transplantation. Following the diagnosis of BKVN, MMF therapy was discontinued to reduce immunosuppression. After this intervention, a temporary decline in BKV levels was observed (urine: 4,666,376,864 copies/mL; plasma: 202,969 copies/mL). However, in subsequent follow-ups, the patient's BKV viral load increased again (urine: 6,656,038,735 copies/mL; plasma: 413,255 copies/mL), and renal function tests progressively deteriorated (creatinine: 3.15 mg/dL).

As this condition remained unresponsive to immunosuppression reduction, the patient was administered IVIG at a dose of 300 mg/kg for a total of four doses as adjuvant therapy. A decline in BKV levels was observed after IVIG treatment (urine: 97,361,574 copies/mL; plasma: 162,790 copies/mL), and partial

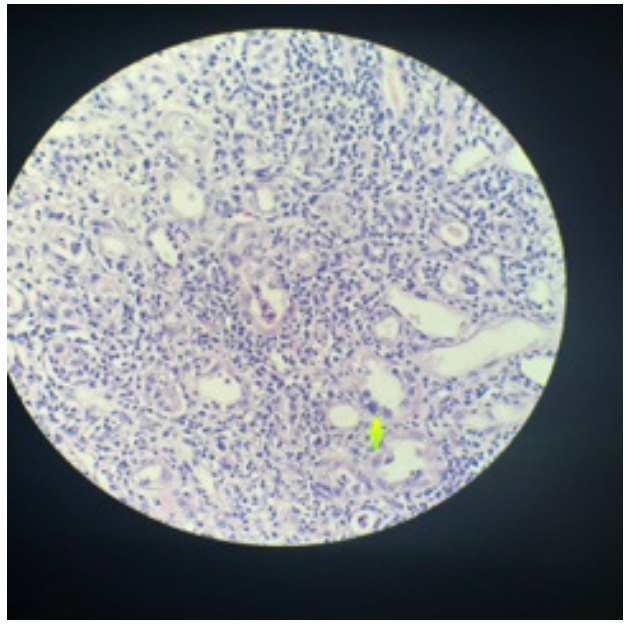

Figure 1. Hematoxylin and eosin–stained section showing viral infection, characterized by inclusion bodies in large-nucleated cells lining the tubular epithelium (original magnification × 400).

improvement was achieved in renal function (creatinine: 2.8 mg/dL). The patient's laboratory results are presented in Table 1.

Discussion

This case demonstrates the complexity and challenges involved in managing BKVN after kidney transplantation. In our patient, the failure to control BKV replication and the progressive deterioration of renal function despite discontinuation of MMF, one of the mainstays of immunosuppressive therapy, highlight how difficult the treatment of this disease can be. The most fundamental challenge in BKV treatment is the need to suppress viral replication by allowing an immune response while simultaneously avoiding allograft rejection[5].

Another important factor that negatively affected the course of this case was the patient's late presentation, after significant deterioration in renal function and lack of routine follow-up. Current clinical guidelines recommend regular screening of kidney transplant recipients, particularly within the first 1–2 years, to detect BKV reactivation at an early stage[4]. This screening is usually performed using plasma BKV PCR testing, with the aim of detecting viremia before graft dysfunction develops and initiating preemptive immunosuppression reduction. Early intervention significantly reduces progression to BKVN and the associated risk of irreversible fibrosis[6].

Reduction of immunosuppression, particularly discontinuation of antiproliferative agents, is considered the first-line treatment for BKVN. However, this approach places patients at

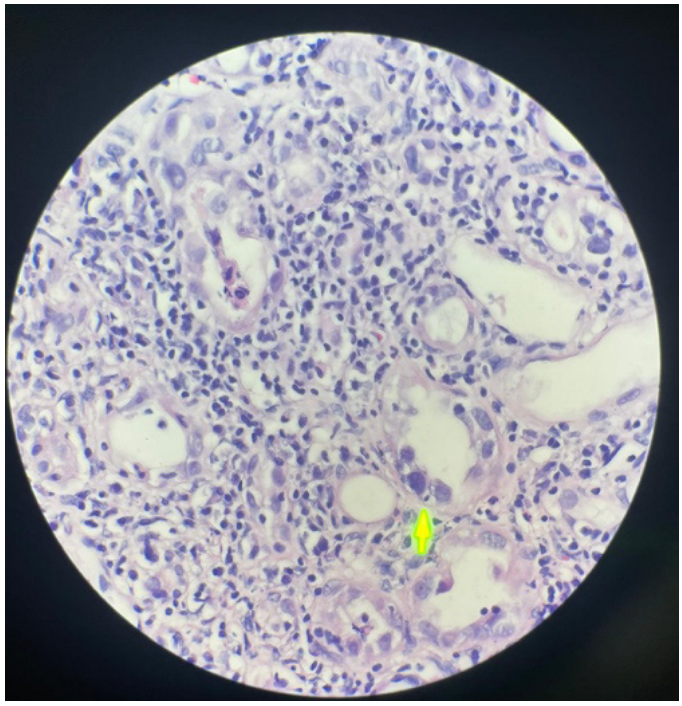

Figure 2. Hematoxylin and eosin–stained section demonstrating inclusion bodies in large-nucleated cells affecting the tubular epithelium in viral infection (original magnification × 600).

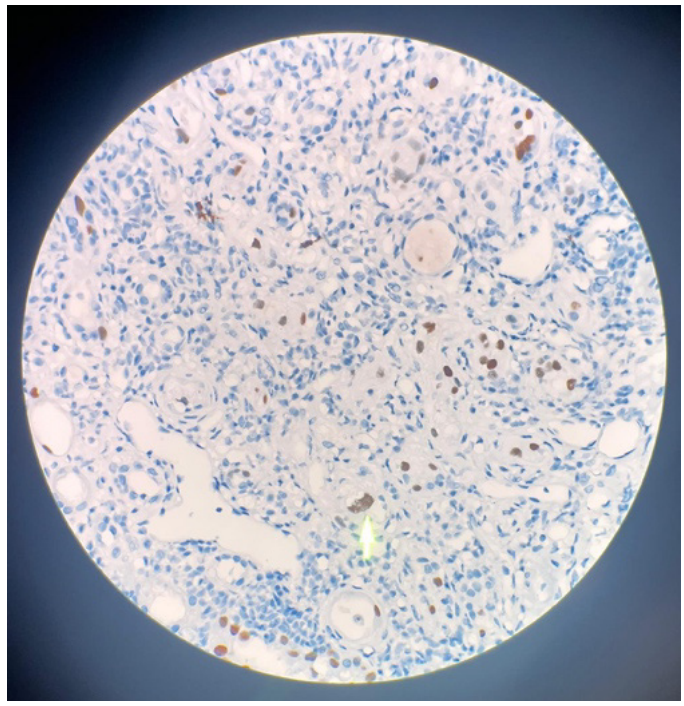

Figure 3. BK virus (SV40) immunohistochemical staining showing nuclear positivity in viral structures (original magnification × 600).

Table 1. Patient's laboratory results.

	Admission	First month follow-up	Second month follow-up	Before IVIG	After IVIG
BKV DNA (PCR) Plasma	987,870 copies/mL	704,587 copies/mL	202,969 copies/mL	413,255 copies/mL	162,790 copies/mL
BKV DNA (PCR) Urine	12,747,732,633 copies/mL	4,666,376,864 copies/mL	6,656,038,735 copies/mL	351,117,772 copies/mL	97,361,574 copies/mL
Creatinine	1.93 mg/dL	1.94 mg/dL	2.42 mg/dL	3.15 mg/dL	2.83 mg/dL
eGFR	36 mL/min/1.73 m^2	36 mL/min/1.73 m^2	28 mL/min/1.73 m^2	21 mL/min/1.73 m^2	24 mL/min/1.73 m^2
Tacrolimus drug level	3.42 µg/L	6.47 µg/L	13.9 µg/L	9.22 µg/L	7.84 µg/L
Protein/creatinine (spot urine)	291.67 µg/mg creatinine	317.99 µg/mg creatinine	516.74 µg/mg creatinine	371.26 µg/mg creatinine	387.23 µg/mg creatinine

IVIG, intravenous immunoglobulin; eGFR, estimated glomerular filtration rate; BKV, BK virus; PCR, polymerase chain reaction.

risk of T-cell-mediated or antibody-mediated rejection. Studies have reported that acute rejection rates in patients whose immunosuppression was reduced after BKVN diagnosis range from 8% to 12%[7]. Therefore, viral load and graft function should be closely monitored.

In cases unresponsive to standard treatment, various adjuvant therapies, whose efficacy and safety have not been established in randomized controlled trials, have been explored. Although agents such as leflunomide, cidofovir, and fluoroquinolones have been used in different studies, their inconsistent success rates and potential side effects (e.g., nephrotoxicity associated with cidofovir) limit their use[4]. In this context, IVIG therapy emerges as a potential option, particularly in cases where immunosuppression cannot be further reduced or remains ineffective. Although the mechanism of action of IVIG is not fully understood, it is thought to exert a direct antiviral effect through neutralizing antibodies against BKV and to modulate T- and B-cell responses through its broad immunomodulatory properties[8]. In our case, a decrease in BKV loads in plasma and urine was observed after IVIG treatment. This virological response supports the potential role of IVIG in suppressing BKV replication.

However, despite the virological response, only minimal improvement in renal function was observed in our patient. This suggests that irreversible chronic damage, such as interstitial fibrosis and tubular atrophy, may have already developed in the kidney by the time BKVN was diagnosed and treatment initiated. The occurrence of BKVN against a background of significant tubulointerstitial damage explains why functional improvement may be limited even when virological control is achieved.

Conclusion

In conclusion, this case demonstrates that IVIG may serve as an adjuvant therapy capable of providing virological control in BKVN that is resistant to immunosuppression reduction, but it may be insufficient to reverse established graft damage. Furthermore, the case highlights the critical importance of proactive screening strategies and patient adherence in preventing irreversible graft injury in the management of BKVN.

Ethics

Informed Consent: Written informed consent was obtained from the patient for this case report.

Footnotes

Authorship Contributions

Surgical and Medical Practices: D.E., S.K., F.T., E.E.E., R.Ö., K.U., Concept: D.E., S.K., E.E.E., K.U., Design: D.E., S.K., F.T., R.Ö., K.U., Data Collection or Processing: D.E., S.K., F.T., R.Ö., K.U., Analysis or Interpretation: D.E., E.E.E., R.Ö., K.U., Literature Search: D.E., F.T., E.E.E., Writing: D.E., E.E.E.

Conflict of Interest: Esma Eryılmaz Eren, the author of this article, is a member of the editorial board of the Mediterranean Journal of Infection, Microbes and Antimicrobials. However, she was not involved in any stage of the editorial review or decision-making process for this manuscript.

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A Rare Cause of Fever of Unknown Origin: A Case of Secondary Adrenal Insufficiency

Nedeni Bilinmeyen Ateşin Nadir Sebebi: Sekonder Adrenal Yetmezlik Olgusu

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Abstract

Fever is one of the most common symptoms observed in infectious diseases. However, non-infectious causes should also be considered in the differential diagnosis. In patients presenting with a common symptom such as fever, it is important to keep adrenal insufficiency in mind as a rare but possible underlying cause. A 54-year-old woman with a history of coronary artery disease, hypertension, and hypothyroidism presented with complaints of fatigue, weakness, nausea, vomiting, abdominal pain, headache, and fever. Her past medical history was notable for a previous pituitary adenoma surgery. Laboratory tests revealed elevated C-reactive protein (65 mg/L) and procalcitonin levels (33.2 ng/mL). To investigate a possible infectious etiology, neck, thoracic, and abdominal computed tomography, transthoracic echocardiography, blood and urine cultures, and other infectious disease workups were performed, but no pathology was identified. Pituitary magnetic resonance imaging revealed a macroadenoma. Laboratory findings showed hyponatremia and decreased early morning cortisol levels (1.82 µg/dL), while adrenocorticotropic hormone (ACTH) levels were within the normal range. A high-dose ACTH stimulation test confirmed the diagnosis of secondary adrenal insufficiency. The patient showed a dramatic clinical improvement after glucocorticoid replacement therapy and was discharged with an appropriate treatment plan. When investigating the cause of fever, a thorough medical history, repeated physical examinations, and appropriate use of laboratory and imaging studies are essential. Common etiologies should be considered first, and if no diagnosis is established, rare causes must be carefully evaluated.

Keywords: Adrenal insufficiency, case report, fever, fever of unknown origin (FUO)

Öz

Ateş, enfeksiyon hastalıklarının sık görülen semptomlarından birisidir. Ancak enfeksiyon dışı nedenlerinin de olduğu unutulmamalıdır. Ateş gibi yaygın bir semptom ile başvuran hastalarda nadir de olsa adrenal yetmezliğin tanıda akla gelmesi önem taşımaktadır. Koroner arter hastalığı, hipertansiyon ve hipotiroidi tanıları olan 54 yaşında kadın hasta halsizlik, yorgunluk, bulantı-kusma, karın ağrısı, baş ağrısı ve ateş nedeniyle başvurdu. Özgeçmişinde hipofiz adenomu nedeniyle operasyon öyküsü mevcuttu. C-reaktif protein (65 mg/L) ve prokalsitonin (33,2 ng/mL) yüksekliği dikkat çekmekteydi. Olası enfeksiyöz patolojilerin araştırılması için boyun, toraks, abdomen bilgisayarlı tomografi, transtorasik ekokardiyografi, kan, idrar kültürleri ve bulaşıcı hastalıklar açısından yapılan diğer testlerde patoloji saptanmadı. Hipofiz manyetik rezonans görüntülemede makroadenom olduğu tespit edildi. Hiponatremi ve sabah erken kortizol seviyesinde düşüklük (1,82 µg/dL) tespit edildi. Adrenokortikotropik hormon (ACTH) ise normal düzeylerdeydi. Yüksek doz ACTH stimülasyon testi ile sekonder adrenal yetmezlik tanısı konuldu. Glukokortikoid tedavisine dramatik yanıt alınan hasta, tedavi planı yapılarak taburcu edildi. Ateşin nedeni araştırılırken iyi bir anamnez, tekrarlayan fizik muayeneler, uygun laboratuvar ve görüntülemeler akıllıca kullanılmalıdır. Öncelikle sıklıkla görülen nedenler düşünülmeli ve tanı konulamıyorsa nadir sebepler açısından değerlendirmeler yapılmalıdır.

Anahtar Kelimeler: Adrenal yetmezlik, vaka sunumu, ateş, nedeni bilinmeyen ateş (NBA)

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Introduction

Fever is one of the most common symptoms encountered in infectious diseases; however, it should be kept in mind that there are also numerous non-infectious causes[1]. These include malignancies, autoinflammatory and autoimmune diseases, medications, and various other conditions[2]. In patients presenting with fever, a thorough medical history, detailed physical examination, and appropriate laboratory and imaging studies should be systematically utilized to elucidate the underlying etiology[1].

Adrenal insufficiency presents with non-specific symptoms such as weight loss, loss of appetite, fatigue, abdominal pain, nausea, vomiting, fever, and hypotension[3]. In primary adrenal insufficiency, the adrenal glands are affected; in secondary adrenal insufficiency, the pituitary gland; and in tertiary adrenal insufficiency, the hypothalamus. The diagnosis is established through evaluation of adrenal-related hormones and stimulation tests. Treatment is achieved by hormone replacement therapy[4].

In this case report, we present a patient with secondary adrenal insufficiency as a rare cause of a common symptom, fever. It is important to consider adrenal insufficiency in the differential diagnosis, even though it is uncommon, in patients presenting with a common symptom such as fever.

Case Report

A 54-year-old woman with a medical history of coronary artery disease, hypertension, and hypothyroidism presented with complaints of fatigue, nausea, vomiting, headache, and abdominal pain that had persisted for approximately three weeks, accompanied by high-grade fever for the past 10 days. Her regular medications included acetylsalicylic acid 100 mg, rivaroxaban 20 mg, and levothyroxine 100 µg.

Her medical history was notable for four previous surgeries due to pituitary adenoma, the most recent occurring three months prior to admission. The patient's long-term low-dose glucocorticoid therapy (prednisolone 5 mg) had been discontinued about 4 months prior. She had also been hospitalized two months earlier with a diagnosis of acute cholecystitis and biliary pancreatitis. During that hospitalization, she developed deep vein thrombosis, pulmonary embolism, and a cerebrovascular event. The patient reported receiving amoxicillin/clavulanate 1000 mg every 12 hours during the past seven days without clinical improvement. She denied any history of animal contact, consumption of well water, or recent travel.

On physical examination, the patient was in moderate general condition. Her blood pressure was 100/70 mmHg, and body temperature was 38.5 °C. Tachycardia was present during febrile episodes. She was conscious and fully oriented to time and place. No neck stiffness or other meningeal irritation signs were observed. Head and neck examination was unremarkable. Breath sounds were equal bilaterally, with no rales, rhonchi, or additional sounds. Cardiac auscultation revealed a regular rhythm without murmurs or extra heart sounds. Abdominal examination was normal, and no abnormalities were detected in the extremities. No prosthetic devices or indwelling catheters were present.

At admission, the patient's C-reactive protein (CRP) level was 65 mg/L and procalcitonin was 33.2 ng/mL, both markedly elevated. Leukocytosis was not present, and urinalysis showed no pyuria. No infectious focus explaining the elevated acute-phase reactants was identified during the initial examination. Two sets of blood cultures and a urine culture were obtained, and empirical ceftriaxone therapy (1 g every 12 hours) was initiated. Subsequently, contrast-enhanced computed tomography (CT) scans of the neck, thorax, and abdomen were performed to identify a possible infectious focus. Imaging revealed only a 3-mm hypodense lesion in the gallbladder consistent with a cholesterol stone. Additionally, various blood tests were ordered to investigate both infectious and non-infectious causes, and the requested tests and their results are presented in Table 1.

On the 48th hour of hospitalization, the patient continued to have a fever of 38.5 °C. Follow-up blood cultures were obtained, and empirical antibiotic therapy was changed to piperacillin–tazobactam 4.5 g every 6 hours. The initial urine culture showed no bacterial growth. *Staphylococcus epidermidis* was isolated twice from blood cultures; therefore, transthoracic echocardiography was performed to evaluate for possible endocarditis, but no vegetations or findings suggestive of endocarditis were detected.

Considering the patient's history of pituitary surgery, a contrast-enhanced pituitary magnetic resonance imaging (MRI) was performed, which revealed a 2-cm macroadenoma within the pituitary fossa. Laboratory evaluation showed thyroid-stimulating hormone <0.02 mIU/L, morning cortisol 1.82 µg/dL, adrenocorticotropic hormone (ACTH) 26.3 pg/mL, and serum sodium 130 mmol/L. To confirm the diagnosis of secondary adrenal insufficiency, a high-dose ACTH stimulation test was performed. After intravenous administration of 250 µg of synthetic ACTH (cosyntropin), cortisol levels were measured at 30 and 60 minutes, with a peak value of 4.2 µg/dL. These findings confirmed the diagnosis of secondary adrenal insufficiency.

On the fourth day of hospitalization, the patient developed neutropenia (neutrophil count: 1.19×10^9/L). Peripheral blood smear revealed no abnormal cell morphology or pathological

Table 1. Initial diagnostic evaluation.

Parameters	Result	Reference range
Leukocyte	4.26	4–10 $\times 10^9$/L
Neutrophil (n, %)	2.62 (61.6%)	2–7 $\times 10^9$/L
Lymphocyte (n, %)	1.38 (32.3%)	0.8–4 $\times 10^9$/L
Haemoglobin	11.9	11–16 g/dL
Platelet	228	150–400 $\times 10^9$/L
Creatinine	0.8	0.51–0.95 mg/dL
Urea	15.6	17–43 mg/dL
Glucose	85	74–106 mg/dL
Sodium (Na)	130	136–145 mmol/L
Potassium (K)	4.7	3.5–5.1 mmol/L
Lactate dehydrogenase (LDH)	254	0–247 U/L
Aspartate aminotransferase (AST)	32	0–35 U/L
Alanine aminotransferase (ALT)	8.5	0–35 U/L
Total bilirubin	1.22	0.3–1.2 mg/dL
Direct bilirubin	0.22	0–0.2 mg/dL
Alkaline phosphatase (ALP)	35.1	30–120 U/L
Gamma-glutamyl transferase (GGT)	12.7	0–38 U/L
Lipase	31.5	0–67 U/L
Amylase	36.3	28–100 U/L
C-reactive protein (CRP)	65	0–5 mg/L
Procalcitonin	33.2	ng/mL
Erythrocyte sedimentation rate (ESR)	29	mm/hour
INR	1.51	0.8–1.24
Prothrombin time (PT)	19.2	11–16 s
Activated partial thromboplastin time (aPTT)	53.7	27–45 s
D-dimer	0.86	0–0.5 µg/mL
Fibrinogen	401	200-400 mg/dL
Brucella Rose Bengal test	Negative	
Brucella Coombs gel test	Negative	
HBsAg	0.22 (negative)	0-1
Anti-HCV	0.05 (negative)	0-1
Anti-HIV	0.24 (negative)	0-1
Syphilis Ig total	0.06 (negative)	0-1
Anti-nuclear antibody (ANA)	0.1 (negative)	0–1.2 index
Rheumatoid factor (RF)	8.3	0–14 IU/mL
Complement C3	1.19	0.9–1.8 g/L
Complement C4	0.41	0.1–0.4 g/L

HCV, hepatitis C virus; HIV, human immunodeficiency virus; Ig, immunoglobulin; dL, deciliter; g, gram; INR, international normalized ratio; IU, international unit; L, liter; mg, milligram; milliliter; millimeter; ng, nanogram; n, number; U, unit; µg, microgram.

findings. Follow-up blood cultures obtained during the febrile period showed no bacterial growth. Based on the available findings, infectious pathology was deemed unlikely, and antibiotic therapy was discontinued due to concern for antibiotic-associated neutropenia.

Following confirmation of secondary adrenal insufficiency, methylprednisolone therapy was initiated at a dose of 60 mg daily. The patient showed a dramatic clinical response to glucocorticoid replacement, with rapid improvement in fatigue and malaise and resolution of fever. The methylprednisolone dose was gradually tapered (60 mg for three days, followed by 30 mg, and subsequently reduced over one month until discontinuation). On the fourth day of glucocorticoid therapy, laboratory results showed a neutrophil count of 2.1 $\times$ 10^9/L, serum sodium of 141 mmol/L, CRP of 9 mg/L, and

procalcitonin of 0.7 ng/mL. After a total hospital stay of seven days, the patient demonstrated both clinical and laboratory improvement and was discharged with a recommendation for outpatient endocrinology follow-up.

At the three-month follow-up visit after discharge, the patient was clinically stable and had experienced no further febrile episodes. She reported regular adherence to her medications with good tolerance and was being followed on a maintenance dose of 5 mg prednisolone daily.

Discussion

While infectious diseases are often the leading causes of fever of unknown origin (FUO), non-infectious causes should not be overlooked.

The definition of FUO has evolved over time. It was first described in 1961 by Petersdorf and Beeson as cases presenting with a fever of ≥38.3 °C lasting for at least three weeks and remaining undiagnosed after one week of inpatient evaluation[5]. In 1991, Durack and Street proposed modifying the definition by replacing the inpatient duration with either three days of hospitalization or three outpatient visits[6] . Later, in 1997, De Kleijn et al.[7,8] suggested defining FUO based on the failure to establish a diagnosis after a set of specific investigations, regardless of hospitalization duration or visit number. These investigations include a complete blood count, serum calcium, liver function tests, a comprehensive metabolic panel, erythrocyte sedimentation rate, CRP, ferritin, at least three sets of blood cultures (incubated for a minimum of five days), urinalysis and urine culture, tuberculin skin test (TST) or interferon-gamma release assay (IGRA), abdominal ultrasonography, chest radiography, and CT of the thorax, abdomen, and pelvis[7,8].

Recent studies have reported that the etiologies of FUO comprise infections in 12–59% of cases, non-infectious inflammatory diseases in 11–34%, malignancies in 3–32%, miscellaneous causes in 0–23%, and undiagnosed conditions in 2–51%[9]. In our patient, who presented with a 10-day history of fever, we performed most of the initial diagnostic investigations mentioned above but could not establish a definitive diagnosis. Therefore, further evaluation was undertaken for less common causes.

Adrenal insufficiency is a clinical condition characterized by impaired cortisol secretion from the adrenal cortex. It typically presents with non-specific symptoms such as fatigue, weakness, and hypotension. Laboratory findings may include hyponatremia and hyperkalemia. The diagnostic evaluation begins with measurement of early morning serum cortisol levels (between 6:00 and 9:00 a.m.). A low morning cortisol level should prompt further investigation for adrenal insufficiency. A cortisol level ≥18 µg/dL effectively rules out adrenal insufficiency, whereas a value ≤3 µg/dL in the presence of compatible clinical findings is diagnostic without the need for an ACTH stimulation test. For cortisol levels between 3 and 18 µg/dL, an ACTH stimulation test is required to confirm the diagnosis. The standard high-dose ACTH stimulation test involves intravenous administration of 250 µg synthetic ACTH (cosyntropin), followed by measurement of serum cortisol at 30 and 60 minutes. A peak cortisol value <18 µg/dL confirms adrenal insufficiency. Measurement of plasma ACTH levels helps differentiate between primary and secondary adrenal insufficiency: If the plasma ACTH level is above the reference range, primary adrenal insufficiency is diagnosed, whereas values within the lower half of the reference range indicate secondary adrenal insufficiency. The cornerstone of adrenal insufficiency treatment is glucocorticoid replacement therapy[10]. In our case, a low early morning cortisol level raised suspicion for adrenal insufficiency, prompting a high-dose ACTH stimulation test, which confirmed the diagnosis. Plasma ACTH levels were within the reference range, suggesting secondary adrenal insufficiency. Subsequent pituitary MRI revealed a macroadenoma, supporting the diagnosis. Glucocorticoid therapy was initiated, resulting in a rapid clinical improvement. The patient's history of previous pituitary surgery played a crucial role in guiding the diagnostic process.

Adrenal insufficiency is among the rare causes of FUO. In a 2019 systematic review, Fusco et al.[11] reported that conditions categorized as "other causes," including adrenal insufficiency, accounted for 6.5% of FUO cases. In a 2017 study, Hung et al.[12] evaluated 58 patients using nuclear imaging methods and identified adrenal insufficiency in 2 cases (3.4%). More recently, Ko et al.[13] assessed 202 patients and diagnosed adrenal insufficiency in 61 of them (30.1%).

Patients with adrenal insufficiency may present with fever. In a 2017 study, Lee et al.[14] reported that 33 (18%) of 183 patients with adrenal insufficiency presented with fever. In their 2019 systematic review, Lee et al.[15] also found that fever occurred in 33.3% of adrenal insufficiency cases. Similarly, Jang et al.[16] analyzed 150 patients with adrenal insufficiency and observed that 45 (30%) presented with fever.

Glucocorticoids released under the influence of cortisol suppress proinflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha. In adrenal insufficiency, cortisol deficiency can lead to increased levels of these cytokines. The elevation of proinflammatory cytokines may, in turn, cause an increase in inflammatory markers such as CRP and procalcitonin. Elevated inflammatory markers often suggest an infectious etiology, thereby complicating the diagnostic process[17]. Procalcitonin is now widely recognized as a clinically valuable biomarker for evaluating systemic

inflammatory responses, particularly in distinguishing bacterial infections from non-infectious inflammation[18]. Although elevated procalcitonin levels are typically associated with infections, they may also be seen in various non-infectious conditions (e.g., trauma, burns, surgery, malignancy, acute pancreatitis)[19-21]. In our case, markedly elevated CRP and procalcitonin levels initially led to an extensive infectious workup. However, no infectious pathogens were identified, and subsequent hormonal testing and imaging confirmed the diagnosis of secondary adrenal insufficiency. Despite receiving broad-spectrum antibiotic therapy for approximately four days, the patient showed no clinical or laboratory improvement, whereas a dramatic response was observed following the initiation of glucocorticoid therapy.

A peripheral blood smear for malaria was not performed due to the absence of a travel history. TST or IGRA could not be performed because these tests were not available at our institution.

Conclusion

In the evaluation of FUO, a thorough medical history, repeated physical examinations, and appropriate laboratory and imaging studies should be used judiciously. Common causes should be considered first, and when a diagnosis cannot be established, rare etiologies must also be evaluated. In our case, the patient's history of pituitary surgery prompted suspicion, and subsequent diagnostic testing led to the identification of secondary adrenal insufficiency—a rare cause of FUO.

Ethics

Informed Consent: Written informed consent was obtained from the patient for the anonymous publication of their medical data and images in this case report.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.E., B.E., Concept: E.E., B.E., Design: E.E., B.E., Data Collection or Processing: E.E., B.E., Analysis or Interpretation: E.E., Literature Search: E.E., Writing: E.E., B.E.

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Lupus Vulgaris Treated Successfully with a Modified Anti-Tuberculosis Regimen in a Patient with Drug-Induced Liver Injury Patient: A Case Report

İlaca Bağlı Karaciğer Hasarı Olan Bir Hastada Modifiye Antitüberküloz Rejimi ile Başarıyla Tedavi Edilen Lupus Vulgaris: Olgu Sunumu

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Abstract

Lupus vulgaris is a form of cutaneous tuberculosis (TB) that commonly affects the face and neck. It is typically treated with standard anti- TB therapy (ATT). However, adverse drug reactions may alter treatment strategies. We report a case of a female patient with a red plaque on the left cheek, diagnosed as lupus vulgaris. Standard ATT was initiated but subsequently discontinued due to signs and symptoms of drug-induced liver injury (DILI). The treatment regimen was modified to levofloxacin, ethambutol, and streptomycin, with the gradual reintroduction of rifampicin. Reintroduction of isoniazid led to intolerance symptoms and was therefore discontinued. The patient declined reintroduction of pyrazinamide due to fear of recurrence of the same symptoms. Consequently, a modified regimen of levofloxacin, streptomycin, ethambutol, and rifampicin was continued in intensive phase, followed by rifampicin and ethambutol in continuation phase. Marked clinical improvement was observed. This case demonstrates successful individualized management of lupus vulgaris with a modified regimen in the setting of DILI, representing the first reported use of this approach.

Keywords: Antituberculous drugs, cutaneous tuberculosis, drug-induced liver injury, hepatotoxicity, lupus vulgaris

Öz

Lupus vulgaris, genellikle yüz ve boyun bölgelerini tutan bir kutanöz tüberküloz formudur. Bu durum genellikle standart anti-tüberküloz tedavisi (ATT) ile tedavi edilir. Ancak, advers ilaç reaksiyonları tedavi stratejisinin değiştirilmesini gerektirebilir. Sol yanakta eritematöz plak bulunan bir kadın hastada lupus vulgaris tanısı konulan bir olguyu sunuyoruz. Standart ATT başlatılmış, ancak ilaca bağlı karaciğer hasarı (drug-induced liver injury, DILI) belirti ve bulgularının ortaya çıkması üzerine tedavi sonlandırılmıştır. Tedavi rejimi daha sonra levofloksasin, etambutol ve streptomisin içerecek şekilde modifiye edilmiş, rifampisin ise kademeli olarak yeniden tedaviye dahil edilmiştir. İzoniazide yeniden başlanması intolerans semptomlarına yol açmış ve bu nedenle tedavi kesilmiştir. Hasta, pirazinamidin yeniden başlanmasını reddetmiştir. Bu nedenle, yoğun fazda levofloksasin, streptomisin, etambutol ve rifampisinden oluşan modifiye rejim sürdürülmüş; ardından idame fazında rifampisin ve etambutol ile tedaviye devam edilmiştir. Belirgin klinik iyileşme gözlenmiştir. Bu olgu, DILI varlığında modifiye edilmiş bir rejim ile lupus vulgarisin bireyselleştirilmiş tedavisinin başarısını vurgulamaktadır.

Anahtar Kelimeler: Anti-tüberküloz tedavi, kutanöz tüberküloz, ilaca bağlı karaciğer hasarı, hepatotoksisite, lupus vulgaris

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Introduction

Lupus vulgaris is a chronic, progressive form of cutaneous tuberculosis (TB) that typically develops in individuals with moderate to high immune competence[1]. Predilection sites of lupus vulgaris include the face and neck. Lupus vulgaris can be caused by the spread of TB through the bloodstream, lymphatic system or by directly extending from an adjacent infected organ[2]. The typical manifestations are well-demarcated reddish-brown plaques or nodules with an apple-jelly sign. The treatment of lupus vulgaris typically involves the standard anti-TB therapy (ATT). However, this case reports the first successful use of a combination regimen of levofloxacin, ethambutol, streptomycin, and rifampicin for lupus vulgaris in the setting of drug-induced liver injury (DILI).

Case Report

A 53-year-old female presented with a thick, red plaque on her left cheek. The patient reported occasional pruritus. The lesion had been present for one year prior to hospital admission. The lesion did not worsen with sun exposure. There was no associated numbness. The patient denied any history of irritation from topical substances and had no history of chronic cough, weight loss, or a diagnosis of pulmonary TB. There was no family history of similar symptoms. Physical examination was unremarkable, except for multiple erythematous plaques with telangiectasia on the left cheek. Histopathological examination revealed epidermal atrophy with parakeratosis and focal lymphocytic exocytosis. The superficial to deep dermis and superficial subcutis showed confluent granulomatous infiltrates composed of epithelioid histiocytes and Langhans-type giant cells, surrounded by lymphocytes. No caseating necrosis was observed. These findings were consistent with lupus vulgaris.

The patient was referred to the pulmonology clinic and started on standard multidrug regimen. The intensive phase regimen consists of rifampicin, isoniazid, pyrazinamide, and ethambutol. After two weeks of therapy, the patient developed severe nausea, and liver function test levels were found to be elevated more than five times the upper reference limit (Table 1). The bilirubin and aspartate aminotransferase (AST) values were not obtained before the first line regiment initiation due to limited coverage from insurance. The patient had no history of alcohol consumption. She was diagnosed with DILI, and the standard regimen was discontinued. The treatment was modified to a non-hepatotoxic regimen consisting of levofloxacin 750 mg (11.7 mg/kg), ethambutol 1000 mg, and streptomycin 1000 mg intramuscularly (15.62 mg/kg).

Rifampicin was subsequently reintroduced after two weeks of the alternative regimen, with close monitoring for signs of hepatotoxicity and serial assessment of liver function parameters to evaluate for DILI associated with first-line standard ATT. The initial dose of rifampicin was 450 mg daily for one week. During this period, liver function tests improved and approached the normal range. The rifampicin dose was then increased to 600 mg daily, followed by the addition of isoniazid at a dose of 360 mg daily for one week. However, shortly after the reintroduction of isoniazid, the patient developed palpitations, nausea, and vomiting. The drug was discontinued based on suspicion of isoniazid allergy or intolerance. The patient declined reintroduction of pyrazinamide due to concerns about potential adverse effects.

The intensive phase of treatment was completed using levofloxacin, streptomycin, ethambutol, and rifampicin. The continuation-phase regimen was comprised of rifampicin 600 mg and ethambutol 1000 mg, which was given for 7 months, bringing the total treatment duration to 9 months. There were no adverse events during this phase. By the end of the treatment, the patient demonstrated significant clinical improvement with thinning of the lesion and resolution of erythema (Figure 1). This case illustrates a successful alternative regimen for lupus vulgaris in a patient who developed DILI from the standard ATT.

Table 1. Course of liver function tests.

Parameters	Upper reference limit	Before initiation of the first-line regimen	After initiation of the first-line	After 1-week of levofloxacin, ethambutol, and streptomycin for a week	After 1-week of additional rifampicin
ALT	55	34	142	95	50
AST	34	-	194	67	33
Total bilirubin	1.2	-	0.9	0.36	0.45
Indirect bilirubin	0.8	-	0.48	0.18	0.21
Direct bilirubin	0.5	-	0.42	0.18	0.24

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

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Figure 1. Skin lesion before and after treatment.

Discussion

Lupus vulgaris is a paucibacillary form of cutaneous TB. Histopathology of lupus vulgaris shows epithelioid cells, macrophages, and Langhans giant cells[3]. Caseating granuloma is rarely seen in lupus vulgaris. These findings are in accordance with the result of patient histopathology. Cutaneous TB, including lupus vulgaris, is treated with standard fixed-dose combination ATT. The first line treatment should be taken for at least 9 months which is divided into two phases, intensive and continuation. The intensive phase consists of a two-month regimen comprising rifampicin, isoniazid, pyrazinamide, and ethambutol. However, liver injury is one of the possible side effects. The incidence rate of DILI is 2-28%[4]. Pyrazinamide is the most common causative drug for DILI among the other drugs in first line ATT[5]. In one study assessing serious adverse events associated with pyrazinamide during first-line ATT, hepatotoxicity was identified as the most frequent adverse effect, accounting for 44.5% of cases[6].

Elevation of liver enzymes of more than five times is an indication to stop the hepatotoxic medicine (rifampicin, isoniazid, pyrazinamide). Rifampicin should be reintroduced at a low dose and slowly titrated. The patient tolerated the full dose of rifampicin; therefore, rifampicin was continued, and isoniazid was subsequently reintroduced. However, following the reintroduction of isoniazid, the patient exhibited signs of drug intolerance, including palpitations, nausea, and vomiting. Based on the symptoms, isoniazid hypersensitivity was suspected. According to the literature, 73.7% of hypersensitivity reactions with first-line ATT occurred within the first week of treatment and presentation may vary. Nausea and vomiting are found in immediate-type hypersensitivity[7]. However, the data regarding skin rash, eosinophilia, and specific immunological test results were not available. Thus, the palpitations, nausea, and vomiting should be categorized as intolerance to the side effects of isoniazid.

Second-line drugs of cutaneous TB include aminoglycosides and quinolones[8]. According to Indonesian National Guidelines for TB, regimen of fluoroquinolone, ethambutol, and streptomycin may be administered when the patient has liver impairment[9]. This is consistent with other literature, which states that if discontinuing ATT is not safe, an alternative regimen can be administered. The regimen consists of non-hepatotoxic drugs: streptomycin, ethambutol, and fluoroquinolone[4]. The dose of streptomycin and levofloxacin are based on patient's body weight. According to the Kementerian Kesehatan Republik Indonesia guidelines for the management of TB, streptomycin is administered at a recommended dosage of 10–15 mg/kg body weight per day. The standard therapeutic dose is 15 mg/kg, with a maximum daily dose not exceeding 1,000 mg[9]. A previous case report has demonstrated successful treatment of multidrug-resistant lupus vulgaris using regimens including kanamycin, cycloserine, moxifloxacin, linezolid, clofazimine, para-aminosalicylic acid, and pyridoxine[10]. To the best of the authors' knowledge, this is the first case report to describe the successful treatment of lupus vulgaris using an alternative regimen consisting of levofloxacin, ethambutol, streptomycin, and rifampicin. This regimen may be beneficial for patients with similar experiences of liver injury associated with standard ATT and suspected isoniazid hypersensitivity.

Conclusion

Hepatotoxicity caused by anti-TB treatment may alter the management course of lupus vulgaris. A modified regimen consisting of levofloxacin, streptomycin, ethambutol, and rifampicin resulted in satisfactory resolution of the cutaneous lesion in a patient with DILI.

Ethics

Informed Consent: The patient was informed in detail about the study, and written informed consent was obtained for the publication of this case report and any associated images for scientific purposes.

Footnotes

Authorship Contributions

Surgical and Medical Practices: F.D.S.F.A., M.M., E.M., Concept: F.D.S.F.A., Design: F.D.S.F.A., Data Collection or Processing: F.D.S.F.A., M.M., E.M., Analysis or Interpretation: F.D.S.F.A., M.M., E.M., Literature Search: F.D.S.F.A., Writing: F.D.S.F.A., M.M., E.M.

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CASE REPORT / OLGU SUNUMU

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Early-Onset Methemoglobinemia in an HIV Patient Receiving Dapsone for PCP Prophylaxis Without G6PD Deficiency: A Case Report

G6PD Eksikliği Olmayan ve PCP Profilaksisi için Dapson Alan Bir HIV Hastasında Erken Başlangıçlı Methemoglobinemi: Bir Vaka Raporu

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Abstract

Dapsone is used for *Pneumocystis jirovecii* pneumonia (PCP) prophylaxis in patients with human immunodeficiency virus (HIV) infection who cannot tolerate trimethoprim–sulfamethoxazole (TMP-SMX), the first-line agent. Methemoglobinemia is a known adverse effect of dapsone, usually associated with glucose-6-phosphate dehydrogenase (G6PD) deficiency, but it may also occur in patients with normal enzyme activity. We report a case of early-onset methemoglobinemia in a newly diagnosed HIV-infected patient without G6PD deficiency receiving dapsone for PCP prophylaxis. A 24-year-old male with a CD4 T-cell count of 32/mm^3 developed decreased oxygen saturation approximately 72 hours after starting dapsone. Despite minimal symptoms, methemoglobin levels increased up to 11.5%. Dapsone was discontinued, and the patient was treated with methylene blue with rapid improvement. Although dapsone-induced methemoglobinemia is well described, reports in HIV-infected patients receiving dapsone for PCP prophylaxis are limited. This case highlights that methemoglobinemia may occur without G6PD deficiency and emphasizes early recognition.

Keywords: Dapsone, methemoglobinemia, HIV, PCP prophylaxis, G6PD

Öz

Dapson, trimetoprim–sülfametoksazol (TMP-SMX)'i tolere edemeyen HIV enfeksiyonu olan hastalarda, birinci basamak ajan olan TMP-SMX'e alternatif olarak Pneumocystis jirovecii pnömonisi (PCP) profilaksisinde kullanılmaktadır. Methemoglobinemi, dapsonun bilinen bir yan etkisi olup genellikle glukoz-6-fosfat dehidrogenaz (G6PD) eksikliği ile ilişkilidir; ancak normal enzim aktivitesine sahip hastalarda da görülebilir. Bu çalışmada, PCP profilaksisi amacıyla dapson kullanan ve G6PD eksikliği olmayan yeni tanı almış HIV enfekte bir hastada gelişen erken başlangıçlı methemoglobinemi olgusu sunulmuştur. CD4 T hücre sayısı 32/mm^3 olan 24 yaşındaki erkek hastada, dapson başlanmasından yaklaşık 72 saat sonra oksijen satürasyonunda düşüş gelişti. Minimal semptomlara rağmen, methemoglobin düzeylerinin %11,5'e kadar arttığı saptandı. Dapson tedavisi kesildi ve hasta metilen mavisi ile tedavi edilerek hızlı düzelme sağlandı. Dapsona bağlı methemoglobinemi iyi tanımlanmış olmakla birlikte, PCP profilaksisi amacıyla dapson kullanan HIV enfekte hastalarda bildirilen olgular sınırlıdır. Bu olgu, methemoglobineminin G6PD eksikliği olmaksızın da gelişebileceğini ve erken tanının önemini vurgulamaktadır.

Anahtar Kelimeler: Dapson, methemoglobinemi, HIV, PCP profilaksisi, G6PD

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Introduction

Dapsone is a sulfone antibiotic widely used in the prophylaxis of *Pneumocystis jirovecii* pneumonia (PCP), particularly in patients intolerant to trimethoprim–sulfamethoxazole (TMP-SMX)[1]. One of its well-recognized adverse effects is methemoglobinemia, which results from oxidative stress on erythrocytes[2]. Although the risk is increased in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, it may also occur in individuals with normal enzyme activity[3].

Methemoglobinemia can present with non-specific symptoms and may be overlooked, particularly in patients without overt cyanosis or respiratory distress. Early identification is crucial, as delayed diagnosis may lead to unnecessary investigations and potential clinical deterioration[4].

Here, we present a case of early-onset, mild methemoglobinemia in a patient with human immunodeficiency virus (HIV) infection receiving dapsone for PCP prophylaxis, emphasizing the importance of clinical suspicion even in low-risk individuals.

Case Report

A 24-year-old male patient, with no known history of chronic disease, was under follow-up for newly diagnosed acquired immunodeficiency syndrome (AIDS), with a CD4 T lymphocyte count of 32/mm^3. As the patient had no clinical signs of pneumonia, PCP prophylaxis was initiated according to the European AIDS Clinical Society guideline version 12.1 with TMP-SMX 960 mg once daily orally [5]. However, the patient developed severe dyspnea on the second day of TMP-SMX prophylaxis, and TMP-SMX prophylaxis was discontinued, considering the drug side effect. Current guidelines recommend agents such as dapsone, atovaquone, and pentamidine as alternatives to TMP-SMX for the primary prophylaxis of opportunistic infections[5]. Since G6PD deficiency was not detected in the patient, dapsone prophylaxis was initiated at a dose of 100 mg per day.

Approximately 72 hours after initiation of dapsone therapy, oxygen saturation decreased to 87% on room air. The patient did not exhibit cyanosis and reported no significant dyspnea. Physical examination was otherwise unremarkable. Electrocardiography revealed sinus tachycardia, and chest radiography was normal.

Arterial blood gas analysis demonstrated methemoglobin levels of 5.3%, 7.3%, 9.8% and 11.5% over serial measurements. The hemoglobin level was 7.7 g/dL. Given the progressive increase in methemoglobin levels and the associated decrease in oxygen saturation, dapsone-induced methemoglobinemia was suspected. Dapsone was discontinued immediately. The patient received supplemental oxygen and a single dose of methylene blue (1 mg/kg). He was monitored in the intensive care unit.

At approximately 52 hours after initiation of methylene blue therapy, oxygen saturation was 98% on room air, with normalization of methemoglobin levels on arterial blood gas analysis (Figure 1). The patient's clinical condition remained stable. After ruling out opportunistic infections, antiretroviral therapy was initiated with a regimen of tenofovir disoproxil fumarate, emtricitabine, and dolutegravir. After discontinuation of dapsone treatment, primary prophylaxis was continued with 960 mg TMP-SMX three times a week, and no new side effects were observed in the patient.

Discussion

Dapsone-induced methemoglobinemia is a well-documented adverse effect mediated by its hydroxylamine metabolite, which oxidizes hemoglobin[2]. While G6PD deficiency is a known risk factor, cases in patients with normal enzyme activity have also been reported[3,4].

This case is notable for the early onset and relatively mild presentation of methemoglobinemia, which occurred within days of initiating standard-dose dapsone. Despite relatively low methemoglobin levels, the patient exhibited decreased oxygen saturation, illustrating that even mild elevations may have clinical relevance.

A key diagnostic feature is the discrepancy between pulse oximetry and arterial oxygen measurements (the so-called "saturation gap") and clinical presentation [1]. In such cases, co-oximetry or blood gas analysis is essential for accurate diagnosis. Recognizing this pattern can prevent unnecessary investigations for pulmonary or cardiac causes of hypoxia[4,6].

Although dapsone-induced methemoglobinemia is well described, a focused literature search was performed in PubMed and Google Scholar using the terms "dapsone", "methemoglobinemia", "HIV", and "PCP prophylaxis". This search identified only a limited number of reports specifically describing this complication in HIV-infected patients receiving dapsone for PCP prophylaxis. In contrast, most published cases, including recent reports such as those summarized in Table 1, describe dapsone-induced methemoglobinemia in patients without G6PD deficiency but in non-HIV clinical settings, rather than in the context of HIV infection. In clinical practice, hypoxemia in people living with HIV (PLWH) is often primarily attributed to opportunistic infections such as PCP, bacterial pneumonia, or other pulmonary complications. This diagnostic bias may delay consideration of drug-related causes such as methemoglobinemia.

Our case highlights the importance of maintaining a high index of suspicion for methemoglobinemia in PLWH receiving dapsone, even in the absence of G6PD deficiency and in the presence of mild or non-specific symptoms. Early recognition in this population is particularly important to avoid unnecessary diagnostic procedures and delays in appropriate management.

Management involves prompt discontinuation of the offending agent. Methylene blue is indicated in symptomatic patients or those with higher methemoglobin levels and typically results in rapid clinical improvement, as observed in our case[1]. Compared to previously reported cases, our patient presented with lower methemoglobin levels but clinically relevant hypoxemia, highlighting the importance of early recognition even in mild cases.

Previous reports have described dapsone-induced methemoglobinemia in various clinical contexts, including patients with G6PD deficiency, malignancy, renal disease, or overdose[2,7,8]. Compared to these cases, our patient had no significant comorbidities, highlighting that this adverse effect can occur even in relatively uncomplicated clinical scenarios (Table 1).

Conclusion

It is known that the number of PLWH is increasing. Although we are in the antiretroviral era, it is anticipated that the need for TMP-SMX use for PCP prophylaxis in PLWH may increase secondary to the increase in the number of patients. However, TMP-SMX treatment or prophylaxis is associated with serious adverse effects that may necessitate discontinuation of the drug. In this context, the use of dapsone, recommended as an alternative to TMP-SMX, may become more common.

With this case report, we aim to emphasize that patients receiving dapsone should be closely monitored for adverse effects even if asymptomatic, that screening for G6PD deficiency should be performed prior to initiation, and that methemoglobinemia may develop even in the absence of G6PD deficiency. Furthermore, we highlight that this condition can range from an asymptomatic course to severe respiratory failure. Early recognition and treatment are essential to prevent complications and avoid unnecessary diagnostic procedures.

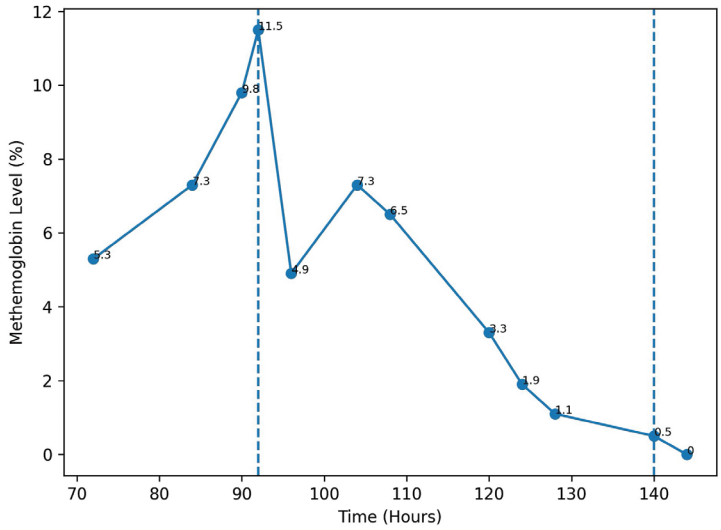

Figure 1. Serial measurements of methemoglobin levels following dapsone exposure. Methemoglobinemia developed after 72 hours of therapy, with levels increasing progressively and reaching a peak of 11.5% at 92 hours, when methylene blue (MB) was initiated. MB therapy was discontinued at 140 hours, with normalization by 144 hours.

Table 1. Reported cases of dapsone-induced methemoglobinemia without G6PD deficiency.

Study	Patient characteristics	Indication	Dapsone dose	MetHb (%)	Outcome
Bai et al.[3]	49F, multiple myeloma, CKD	PCP prophylaxis	100 mg/day	~20%	Recovered
Shenouda et al.[4]	64M, Behçet's syndrome	Behçet's syndrome	100 mg/day	15–25%	Recovered
Present case	24M, HIV (CD4: 32)	PCP prophylaxis	100 mg/day	11.5%	Recovered

G6PD, glucose-6-phosphate dehydrogenase; CKD, chronic kidney disease; HIV, human immunodeficiency virus; PCP, *Pneumocystis jirovecii* pneumonia; MetHb, methemoglobin.

Ethics

Informed Consent: Written informed consent was obtained from the patient.

Footnotes

Authorship Contributions

Concept: D.K.Ö., F.P., Design: D.K.Ö., F.P., Data Collection or Processing: D.K.Ö., C.C., Analysis or Interpretation: D.K.Ö., E.Ö.Ç., Literature Search: D.K.Ö., Writing: D.K.Ö.

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Drug–Drug Interactions in Patients with Brucellosis: A Retrospective Analysis

Bruselloz Hastalarında İlaç–İlaç Etkileşimleri: Retrospektif Bir Analiz

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Abstract

Introduction: The prolonged duration of drug treatment and the need for combination therapy in brucellosis increase the risk of drug-related problems. One of the most significant drug-related problems is drug–drug interactions. The aim of this study was to identify potential drug interactions that may occur during the treatment of patients with brucellosis, investigate their causes, and contribute to clinical practice.

Materials and Methods: This retrospective study included 76 patients admitted to the Infectious Diseases and Clinical Microbiology Clinic of Ege University Faculty of Medicine Hospital between January 2016 and November 2022 because of brucellosis and/or its complications. Drug interactions among the antibiotics used by the patients and between these antibiotics and other medications were investigated. Lexicomp® and Micromedex® were used as interaction databases. The severity and number of drug interactions identified in each database were assessed. Additionally, gender, age, occupation, the presence of comorbidities, and comparisons of database results across other medication groups were evaluated. Data were analyzed using SPSS version 27. Ethics committee approval was obtained, and verbal informed consent was obtained from all patients.

Results: Of the 76 patients included in the study, 49 were male, the mean age was 47.23 ± 14.16 years, and 53.95% had comorbidities. The mean number of drug interactions per patient was 4.88 ± 2.88 in LC and 2.63 ± 1.81 in MM. Significant associations were found between age, comorbidities, ciprofloxacin use, and the number of drug interactions in both databases ($p < 0.05$). When the interactions between antibiotics used for brucellosis and other medications were examined, the most common were decreased efficacy of doxycycline (97.30%), increased efficacy of gentamicin (54.17%), and decreased efficacy of rifampin (58.05%). The medications most commonly associated with decreased efficacy were statins, neurologic drugs, and antihypertensive agents ($p = 0.001$).

Conclusion: In this study, we focused on drug interactions during antibiotic treatment. Long-term treatment and polypharmacy can lead to drug interactions. This can negatively affect treatment effectiveness and prolong the duration of therapy.

Keywords: Antibiotics, drug interactions, brucellosis

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Öz

Giriş: Bruselloz tedavisinde ilaç kullanım süresinin uzun olması ve kombine tedavi gerekliliği, ilaçla ilişkili sorunlara yol açabilmektedir. Bu sorunlardan en önemlilerinden biri ilaç etkileşimleridir. Bu çalışmanın amacı, bruselloz hastalarının tedavisi sırasında ortaya çıkabilecek ilaç etkileşimlerini belirlemek, nedenlerini araştırmak ve klinik uygulamaya katkı sağlamaktır.

Gereç ve Yöntem: Bu retrospektif çalışmaya, Ocak 2016 ile Kasım 2022 tarihleri arasında Ege Üniversitesi Tıp Fakültesi Hastanesi Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Kliniği'ne bruselloz ve/veya komplikasyonları nedeniyle başvuran 76 hasta dahil edildi. Hastaların kullandığı antibiyotikler arasındaki ve antibiyotiklerle diğer ilaçlar arasındaki etkileşimler incelendi. Etkileşim veritabanı olarak Lexicomp® ve Micromedex® kullanıldı. Veritabanlarında saptanan etkileşimlerin düzeyi ve sayısı değerlendirildi. Ayrıca cinsiyet, yaş, meslek, komorbidite varlığı ve veritabanı sonuçlarının diğer ilaç gruplarıyla karşılaştırılması analiz edildi. Veriler SPSS 27 ile analiz edildi. Etik kurul onayı alındı ve tüm hastalardan sözlü bilgilendirilmiş onam alındı.

Bulgular: Çalışmaya dâhil edilen 76 hastanın 49'u erkek olup, yaş ortalaması 47,23 ± 14,16 idi ve hastaların %53,95'inde komorbidite mevcuttu. Hasta başına düşen ilaç etkileşimi sayısı LC'ta 4,88 ± 2,88; MM'te 2,63 ± 1,81 olarak bulundu. Her iki veritabanında da yaş, komorbidite ve siprofloksasin kullanımı ile ilaç etkileşimi sayısı arasında anlamlı ilişki saptandı ($p < 0,05$). Bruselloz tedavisinde kullanılan antibiyotiklerin diğer ilaçlarla etkileşimleri incelendiğinde, en sık görülenler doksisiklin etkinliğinde azalma (%97,30), gentamisin etkinliğinde artış (%54,17) ve rifampisin etkinliğinde azalma (%58,05) idi. Etkinliği azalan ilaçlar arasında en sık statinler, nörolojik ilaçlar ve antihipertansifler yer aldı ($p = 0,001$).

Sonuç: Çalışmamızda antibiyotik tedavisi sırasında gelişen ilaç etkileşimleri üzerinde durulmuştur. Uzun süreli tedavi ve çoklu ilaç kullanımı ilaç etkileşimlerine neden olabilir. Bu durum tedavinin etkinliğini ve süresini olumsuz etkileyebilir.

Anahtar kelimeler: Antibiyotikler, ilaç etkileşimleri, bruselloz

Introduction

Brucellosis, also known as "undulant fever," "Mediterranean fever," or "Malta fever," is a zoonotic disease caused by *Brucella* species. The infection is almost always transmitted through direct or indirect contact with infected animals or animal products[1,2]. Brucellosis is commonly observed in Mediterranean countries and neighboring regions, including our country[3,4]. Although the highest prevalence in our country is reported in Eastern Anatolia, the exact incidence and prevalence remain unknown because of inadequate diagnosis and reporting[5]. Worldwide, more than 500,000 new cases of brucellosis occur each year, and an estimated 2.4 billion people are at risk[6,7]. This disease, which often causes prolonged fever, can affect multiple organs and organ systems, primarily involving the bones and joints, and can result in diverse clinical manifestations[8].

Brucellosis is often chronic in nature and can recur even when antimicrobial agents are administered according to the standard recommendations of the World Health Organization, thereby requiring long-term combination therapy[9,10]. The treatment duration is generally six weeks but may extend to three months in cases of complications or poor prognosis[10].

The prolonged treatment duration and the need for combination therapy also increase the risk of drug-related problems. One of the most important drug-related problems is drug–drug interactions[11]. Drug–drug interactions refer to changes in the effects of a drug that may occur because of the concomitant or closely timed use of another drug. The combined use of two drugs can lead to toxicity because of increased drug effects or to therapeutic failure because of reduced drug efficacy[12]. Inhibition or induction of cytochrome P450 drug-metabolizing isoenzymes is the most common mechanism by which clinically significant drug interactions occur[13,14]. Identifying clinically relevant drug interactions is essential for patient safety. Strategies to reduce the risk of drug interactions include minimizing the number of prescribed medications, regularly reassessing treatment, considering non-pharmacologic options, and monitoring for signs and symptoms of toxicity or therapeutic effectiveness.

In this study, we aimed to contribute to clinical practice by examining the drug interactions between antimicrobial agents (e.g., rifampin, doxycycline, ciprofloxacin, and gentamicin) used by patients diagnosed with brucellosis and other medications prescribed by physicians. We believe that this is the first study to investigate drug interactions in patients with brucellosis in our country.

Materials and Methods

Seventy-six patients admitted to the Infectious Diseases and Clinical Microbiology Clinic of Ege University Faculty of Medicine Hospital between January 2016 and November 2022 because of brucellosis and/or its complications were included in the study. Patients who met the inclusion criteria were selected from retrospective patient records (Table 1). Drug interactions between the antibiotics used by the patients and other medications were examined.

Lexicomp® (LC) and Micromedex® (MM) were used as the interaction databases (Table 2). The severity and number of drug interactions identified in the databases were evaluated. In addition, comparisons were made between the database

results and patients' gender, age, occupation, presence of comorbidities, and other medication groups used.

Verbal informed consent was obtained from all patients.

Statistical Analysis

The data obtained in this study were analyzed using the licensed SPSS version 27 statistical software package. Initially, frequency analyses were conducted for demographic characteristics, and frequencies (n) and percentages (%) were calculated for each group. Subsequently, descriptive statistics for continuous variables were calculated, including the mean, standard deviation, minimum and maximum values.

The normality of the variables was assessed using skewness and kurtosis coefficients, and a normal distribution was assumed when the skewness and kurtosis values fell between –1.50 and +1.50. Accordingly, the variables were determined to be normally distributed. The assumption of homogeneity of variances was evaluated using Levene's test.

Because the variables were normally distributed, differences between groups were analyzed using the independent samples t-test for comparisons between two independent groups and analysis of variance (ANOVA) for comparisons among three or more independent groups. When the assumption of homogeneity of variances was violated in the parametric tests, the results of Welch's test were considered. For multiple comparisons following a significant ANOVA result, Tukey's or Tamhane's post-hoc tests were applied depending on the homogeneity of variances.

Relationships between continuous variables were examined using Pearson's correlation analysis, whereas associations between categorical variables were evaluated using the chi-square test. For the interpretation of the results, the significance level was set at 0.05, and results with $p < 0.05$ were considered statistically significant.

Ethical Approval

The study was approved by the Ege University Medical Research Ethics Committee (approval date: December 15, 2022; approval number: 22-12.1T/3).

Results

Among the 76 patients included in the study, 49 were male, the mean age was 47.23 ± 14.16 years, and 30.26% were involved in animal husbandry. While 30.26% of the patients had a history of suspected food consumption, the most common symptom was fever (75%). A total of 53.95% of the patients included in the study had comorbidities, the most common of which were cardiovascular diseases (Table 3).

When the distribution of antibiotics used for brucellosis was examined, rifampin (98.68%) and doxycycline (97.37%) were the most frequently prescribed antibiotics, as expected. According to the interaction databases, the mean number of drug interactions per patient was 4.88 ± 2.88 in LC and 2.63 ± 1.81 in MM (Table 4).

The antibiotics used by the patients and the other medications prescribed for their illnesses were grouped according to their pharmacological properties and/or effects. The distribution of drug interaction counts for all medications in both drug interaction databases was analyzed. Rifampin was the antibiotic with the highest number of drug interactions, whereas non-steroidal anti-inflammatory drugs antihypertensive agents, and antiemetics were the most common medications in the other drug groups. The similarity of drug interactions between the two databases and the distribution of potential adverse effect profiles associated with antibiotics were also examined (Table 5).

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
18 years and older (adults)	Patients under 18 years of age
Use of two or more medications	Patients with incomplete data in the Electronic Patient Record
Complete patient information in electronic and/or manual patient records	Those with pregnancy or suspected pregnancy

Table 2. Drug–drug interactions according to the Lexicomp® and Micromedex® databases.

Lexicomp®		Micromedex®	
X	Avoid concomitant use	Contraindicated	Concurrent use of medications is contraindicated
D	Consideration of treatment modification	Major	Serious interaction level, intervention required
C	Treatment monitoring should be ensured	Moderate	Treatment may need to be modified
B	No intervention required	Minor	No change in treatment is required
A	No known drug interactions		

Table 3. Frequency distribution of demographic characteristics, disease symptoms, and comorbidities.

Variable	Category	n	%
Gender	Female	27	35.53
	Male	49	64.47
Occupation	Farmer	8	10.53
	Livestock	23	30.26
	Other	45	59.21
Suspicious food consumption		23	30.26
Variable		Mean ± SD	Min–max
Age		47.23 ± 14.16	18–71
Symptoms		n	%
Fever		57	75
Joint pain		46	60.53
Muscle pain		51	67.11
Variable		n	%
Comorbidities		41	53.95
Cardiovascular disease		26	34.21
COPD		4	5.26
Psychiatric and neurological disorders		12	15.79
Diabetes		11	14.47
Other		28	36.84

SD, standard deviation; Min, minimum; Max, maximum; COPD, chronic obstructive pulmonary disease.

Table 4. Distribution of antibiotics used and number of drug interactions.

Variable		n	%
Doxycycline		74	97.37
Rifampin		75	98.68
Gentamicin		39	51.32
Ciprofloxacin		24	31.58
Variable	Mean ± SD	Min–max	
LC drug interaction count	4.88 ± 2.88	1–12	
MM drug interaction count	2.63 ± 1.81	1–9	

SD, standard deviation; Min, minimum; Max, maximum; LC, Lexicomp®; MM, Micromedex®.

To make the interaction descriptions in the databases easier to understand with respect to antibiotics, they were classified into five groups according to the antibiotic's susceptibility to interactions. These groups were "Antibiotic Effect Decreases," "Antibiotic Effect Increases," "Antibiotic Not Affected, Side Effect May Occur," "Antibiotic Not Affected, Other Drug Effect Decreases," and "Antibiotic Not Affected, Other Drug Effect Increases." Regarding the distribution of database categories, category C was the most common in LC, whereas the moderate category was the most common in MM. In terms of interaction status, the group characterized by a decrease in antibiotic effect was the most frequently observed (Table 6).

When the levels of drug interactions in the drug interaction databases were compared with the study variables, a significant association was found in LC, and in both databases, between the number of drug interactions and age, the presence of comorbidities, and ciprofloxacin use (Tables 7 and 8).

Only seven patients in our study experienced recurrence. When the number of drug interactions was compared between patients who experienced recurrence and those who did not, no statistically significant difference was found (Table 9).

When the drugs, database categories, and frequency distributions of interaction statuses in the drug interaction databases were examined, the most common interaction

category was "decreased antibiotic effect." When the interactions between antibiotics used for brucellosis and other medications were evaluated, the most common findings were decreased efficacy of doxycycline (97.30%), increased efficacy of gentamicin (54.17%), and decreased efficacy of other drugs associated with rifampin (58.05%). The medications most frequently associated with decreased efficacy were statins, neurologic drugs, and antihypertensive agents ($p = 0.001$). Statistically significant differences were found between the antibiotic adverse effect profiles that could result from drug interactions and the similarity of drug interactions between the databases (Table 10).

Table 5. Distribution of drugs involved in drug interactions.

Variable	Category	n	%
Antibiotics used for brucellosis	Rifampin	213	56.95
	Doxycycline	75	20.05
	Gentamicin	24	6.42
	Ciprofloxacin	38	10.16
	Other	24	6.42
Medications used for other reasons	Antihypertensives	53	14.17
	Antidiabetics	10	2.67
	NSAIDs and other analgesics	78	20.86
	Antiemetics	54	14.44
	PPIs	43	11.50
	Statins	5	1.34
	NPM	12	3.21
	Other medications	20	5.35
	Other antibiotics	99	26.47
	QT prolongation	9	2.41
	Nephrotoxicity	9	2.41
Antibiotic side effects	Ototoxicity	2	0.53
	Hypoglycemia	1	0.27
	None	353	94.39
LC-MM same situation	Different	255	68.18
	Same	119	31.82

NSAIDs, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors; NPM, neurological and psychiatric medications; LC, Lexicomp®; MM, Micromedex®.

Table 6. Frequency distribution of drug interactions.

Variable	Category	n	%
LC interaction level	B	21	5.61
	C	307	82.09
	D	24	6.42
	X	22	5.88
MM interaction category	Major	65	17.38
	Moderate	137	36.63
	None	172	45.99
Interaction status	Antibiotic effect decreasing	165	44.12
	Antibiotic effect increasing	40	10.70
	Antibiotic effect not affected side effects may occur	21	5.61
	Antibiotic not affected, effect of other medications reduced	131	35.03
	Antibiotic unaffected, other drug effects increasing	17	4.55

LC, Lexicomp®; MM, Micromedex®.

Table 7. Comparison of Lexicomp drug interaction levels with variables.

Variable	Level	LC-DI		Statistics		
		Mean	SD	Test value	p	d
Occupation	Farmer(1)	7.13	3.52	2.902[A]	0.041*	1>3
	Livestock farmer(2)	4.78	2.15			
	Other(3)	4.53	2.97			
Additional medical condition	None	3.14	1.57	−6.088[t]	0.001*	-
	Present	6.37	2.93			
Doxycycline	None	4.50	4.95	−0.188[t]	0.851	-
	Yes	4.89	2.86			
Rifampin	None	8.00	1.00	1.091[t]	0.279	-
	Yes	4.84	2.88			
Gentamicin	None	4.57	2.81	−0.924[t]	0.359	-
	Yes	5.18	2.95			
Ciprofloxacin	None	4.23	2.48	−3.052[t]	0.003*	-
	Yes	6.29	3.24			
Age				0.281[r]	0.014*	-

*p < 0.05; F: ANOVA test; t: independent samples t-test; r: correlation test; d: post hoc Tukey comparison; (1) Farmer, (2) Livestock farmer, (3) Other.

Table 8. Comparison of Micromedex drug interaction levels with variables.

Variable	Level	MM-DI		Statistics		
		Mean	SD	Test value	p	d
Occupation	Farmer(1)	3.63	2.50	1.409	0.251	-
	Livestock(2)	2.61	1.67			
	Other(3)	2.47	1.73			
Additional medical condition	None	1.49	0.74	−6.645	0.001*	-
	Present	3.61	1.88			
Doxycycline	None	1.50	0.71	−0.895	0.374	-
	Yes	2.66	1.82			
Rifampin	None	2.00	1.00	−0.349	0.728	-
	Yes	2.64	1.82			
Gentamicin	None	2.81	1.84	0.839	0.404	-
	Yes	2.46	1.79			
Ciprofloxacin	None	2.31	1.46	−2.022	0.021*	-
	Yes	3.33	2.28			
Age				0.312	0.006*	-

*p < 0.05; F, ANOVA test, difference; Tukey test, t, independent samples t-test; r,: correlation test; d, difference; MM-DI, Micromedex drug interaction count; SD, standard deviation.

Table 9. Relationship between recurrence and the number of drug interactions.

Variable	Category	Disease relapse status			p
		Mean	SD	Median	
Lexicomp drug interaction count	Yes	5.29	3.64	5.00	0.763
	No	4.84	2.83	4.00	
Micromedex drug interaction count	Yes	2.71	1.70	2.00	0.897
	No	2.62	1.83	2.00	

*p <0.05; independent samples t-test; SD: standart deviation.

Table 10. Chi-square test results regarding the relationship between medications used and interaction status.

Variable	Category	Interaction					Chi-square Test
		AED	AEI	AA-SE	AEU-ODE	AEU-ODI	
		n	n	n	n	n	z
Antibiotics used for *Brucellosis*	Rifampin	78 (38.05%)	5 (2.44%)	0 (0.00%)	119 (58.05%)	3 (1.46%)	345.777 (p = 0.001*)
	Doxycycline	72 (97.30)	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (2.70%)	
	Gentamicin	4 (16.67%)	13 (54.17%)	6 (25.00%)	1 (4.17%)	0 (0.00%)	
	Ciprofloxacin	6 (21.43%)	11 (39.29%)	1 (3.57%)	5 (17.86%)	5 (17.86%)	
	Other	2 (9.09%)	9 (40.91%)	7 (31.82%)	0 (0.00%)	4 (18.18%)	
Antibiotics and medications used for other reasons	AHT	10 (21.28%)	5 (10.64%)	5 (10.64%)	23 (48.94%)	4 (8.51%)	277.053 (p = 0.001*)
	Antidiabetics	0 (0.00%)	0 (0.00%)	0 (0.00%)	5 (50.00%)	5 (50.00%)	
	NSAID	7 (9.09%)	22 (28.57%)	1 (1.30%)	47 (61.04%)	0 (0.00%)	
	Antiemetics	31 (67.39%)	0 (0.00%)	2 (4.35%)	12 (26.09%)	1 (2.17%)	
	PPI	28 (68.29%)	2 (4.88%)	0 (0.00%)	11 (26.83%)	0 (0.00%)	
	Statins	0 (0.00%)	0 (0.00%)	0 (0.00%)	4 (80.00%)	1 (20.00%)	
	NPM	0 (0.00%)	0 (0.00%)	0 (0.00%)	10 (83.33%)	2 (16.67%)	
	OM	2 (11.11%)	1 (5.56%)	1 (5.56%)	13 (72.22%)	1 (5.56%)	
	OA	84 (86.60%)	8 (8.25%)	5 (5.15%)	0 (0.00%)	0 (0.00%)	
Antibiotic side effect	QT-p	0 (0.00%)	0 (0.00%)	2 (100.00%)	0 (0.00%)	0 (0.00%)	117.805 (p 0.001*)
	Nephrotoxicity	0 (0.00%)	0 (0.00%)	9 (100.00%)	0 (0.00%)	0 (0.00%)	
	Ototoxicity	0 (0.00%)	0 (0.00%)	2 (100.00%)	0 (0.00%)	0 (0.00%)	
	Hypoglycemia	0 (0.00%)	0 (0.00%)	1 (100.00%)	0 (0.00%)	0 (0.00%)	
	None	162 (47.79%)	38 (11.21%)	0 (0.00%)	125 (36.87%)	14 (4.13%)	
LC-MM	Different	85 (34.84%)	38 (15.57%)	14 (5.74%)	97 (39.75%)	10 (4.10%)	48.522 (p 0.001*)
	Same	77 (70.64%)	0 (0.00%)	0 (0.00%)	28 (25.69%)	4 (3.67%)	

*p < 0.05; chi-square test AED, antibiotic effect decreasing; AEI, antibiotic effect increasing; AA-SE, antibiotic not affected, side effects may occur; AEU-ODE, antibiotic effect remains unchanged, other drug effect decreases; AEU-ODI, antibiotic effect not affected, other drug effect increasing; AHT, antihypertensives; NPM, neurological and psychiatric medications; OM, other medications; OA, other antibiotics; LC-MM: Lexicomp-Micromedex

Discussion

Lexicomp® and Micromedex® databases were used in our study to identify drug interactions. Statistical analyses revealed that the number of drug interactions identified in both databases was significantly associated with age, the presence of comorbidities, and ciprofloxacin use, whereas occupation was significantly associated only in the Lexicomp database. A review of the literature indicates that, although studies investigating drug interactions in patients with brucellosis are limited, numerous studies have examined drug interactions involving currently available antibiotics.

In a study published in 2021, drug interactions, particularly those involving HIV medications and antimicrobial agents, were examined using three different databases (Micromedex, Drugs.com, and the Liverpool HIV Interactions Programme). The Liverpool database reported 10% contraindications, Micromedex reported 14% contraindications and 59% major interactions, and Drugs.com reported 21% major interactions[15]. These findings demonstrate that the frequency and severity of drug interactions may vary considerably depending on the database used.

A study conducted at Augusta University's Georgia Medical School examined interactions between antimicrobial agents and anticoagulants. It found that certain antimicrobial agents inhibit warfarin metabolism, particularly in patients receiving warfarin. The antimicrobials most likely to cause these interactions include trimethoprim/sulfamethoxazole, metronidazole, and fluconazole. Other antimicrobials, such as ciprofloxacin, levofloxacin, azithromycin, and clarithromycin, have also been shown to have similar effects[16]. In a multicenter study conducted in our country, the Micromedex database was used to evaluate drug interactions. Potential antimicrobial-related drug interactions accounted for 26.4% of all interactions[17].

Unlike previous studies, our study evaluated the number of drug interactions in relation to several patient-related parameters. The increase in the number of comorbidities and medications used with advancing age was found to affect the number of drug interactions, which was considered an expected finding.

Our results showed that drug interactions were significantly more frequent in both databases among patients treated with ciprofloxacin. This finding is consistent with the pharmacological properties of ciprofloxacin reported in the literature. Tamma et al.[18] emphasized that ciprofloxacin inhibits the CYP1A2 enzyme, thereby increasing the risk of metabolic interactions with drugs such as theophylline, clozapine, tizanidine, and warfarin, potentially leading to toxicity. Owens and Ambrose[19] also reported that fluoroquinolones may cause clinically significant pharmacokinetic and pharmacodynamic interactions, particularly in patients receiving multiple medications, and highlighted the need for careful monitoring of cardiovascular, neurologic, and gastrointestinal adverse effects. Similarly, in our study, interactions associated with an increased risk of QT prolongation related to ciprofloxacin were identified in both databases.

In a recent review by Zhou et al.[20], clinically significant drug–drug interactions between quinolones and statins were highlighted, and close monitoring of creatine kinase levels and muscle-related symptoms was recommended, particularly in patients receiving high-dose atorvastatin or simvastatin in combination with ciprofloxacin.

In this study, rifampin, a potent inducer of cytochrome P450 enzymes, was shown to significantly reduce the efficacy of other medications, particularly antihypertensive agents, statins, and neurologic drugs. Conversely, doxycycline was found to be associated with reduced antibiotic efficacy through drug–drug interactions. A significant association was observed in the drug interaction data, as concomitantly administered medications, especially proton pump inhibitors, reduced the bioavailability of doxycycline. Furthermore, in our study, statins and neurologic drugs were the other medication classes whose efficacy was reduced as a result of antibiotic-related drug interactions, in descending order of frequency.

In a case report by Han et al.[21], a patient diagnosed with HIV/AIDS was reported to have concurrent pulmonary tuberculosis, chronic hepatitis C, and systemic brucellosis. A standard antituberculosis regimen containing rifampin was initiated for tuberculosis treatment, whereas doxycycline-based antibiotic therapy was used for brucellosis. In the management of chronic hepatitis C infection, the selection and timing of antiviral therapy were carefully adjusted because of the potential risk of hepatotoxicity. Antiretroviral therapy was continued for HIV/AIDS; however, clinical modifications were made in consideration of the potential reduction in antiretroviral drug levels caused by the cytochrome P450 enzyme-inducing effect of rifampin. Throughout the course of treatment, close clinical and laboratory monitoring was emphasized as essential because of both drug–drug interactions and the risk of hepatotoxicity[21]. Considering the prolonged duration of brucellosis treatment, close monitoring of medications used for concomitant diseases is of great importance throughout the treatment period, and clinically significant potential drug–drug interactions should not be overlooked.

Study Limitations

Because of the retrospective study design based on medical record review, patients with incomplete data were excluded, which may have limited the sample size. As a result of the

retrospective design, the clinical outcomes of the identified potential drug–drug interactions, such as adverse effects or their impact on treatment effectiveness, could not be directly observed. In addition, changes in patients' medication regimens after hospital discharge and the potential drug–drug interactions arising from these changes could not be evaluated. Furthermore, the single-center design and the relatively small sample size limit the generalizability of the findings. Therefore, prospective, multicenter studies incorporating post-discharge follow-up and clinical outcome assessment are warranted to provide more comprehensive evidence.

Conclusion

Long-term antibiotic regimens used to treat brucellosis pose a substantial risk of clinically significant drug–drug interactions. These interactions may not only compromise treatment efficacy but also prolong treatment duration and create considerable challenges in the management of comorbid conditions. Our findings demonstrate that the identification of drug interactions varies significantly depending on the drug interaction database used, indicating that potential interactions should be evaluated not only according to their frequency but also in terms of their clinical significance. In particular, treatment regimens containing ciprofloxacin or rifampin warrant careful review of concomitant medications, with a patient-specific assessment of potential interactions. In this context, the active involvement of clinical pharmacists as part of the multidisciplinary health care team is essential for the early identification, prevention, and effective management of clinically meaningful drug–drug interactions, thereby ensuring safe and optimized treatment strategies for patients with brucellosis.

Ethics

Ethics Committee Approval: The study was approved by the Ege University Medical Research Ethics Committee (approval date: December 15, 2022; approval number: 22-12.1T/3).

Informed Consent: The verbal informed consent was obtained from all patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.T.C., M.T., Concept: B.Ç., F.F.Y., Design: B.Ç., M.T., Data Collection or Processing: F.F.Y., Analysis or Interpretation: M.T.C., Literature Search: B.Ç., M.T.C., Writing: B.Ç., M.T.C., M.T.

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Conservative Management of Diabetic Foot Infections: Real-world Outcomes

Diyabetik Ayak Enfeksiyonlarında Konservatif Tedavi: Gerçek Yaşam Sonuçları

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Abstract

Introduction: Diabetic foot infections (DFIs) remain a significant clinical problem despite advances in the treatment of diabetes mellitus. This study aims to contribute to the body of knowledge in this field by evaluating the epidemiological characteristics, treatment processes, and outcomes of patients diagnosed with DFIs.

Materials and Methods: We retrospectively analyzed the characteristics, treatments, and outcomes of patients with DFIs who were admitted between October 2020 and November 2023. All data were analyzed using Statistical Package for the Social Sciences version 22.0, and statistical significance was set at $p < 0.05$.

Results: The mean age of the 129 patients included in the study was 61.27 ± 9.75 years, and 74.4% were male. Overall, 90.7% of diabetic foot ulcers were classified as perfusion, extent/size, depth/tissue loss, infection, and sensation (PEDIS) grades 3 and 4. *Staphylococcus aureus* was the most frequently detected pathogen among patients with positive culture results. Osteomyelitis was detected in 85.3% of the patients, and 55.8% of the cases were treated with antibiotic therapy and/or debridement without amputation. The rate of piperacillin-tazobactam use was higher among patients with osteomyelitis who underwent surgical treatment. A higher rate of surgical treatment was observed in patients with higher PEDIS scores ($p = 0.004$).

Conclusion: The results of our study indicate that most patients recovered with antibiotic therapy and/or debridement without amputation.

Keywords: Amputation, diabetic foot infection, osteomyelitis, treatment

Öz

Giriş: Diyabetik ayak enfeksiyonları (DAE), diyabetes mellitus tedavisindeki ilerlemelere rağmen önemli bir klinik sorun olmaya devam etmektedir. Bu çalışmada, DAE tanılı hastaların epidemiyolojik özellikleri, tedavi süreçleri ve sonuçlarını değerlendirerek bu alandaki bilgi birikimine katkı sağlanması amaçlanmıştır.

Gereç ve Yöntem: Bu retrospektif çalışmada, Ekim 2020-Kasım 2023 tarihleri arasında kliniğimizde DAE tanısıyla yatırılarak takip ve tedavi edilen hastaların demografik, klinik ve laboratuvar verileri, tedavi yöntemleri ve sonuçları kaydedildi. Tüm veriler SPSS 22.0 programı kullanılarak analiz edildi ve istatistiksel anlamlılık düzeyi $p < 0,05$ olarak kabul edildi.

Bulgular: Çalışmaya dahil edilen 129 hastanın yaş ortalaması 61,27 ± 9,75 yıl olup %74,4'ü erkekti. Diyabetik ayak ülserlerinin %90,7'si perfüzyon, yaygınlık, derinlik, enfeksiyon ve duyu (PEDIS) 3 ve 4 idi. Yara yüzeyel sürüntü/derin doku kültür üremesi olan hastalarda en sık *Staphylococcus aureus*

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saptandı. Hastaların %85,3'ünde osteomiyelit saptandı ve olguların %55,8'inin amputasyon uygulanmaksızın antibiyotik tedavisi ve/veya debritman ile tedavi edildiği belirlendi. Cerrahi tedavi uygulanan osteomiyelit hastalarında piperasilin-tazobaktam kullanım oranı anlamlı düzeyde daha yüksekti. PEDIS skoru yüksek olan hastalarda daha yüksek oranda cerrahi tedavi uygulandığı bulundu (p = 0,004).

Sonuç: Çalışmamızın en dikkat çekici sonucu, hastaların çoğunun amputasyon uygulanmadan antibiyotik tedavisi ve/veya debritman ile iyileşmiş olmasıdır. Bu bulgular, ağır DAE ve osteomiyeliti olan hastalarda tedavi stratejilerinin iyileştirilmesine katkıda bulunabilir.

Anahtar Kelimeler: Amputasyon, diyabetik ayak enfeksiyonu, osteomiyelit, tedavi

Introduction

Diabetes affects 828 million adults worldwide, and its age-standardized global prevalence among adults is estimated to be approximately 14%[1]. The prevalence of diabetes mellitus (DM) in Türkiye is rapidly increasing. While the prevalence among individuals older than 20 years was 7.8% in the TURDEP-I study conducted in 2000, it increased to 14–16% in studies conducted in 2009 and 2010[2]. According to the latest data, Türkiye has the second-highest prevalence of diabetes among upper-middle-income countries[3].

Diabetic foot infection (DFI) is one of the major complications of DM. The pathogenesis of DFI is a complex process resulting from the interaction of neuropathy, vasculopathy, immune dysfunction, and secondary infections[4]. According to estimates, 15% of patients with diabetes develop diabetic neuropathy and DFI, a complication of diabetes, at least once during their lifetime[5]. DFI increases the risk of hospitalization by 50-fold compared with that in individuals without diabetes[6]. In 2016, DFI led to more than 130,000 lower-extremity surgeries in the United States. The 5-year mortality rate after amputation is approximately 50%, and the first few years after surgery are considered the period with the highest mortality risk[7,8].

The management of DFI is complex and requires a multidisciplinary approach. Treatment varies depending on whether the infection is limited to soft tissue or whether osteomyelitis is present. Antibiotic therapy and surgical debridement form the cornerstone of treatment in patients with severe infections, and patients often require several weeks of antibiotic therapy along with long-term wound care[9].

The aim of this study is to contribute to the body of knowledge in this field by evaluating the epidemiological characteristics, treatment processes, and outcomes of patients diagnosed with DFI.

Materials and Methods

This retrospective study included patients aged 18 years and older who were hospitalized and received follow-up and treatment for DFI between October 2020 and November 2023. Patients younger than 18 years, pregnant women, patients with wounds caused by other inflammatory conditions, such as trauma, fracture, or thrombosis, and patients with missing data were excluded from the study. Approval for the study was obtained from Necmettin Erbakan University's Ethics Committee (date: 03.11.2023; decision no: 2023/4622). The requirement for obtaining informed consent from individual participants was waived by the Ethics Committee due to the retrospective nature of the study. The patients' age, sex, comorbidities, presence of DM-related complications, medical history, physical examination findings, clinical characteristics, and laboratory findings, including leukocyte count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), glycated hemoglobin A1c (HbA1c) levels measured at hospital admission, superficial wound swab/deep tissue culture results, treatment methods, and outcomes, were recorded using a data collection form developed by the researcher based on the literature.

Ulcerative lesions were defined as cellulitis-type infections in which the infection was limited to the skin and subcutaneous tissue, the muscle tissue remained intact, and there were no signs of systemic toxicity or ischemia. Osteomyelitis was diagnosed based on clinical, radiological, and microbiological findings. Plain radiography and/or magnetic resonance imaging were used for radiological evaluation. Acute osteomyelitis was diagnosed based on increasing pain at the base of the ulcer, erythema, increased local warmth, and a sausage toe appearance, whereas chronic osteomyelitis was diagnosed based on persistent or recurrent discharge, a sinus tract, minimal systemic symptoms, and typically mild to moderate pain, along with findings such as cellulitis and lymphangitis[9].

DFIs were classified according to the perfusion, extent/size, depth/tissue loss, infection, and sensation (PEDIS) classification, which is based on five basic features (PEDIS) and has been widely used in recent years[9].

The presence of microbial growth in superficial wound swabs or deep tissue cultures, the type of microorganisms isolated, the antibiotics used for medical treatment and their duration, and the treatment methods applied (vacuum-assisted wound closure, major surgery, or minor surgery) were recorded. Lower-extremity amputations were categorized as minor (at or below the ankle) or major (above the ankle). Microbiological examinations (Gram staining and culture) were performed on debridement or aspiration specimens obtained from the lesions. Clinical samples were cultured on blood agar and eosin methylene blue agar plates. Identification of microorganisms

and antimicrobial susceptibility testing were performed using the VITEK-2 Compact, Phoenix BD100, and M50 automated systems.

Clinical success was defined as improvement in clinical and radiological findings along with laboratory parameters at the end of treatment.

Statistical Analysis

The obtained data were analyzed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). In the descriptive analyses, categorical data were presented as frequencies (n) and percentages (%), whereas continuous data were presented as the mean ± standard deviation (SD) and median (minimum–maximum).

The Pearson chi-square (χ^2) test and Fisher's exact test were used to compare categorical variables. The normality of the distribution of continuous data was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. For comparisons of continuous data between two independent groups, the independent-samples t-test was used for parametric data, whereas the Mann–Whitney U test was used for non-parametric data. For comparisons among more than two independent groups, one-way analysis of variance was used for parametric data, whereas the Kruskal–Wallis test was used for non-parametric data. Variables showing statistically significant differences in the Kruskal–Wallis test were evaluated using post-hoc pairwise comparisons with the Mann–Whitney U test, and the Dunn–Bonferroni correction was applied. Results were evaluated at a 95% confidence interval, with statistical significance set at $p < 0.05$.

Results

A total of 129 patients diagnosed with DFI were included in the study. The mean age of the patients was 61.27 ± 9.75 years, and 74.4% (n = 96) were male. Overall, 55.0% (n = 71) of the patients had additional comorbidities and/or at least one diabetes-related complication in addition to DM (Table 1).

Table 1. Demographic and clinical characteristics of the patients.

	Mean ± SD	**Median (min–max)**
Age (years)	61.27 ± 9.75	60 (37–86)
	Number (n)	**Percent (%)**
Gender		
Female	33	25.6
Male	96	74.4
Comorbidity or DM complication*	71	55
Retinopathy	29	22.5
Chronic renal disease	20	15.5
Hypertension	21	16.3
Coronary heart disease	13	10.1
Hyperlipidemia	-	7.5
Cerebrovascular diseases	7	5.4
Presence of an infected ulcer		
No ulcer or sausage finger	15	11.6
0–2 cm open wound + discharge	44	34.1
≥2 cm open wound + discharge	70	54.3
Presence of osteomyelitis		
Ulcered infected lesion	19	14.7
Phalangeal bone osteomyelitis	58	45
Tarsal bone osteomyelitis	52	40.3
PEDIS score		
2	12	9.3
3	60	46.5
4	57	44.2

*, some patients had multiple comorbidities and/or diabetes-related complications; DM, diabetes mellitus, PEDIS, perfusion, extent/size, depth/tissue loss, infection, and sensation; SD, standard deviation; min, minimum; max, maximum.

The most frequent physical examination finding was an open wound measuring ≥2 cm with discharge, which was observed in 54.3% (n = 70) of the patients. When diabetic foot ulcers (DFUs) were classified according to the PEDIS classification, 46.5% of the patients were classified as PEDIS grade 3 and 44.2% as PEDIS grade 4 (Table 1).

Among patients with positive superficial wound swab/deep tissue culture results, *Staphylococcus aureus* was the most frequently isolated microorganism (n = 21, 16.3%) (Table 2). Osteomyelitis was detected in the phalangeal bones in 45.0% (n = 58) of the patients and in the tarsal bones in 40.3% (n = 52). Overall, 55.8% (n = 72) of the patients were treated with antibiotic therapy and/or debridement without amputation. The most frequently used antibiotics were fluoroquinolones, which were prescribed in 72.9% of the cases. The mean duration of antibiotic therapy was 33.6 ± 21.6 days (Table 2).

There was a statistically significant association between an increased ulcer diameter and leukocyte count and the presence of osteomyelitis ($p = 0.043$ and $p < 0.001$, respectively).

Table 2. Distribution of superficial wound swab/deep tissue culture results, antibiotic therapies, laboratory parameters, and treatment durations.

	Number (n)	**Percent (%)**
Wound/deep tissue culture		
Culture not taken or no growth	23	17.8
Polymicrobial	20	15.5
Gram-positive bacteria	45	34.9
Staphylococcus aureus (7 MRSA/14 MSSA)	21	16.3
Coagulase-negative *staphylococci*	12	9.3
Streptococcus pyogenes/Streptococcus agalactiae	3	1.6
Other Gram-positives	9	7.0
Gram-negative bacteria	41	31.8
Enterobacter spp.	14	10.9
Escherichia coli	12	9.3
Pseudomonas aeruginosa	8	6.2
Proteus spp.	5	3.9
Klebsiella spp.	2	1.6
Antibiotic therapy*		
Fluoroquinolones	94	72.9
Teicoplanin	68	52.7
Piperacillin–tazobactam	38	29.5
Meropenem	17	13.2
Linezolid	13	10.1
Amoxicillin	5	3.9
Ceftriaxone	5	3.9
Tigecycline	3	2.3
Cefazolin	3	2.3
Other	11	8.5
Laboratory parameters	**Mean ± SD**	**Median (min–max)**
Leukocyte count ($10^3/\mu L$)	11.70 ± 5.28	10.65 (4–33.4)
Sedimentation rate (mm/h)	60.82 ± 32.11	57 (2–140)
CRP (mg/L)	103.65 ± 88	75 (2–341)
HbA1c (%)	9.61 ± 2.44	9.5 (5–16.7)
Treatment duration (days)	33.64 ± 21.6	28.5 (7–120)

*, multiple antibiotics were administered to many patients; CRP, C-reactive protein; HbA1c, glycated hemoglobin A1c; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; n, number; min, minimum; max, maximum.

Table 3. Comparison of patient characteristics according to diabetic foot ulcer status.

	No ulcers or sausage fingers were present (n = 15)	0–2 cm open wound (n = 44)	≥2 cm open wound + discharge (n = 70)	p-value
Gender n (%)				
Female	3 (20)	10 (22.7)	20 (28.6)	0.683*
Male	12 (80)	34 (77.3)	50 (71.4)	
Age (years) median (min–max)	65 (51–80)	59.5 (37–86)	59.5 (45–85)	0.056**
Comorbidity and/or DM complications n (%)				
Absent	6 (40)	17 (38.6)	35 (50)	0.454*
Present	9 (60)	27 (61.4)	35 (50)	
Presence of osteomyelitis n (%)				
No osteomyelitis or ulcerated infected lesion	4 (26.7)	6 (13.6)	9 (12.9)	<0.001*
Phalangeal osteomyelitis	7 (46.7)	30 (68.2)	21 (30)	
Tarsal osteomyelitis	4 (26.7)	8 (18.2)	40 (57.1)	
Surgical treatment n (%)				-
Medical treatment or medical treatment + debridement	10 (66.7)	22 (50)	40 (57.1)	
Finger amputation	4 (26.7)	20 (45.5)	5 (7.1)	
Amputation including the metatarsal	0	1 (2.3)	5 (7.1)	
Foot amputation	1 (6.7)	0	2 (2.9)	
Below-knee amputation	0	1 (2.3)	18 (25.7)	
PEDIS score				
2	4 (26.7)	5 (11.4)	3 (4.3)	0.054*
3	6 (40)	23 (52.3)	31 (44.3)	
4	5 (33.3)	16 (36.4)	36 (51.4)	
Antibiotic therapy				
Fluoroquinolone	13 (86.7)	37 (84.1)	44 (62.9)	0.020*
Teicoplanin	9 (60)	20 (45.5)	39 (55.7)	0.472*
Piperacillin–tazobactam	3 (20)	7 (15.9)	28 (40.0)	0.016*
Meropenem	1 (6.7)	2 (4.5)	14 (20.0)	0.044*
Linezolid	2 (13.3)	3 (6.8)	8 (11.4)	-
Amoxicillin	1 (6.7)	2 (4.5)	2 (2.9)	-
Ceftriaxone	0 (0)	3 (6.8)	2 (2.9)	-
Tigecycline	0 (0)	0 (0)	3 (4.3)	-
Cefazolin	0 (0)	0 (0)	3 (4.3)	-
Other	1 (6.7)	4 (9.1)	6 (8.6)	-
Laboratory findings				
Leukocyte count ($10^3/\mu L$)	10.5 (6.05–23.0)	9 (4.7–18.03)	11.03 (4–33)	0.043**
Sedimentation rate (mm/h)	53.53 ± 33.79	57.63 ± 33.01	64.41 ± 31.17	0.368***
CRP (mg/L)	43 (4–244)	80 (2–253)	76 (5–341)	0.440**
HbA1c (mmol/mol) mean ± SD	9.44 ± 2.49	9.49 ± 2.20	9.71 ± 2.59	0.861***
Treatment duration (days) median (min–max)	29.0 (15–60)	30 (10–120)	22.0 (7–90)	0.751**

*, Pearson chi-square test; **, Kruskal–Wallis test; ***, One-way analysis of variance; CRP: C-reactive protein; n, number; DM, diabetes mellitus; HbA1c, glycated hemoglobin A1c; min, minimum; max, maximum.

The rate of fluoroquinolone use was lower among patients with open wounds measuring ≥2 cm than among the other groups, whereas the rates of piperacillin–tazobactam and meropenem use were significantly higher (p = 0.020, p = 0.016, and p = 0.044, respectively) (Table 3).

The rate of piperacillin–tazobactam use was significantly higher in patients with osteomyelitis (p = 0.012). No statistically significant differences were observed between the presence of osteomyelitis and treatment duration, leukocyte count, ESR, or CRP level (p > 0.05) (Table 4).

Surgical treatment was performed at a higher rate in patients with higher PEDIS scores (p = 0.004). In addition, the rate of piperacillin–tazobactam use and ESR values were significantly higher among patients who underwent surgery (p = 0.043 and p = 0.044, respectively). No statistically significant differences were observed in sex, age, comorbidities and/or DM-related complications, or HbA1c levels according to whether surgery was performed (p > 0.05).

Discussion

In the coming years, as the prevalence of DM increases, a substantial increase in the incidence of diabetes-related complications, such as DFI, is expected. Given that DFI treatment requires prolonged antibiotic use and considering the global

Table 4. Comparison of patient characteristics according to the presence of osteomyelitis.

	Without osteomyelitis (n = 19)	With osteomyelitis (n = 110)	p-value
Gender n (%)			
Female	8 (42.1)	25 (22.7)	0.090*
Male	11 (57.9)	85 (77.3)	
Age (years) median (min–max)	61 (47–77)	60 (37–86)	0.561**
Comorbidity and/or DM complications n (%)			
None	6 (31.6)	52 (47.3)	0.204***
Yes	13 (68.4)	58 (52.7)	
Surgical treatment n (%)			
Medical treatment or medical treatment + debridement	19 (100)	53 (48.2)	
Finger amputation	-	29 (26.4)	-
Amputation including the metatarsal	-	6 (5.5)	
Foot amputation	-	3 (2.7)	
Below-knee amputation	-	19 (17.3)	
Antibiotic therapy			
Fluoroquinolone	15 (78.9)	79 (71.8)	0.519***
Teicoplanin	9 (47.4)	59 (53.6)	0.613***
Piperacillin–tazobactam	1 (5.3)	37 (33.6)	**0.012***
Meropenem	3 (15.8)	14 (12.7)	0.716*
Linezolid	0	13 (11.8)	0.213*
Amoxicillin	0	5 (4.5)	1.000*
Ceftriaxone	0	5 (4.5)	1.000*
Tigecycline	0	3 (2.7)	1.000*
Cefazolin	0	3 (2.7)	1.000*
Other	0	11 (10)	0.367*
Laboratory findings			
Leukocyte count ($10^3/\mu L$)	8.9 (4–21.3)	10.70 (4.7–33.4)	0.182**
Sedimentation rate (mm/hour)	50.27 ± 34.35	62.63 ± 31.52	0.132****
CRP (mg/L)	65 (4–340)	78 (2–341)	0.396**
HbA1c (mmol/mol)	9.30 (5.7–16.4)	9.60 (5–16.7)	0.218**
Treatment duration (days) median (min–max)	24 (10–53)	29 (7–120)	0.399**

*, Fisher's exact test; **, Mann–Whitney U test; ***, Pearson chi-square test; ****, independent-samples t-test; CRP, C-reactive protein; n, number; DM, diabetes mellitus; HbA1c, glycated hemoglobin A1c; min, minimum; max, maximum.

increase in antibiotic resistance, comprehensive and up-to-date research is needed on the etiology, prognostic factors, and response rates to different antibiotic regimens for DFI.

Being older than 65 years is a known risk factor for the development of DFUs[10]. A global study reported the mean age of patients with DFUs as 61.86 ± 12.10 years[11]. Similarly, an international meta-analysis reported the mean age of patients with DFUs as 61.7 ± 3.7 years[12]. Studies from Türkiye also support this demographic profile. While one study reported the mean age of patients with DFI as 71.5 ± 12 years[13], another multicenter study reported a median age of 61 years[14]. The mean age observed in our study (61.27 ± 9.75 years) is consistent with the published literature, reflecting the global demographic profile of our patient population and supporting the generalizability of our findings.

Studies on DFI consistently show a predominance of male patients. In the study by Lynar et al.[15], the proportion of male patients was reported as 61.3%, whereas another study reported a rate of 76.7%[16]. In our study, 74.4% of the patients were male. These findings support the hypothesis that male sex is a predisposing factor for the development of DFI. This may be explained by greater exposure to outdoor working conditions, poorer adherence to foot care practices, and sex-related lifestyle differences.

Approximately 40–60% of patients with DFUs have chronic kidney disease (CKD). CKD is an established risk factor for the development and recurrence of DFUs and is a significant predictor of adverse outcomes, such as poor healing, amputation, and death[17]. In our study, 15.5% of the patients had CKD.

Significant coronary artery disease (CAD) is observed in approximately 60% of patients with neuroischemic DFUs, and CAD is the leading cause of death in patients with DFUs[17]. Previous studies have also reported that the prevalence of diabetic retinopathy ranges from 22.5% to 95.6% among patients with DFI[18,19]. In our study, retinopathy was detected in 22.5% of the patients, whereas CAD was identified in 10.1%. The finding that 55% of the patients had DM-related complications and/or comorbidities indicates that DFI management involves a complex clinical scenario in which multiple factors can influence treatment outcomes. Therefore, in patients with DFI, the treatment plan should be determined using a multidisciplinary approach that also considers accompanying comorbidities and complications.

Osteomyelitis is present at the time of admission in approximately 20% of outpatients and 40–60% of hospitalized patients with DFI. Therefore, patients with moderate-to-severe infections should be hospitalized, at least initially[20]. In our study, 62.8% (n = 81) of the patients were hospitalized, and osteomyelitis was detected in 85.3% of these patients. Because osteomyelitis is a common and serious complication of DFI, its presence should always be considered in this patient population and evaluated using appropriate diagnostic methods.

Several studies have reported ulcer size and depth as independent risk factors for the development of osteomyelitis, with larger and deeper ulcers being more likely to progress to osteomyelitis[21]. In our study, a statistically significant association was observed between ulcer size and the presence of osteomyelitis ($p < 0.001$). Based on these findings, patients with ulcers measuring ≥2 cm should be evaluated more thoroughly for osteomyelitis.

One study reported that parameters such as ulceration and perfusion, which are included in the PEDIS classification, were significantly associated with amputation and that all patients who underwent major or minor amputation had high PEDIS scores[22]. Similarly, in our study, the rate of surgical intervention was higher among patients with higher PEDIS scores. These findings demonstrate that the PEDIS classification is an important tool for determining the treatment needs of patients with DFI and that high-risk patients should be monitored more closely.

HbA1c levels are closely associated with diabetic complications. Elevated HbA1c levels reflect poor glycemic control and are associated with an increased risk of developing vasculopathy and neuropathy. In the meta-analysis by Tang et al.[23], HbA1c level was reported to be one of the factors predicting the development of DFUs. In our study, HbA1c values >9 measured at the time of DFI diagnosis suggest that patients may be at an increased risk of developing moderate-to-severe DFI.

CRP and ESR are important laboratory parameters used to detect infection and inflammation. CRP is an acute-phase reactant synthesized by the liver, whereas ESR is a non-specific marker of inflammation. Both parameters are widely used in clinical practice for the diagnosis, follow-up, and evaluation of treatment response in various diseases. In one study, the mean CRP values were significantly higher in patients who underwent major or minor amputation than in those who did not undergo amputation[24]. Another study reported that ESR has the highest sensitivity for the diagnosis of DFI and that low ESR values can be used to rule out the diagnosis[25]. In our study, CRP and ESR values were significantly higher in patients who underwent surgery ($p = 0.043$). These results suggest that CRP and ESR may be clinically valuable in predicting the need for surgery in patients with DFI.

In a study conducted at a tertiary care center involving patients with DFI, the most frequently isolated pathogen was *Staphylococcus aureus*; 57% of the isolates were identified as methicillin-susceptible *Staphylococcus aureus* (MSSA) and

43% as methicillin-resistant *Staphylococcus aureus* (MRSA)[26]. Similarly, in our study, *Staphylococcus aureus* was the most frequently isolated microorganism from wound and deep tissue cultures (16.3%), with 66.7% of the isolates identified as MSSA and 33.3% as MRSA. Consistent with data from developed countries, the predominance of Gram-positive bacteria as causative agents in previously untreated patients with DFI may reflect improvements in hygiene standards, foot care practices, and healthcare services in our country. Studies conducted in our country have shown that Gram-negative bacteria generally predominate in the etiology of DFI; however, Gram-positive microorganisms, particularly *Staphylococcus aureus*, also account for a substantial proportion of cases[27]. In our study, *Staphylococcus aureus*, polymicrobial pathogens, *Enterobacter* spp., and coagulase-negative *staphylococci* were identified in descending order of frequency. These findings are consistent with previous reports and support the frequent occurrence of polymicrobial infections in chronic wounds[28,29].

Although amputation has traditionally been the standard approach for moderate-to-severe DFI and osteomyelitis, current evidence supports the effectiveness of antibiotic therapy. Recent randomized controlled trials have demonstrated similar success rates for medical and surgical treatment. In the study by Lázaro-Martínez et al.[30], patients were randomized to an antibiotic group [(AG), n = 24] or a surgical group [(SG), n = 22]. The AG received antibiotic therapy alone, whereas the SG received antibiotic therapy for 10 days following conservative surgery. The primary cure rates were 75.0% in the AG and 86.3% in the SG ($p = 0.33$)[30]. In our study, 55.8% of the patients diagnosed with osteomyelitis achieved clinical success without amputation following antibiotic therapy combined with debridement and/or vacuum-assisted closure therapy.

Moxifloxacin is a broad-spectrum fluoroquinolone indicated for the treatment of complicated skin and soft tissue infections and can be administered intravenously (IV) or orally. In a clinical study evaluating the efficacy of moxifloxacin for the treatment of moderate-to-severe DFI, it was found to be as effective as sequential therapy with amoxicillin-clavulanate followed by IV piperacillin–tazobactam[31]. Currently, because of the availability of orally administered antibiotics with high bioavailability, such as fluoroquinolones, co-trimoxazole, and linezolid, many patients with DFI can be managed on an outpatient basis[9]. In our study, fluoroquinolones, most commonly moxifloxacin, were the most frequently prescribed antibiotics for the treatment of DFI, accounting for 72.9% of cases.

The frequency of resistant pathogens, such as *Staphylococcus aureuss* and *Pseudomonas aeruginosa*, is increasing in DFIs[32,33]. The IWGDF/IDSA recommends the use of oral, narrow-spectrum agents for mild superficial infections and IV broad-spectrum regimens covering Gram-negative and anaerobic microorganisms for moderate-to-severe infections. In deep and/or very large ulcers, the use of IV piperacillin–tazobactam or broad-spectrum agents such as carbapenems is recommended in patients at risk of multidrug-resistant infections[9]. In our study, fluoroquinolones (72.9%), teicoplanin (52.7%), piperacillin–tazobactam (29.5%), and meropenem (13.2%) were the most frequently prescribed antibiotics. However, fluoroquinolone use was lower in patients with ulcers measuring ≥2 cm, whereas the use of piperacillin–tazobactam and meropenem was significantly higher. Similarly, piperacillin–tazobactam use was higher in patients with osteomyelitis who underwent surgical treatment ($p = 0.012$ and $p = 0.043$, respectively). The significantly higher use of piperacillin–tazobactam suggests that broad-spectrum antipseudomonal antibiotic therapy may be particularly beneficial for patients with osteomyelitis requiring major surgery. In patients with moderate-to-severe DFI, because osteomyelitis is more frequently encountered, treatment should be initiated with IV broad-spectrum agents, followed by de-escalation based on culture results.

The recommended treatment duration for osteomyelitis developing after DFI is 4–6 weeks[9], and the mean treatment duration in our study (33.6 ± 21.6 days) appears to be consistent with these recommendations.

Study Limitations

Our study was a single-center, retrospective study. Its retrospective design resulted in some missing or incomplete data.

Conclusion

The most notable finding of our study is that most patients achieved clinical success with antibiotic therapy and/or debridement without undergoing amputation. Although our study contributes to the current epidemiological understanding of DFI, it also provides insights for improving treatment strategies and antimicrobial selection in patients with severe DFI and osteomyelitis.

The prevalence of diabetes is expected to increase in the coming years, and consequently, the incidence of complications such as DFI is also likely to increase. Long-term antibiotic therapy is required for the treatment of DFI. Given the increasing prevalence of antibiotic resistance and the resulting reduction in available treatment options, comprehensive and up-to-date studies are needed on the etiology, prognosis, and response to various antibiotic therapies for DFI.

Ethics

Ethics Committee Approval: Approval for the study was obtained from Necmettin Erbakan University's Ethics Committee (date: 03.11.2023; decision no: 2023/4622).

Informed Consent: The requirement for obtaining informed consent from individual participants was waived by the Ethics Committee due to the retrospective nature of the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: R.Ç.K., B.K., R.B., İ.E., Concept: B.K., Design: B.K., Data Collection or Processing: R.Ç.K., Analysis or Interpretation: R.Ç.K., B.K., R.B., Literature Search: R.Ç.K., B.K., R.B., Writing: R.Ç.K., B.K., R.B., İ.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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Perceptions, Attitudes, and Preferences Toward Long-acting Injectable HIV Treatments: A Cross-sectional Survey of People Living with HIV in Türkiye

Uzun Etkili Enjekte Edilebilir HIV Tedavilerine Yönelik Algılar, Tutumlar ve Tercihler: Türkiye'de HIV ile Yaşayan Bireyler Arasında Kesitsel Bir Anket Çalışması

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Abstract

Introduction: Long-acting injectable antiretroviral therapy (LAI-ART) offers an alternative to daily oral treatment and represents an important innovation in human immunodeficiency virus (HIV) care. Although LAI-ART has recently been approved in Türkiye, it has not yet been integrated into clinical practice because of the lack of reimbursement. This study explored awareness, attitudes, and preferences regarding LAI-ART among people living with HIV in Türkiye.

Materials and Methods: This cross-sectional survey was conducted between April and August 2025 among adults with HIV receiving ART at a university-affiliated infectious diseases outpatient clinic in Türkiye. Eligible participants (≥18 years) attending routine follow-up visits were invited to complete a structured, anonymous questionnaire assessing sociodemographic and clinical characteristics, as well as awareness, attitudes, and preferences regarding LAI-ART. The primary outcome was willingness to accept LAI-ART if recommended by a physician, whereas secondary outcomes included awareness, preferred administration setting, and perceived advantages and disadvantages.

Results: The mean age of the participants was 37.8 years, 91% were male, and the total sample comprised 200 participants. Most participants (92%) reported that their condition was well controlled, 66% were receiving single-tablet regimens, and a greater pill burden was associated with lower adherence ($p = 0.045$). Awareness of LAI-ART was moderate, with 30% of participants unaware of its existence; men were more informed than women. If recommended by their physicians, 79% indicated a willingness to adopt injectable therapy. The perceived advantages included freedom from daily pills (85%) and reduced concerns about forgetfulness (72%), whereas the primary concern was fear of side effects (51%). No significant association was found between recent missed doses and willingness to switch ($p = 0.57$).

Conclusion: Overall, the participants demonstrated strong interest in LAI-ART, highlighting the need for targeted educational initiatives, particularly for women, as well as strategies to address concerns regarding adverse effects and injection scheduling.

Keywords: Adherence, antiretroviral treatment, long-acting injectables, preference, questionnaire

Öz

Giriş: Uzun etkili enjekte edilebilir antiretroviral tedavi (LAI-ART), günlük oral tedaviye bir alternatif sunmakta ve insan bağışıklık yetmezliği virüsü (HIV) bakımında önemli bir yeniliği temsil etmektedir. Türkiye'de yakın zamanda ruhsatlandırılmış olmasına rağmen, geri ödeme eksikliği nedeniyle henüz klinik uygulamaya entegre edilmemiştir. Bu çalışma, Türkiye'de HIV ile yaşayan bireyler arasında LAI-ART'a yönelik farkındalık, tutum ve tercihleri değerlendirmeyi amaçlamıştır.

Gereç ve Yöntem: Bu kesitsel anket çalışması, Nisan–Ağustos 2025 tarihleri arasında Türkiye'de üniversiteye bağlı bir enfeksiyon hastalıkları polikliniğinde antiretroviral tedavi almakta olan HIV pozitif erişkinler arasında yürütülmüştür. Rutin takip ziyaretlerine gelen uygun katılımcılar (≥18 yaş), sosyodemografik ve klinik özellikler ile LAI-ART ilişkin farkındalık, tutum ve tercihleri değerlendiren yapılandırılmış ve anonim bir anketi

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doldurmaya davet edilmiştir. Birincil sonlanım noktası, hekim tarafından önerilmesi durumunda LAI-ART'ı kabul etme istekliliği olup; ikincil sonlanım noktaları farkındalık düzeyi, tercih edilen uygulama ortamı ve algılanan avantajlar ile dezavantajları içermektedir.

Bulgular: Toplam 200 katılımcının ortalama yaşı 37,8 olup, %91'i erkekti. Katılımcıların büyük çoğunluğu (%92) hastalığının iyi kontrol altında olduğunu düşünmekteydi; %66'sı tek tablet rejim kullanmaktaydı ve daha yüksek ilaç yükü daha düşük tedavi uyumu ile ilişkiliydi (p = 0,045). LAI-ART'a yönelik farkındalık orta düzeydeydi ve katılımcıların %30'u bu tedaviden habersizdi; erkekler kadınlara göre daha fazla bilgi sahibiydi. Hekim önerisi durumunda katılımcıların %79'u enjekte edilebilir tedaviyi kabul etmeye istekli olduğunu belirtti. Algılanan başlıca avantajlar günlük ilaç alma zorunluluğundan kurtulma (%85) ve unutkanlığın ortadan kalkması (%72) iken, en önemli endişe yan etki korkusuydu (%51). Son dönemde ilaç dozunu atlama ile tedaviye geçiş istekliliği arasında anlamlı bir ilişki saptanmadı (p = 0,57).

Sonuç: Katılımcılar genel olarak LAI-ART'a güçlü bir ilgi göstermiştir. Bu bulgular, özellikle kadınlar arasında hedefe yönelik eğitim ihtiyacını ve yan etkiler ile enjeksiyon planlamasına ilişkin endişelerin giderilmesine yönelik stratejilerin önemini ortaya koymaktadır.

Anahtar Kelimeler: Uzun etkili enjekte edilebilir tedaviler, antiretroviral tedavi, tercih, tedavi uyumu, anket

Introduction

Human immunodeficiency virus (HIV) remains a major global public health challenge despite substantial advances in diagnosis, treatment, and prevention over the past four decades. The advent of combination antiretroviral therapy (ART) has transformed HIV infection from a fatal disease into a manageable chronic condition, with life expectancy approaching that of the general population among individuals who achieve sustained viral suppression[1,2]. Nevertheless, the requirement for lifelong daily pill intake remains a major challenge. Suboptimal adherence contributes to virologic failure, drug resistance, and disease progression while also negatively affecting patients' quality of life[3]. Factors such as pill fatigue, stigma, concerns about confidentiality, and the daily reminder of illness underscore the need for simplified treatment modalities[4].

Long-acting injectable-ART (LAI-ART), most notably the combination of cabotegravir and rilpivirine, has emerged as a promising alternative to daily oral regimens. Administered intramuscularly at monthly or bimonthly intervals, these regimens have demonstrated non-inferior efficacy compared with standard oral ART in pivotal phase III trials (ATLAS, FLAIR, and ATLAS-2M), maintaining virologic suppression in appropriately selected patients[5-7]. Beyond virologic outcomes, injectable therapy may reduce treatment burden, improve adherence, and enhance patient satisfaction. However, successful implementation requires consideration of barriers such as injection-site reactions, the need for regular clinic visits, and the implications of missed doses[8].

Understanding the perceptions and preferences of people living with HIV (PLWH) is critical for the real-world adoption of LAI-ART. Studies conducted in Europe and the United States have demonstrated high levels of interest, particularly among individuals who struggle with adherence to daily oral therapy[9,10]. However, evidence from low- and middle-income countries remains limited, and cultural and health care system factors may significantly influence acceptance and uptake. In Türkiye, LAI-ART has recently been approved by the Ministry of Health but has not yet been integrated into routine clinical practice because of the lack of reimbursement coverage[11]. This situation presents both opportunities and challenges for its potential implementation.

Therefore, this study aims to evaluate the awareness, attitudes, and preferences of PLWH in Türkiye regarding LAI-ART. Specifically, it seeks to explore the perceived advantages and disadvantages, preferred administration intervals, and concerns related to transitioning from oral to injectable therapy. By identifying facilitators and barriers from the patient perspective, the findings will provide valuable insights to guide clinical decision-making, inform patient counseling, and support the future integration of LAI-ART into HIV care in Türkiye.

Materials and Methods

Study Design and Setting

This study was designed as a cross-sectional survey to assess awareness, attitudes, and preferences regarding LAI-ART among PLWH in Türkiye. The survey was conducted between April and August 2025 at the infectious diseases and clinical microbiology outpatient clinic of a large university-affiliated health care center providing comprehensive HIV care.

Participants and Recruitment

Eligible participants were adults (≥18 years) with a confirmed diagnosis of HIV who were receiving ART and attending routine follow-up visits during the study period. Exclusion criteria included the inability to provide informed consent, cognitive impairment that precluded completion of the questionnaire, and refusal to participate. Informed consent was obtained from all participants before enrollment. A total of 200 individuals were included in the analysis.

We targeted a sample size of n = 200 to ensure adequate statistical power for detecting small-to-moderate associations. Based on Cohen's effect size framework for the chi-square test (w), a total sample of 200 provides approximately 80% power to

detect an effect size of $w \approx 0.20$ and >95% power to detect an effect size of $w \approx 0.30$. Thus, the study was powered to detect small-to-moderate associations between key binary variables (e.g., adherence-related perceptions and concerns).

Survey Instrument

A structured, anonymous questionnaire was developed by the study team based on the existing literature[9,10,12-15] on LAI-ART and adapted to the Turkish context (Supplementary Material 1). The instrument consisted of closed-ended and multiple-choice questions covering four domains. Sociodemographic and clinical characteristics included age, gender, marital status, comorbidities, and lifestyle factors such as smoking, alcohol consumption, and recreational drug use. HIV-related variables included the duration since diagnosis, duration on the current regimen, number of tablets taken per day, treatment adherence (missed doses during the previous 30 days), and perceived treatment control. Additional domains assessed awareness of LAI-ART and sources of information, as well as attitudes and preferences, including willingness to switch if recommended by a physician and the preferred site of administration (home, physician's office, or health care facility). Finally, the questionnaire explored the perceived advantages and disadvantages of LAI-ART. The questionnaire was pilot-tested in a small group of PLWH (n = 10) to assess clarity and was revised accordingly.

Data Collection

Participants completed the questionnaire either in paper form at the clinic or electronically through a secure online platform. No identifying information was collected. Completed questionnaires were reviewed for completeness by the study staff, and missing or inconsistent responses were excluded from the relevant analyses.

Outcomes

The primary outcome was willingness to accept LAI-ART if recommended by a physician. Secondary outcomes included awareness of LAI-ART, preferred administration setting, and reported advantages and disadvantages. Additional exploratory outcomes included associations between sociodemographic and clinical characteristics and treatment acceptance, adherence, or awareness.

Ethical Considerations

The study protocol was approved by the Institutional Ethics Committee of University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital (approval no: 11/25.06.205.230, date: 19.08.2025). Participation was voluntary, and all participants provided written informed consent. The study adhered to the principles of the Declaration of Helsinki.

Statistical Analysis

Data were entered into SPSS (IBM SPSS Statistics, version XX) for analysis. Descriptive statistics were reported as means ± standard deviation for continuous variables and frequencies with percentages for categorical variables. Between-group comparisons were performed using the Mann-Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables. One-way analysis of variance was used for comparisons involving more than two groups, where appropriate. Statistical significance was defined as $p < 0.05$.

Results

A total of 200 individuals living with HIV participated in the survey, with a mean age of 37.8 ± 10.7 years. The vast majority were male (91%), while 8% were female, and one participant each identified as transgender or chose not to disclose their gender. Sexual preference was predominantly heterosexual: 59% of male participants reported preferring women, 20% preferred men, 5% preferred both sexes, and 16% chose not to disclose their preference. Among women, 88% preferred men, while smaller proportions preferred women or declined to respond. Both the transgender participant and the participant who did not disclose gender exclusively reported a preference for men.

Family awareness and marital status varied considerably. Overall, 60% of participants reported that their families or sexual partners were aware of their HIV status, whereas 39% reported that they were not. Slightly more than half of the cohort was single, 41% was married, and 6% was divorced. Chi-square analysis demonstrated a significant association between marital status and family awareness ($p < 0.001$), indicating that married individuals were significantly more likely to have disclosed their HIV status to their families than single or divorced participants.

Chronic comorbidities were relatively uncommon, with hypertension and hypercholesterolemia (4.5% each) and diabetes (4%) being the most frequently reported conditions. Psychiatric disorders, asthma, and other rare diseases were reported less frequently. Regarding lifestyle factors, 47% of participants currently smoked, 29% consumed alcohol, and 8% reported recreational drug use.

Regarding HIV-related variables, nearly half of the respondents had been living with HIV for 1–5 years, whereas one-third had been diagnosed within the previous year. Only 16% had been living with HIV for more than 5 years. Similarly, current antiretroviral regimens had most commonly been used for less than 5 years, and only 9% of participants reported longer treatment durations. Most participants were receiving a single-tablet regimen (66%), whereas smaller proportions took two or

three tablets, and only 1.5% reported taking more than three tablets. Treatment adherence was high, with more than three-quarters of participants reporting no missed doses during the previous 30 days. Almost all participants (92%) believed that their disease was well controlled with their current therapy. Participants taking a greater number of tablets were more likely to report missed doses ($p = 0.045$). In contrast, no association was observed between daily tablet burden and willingness to initiate injectable treatment ($p = 0.78$), suggesting that pill burden influenced adherence but not acceptance of new treatment regimens. Likewise, the duration of HIV infection was not significantly associated with acceptance of injectable therapy ($p = 0.08$).

Awareness of LAI-ART was limited. Nearly half of the participants reported first hearing about it from healthcare professionals, while 30% had no prior knowledge of the therapy. The internet and social media accounted for 20% of information sources, followed by HIV support groups (14%) and television or radio (1%). Men were more likely than women to have received information from healthcare professionals, whereas more than half of the women reported having no knowledge of the therapy, highlighting a gender disparity in awareness.

Acceptance of LAI treatment, if recommended by a physician, was high. Nearly four in five participants (80%) indicated that they would accept the treatment, whereas 15% were undecided and only 5% declined (Figure 1). We explored potential associations between acceptance of LAI treatment and various demographic and clinical parameters. Acceptance of treatment, measured by the response to the question, "If your doctor offered this treatment, would you accept it?", showed no significant associations with gender, marital status,

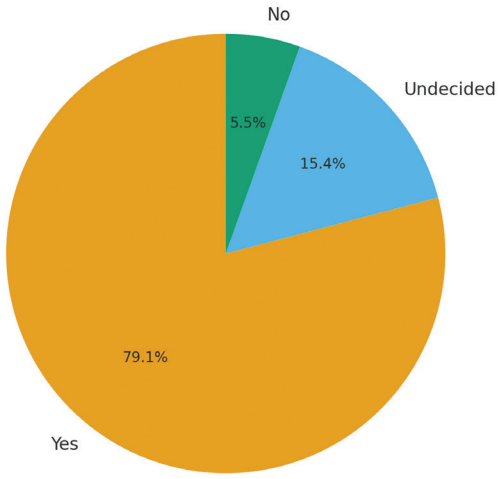

Figure 1. Acceptance of LAI-ART among people living with HIV in Türkiye, if recommended by a physician. LAI-ART, long-acting injectable antiretroviral therapy; HIV, human immunodeficiency virus.

family awareness of HIV status, number of daily tablets, or duration of HIV infection. There was also no statistically significant association between missing at least one dose during the previous 30 days and willingness to accept LAI HIV treatment ($p > 0.57$). In other words, adherence level (perfect vs. imperfect) did not significantly influence acceptance of the new therapy. Perceived benefits of injectable therapy centered on relief from the daily burden of pill taking (85%), reduced anxiety about forgetting medication (72%), and the opportunity to avoid thinking about HIV every day (61%) (Figure 2; Table 1). Concealment of HIV status from others was cited by nearly half of the participants, whereas fewer respondents emphasized guaranteed efficacy or freedom from side effects. The most commonly perceived disadvantages were concerns about potential side effects (51%) and missing an injection appointment (37%), followed by doubts about treatment effectiveness (21%), feeling like a test subject (20%), and fear of injections (6%) (Figure 3; Table 2). Notably, a significant association was observed between relief from

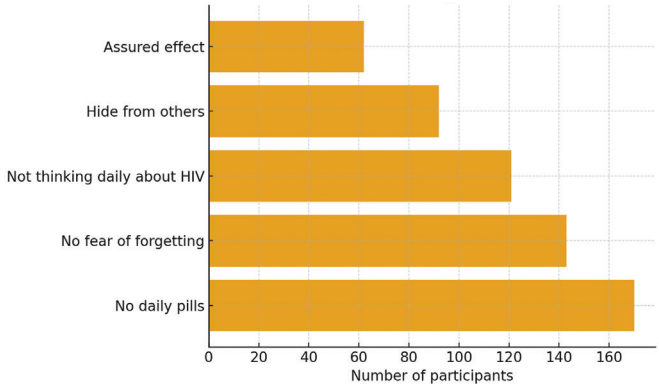

Figure 2. Perceived advantages of LAI-ART among people living with HIV in Türkiye. LAI-ART, long-acting injectable antiretroviral therapy; HIV, human immunodeficiency virus.

Table 1. Top five most frequently co-selected advantages of LAI-ART.

Rank	Advantage pair	n (participants)
1	Not having to take medication every day × no fear of forgetting doses	129
2	Not having to take medication every day × not thinking about HIV daily	105
3	No fear of forgetting doses × not thinking about HIV daily	101
4	Not having to take medication every day × ability to hide medication use	89
5	No fear of forgetting doses × ability to hide medication use	81

LAI-ART, long-acting injectable antiretroviral therapy; HIV, human immunodeficiency virus.

concerns about forgetting medication and fear of missing an injection appointment, highlighting adherence-related concerns ($p = 0.003$). When asked about their preferred site of administration, more than half of the participants favored healthcare facilities such as hospitals or primary care centers, one-third preferred a doctor's office, and only one in ten preferred self-administration at home (Figure 4).

Regarding healthcare utilization, 43% of respondents welcomed the more frequent hospital visits associated with injectable treatment, 32% were indifferent, and 28% preferred not to attend more frequently. Finally, when asked about their willingness to initiate injectable therapy, 43% reported that they would accept the treatment despite increased hospital visits, 37% were willing but expressed concerns about side effects, 22% would accept the treatment only if it could be administered at home, and 10% did not want to change their current regimen.

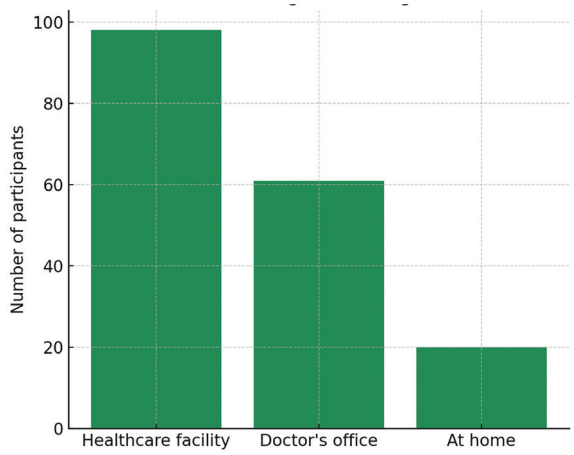

Figure 4. Preferred settings for receiving LAI-ART among people living with HIV in Türkiye. LAI-ART, long-acting injectable antiretroviral therapy; HIV, human immunodeficiency virus.

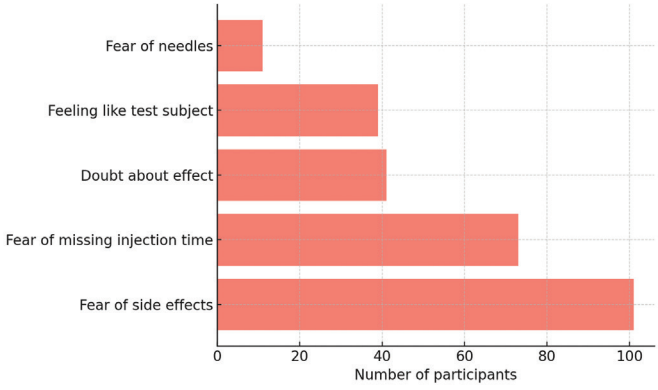

Figure 3. Perceived disadvantages of LAI-ART among people living with HIV in Türkiye. LAI-ART, long-acting injectable antiretroviral therapy; HIV, human immunodeficiency virus.

Table 2. Top five most frequently co-selected disadvantages of LAI-ART.

Rank	Disadvantage Pair	n (participants)
1	Fear of side effects × concern about missing injection timing	28
2	Fear of side effects × feeling like a test subject	27
3	Fear of side effects × belief that illness will not change	20
4	Concern about missing injection timing × feeling like a test subject	14
5	Concern about missing injection timing × belief that illness will not change	13

LAI-ART, long-acting injectable antiretroviral therapy.

Discussion

This study provides insights from Türkiye into the awareness, attitudes, and preferences of PLWH regarding LAI-ART. Our findings demonstrate a high level of willingness to initiate injectable therapy if recommended by a physician (nearly 80%), consistent with previous international studies reporting strong acceptability across diverse populations[12,13]. The main perceived advantages were freedom from daily pill taking and reduced anxiety about forgetting medication, whereas the most prominent disadvantage was fear of side effects. These findings underscore both the promise and the challenges of implementing LAI-ART in routine clinical care.

In our cohort, willingness to initiate LAI-ART if recommended by a physician was high (80%). This level of acceptance is comparable to, although slightly lower than, that reported in the Italian survey by Celesia et al.[10], in which 88.8% of participants expressed interest in switching to long-acting options, and higher than the 56% interest reported in a U.S. Southern clinic sample by Stout et al.[9]. It also aligns with the 74% interest observed among PLWH in the French study by Slama et al.[13]. The perceived advantages identified in our study are consistent with those reported elsewhere. Stout et al.[9] highlighted convenience-related benefits, such as traveling without medications and avoiding daily dosing, which closely parallel our cohort's emphasis on pill-free routines and reduced anxiety about forgetting medication.

One of the most important observations in our study was that treatment burden, reflected by the number of daily tablets, was significantly associated with adherence. Patients receiving multi-tablet regimens were more likely to miss

doses, consistent with previous research linking greater pill burden to suboptimal adherence[14,16,17]. However, acceptance of LAI-ART was not associated with the number of tablets taken, suggesting that psychological and lifestyle benefits motivate interest even among patients receiving single-tablet regimens. By eliminating the daily reminder of illness, LAI-ART may reduce treatment fatigue, stigma, and anxiety, thereby promoting greater treatment satisfaction and engagement in care. This finding is consistent with that of Slama et al.[13], who reported no significant influence of demographic or lifestyle factors on treatment acceptance. The benefits of convenience and privacy appear to be relevant across different settings. Compared with findings from the United States, the higher acceptance rates observed in our study may reflect differences in patient expectations and the influence of physicians' recommendations[9].

The predominant concern in our sample was fear of side effects (51%), followed by anxiety about missing injection appointments (37%), indicating that LAI-ART may shift—rather than eliminate—adherence concerns from daily pill taking to adherence to scheduled clinic visits. This pattern differs from that reported by Stout et al.[9], in which cost and insurance coverage were the primary concerns, likely reflecting system-level differences, such as the more fragmented, privately financed healthcare system in the United States compared with the predominantly social insurance–based model in Türkiye. These differences may influence the relative importance of out-of-pocket costs compared with concerns about safety and treatment logistics. Preferences regarding treatment delivery also differed. Whereas most participants in the Italian survey preferred receiving injections at home (57.9%), and many remained interested even if injections were available only in hospitals (64.4%)[10], more than half of our participants preferred administration in healthcare facilities, with only approximately 10% favoring home administration. This finding suggests greater reliance on institutional healthcare settings during the early stages of LAI-ART implementation. Taken together, international evidence indicates that the primary barriers to LAI-ART adoption are context specific, with cost and insurance coverage predominating in some healthcare systems[9] and concerns about logistics and safety in others[10]. These findings suggest that tailored patient education on safety and tolerability, flexible treatment delivery models, and clear reimbursement pathways will be essential for the successful implementation of LAI-ART. Additional concerns, including doubts about treatment effectiveness and perceptions of being "test subjects," further highlight the need for transparent patient education and trust building. Addressing these concerns through counseling, clear communication of efficacy and safety data, and flexible service delivery models will be critical to maximizing the uptake and sustained use of LAI-ART.

Awareness of LAI-ART was limited, with one-third of the participants reporting no prior knowledge of the therapy. Notably, women were less informed than men and often lacked access to information beyond that provided by healthcare professionals. This gender disparity in awareness is consistent with previous studies highlighting inequalities in HIV-related health literacy and the importance of equitable patient education[15]. Ensuring access to accurate and accessible information will be essential to support informed decision-making and reduce inequities in treatment uptake.

The sample size of 200 participants was selected to provide adequate statistical power while remaining feasible within the constraints of a single-center survey. Previous studies evaluating perceptions of LAI-ART have generally included between 100 and 250 participants, suggesting that this range is sufficient to capture representative attitudes while allowing subgroup analyses based on gender, marital status, and treatment characteristics[9,10,13]. A cohort of 200 participants provided an appropriate balance between statistical robustness and practical feasibility, enabling the detection of significant associations (e.g., between pill burden and adherence or between perceived advantages and disadvantages) while minimizing the risk of a type II error.

Study Limitations

This study has several limitations, including its single-center design and reliance on self-reported data, which may be subject to social desirability and recall bias. In addition, its cross-sectional design precludes causal inference, and the relatively small proportion of female and transgender participants may limit the generalizability of the findings. Nevertheless, this study provides valuable baseline data to inform future implementation strategies for LAI-ART in Türkiye.

Conclusion

Turkish PLWH demonstrated a strong willingness to adopt LAI-ART, primarily motivated by the desire to reduce the daily pill burden and improve quality of life. Addressing concerns about side effects, expanding education regarding injectable treatment options, and ensuring equitable access across gender groups will be essential for the successful integration of this novel treatment modality.

Ethics

Ethics Committee Approval: The study protocol was approved by the Institutional Ethics Committee of University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital (approval no: 11/25.06.205.230, date: 19.08.2025).

Informed Consent: Participation was voluntary, and all participants provided written informed consent.

Footnotes

Authorship Contributions

Concept: B.K., M.M.K., A.G., Ö.A.A., Design: B.K., M.M.K., A.G., Ö.A.A., Data Collection or Processing: B.K., Analysis or Interpretation: B.K., M.M.K., A.G., Ö.A.A., Literature Search: B.K., M.M.K., A.G., Ö.A.A., Writing: B.K., M.M.K., A.G., Ö.A.A.

Conflict of Interest: No conflict of interest was declared by the author(s).

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Clinical Characteristics and the Role of Biomarkers in the Differential Diagnosis of Community-Acquired Central Nervous System Infections: A Five-Year Retrospective Study

Toplum Kökenli Santral Sinir Sistemi Enfeksiyonlarının Ayırıcı Tanısında Klinik Özellikler ve Biyobelirteçlerin Rolü: Beş Yıllık Retrospektif Çalışma

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Abstract

Introduction: Community-acquired central nervous system infections (CA-CNSIs) are associated with substantial morbidity and mortality, and early differentiation between purulent and aseptic meningitis remains a major clinical challenge. Readily available admission biomarkers may facilitate timely clinical differentiation.

Materials and Methods: We conducted a retrospective cohort study including adult patients (≥18 years) hospitalized with CA-CNSIs at Şişli Hamidiye Etfal Training and Research Hospital over a 5-year period. Epidemiological, clinical, and laboratory characteristics were evaluated. The diagnostic performance of routinely available biomarkers was assessed using receiver operating characteristic analysis. Multivariable Firth penalized logistic regression was applied, with internal validation performed using bootstrap resampling.

Results: Seventy patients were included (median age, 45 years; 63% male). Purulent meningitis accounted for 46% of cases, whereas aseptic meningoencephalitis comprised 24%. Patients with purulent meningitis had significantly higher leukocyte counts and C-reactive protein (CRP) and procalcitonin levels, as well as a lower cerebrospinal fluid (CSF)-to-serum glucose ratio. The CSF-to-serum glucose ratio demonstrated the highest discriminative ability for differentiating purulent from aseptic meningitis [area under the curve (AUC), 0.97], followed by CRP (AUC, 0.89). In the multivariable analysis, the CSF-to-serum glucose ratio remained independently associated with purulent meningitis, with CRP providing additional discriminatory value.

Conclusion: In this real-world cohort, the CSF-to-serum glucose ratio was the most robust admission biomarker for differentiating purulent from aseptic meningitis. CRP provided additional discriminatory value, supporting the combined use of routinely available biomarkers to improve early clinical decision-making in patients with suspected CA-CNSIs.

Keywords: Central nervous system infections, meningitis, bacterial, cerebrospinal fluid, biomarkers, C-reactive protein

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Öz

Giriş: Toplum kökenli santral sinir sistemi enfeksiyonları (TK-SSSE), belirgin morbidite ve mortalite ile ilişkilidir. Pürülan menenjit ile aseptik menenjitin erken dönemde ayırt edilmesi klinikte önemli bir sorun olmaya devam etmektedir. Başvuru sırasında rutin olarak elde edilebilen biyobelirteçler, bu ayrımı zamanında yapmaya yardımcı olabilir.

Gereç ve Yöntem: Üçüncü basamak bir eğitim ve araştırma hastanesinde, beş yıllık dönemde TK-SSSE nedeniyle yatırılarak izlenen ≥18 yaş erişkin hastalar retrospektif kohort tasarımında değerlendirildi. Epidemiyolojik, klinik ve laboratuvar veriler incelendi. Rutin biyobelirteçlerin tanısal performansı alıcı işletim karakteristiği eğrisi analizi ile değerlendirildi. Çok değişkenli analizde Firth penalizasyonlu lojistik regresyon kullanıldı; iç doğrulama bootstrap yeniden örnekleme ile yapıldı.

Bulgular: Çalışmaya 70 hasta dahil edildi (medyan yaş 45 yıl; %63 erkek). Olguların %46'sı pürülan menenjit, %24'ü aseptik meningoensefalit idi. Pürülan menenjitli hastalarda lökosit sayısı, C-reaktif protein (CRP) ve prokalsitonin düzeyleri anlamlı olarak daha yüksek; beyin omurilik sıvısı (BOS)/serum glukoz oranı ise daha düşüktü. Pürülan ve aseptik menenjitin ayrımında en yüksek ayırt edici gücü BOS/serum glukoz oranı gösterdi [eğri altındaki alan (AUC) 0,97]; bunu CRP izledi (AUC: 0,89). Çok değişkenli analizde BOS/serum glukoz oranı pürülan menenjit ile bağımsız olarak ilişkili bulunurken, CRP ek ayırt edicilik sağladı.

Sonuç: Bu kohortta pürülan ve aseptik menenjitin ayırıcı tanısında en güçlü başvuru biyobelirteci BOS/serum glukoz oranı olarak saptandı. CRP'nin ek katkısı, TK-SSSE şüphesi olan hastalarda erken klinik karar vermeyi desteklemek amacıyla rutin biyobelirteçlerin birlikte değerlendirilmesini desteklemektedir.

Anahtar Kelimeler: Santral Sinir sistemi enfeksiyonları, bakteriyel menenjit, aseptik menenjit, serebrospinal sıvı, biyobelirteçler, C-reaktif protein

Introduction

Central nervous system infections (CNSIs) are among the most severe infectious diseases, characterized by rapid clinical progression and associated with high morbidity and mortality[1]. The most common clinical presentations include meningitis, encephalitis, meningoencephalitis, and brain abscess. Early diagnosis and timely initiation of appropriate therapy are critical for improving prognosis. However, the initial manifestations are often non-specific and typically include fever, headache, neck stiffness, altered consciousness, and focal neurological deficits. Consequently, the diagnosis of CNSIs requires a comprehensive clinical and laboratory evaluation[2].

Timely recognition of factors that may influence disease course is also crucial for effective patient management[3]. Previous studies have demonstrated that admission variables, such as the level of consciousness, cerebrospinal fluid (CSF) cell count, and protein concentration, are associated with unfavorable outcomes[3-5].

The limited sensitivity of microbiological diagnostic methods frequently results in unidentified pathogens, necessitating empirical therapy[6]. Although studies focusing on specific clinical subgroups are available, there remains a need for comprehensive investigations addressing community-acquired (CA) CNSIs from epidemiological, clinical, microbiological, and therapeutic perspectives[7].

This study aimed to comprehensively evaluate the epidemiological characteristics and the clinical and laboratory findings at presentation in patients with CA-CNSIs. The primary objective was to identify clinical and laboratory parameters that could aid in differentiating purulent from aseptic meningitis. In addition, the study sought to identify risk factors associated with adverse clinical outcomes, thereby contributing to the diagnostic and prognostic process.

Materials and Methods

Study Design and Patient Selection

This retrospective cohort study reviewed the medical records of adult patients hospitalized with a diagnosis of CA CNSI at Şişli Hamidiye Etfal Training and Research Hospital in İstanbul, Türkiye, between January 1, 2020, and December 31, 2024. Due to the retrospective design of the study, informed consent was not required.

Inclusion and Exclusion Criteria

Patients aged ≥18 years who were hospitalized in the Department of Infectious Diseases during the study period with a preliminary diagnosis of CA-CNSI based on clinical and laboratory findings were included. Patients younger than 18 years, those with hospital-acquired CNSI, and those with secondary CNSI resulting from non-primary causes of meningitis (e.g., post-traumatic meningitis) were excluded.

All consecutive patients who met the predefined inclusion criteria during the 5-year study period were included. No sampling was performed.

Clinical Classification

Patients were classified according to predefined clinical and CSF findings. Accordingly, cases were categorized as aseptic meningoencephalitis, purulent meningitis, tuberculous

meningitis (TBM), cryptococcal meningitis (CM), brain abscess, or neurosyphilis.

Purulent meningitis was generally characterized by neutrophil-predominant CSF pleocytosis, biochemical findings suggestive of bacterial infection, and a compatible clinical presentation. Aseptic meningoencephalitis was characterized by lymphocyte-predominant CSF pleocytosis, relatively preserved CSF glucose levels, and the absence of microbiological evidence of bacterial infection.

Cases of purulent meningitis were further classified as laboratory-confirmed or non–laboratory-confirmed based on microbiological evidence [CSF culture, multiplex polymerase chain reaction (PCR) panel, or microscopic examination]. Final diagnoses were established by integrating objective laboratory findings, microbiological results, when available, and clinical assessment.

Data Collection

Epidemiological characteristics, clinical symptoms, laboratory findings, microbiological diagnostic methods, empirical and targeted treatment strategies, and clinical outcomes (recovery, sequelae, and mortality) were assessed. Laboratory data were retrieved from existing records maintained by the hospital's microbiology and biochemistry laboratories, including CSF analysis, cultures, commercial multiplex PCR meningitis/encephalitis panels, and antibiograms. Procalcitonin was generally measured at admission as part of the initial infectious disease workup in patients presenting with suspected CNS infection. All data were recorded using a standardized data collection form designed by the investigator, de-identified before analysis, and anonymized. The dataset was securely stored in a digital format and was accessible only to the investigator.

Ethical Approval

Ethical approval was obtained from the Ethics Committee of University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital (approval date: 22.04.2025; approval number: 2980). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

Descriptive statistics are presented as mean ± standard deviation or median (first–third quartile) for continuous variables, depending on the data distribution, and as counts and percentages for categorical variables. Group comparisons were performed using the chi-square test or Fisher's exact test, as appropriate, and the Mann–Whitney U test for non-parametric data.

All analyses were performed using R software (R Foundation for Statistical Computing)[8]. Multivariable logistic regression analysis was initially performed to identify candidate predictors for differentiating purulent from aseptic meningitis. Model simplification was conducted using stepwise selection based on the Akaike Information Criterion to obtain the most parsimonious model. The final model was then re-estimated using Firth penalized logistic regression method to mitigate potential separation issues related to the limited sample size and low event frequency[9].

Receiver operating characteristic (ROC) curve analyses were performed to evaluate the discriminative ability of individual laboratory parameters to differentiate purulent from aseptic meningitis. Each biomarker was analyzed separately. Optimal cut-off values were determined using the Youden index (maximum sensitivity + specificity – 1). ROC analysis was also performed for the final penalized logistic regression model using model-predicted probabilities. The area under the curve (AUC) and corresponding 95% confidence intervals (CIs) were calculated using DeLong's method, and AUC values were compared when appropriate[10].

Model validation was performed using bootstrap resampling (case resampling, B = 1000), with the model reconstructed and performance metrics recalculated during each iteration[11]. Data cleaning and preprocessing were conducted using the tidyverse package. To ensure reproducibility, a fixed random seed was applied (set.seed = 123). Missing data were handled using complete-case analysis. Among the variables included in the final multivariable model, missing data were observed for the CSF/serum glucose ratio in seven patients and for procalcitonin in three patients, whereas no missing data were observed for C-reactive protein (CRP). Bootstrap samples without representation from both outcome classes were excluded from ROC/AUC calculations and bootstrap iterations. For multiple comparisons, the Benjamini–Hochberg correction was applied.

Results

A total of 70 patients were included in the study. The median age was 45 years (first–third quartile, 35–62; range, 18–92 years), and 63% were male. At least one comorbidity was present in 54% of the patients, with hypertension (23%) and diabetes mellitus (16%) being the most common. The rate of immunosuppression was 23%, and predisposing factors were identified in 40% of the cases. More than half of the patients (56%) had sought medical care before admission, and 33% had received antibiotics. None of the patients had a documented history of pneumococcal vaccination. The median time from symptom onset to hospitalization was 3 days. The most

common presenting symptoms were headache (77%), altered consciousness (57%), and fever (56%). The demographic and clinical characteristics of the patients are summarized in Table 1.

Laboratory investigations revealed a median leukocyte count of 11,260/µL, a CRP level of 24 mg/L, a CSF cell count of 330/mm^3, and a CSF/serum glucose ratio of 0.37. Gram staining was positive in 18% of the cases. The most frequently isolated pathogen was *Streptococcus pneumoniae*, followed by *Listeria monocytogenes*. Multiplex PCR identified causative pathogens more frequently than culture. Tuberculosis (TB) PCR was performed in 55 patients, of whom four (7.3%) tested positive. TB culture was performed in 57 patients, yielding four (7.0%) positive results. In addition, acid-resistant bacilli testing was performed in 57 patients, with five (8.8%) positive results. Detailed laboratory findings are presented in Table 2.

Table 1. Demographic and clinical characteristics of the patients.

Characteristic	n = (%) or Median [IQR]; [min–max]
Age (years)	45 [35–62]; [18–92]
Sex	
Female	26 (37)
Male	44 (63)
Comorbidity	38 (54)
Hypertension	16 (23)
Diabetes mellitus	11 (16)
Other (cardiac, renal, and hepatic diseases)	11 (16)
Immunosuppression	16 (23)
Predisposing factor	28 (40)
History of head trauma or surgery	2 (3)
Splenectomy	1 (1)
Chronic ENT infection	2 (3)
Chronic alcohol use	3 (4)
Smoking	12 (17)
Prior healthcare visit	39 (56)
Prior antibiotic use	23 (33)
History of vaccination	0 (0)
Symptom-to-admission interval (days)	3 [1–7]; [1–365]
Presenting symptoms	
Fever	39 (56)
Headache	54 (77)
Nausea/vomiting	26 (37)
Altered consciousness	40 (57)
Seizure	9 (13)
Personality change	13 (19)
Neck stiffness	28 (40)
Kernig and/or Brudzinski sign	9 (13)
Focal neurological deficit	8 (11)
Cranial nerve involvement	12 (17)

Data are presented as n (%) or median [1st–3rd quartile]; [min–max]. IQR, interquartile range; ENT, ear, nose, and throat; min, minimum; max, maximum.

The most common clinical diagnoses were purulent meningitis (46%) and aseptic meningoencephalitis (24%). In addition, 12 patients (17%) were diagnosed with TBM, and three patients (4%) were diagnosed with CM. Among patients with purulent meningitis, 53% (n = 21) had microbiologically confirmed disease. Compared with clinically diagnosed cases, confirmed purulent meningitis cases had significantly lower CSF/serum glucose ratios (p = 0.007) and were more likely to present with focal neurological deficits (p = 0.033). During follow-up, neurological sequelae developed in 12 patients (18%), and the overall mortality rate was 13%.

To explore prognostic factors, patients who died were compared with survivors. Mortality was associated with a higher frequency of renal or hepatic disease (p = 0.006) and lower lymphocyte counts (p = 0.007). Older age, altered consciousness, focal neurological deficits, and lower CSF/serum glucose ratios were also more common among non-survivors, although these findings reached only borderline significance. No other significant demographic, clinical, or microbiological differences were observed. Key comparisons are presented in Table 3. Given the limited number of deaths, multivariable regression analysis for mortality prediction was not performed to avoid overfitting.

Patients were classified into three groups: purulent meningitis, aseptic meningoencephalitis, and TBM. Cases of CM (n = 3)

Table 2. Laboratory findings.

Parameter	Median [IQR]; [min–max]
Blood parameters	
Leukocyte (cells/µL)	11,260 [7,570–15,600]; [1,610–37,840]
Neutrophil (cells/µL)	8,235 [5,220–13,180]; [1,160–34,570]
Lymphocyte (cells/µL)	1,155 [700–1,920]; [180–7,730]
Hemoglobin (g/dL)	13.0 [11.6–14.0]; [7.7–18.4]
Platelet (×10^3/µL)	224 [163–289]; [95–615]
CRP, mg/L	24 [4–156]; [0–528]
Procalcitonin (ng/mL)	0 [0–2]; [0–100]
CSF parameters	
Cell count (cells/mm^3)	330 [130–873]; [0–9,800]
Protein (mg/dL)	291 [95–890]; [14–10,084]
CSF/serum glucose ratio	0.37 [0.13–0.50]; [0.00–0.83]
Gram stain positivity	11 (18%)

Data are presented as n (%) or median [1st–3rd quartile]; [minimum–maximum], as appropriate. CSF, cerebrospinal fluid; CRP, C-reactive protein; IQR, interquartile range; min, minimum; max, maximum.

were excluded from the comparative subgroup analyses because the small sample size precluded meaningful statistical comparisons. The median age was significantly higher in the purulent meningitis group than in the TBM group ($p = 0.003$). Leukocyte, neutrophil, CRP, and procalcitonin levels were markedly higher in patients with purulent meningitis than in those with aseptic meningoencephalitis or TBM (all $p \leq 0.003$). Conversely, lymphocyte counts were higher in the aseptic meningoencephalitis group ($p = 0.007$), and the CSF/serum glucose ratio was significantly higher in the aseptic meningoencephalitis group than in both of the other groups ($p < 0.001$). CSF cell counts were also higher in the purulent meningitis group than in the other groups ($p \leq 0.008$), whereas CSF protein levels were higher only in comparison with the aseptic meningoencephalitis group ($p = 0.004$). Clinically, neck stiffness was more common in patients with purulent meningitis than in those with aseptic meningoencephalitis ($p < 0.001$). Human immunodeficiency virus (HIV) positivity

Table 3. Comparison of clinical and laboratory characteristics between survivors and non-survivors.

Variable	Survivors (n = 61[1])	Non-survivors (n = 9[1])	p-value[2]
Age, years	44 [30–58]	49 [42–63]	0.065
Male sex, n (%)	34 (55.7)	5 (55.6)	0.99
Renal/hepatic disease, n (%)	1 (1.6)	3 (33.3)	**0.006**
Altered consciousness, n (%)	32 (52.5)	8 (88.9)	0.068
Focal neurological deficit, n (%)	5 (8.2)	3 (33.3)	0.060
Lymphocyte count (/μL)	1,280 [730–2,030]	800 [480–950]	**0.007**
WBC (/μL)	10,200 [7,900–12,800]	11,300 [8,400–13,500]	0.41
CRP (mg/L)	38 [15–92]	45 [21–110]	0.57
CSF cell count (/mm^3)	210 [90–540]	280 [100–640]	0.49
CSF protein (mg/dL)	135 [90–220]	150 [95–230]	0.62
CSF/serum glucose ratio	0.38 [0.24–0.50]	0.07 [0.02–0.25]	0.068

[1]Data are presented as median [IQR] or n (%), as appropriate.

[2]Wilcoxon rank-sum test was used for continuous variables and Fisher's exact test for categorical variables.

CSF, cerebrospinal fluid; CRP, C-reactive protein; WBC, white blood cell; IQR, interquartile range.

Table 4. Variables showing statistically significant differences in pairwise comparisons according to clinical diagnosis.

Variable	Comparison	Higher in	Adjusted p-value
Age	Purulent vs. TBM	Purulent	**0.003**
WBC	Aseptic vs. purulent	Purulent	**0.013**
WBC	Purulent vs. TBM	Purulent	**0.001**
Neutrophil count	Aseptic vs. purulent	Purulent	**0.012**
Neutrophil count	Purulent vs. TBM	Purulent	**0.002**
Lymphocyte count	Aseptic vs. purulent	Aseptic	**0.007**
CRP	Aseptic vs. purulent	Purulent	**<0.001**
CRP	Purulent vs. TBM	Purulent	**0.002**
Procalcitonin	Aseptic vs. purulent	Purulent	**0.001**
Procalcitonin	Purulent vs. TBM	Purulent	**0.003**
CSF cell count	Aseptic vs. purulent	Purulent	**0.008**
CSF cell count	Purulent vs. TBM	Purulent	**0.001**
CSF protein	Aseptic vs. purulent	Purulent	**0.004**
CSF/serum glucose ratio	Aseptic vs. purulent	Aseptic	**<0.001**
CSF/serum glucose ratio	Aseptic vs. TBM	Aseptic	**<0.001**
Neck stiffness	Aseptic vs. purulent	Purulent	**<0.001**
HIV positivity	TBM vs. purulent	TBM	**0.011**

[1]Post-hoc Dunn and Fisher's exact tests; p-values adjusted for multiple comparisons. Aseptic meningoencephalitis (n = 17), purulent meningitis (n = 32), and TBM (n = 12). CSF, cerebrospinal fluid; CRP, C-reactive protein; WBC, white blood cell; TBM, tuberculous meningitis; HIV, human immunodeficiency virus.

was significantly more frequent in the TBM group than in the purulent meningitis group ($p = 0.011$). These comparisons are summarized in Table 4.

In the differential diagnosis of aseptic and purulent meningitis, ROC curve analysis showed that the CSF/serum glucose ratio had the highest discriminative ability (AUC = 0.971), followed by CRP (AUC = 0.885) and procalcitonin (AUC = 0.838). According to the DeLong test, the CSF/serum glucose ratio demonstrated significantly greater discriminative performance than all other parameters (all $p < 0.001$). Detailed diagnostic performance metrics are presented in Table 5.

The multivariable analysis was restricted to patients with purulent and aseptic meningitis. After excluding other diagnostic categories and applying complete-case analysis, 43 patients were included in the final model. Multivariable Firth penalized logistic regression analysis was performed to differentiate purulent from aseptic meningitis because this approach reduces small-sample bias and mitigates potential separation in limited datasets. The model included the CSF/serum glucose ratio, CRP, and procalcitonin. The model was statistically significant [likelihood ratio test: $\chi^2(3) = 36.42$, $p < 0.001$] and demonstrated excellent discriminative ability (AUC = 0.990, 95% CI: 0.972–1.000). The CSF/serum glucose ratio showed a strong and significant negative association, whereas CRP showed a significant positive association. Procalcitonin was not statistically significant. Detailed results are presented in Table 6.

The reported odds ratio for the CSF/serum glucose ratio reflects the effect of a one-unit increase. Because the physiological range of this variable is between 0 and 1, a one-unit increase represents a theoretical extreme rather than a clinically typical change. Therefore, smaller incremental differences (e.g., 0.1-unit increases) are more clinically meaningful when interpreting the effect size.

Internal validation using bootstrap resampling confirmed the stability of the model. The 95% CI for the AUC was 0.969–1.000. The mean optimism was 0.055, yielding an optimism-corrected AUC of 0.936, suggesting that the high apparent AUC was not solely attributable to overfitting. Bootstrap CIs for the odds ratios were consistent with the primary estimates. Calibration analysis further supported the predictive performance of the model, with a Brier score of 0.039, an apparent Emax of 0.100, and a bias-corrected Emax of 0.148. These findings indicate that the model demonstrated high discriminative ability and good calibration.

Discussion

In this study, CA-CNSIs were evaluated from both epidemiological and clinical perspectives. The high prevalence of comorbidities and an overall mortality rate of 13% highlight the substantial clinical burden associated with this patient population. Among the clinical presentations, purulent meningitis was the most common, and the CSF/serum glucose ratio emerged as the most effective biomarker for differential diagnosis. These findings

Table 5. ROC curve analysis for the determination of optimal cut-off values in differentiating purulent and aseptic meningitis.

Variable	AUC	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
CRP	0.885	16.75	0.84	0.82	0.90	0.74	0.84
Procalcitonin	0.838	0.39	0.78	0.94	0.96	0.68	0.83
CSF cell count	0.785	578.00	0.57	0.94	0.94	0.56	0.70
CSF/serum glucose ratio	0.971	0.49	1.00	0.88	0.93	1.00	0.95
CSF protein	0.792	195.00	0.79	0.81	0.88	0.68	0.80
Neutrophil count	0.761	11,115.00	0.59	0.88	0.90	0.54	0.69
Symptom-to-admission interval (days)	0.572	4.50	0.74	0.47	0.72	0.50	0.65
Lymphocyte count	0.781	1,745.00	0.84	0.65	0.82	0.69	0.78
ALT (U/L)	0.537	27.50	0.34	0.82	0.79	0.40	0.51

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; CSF, cerebrospinal fluid; ROC, receiver operating characteristic; ALT, alanine aminotransferase.

Table 6. Multivariable Firth penalized logistic regression analysis for the differential diagnosis between purulent and aseptic meningitis.

Variable	OR [exp(β)]	95% CI	p-value
CSF/serum glucose ratio	0.000002	0.000000–0.002619	<0.001
CRP	1.021	1.003–1.053	0.013
Procalcitonin	0.962	0.896–1.031	0.200

OR, odds ratio; CI, confidence interval; CSF, cerebrospinal fluid; CRP, C-reactive protein.

underscore the importance of integrating epidemiological insights with biomarker-based approaches for the diagnosis and prognostic assessment of CNSIs.

In our cohort, nearly half of the patients had at least one comorbidity, and 23% were immunosuppressed. Compared with a large prospective European cohort, our study showed a similar prevalence of comorbidities (48%), whereas the rate of immunosuppression was notably higher (23% vs. 16%)[4]. The most common comorbidities were hypertension and diabetes mellitus. Notably, immunosuppression was not significantly associated with mortality, suggesting that it may not directly influence the clinical course. Nevertheless, several multicenter studies have reported that immunosuppression adversely affects prognosis[12]; this discrepancy may be attributable to differences in patient populations and pathogen distribution. The relatively small sample size of our study may also have contributed to this finding. Furthermore, the rate of prior antibiotic use was 33%, substantially higher than the 9% reported in the European cohort[4]. This difference may reflect community-level antibiotic use as well as structural characteristics of healthcare access in Türkiye. Collectively, these findings underscore the regional epidemiological burden of CA-CNSIs.

In our study, the most common clinical presentation was purulent meningitis (46%), followed by aseptic meningoencephalitis (24%) and TBM (17%). This distribution is largely consistent with that reported in a large multicenter study involving 37 centers across 20 countries, in which 50% of cases were classified as meningitis and 34% as meningoencephalitis[13]. Similarly, a retrospective study from Türkiye reported purulent meningitis (44%) and aseptic meningoencephalitis (39%) as the predominant clinical presentations[14]. In our cohort, patients with microbiologically confirmed purulent meningitis had lower CSF/serum glucose ratios and were more likely to present with focal neurological deficits. Consistent with these findings, previous studies have demonstrated significantly lower CSF/serum glucose levels in patients with confirmed bacterial meningitis[15]. Although some reports suggest that culture-positive cases are associated with more severe disease and poorer prognosis[16], this association was not observed in our cohort. This discrepancy may be explained by the limited sample size or differences in pathogen distribution.

In our cohort, the median time from symptom onset to hospital admission was 3 days, which was longer than the median of 1 day reported in a nationwide Danish cohort[17]. Delayed presentation has been associated with increased mortality and the development of neurological sequelae[18], and the 13% mortality rate observed in our study is consistent with these findings. The delay in presentation may reflect patterns of healthcare access in our country as well as the widespread use of antibiotics in the community.

The most common presenting symptoms were headache (77%), altered consciousness (57%), and fever (56%). Neck stiffness (40%) and other meningeal signs, such as Kernig and Brudzinski signs, were observed less frequently. Although this proportion may appear relatively low compared with that reported in studies focusing exclusively on bacterial meningitis, our cohort included a heterogeneous spectrum of CNS infections. Therefore, the frequency of meningeal signs may vary depending on the underlying etiology, disease stage, and clinical context at presentation. Consequently, the absence of neck stiffness does not exclude the diagnosis of CNS infection and likely reflects the overall clinical heterogeneity of this population[4,19]. Furthermore, the frequencies of seizures (13%) and focal neurological deficits (11%) in our cohort were comparable to those reported in large international studies[17], highlighting the consistency of clinical manifestations across different populations.

In our study, the causative pathogen could not be identified in approximately half of the cases, a proportion consistent with that reported in international cohorts[13,14]. In addition, 33% of the patients had received antibiotics before specimen collection, which may have reduced culture positivity, contributed to the high proportion of microbiologically unconfirmed cases, and potentially influenced the interpretation of biomarker findings. Among the culture-positive specimens, *Streptococcus pneumoniae* was the most frequently isolated pathogen, followed by *Listeria monocytogenes*. Multiplex PCR detected pathogens more frequently than conventional culture, highlighting the limited sensitivity of culture, as reported previously[16]. Notably, PCR has been shown to provide substantial diagnostic value, particularly in culture-negative cases following prior antibiotic use[20]. More recently, studies have suggested that metagenomic next-generation sequencing may further improve pathogen detection by identifying organisms that remain undetected using conventional methods[21]. Therefore, our findings support the expanding role of molecular diagnostic techniques in the evaluation of CA-CNSIs.

Our findings indicate that renal or hepatic disease and low lymphocyte counts were significant risk factors for mortality. Similar findings have been reported in large cohorts of CA bacterial meningitis, in which comorbidities were associated with an increased risk of mortality[4,22]. Previous studies have also reported associations between inflammatory markers, such as the neutrophil-to-lymphocyte ratio, and mortality in adults with bacterial meningitis[23]. However, this parameter was not evaluated directly in our study. In addition, low CSF glucose levels have been identified as an independent

predictor of poor prognosis[24]. Altered consciousness and focal neurological deficits have also been strongly associated with mortality in previous studies[4,17]. Although these factors were more common among patients who died in our study, they reached only borderline statistical significance, likely owing to the limited sample size and heterogeneity of the causative pathogens. Overall, these findings reinforce that mortality prediction in CNSIs cannot rely on a single parameter but requires the integrated assessment of multiple clinical and laboratory indicators. However, because of the limited number of deaths (n = 9), these findings should be interpreted with caution, as the small number of events limits the robustness of the mortality analysis.

Patients with purulent meningitis exhibited significantly higher leukocyte, neutrophil, CRP, and procalcitonin levels than those with aseptic meningoencephalitis or TBM. These findings are consistent with the literature, which demonstrates the more pronounced inflammatory response associated with bacterial infections[16]. In contrast, the higher CSF/serum glucose ratios and lymphocyte counts observed in patients with aseptic meningoencephalitis support the utility of these parameters in the differential diagnosis of viral infections[25]. The greater frequency of neck stiffness in patients with purulent meningitis and the higher prevalence of HIV positivity in those with TBM suggest that clinical features may aid the diagnostic process. However, as emphasized in previous studies, these findings alone do not provide reliable discriminatory criteria[17,26].

ROC analysis identified the CSF/serum glucose ratio as the parameter with the highest discriminative ability (AUC = 0.971). This finding is consistent with recent clinical studies identifying CSF glucose as an important diagnostic parameter in the evaluation of CNSIs[24]. Although CRP and procalcitonin contributed to diagnostic performance, their discriminative ability was inferior to that of the CSF/serum glucose ratio. In the Firth penalized logistic regression model, the CSF/serum glucose ratio remained an independent and robust predictor, whereas CRP provided significant incremental value and procalcitonin was no longer statistically significant. Although previous studies have emphasized the diagnostic utility of serum CRP and procalcitonin for distinguishing purulent meningitis[28,29], their contribution in our cohort was more limited. Notably, the inclusion of CRP improved the overall diagnostic performance, supporting evidence that the combined assessment of multiple biomarkers provides more reliable diagnostic information than reliance on a single parameter[29]. The optimal cut-off values were determined using the Youden index to maximize combined sensitivity and specificity. Notably, the identified cut-off value for the CSF/serum glucose ratio (0.49) is consistent with values reported in previous studies of bacterial meningitis[25]. This agreement supports the potential clinical applicability of the cut-off identified in our cohort, although external validation in larger populations remains necessary.

Bootstrap analysis and calibration assessment supported the methodological robustness of the model, with a low Brier score indicating good predictive accuracy. These findings are consistent with the recommended methodological standards for the development and validation of clinical prediction models[30]. Our results highlight the importance of integrating multiple biomarkers, particularly the CSF/serum glucose ratio, in the differential diagnosis of CNS infections. Furthermore, the validated model demonstrated strong methodological performance, suggesting that it may provide a valuable foundation for the development of more reliable diagnostic tools. Although external validation in larger cohorts remains essential, the internal validation procedures applied in this study increase confidence in the model's performance.

From a clinical perspective, the proposed multivariable model may support clinical decision-making by providing an integrated probability estimate rather than relying on individual biomarkers alone. Although the CSF/serum glucose ratio demonstrated excellent standalone performance, the combined assessment of multiple biomarkers improved overall discrimination and may increase diagnostic confidence, particularly in borderline cases. These findings are consistent with current international guidelines for CNS infections, which emphasize the role of CSF biochemical parameters in early differential diagnosis[6,26]. Our results primarily reinforce current clinical practice while suggesting that the combination of biomarkers may provide additional support for diagnostic decision-making. However, prospective multicenter studies and external validation in diverse populations are required before routine clinical implementation. Further comparisons with emerging molecular diagnostic approaches may also help define the incremental value of predictive modeling in clinical practice.

Study Limitations

This study has several limitations. It was conducted at Şişli Hamidiye Etfal Training and Research Hospital using a retrospective design, which may have introduced selection bias related to referral patterns and limited the generalizability of the findings to other clinical settings. The relatively small sample size may also have reduced statistical precision. In addition, complete-case analysis was applied in the multivariable model, which may have reduced statistical power and introduced bias if the missing data were not completely at random. As an observational study, the possibility of unmeasured confounding cannot be excluded. Furthermore, the absence of long-term follow-up limited the assessment

of late neurological sequelae. The study population consisted exclusively of adult patients; therefore, the findings may not be directly applicable to pediatric populations, in whom CNSIs differ in epidemiology and clinical presentation. Future prospective multicenter studies are warranted to validate these findings.

Conclusion

This study highlights the epidemiological and clinical characteristics of CA-CNSIs and demonstrates that the CSF/serum glucose ratio is a particularly strong diagnostic biomarker. Although CRP provided additional diagnostic value and procalcitonin showed limited utility, the findings suggest that the combined assessment of multiple biomarkers offers greater diagnostic reliability than reliance on individual markers alone. The proposed model, supported by bootstrap and calibration analyses, demonstrated methodological robustness, suggesting that it may provide a valuable foundation for the development of diagnostic approaches for clinical practice. Future studies involving larger cohorts are warranted to validate these findings and facilitate the development of more robust prediction models that may improve the early diagnosis of CNSIs.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ethics Committee of University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital (approval date: 22.04.2025; approval number: 2980). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Due to the retrospective design of the study, informed consent was not required.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: H.M.T., O.D., C.A.T., N.D.D., D.Y.S., İ.D., Concept: H.M.T., O.D., C.A.T., D.Y.S., Design: H.M.T., O.D., C.A.T., D.Y.S., İ.D., Data Collection or Processing: H.M.T., S.A., Analysis or Interpretation: O.D., Literature Search: H.M.T., N.D.D., D.Y.S., İ.D., Writing: H.M.T.

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Temporal Trends in the Seroprevalence of *Toxoplasma gondii*, Rubella, and Cytomegalovirus Infections among Pregnant Women: An 11-Year Retrospective Study

Gebe Kadınlarda *Toxoplasma gondii*, Rubella ve Sitomegalovirüs Enfeksiyonlarının Seroprevalansındaki Zamansal Eğilimler: 11 Yıllık Retrospektif Bir Çalışma

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Abstract

Introduction: Maternal infection with *Toxoplasma gondii (T. gondii)*, rubella virus, and cytomegalovirus (CMV) during pregnancy may result in vertical transmission and adverse fetal or neonatal outcomes. Although these pathogens differ in their microbiology and prevention strategies, they are commonly evaluated together in antenatal care because serological testing plays a central role in assessing immunity, susceptibility, and potential recent infection. This study aimed to describe the seroprevalence of *T. gondii*, rubella virus, and CMV antibodies among pregnant women and evaluate temporal trends.

Materials and Methods: This retrospective study reviewed the antenatal records of pregnant women who attended a tertiary care obstetrics clinic in Türkiye between January 2012 and December 2022. Maternal age, gestational age at testing, and immunoglobulin G (IgG) and immunoglobulin M (IgM) serological results for *T. gondii*, rubella virus, and CMV were extracted from laboratory records. Annual seropositivity rates were calculated. Temporal trends in annual IgG seroprevalence were evaluated using binomial generalized linear models, with year treated as a continuous predictor.

Results: A total of 16,877 pregnant women were included (mean age, 28.72 ± 5.21 years; range, 15–49 years; mean gestational age at testing, 14.0 ± 5.5 weeks). Overall IgG seroprevalence was 31.5% for *T. gondii*, 94.8% for rubella, and 96.9% for CMV, whereas IgM positivity rates were 0.5%, 0.1%, and 0.1%, respectively. Annual IgG seroprevalence showed moderate year-to-year variation but no statistically significant linear trend over the 11-year study period for any of the pathogens (all $p > 0.05$). Analyses of IgM trends were limited because of the very low number of positive cases.

Conclusion: Over the 11-year study period, IgG seroprevalence remained high for rubella and CMV and moderate for *T. gondii*, whereas IgM positivity ranged from 0.1% to 0.5% among pregnant women. The absence of significant linear trends underscores the importance of sustained antenatal serological surveillance and preventive counseling to reduce the risk of congenital infections.

Keywords: Cytomegalovirus, pregnancy, rubella, seroprevalence, *Toxoplasma gondii*

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Öz

Giriş: Gebelik sırasında *Toxoplasma gondii (T. gondii)*, rubella virüsü ve sitomegalovirüs (CMV) ile gelişen maternal enfeksiyonlar, vertikal geçişe ve olumsuz fetal veya neonatal sonuçlara yol açabilir. Bu patojenler mikrobiyolojik özellikleri ve korunma stratejileri bakımından farklılık gösterse de, serolojinin bağışıklık, duyarlılık ve olası yeni enfeksiyonu değerlendirmedeki merkezi rolü nedeniyle antenatal bakımda yaygın olarak birlikte değerlendirilirler. Uzun vadeli seroepidemiyolojik veriler, antenatal tarama uygulamaları ve sürveyans stratejileri hakkında bilgi sağlamak açısından önemlidir.

Gereç ve Yöntemler: Bu retrospektif çalışmada, Ocak 2012 ile Aralık 2022 tarihleri arasında üçüncü basamak bir kadın doğum kliniğine başvuran gebe kadınların antenatal kayıtları incelenmiştir. Laboratuvar kayıtlarından maternal yaş, test sırasındaki gebelik haftası ile *T. gondii*, rubella virüsü ve CMV için immünoglobulün G (IgG) ve immünoglobulün M (IgM) serolojik sonuçları elde edilmiştir. Yıllık seropozitiflik oranları hesaplanmıştır. Yıllık IgG seroprevalansındaki zamansal eğilimler, yılın sürekli bir öngörücü değişken olarak kullanıldığı binomiyal genelleştirilmiş lineer modeller kullanılarak değerlendirilmiştir.

Bulgular: Çalışmaya toplam 16.877 gebe kadın dahil edilmiştir (ortalama yaş 28,7 ± 5,2 yıl; test sırasındaki ortalama gebelik haftası 14,0 ± 5,5 hafta). Genel IgG seroprevalansı *T. gondii* için %31,5, rubella için %94,8 ve CMV için %96,9 olarak saptanırken; IgM pozitifliği her üç enfeksiyon için de düşük kalmıştır (sırasıyla %0,5, %0,1 ve %0,1). Yıllık IgG seroprevalansı yıldan yıla orta dereceli varyasyonlar göstermiş, ancak hiçbir patojen için 11 yıllık dönem boyunca istatistiksel olarak anlamlı bir lineer eğilim göstermemiştir (tümü için $p > 0,05$). IgM eğilim analizleri, vaka sayılarının çok düşük olması nedeniyle sınırlı kalmıştır.

Sonuç: On bir yıllık dönem boyunca gebe kadınlar arasında IgG seroprevalansı rubella ve CMV için yüksek, *T. gondii* için orta düzeyde seyretmiş; IgM pozitifliği ise tutarlı bir şekilde düşük kalmıştır. Anlamlı lineer eğilimlerin olmaması, konjenital enfeksiyon risklerini yönetmek için sürdürülebilir antenatal serolojik sürveyansın ve koruyucu danışmanlığın önemini desteklemektedir.

Anahtar Kelimeler: Sitomegalovirüs, gebelik, rubella, seroprevalans, *Toxoplasma gondii*

Introduction

Maternal infections during pregnancy remain a major concern because primary infection or reactivation can result in vertical transmission and adverse fetal or neonatal outcomes. In this context, *Toxoplasma gondii* (*T. gondii*), rubella virus, and cytomegalovirus (CMV) are of particular clinical relevance, as maternal infection is often asymptomatic, whereas congenital infection may lead to miscarriage, stillbirth, structural anomalies, or long-term neurodevelopmental sequelae[1-3].

Global seroprevalence patterns vary across geographic regions and populations. The global seroprevalence of latent *T. gondii* infection among pregnant women has been estimated at approximately 30%, whereas the seroprevalence of rubella and CMV is influenced by vaccination coverage and population exposure patterns[4-7]. In Türkiye, recent regional cohort studies have reported *T. gondii* immunoglobulin G (IgG) seropositivity rates of 23.0% in İstanbul, 28.3% in Konya, and 35.2% in Diyarbakır, with corresponding rubella IgG seropositivity rates of 89.1%, 98.13%, and 91.2% and CMV IgG seropositivity rates of 98.7%, 99.78%, and 98.7%, respectively[8-10].

T. gondii infection is typically acquired through the ingestion of tissue cysts in undercooked meat or exposure to oocysts in contaminated environments. Congenital toxoplasmosis can result in neurological and ophthalmologic complications[11-13]. Rubella infection during pregnancy may cause congenital rubella syndrome, which is characterized by hearing loss, cardiac defects, ocular abnormalities, and developmental delay; population immunity is strongly influenced by vaccination coverage[3,5,14]. CMV is the most common cause of congenital viral infection worldwide. Although maternal disease is often mild or asymptomatic, congenital CMV infection can result in permanent sequelae, including sensorineural hearing loss and neurodevelopmental impairment, and no licensed vaccine is currently available[15,16].

Seroprevalence studies provide valuable epidemiological information by quantifying immunity and susceptibility patterns among pregnant women, thereby supporting risk communication, preventive counseling, and surveillance strategies. Antenatal testing practices vary across countries and healthcare settings; where available, serological testing may be used to support clinical assessment and public health surveillance of infections associated with congenital disease. Therefore, this study aimed to describe the seroprevalence of *T. gondii*, rubella virus, and CMV antibodies among pregnant women and to examine temporal changes in their distribution over the study period, thereby providing long-term data to support antenatal infectious disease surveillance and prevention.

Materials and Methods

This retrospective study reviewed the antenatal records of pregnant women who attended the outpatient obstetrics clinics of a tertiary care hospital in Türkiye between January 2012 and December 2022.

Pregnant women who attended the antenatal clinic at least once during pregnancy and whose antenatal records contained the required serological test results were included. Records were linked using unique patient identifiers within the hospital information system, enabling the identification and removal

of duplicate entries before screening and analysis. A total of 18,176 de-duplicated antenatal records were initially screened. Of these, 1,299 were excluded because of incomplete key data, including inconsistent maternal age information that could not be reliably verified from hospital records (n = 12), missing gestational age at testing (n = 556), and missing serological results (n = 731). Records with missing serological results were classified under that category even when additional variables were missing. The final analytical cohort consisted of 16,877 pregnant women.

From the eligible records, we extracted maternal age, gestational age at testing, and serological laboratory results for IgG and immunoglobulin M (IgM) antibodies against *T. gondii*, rubella virus, and CMV. The data were entered into a secure study database and checked for completeness and internal consistency.

Serum samples were tested for IgG and IgM antibodies against *T. gondii*, rubella virus, and CMV using routine commercial assays employed by the hospital laboratory. During the earlier years of the study, serological analyses were performed using the Abbott ARCHITECT platforms (i1000SR/i2000SR; Abbott, USA) with the corresponding ARCHITECT Toxo IgG/IgM, Rubella IgG/IgM, and CMV IgG/IgM assays. From 2021 onward, testing was performed using the Roche cobas e801 platform (Roche Diagnostics, Germany) with the corresponding Elecsys Toxo IgG/IgM, Rubella IgG/IgM, and CMV IgG/IgM assays. All assays were performed according to the manufacturers' instructions and interpreted using the contemporaneous kit-specific cutoff values.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Clinical Research Ethics Committee of Kartal Dr. Lütfi Kırdar Training and Research Hospital (approval no.: 2020/514/177/38; date: 13.05.2020). Because this study was a retrospective review of existing records, individual informed consent was not required, as confirmed by the approving ethics committee. All extracted data were de-identified before analysis, stored securely, and accessed only by the study investigators.

Statistical Analysis

Annual seropositivity rates were calculated by dividing the number of positive test results by the total number of tests with available results for each year and are reported as percentages. Descriptive statistics were used, and temporal trends in seropositivity were evaluated using binomial generalized linear models with year treated as a continuous predictor. For the pathogen-specific IgG models, yearly aggregated counts were analyzed, with the number of positive test results relative to the total number of tests performed in each calendar year specified as the dependent outcome. Results are reported as odds ratios (ORs) per one-calendar-year increase. Because of the extremely low number of IgM-positive events, formal trend modeling was not performed for IgM markers, and these results were analyzed descriptively.

Categorical variables are presented as frequencies and percentages, whereas continuous variables are presented as means ± standard deviations or medians, as appropriate. All statistical analyses were performed using IBM SPSS Statistics software (version 24.0; IBM Corp., Armonk, NY, USA).

Results

Following data extraction, the final study dataset comprised records from 16,877 pregnant women. The participants had a mean age of 28.72 ± 5.21 years (range, 15–49 years), and the mean gestational age at testing was 14.0 ± 5.5 weeks (range, 6–28 weeks).

Between 2012 and 2022, laboratory records identified 82 IgM-positive and 5,323 IgG-positive results for anti-*T. gondii*, 28 IgM-positive and 16,003 IgG-positive results for anti-rubella, and 20 IgM-positive and 16,362 IgG-positive results for anti-CMV. The corresponding overall seropositivity rates were 0.5% (IgM) and 31.5% (IgG) for *T. gondii*, 0.1% (IgM) and 94.8% (IgG) for rubella, and 0.1% (IgM) and 96.9% (IgG) for CMV (Table 1).

Table 1. IgG and IgM seroprevalence of anti-*T. gondii*, anti-CMV, and anti-rubella antibodies by year.

	Toxoplasma gondii			**Rubella virus**			**CMV**		
	IgM	IgG	IgM + IgG*	IgM	IgG	IgM + IgG*	IgM	IgG	IgM + IgG*
2012	20 (1.7%)	395 (33.2%)	4 (0.3%)	5 (0.4%)	1151 (96.6%)	4 (0.3%)	4 (0.3%)	1147 (96.3%)	0
2013	6 (0.7%)	277 (30.1%)	1 (0.3%)	3 (0.3%)	911 (99.1%)	1 (0.1%)	1 (0.1%)	879 (95.6%)	0
2014	6 (0.4%)	348 (25.8%)	2 (0.3%)	2 (0.1%)	1319 (97.6%)	0	0	1348 (99.8%)	2 (0.1%)
2015	8 (0.7%)	394 (32.7%)	0	2 (0.2%)	1155 (95.8%)	0	0	1190 (98.7%)	0
2016	2 (0.2%)	271 (25.6%)	0	0	1024 (96.7%)	0	3 (0.3%)	1022 (96.5%)	0
2017	2 (0.2%)	370 (28.3%)	0	0	1285 (98.2%)	0	3 (0.2%)	1261 (96.3%)	0
2018	8 (0.4%)	702 (33.9%)	4 (0.2%)	4 (0.2%)	1935 (93.5%)	3 (0.1%)	3 (0.1%)	2001 (96.7%)	1 (0.0%)
2019	16 (0.8%)	676 (34.9%)	7 (0.4%)	7 (0.4%)	1750 (90.3%)	2 (0.1%)	2 (0.1%)	1808 (93.3%)	0

Table 1. Continued.

	Toxoplasma gondii			Rubella virus			CMV		
	IgM	IgG	IgM + IgG*	IgM	IgG	IgM + IgG*	IgM	IgG	IgM + IgG*
2020	4 (0.3%)	531 (35.1%)	0	2 (0.1%)	1405 (92.8%)	0	2 (0.1%)	1475 (97.4%)	0
2021	4 (0.2%)	683 (32.5%)	0	2 (0.1%)	1988 (94.6%)	0	0	2073 (98.6%)	0
2022	6 (0.3%)	676 (30.5%)	1 (0.0%)	1 (0.0%)	2080 (93.8%)	0	2 (0.1%)	2158 (97.3%)	0
All	82 (0.5%)	5323 (31.5%)	19 (0.1%)	28 (0.1%)	16003 (94.8%)	10 (0.0%)	20 (0.1%)	16362 (96.9%)	3 (0.0%)

*: Concurrent positivity of IgG- and IgM-, values as counts and percentages. *T. gondii, Toxoplasma gondii*; IgG, immunoglobulin G; IgM, immunoglobulin M; CMV, cytomegalovirus.

Annual seroprevalence estimates ranged from 25.6–35.1% for *Toxoplasma* IgG, 90.3–99.1% for rubella IgG, and 93.3–99.8% for CMV IgG over the study period. Annual IgM positivity remained consistently low for all three pathogens, ranging from 0.2–1.7% for *Toxoplasma*, 0.0–0.4% for rubella, and 0.0–0.3% for CMV.

Trend analysis using binomial generalized linear models with year treated as a continuous predictor yielded the following results for the IgG markers: *Toxoplasma* IgG, OR per year = 1.01 [95% confidence interval (CI), 0.99–1.03; $p = 0.25$]; rubella IgG, OR per year = 0.99 (95% CI, 0.97–1.01; $p = 0.18$); and CMV IgG, OR per year = 1.00 (95% CI, 0.99–1.02; $p = 0.62$). These findings indicate that there were no statistically significant linear trends in annual IgG seroprevalence for any of the three pathogens during the 2012–2022 study period.

Yearly trends in IgG seroprevalence for *T. gondii*, rubella virus, and CMV are illustrated in Figure 1. Year-specific IgG and IgM seroprevalence estimates with 95% CIs are provided in Supplementary Tables 1 and 2.

Discussion

This 11-year retrospective study of 16,877 pregnant women provides a comprehensive overview of the seroprevalence trends of three clinically important pathogens: *T. gondii*, rubella virus, and CMV. Our findings showed that although rubella and CMV IgG seroprevalence remained consistently high (94.8% and 96.9%, respectively), *T. gondii* IgG seroprevalence was moderate at 31.5%. Formal trend modeling demonstrated stable IgG seroprevalence throughout the 2012–2022 study period, with no statistically significant linear changes for any of the three pathogens. Furthermore, the low frequency of IgM positivity across all pathogens suggests a low burden of acute maternal infections in this population during the study period.

When interpreted alongside findings from recent regional cohorts in Türkiye, our results suggest regional heterogeneity rather than a uniform national seroepidemiological profile. Compared with the Konya enzyme-linked fluorescent assay (ELFA)-based cohort reported by Ezer et al.[8], which found *T. gondii*, rubella, and CMV IgG seroprevalence rates of 28.3%, 98.13%, and 99.78%, respectively, our cohort showed slightly higher *T. gondii* IgG seroprevalence but somewhat lower rubella and CMV IgG seroprevalence. Conversely, our study demonstrated lower *T. gondii* and CMV IgG seroprevalence but higher rubella IgG seroprevalence than an 8-year enzyme-linked immunosorbent assay (ELISA)-based study conducted in Diyarbakır[9]. An İstanbul cohort using chemiluminescent microparticle immunoassay (CMIA) also highlighted this regional variation, reporting *T. gondii*, rubella, and CMV IgG seroprevalence rates of 23.0%, 89.1%, and 98.7%, respectively[10].

These differences among studies likely reflect a combination of geographic, dietary, and sociodemographic and behavioral factors, as well as important methodological differences. In particular, variation in the analytical sensitivity and specificity of ELISA, ELFA, and CMIA platforms, together with differences in antigen preparations and kit-specific cutoff values, may contribute to discordant serological classifications, particularly for borderline results[8-10]. Our 11-year study provides valuable longitudinal context for these observations. Despite moderate year-to-year variation, no significant linear trends in IgG seroprevalence were identified within our center, indicating a stable seroepidemiological profile over the past decade.

Regarding rubella, the high IgG seroprevalence observed in our study is consistent with previous reports and supports the effectiveness of vaccination programs[3,5,17]. From a public health perspective, persistently high rubella IgG seroprevalence is consistent with sustained population immunity, although periodic fluctuations in vaccine uptake or outbreak dynamics may still occur[18-20]. In our cohort, the absence of a statistically significant temporal trend in rubella IgG seroprevalence suggests a stable immunity profile throughout the study period, which is reassuring for the prevention of congenital rubella.

For CMV, our cohort demonstrated very high IgG seroprevalence, consistent with widespread prior exposure. Although CMV IgG seroprevalence varies among populations, higher rates are commonly reported in settings with greater early-life exposure. Differences in sociodemographic

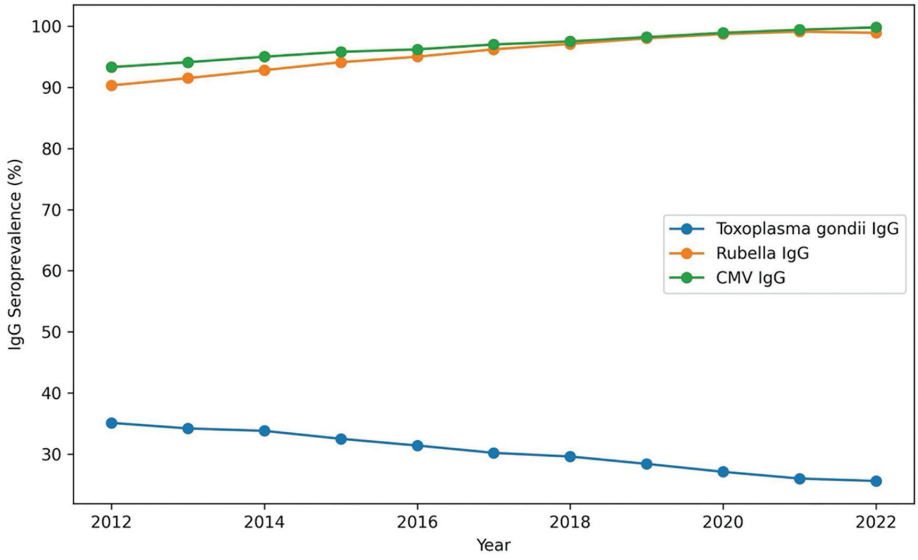

Figure 1. Yearly trends in IgG seroprevalence of *T. gondii*, rubella virus, and CMV among pregnant women between 2012 and 2022. Points represent the annual seroprevalence estimates, and the lines illustrate the overall temporal patterns across the study period. IgG, immunoglobulin G; *T. gondii, Toxoplasma gondii*; CMV, cytomegalovirus.

characteristics and exposure patterns may partially explain the variability observed across studies[6]. CMV IgM positivity remained rare in our cohort, whereas studies from Europe, North America, and Japan have reported higher IgM seroprevalence rates of 1.0–4.6%, 2.3–4.5%, and 0.8%, respectively, potentially reflecting differences in population risk profiles, testing strategies, and assay performance[7]. The combination of very high IgG seroprevalence and low IgM positivity in our cohort is consistent with high background immunity and a low frequency of suspected recent infection during routine antenatal screening.

The absence of statistically significant linear trends in IgG seroprevalence should be interpreted with caution. Rather than indicating a lack of epidemiological significance, these stable estimates may reflect consistently high baseline immunity or persistent exposure patterns that are not expected to change linearly over time. In addition, assay variability and population heterogeneity may have obscured subtle temporal changes. Importantly, these findings highlight the need for continued surveillance, as non-linear fluctuations or cohort-specific changes may still emerge over time.

The moderate year-to-year variation observed in IgG seroprevalence should also be interpreted in the context of assay-related factors. Because different commercial immunoassay platforms were used during the study period, assay-specific cutoff values, borderline classification thresholds, and analytical performance characteristics may have differed across years. Consequently, a small number of samples with results close to the decision thresholds may have been classified differently depending on the assay used. Therefore, some of the observed annual variation in IgG seroprevalence may reflect inter-assay variability in addition to true epidemiological variation.

Study Limitations

The retrospective study design and reliance on IgG and IgM serological testing may not fully capture the timing of infection or the dynamics of the immune response, and pregnancy outcome data were unavailable to correlate serological patterns with clinical outcomes. In addition, different commercial immunoassay platforms were used during the 11-year study period. Although all assays were performed according to the manufacturers' instructions and interpreted using the assay-specific cutoff values applicable at the time of testing, differences in analytical performance among platforms—including variations in sensitivity, specificity, and cutoff definitions—may have contributed to the modest year-to-year variation observed in seroprevalence estimates. Consequently, small annual fluctuations, particularly in IgG seroprevalence, should be interpreted cautiously and should not be assumed to reflect epidemiological changes alone. Other TORCH pathogens were not evaluated; therefore, a more comprehensive assessment of congenital infection profiles was not possible. Despite these limitations, the large sample size, extended study period, and consistent laboratory setting are important strengths that enhance the reliability of the findings.

Future research should prioritize prospective study designs using harmonized assays, more comprehensive clinical outcome data, and, where feasible, confirmatory testing strategies (e.g., avidity testing for *T. gondii* when clinically indicated) to better characterize incident infections and the risk of congenital transmission. Longitudinal follow-up studies could further clarify the relationship between serological screening results, maternal infection status, and pregnancy outcomes, as well as evaluate the effectiveness of targeted preventive counseling and vaccination strategies.

Conclusion

This large antenatal screening study provides long-term seroepidemiological data on *T. gondii*, rubella virus, and CMV infections during pregnancy. IgG seroprevalence remained high for rubella and CMV and moderate for *T. gondii*, whereas IgM positivity remained rare throughout the study period. Formal trend analyses demonstrated no statistically significant linear changes in annual IgG seroprevalence between 2012 and 2022. These findings support the continued value of antenatal serological surveillance, preventive counseling, and vaccination programs where applicable, and underscore the importance of sustained surveillance to inform strategies for the prevention of maternal and congenital infections.

Ethics

Ethics Committee Approval: The study protocol was approved by the Clinical Research Ethics Committee of Kartal Dr. Lütfi Kırdar Training and Research Hospital (approval no.: 2020/514/177/38; date: 13.05.2020).

Informed Consent: Because this study was a retrospective review of existing records, individual informed consent was not required, as confirmed by the approving ethics committee.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.Ö., K.T., Design: K.T., Data Collection or Processing: B.Ö., Analysis or Interpretation: K.T., Literature Search: B.Ö., K.T., Writing: B.Ö., K.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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Supplementary Tables 1 and 2: https://d2v96fxpocvxx.cloudfront.net/4458d962-0fb2-48a3-a23a-e35265f70f9b/content-images/f1800fe4-5c37-4e3f-bd08-127934f57797.pdf

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Age-Specific Anti-HBs Seropositivity Among Individuals Eligible for the Childhood Hepatitis B Vaccination Program: A Single-Center Study

Ulusal Çocukluk Çağı Hepatit B Aşılama Programı Kapsamına Giren Bireylerde Yaşa Göre Anti-HBs Seropozitifliği: Tek Merkezli Bir Çalışma

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Abstract

Introduction: Hepatitis B (HB) vaccination is the most effective strategy for preventing HB virus infection. This study evaluated age-specific HB antibody responses among individuals eligible for the national childhood HB vaccination program to optimize vaccination follow-up policies.

Materials and Methods: This single-center retrospective study included individuals aged 1–22 years who underwent HB serological testing between January 2022 and January 2023. Individuals with positive HBsAg results, missing data, or foreign nationality were excluded. Initial HB surface antibody (anti-HBs) results and age at the time of testing were analyzed. Participants were categorized into five age groups: 1–3, 4–6, 7–12, 13–18, and 19–22 years.

Results: A total of 3,082 individuals were included [47% female; median age, 18 years (interquartile range, 10–20)], of whom 1,766 (57.3%) tested positive for anti-HBs. Higher rates of antibody positivity were observed among children aged 1–3 years. The median age of anti-HBs-negative individuals was significantly higher than that of anti-HBs-positive individuals ($p < 0.001$). Females had a higher anti-HBs seropositivity rate than males (59.6% vs. 55.3%, $p = 0.016$). In the multivariable analysis, older age groups had significantly lower odds of anti-HBs seropositivity than the 1-3-year age group [odds ratio (OR) range = 0.12–0.31], whereas female sex was independently associated with higher odds of seroprotection (OR = 1.26).

Conclusion: The rate of protective antibody detection decreased with age among individuals targeted by the national childhood HB vaccination program. Particularly in settings with intermediate or high HB endemicity, early identification of individuals without detectable antibodies may help optimize immunization monitoring strategies, prevent unnecessary revaccination, and improve resource allocation.

Keywords: Child vaccination schedule, hepatitis B, post-vaccination monitoring, seroprotection

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Öz

Giriş: Hepatit B (HB) aşılaması, HB virüsü enfeksiyonunu önlemenin en etkili stratejisidir. Bu çalışmada, aşılama sonrası takip politikalarını optimize etmek amacıyla, ulusal çocukluk çağı HB aşılama programı kapsamında yer alan bireylerde yaşa özgü HB antikor yanıtları değerlendirilmiştir.

Gereç ve Yöntem: Bu tek merkezli, retrospektif çalışmaya Ocak 2022 ile Ocak 2023 tarihleri arasında HB serolojisi bakılan 1–22 yaş arası bireyler dahil edilmiştir. HBsAg pozitif olan, verileri eksik olan ve yabancı uyruklu bireyler çalışmadan çıkarılmıştır. İlk HB yüzey antikoru (anti-HBs) sonucu ve testin yapıldığı yaştaki değerler analiz edilmiş; yaş, beş kategoriye ayrılmıştır (1–3, 4–6, 7–12, 13–18 ve 19–22 yaş).

Bulgular: Çalışmaya dahil edilen 3082 bireyden (%47'si kadın, ortanca yaş 18 yıl, çeyrekler arası açıklık 10-20), 1766'sında (%57,3) anti-HBs testi pozitif saptandı. En yüksek antikor pozitiflik oranları 1–3 yaş arasındaki çocuklarda gözlendi. Anti-HBs negatif bireylerin ortanca yaşı, pozitif olan bireylere göre anlamlı derecede daha yüksekti ($p < 0,001$). Kadınlarda anti-HBs seropozitifliği erkeklere göre daha yüksekti (%59,6'ya karşı %55,3, $p = 0,016$). Çok değişkenli analizde, 1-3 yaş grubuna kıyasla daha ileri yaş gruplarında anti-HBs seropozitifliği olasılığının anlamlı derecede daha düşük olduğu görüldü [olasılık oranı (OR) aralığı ==0,12-0,31], kadın cinsiyeti ise daha yüksek seroproteksiyon olasılığı ile bağımsız olarak ilişkili bulundu (OR =1,26).

Sonuç: Ulusal aşılama programımızın hedeflediği yaş grubunda koruyucu antikor saptanma oranının yaşla birlikte azaldığı görüldü. Özellikle orta veya yüksek HB endemisitesine sahip bölgelerde, tespit edilebilir antikorları olmayan bireylerin erken dönemde belirlenmesi, aşılama sonrası izlem stratejilerinin optimize edilmesine, gereksiz yeniden aşılamaların önlenmesine ve kaynakların daha etkin kullanılmasına katkı sağlayabilir.

Anahtar Kelimeler: Çocuk aşılama programı, hepatit B, aşılama sonrası izleme, seroproteksiyon

Introduction

Hepatitis B virus (HBV) infection is a major public health problem worldwide and can cause chronic liver disease, cirrhosis, and hepatocellular carcinoma[1]. According to the World Health Organization, over 250 million people were living with chronic hepatitis B infection in 2022, and most infections occurred in individuals over 30 years of age, an age group for which universal hepatitis B vaccination has not been implemented[2]. Although antiviral treatments can suppress viral replication, they cannot eliminate the virus, and HBV remains a major global cause of morbidity and mortality. Consequently, hepatitis B vaccination has proven to be highly effective in preventing HBV infection and its complications[3].

Hepatitis B vaccination programs are an important public health strategy for establishing sustained immunity within the community and reducing the disease burden. The effectiveness of public health systems increases as long-term community immunity is achieved. Hepatitis B vaccination programs targeting infants and young children have been implemented in many countries worldwide[4]. However, routine post-vaccination monitoring of antibody responses is not universally practiced, and the optimal timing for assessing long-term vaccine-induced immunity remains unclear.

Türkiye is considered a country with intermediate hepatitis B endemicity, with an HBsAg prevalence of approximately 4% in the general population[5]. The hepatitis B vaccination program was incorporated into the national childhood immunization schedule in 1998, targeting infants at 0, 1, and 6 months of age[6]. We aimed to evaluate antibody responses across age groups comprising individuals eligible for the national childhood hepatitis B vaccination program. We hypothesized that anti-HBs seropositivity would decline with increasing age and sought to provide evidence to support immunization monitoring policies in countries with intermediate or higher hepatitis B endemicity.

Materials and Methods

Study Design and Population

This single-center, retrospective study was conducted at Recep Tayyip Erdoğan University Hospital. Individuals who underwent HBsAg and hepatitis B surface antibody (anti-HBs) testing at our hospital between January 1, 2022, and January 1, 2023, during either inpatient care or outpatient visits for clinical evaluation or routine screening, were assessed. Among these, individuals aged 1-22 years who were eligible for the national childhood hepatitis B vaccination program in our country were included. Foreign nationals (owing to uncertain vaccination history), patients with missing data, and those who tested positive for HBsAg were excluded. Sex was defined as a biological variable (male/female) as recorded at birth in the hospital electronic medical records.

Data Collection

For eligible individuals, all historical HBV serology results recorded in our system were retrieved. The analysis focused on the earliest available anti-HBs result for each individual, along with the individual's age at the time of testing. Anti-HBs levels were analyzed according to age at the time of testing. Individual vaccination records were not available in the hospital database; therefore, vaccination status was inferred based on eligibility for the national childhood hepatitis B vaccination program introduced in 1998.

Laboratory Methods

Anti-HBs levels were quantitatively measured using a chemiluminescent microparticle immunoassay in a single laboratory. An anti-HBs titer of ≥10 mIU/mL was considered positive.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp.; 2013). The normality of age was assessed using the Kolmogorov-Smirnov test. Because age was not normally distributed, the data are presented as the median and interquartile range (IQR). The Mann-Whitney U test was used to compare the median age between individuals who were anti-HBs positive and those who were anti-HBs negative. Categorical variables were compared using the Pearson chi-square test. Age was categorized into five clinically relevant groups (1-3, 4-6, 7-12, 13-18, and 19-22 years) for binary logistic regression analysis, with anti-HBs serostatus as the outcome variable. Age category (with 1-3 years as the reference group) and sex were included as independent variables. A p-value of <0.05 was considered statistically significant.

Ethics

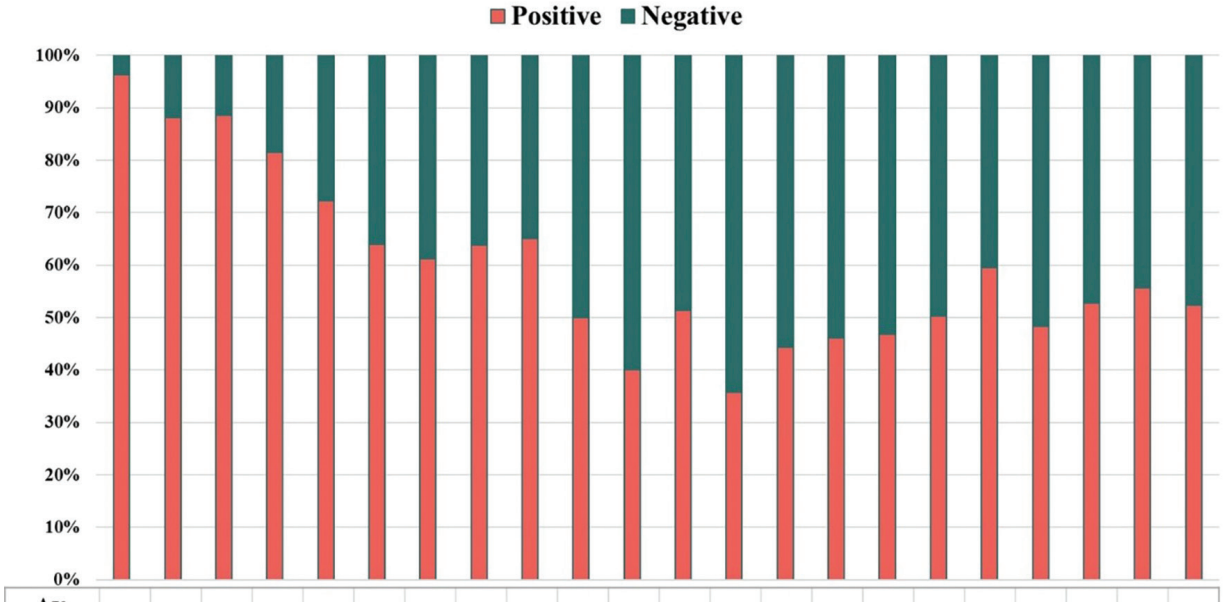
The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Recep Tayyip Erdoğan University (decision no: 2023/237, date: 12.10.2023). Informed consent was waived because of the retrospective nature of the study.

Results

The records of 3,191 individuals who underwent HBV serologic testing within the specified date and age range at our hospital were evaluated. Data from 45 individuals without an HBsAg test, 20 individuals who tested positive for HBsAg, and 44 foreign nationals were excluded, resulting in a final study population of 3,082 individuals. Of these, 1,447 (47.0%) were female, and the median age was 18 years (IQR, 10-20 years).

At the initial hospital visit, 1,766 (57.3%) individuals tested positive for anti-HBs, whereas 1,316 (42.7%) tested negative. Anti-HBs positivity ranged from 35.7% to 96.3%, depending on age, with the highest seropositivity rates observed among individuals aged 1, 3, and 2 years, respectively (Figures 1 and 2A). The median age of individuals with negative anti-HBs results was significantly higher than that of individuals with positive results ($p < 0.001$) (Table 1). The proportion of individuals with protective anti-HBs levels was significantly higher among females than among males (59.6% vs. 55.3%, $p = 0.016$) (Figure 2B).

A binary logistic regression model including age category and sex as predictors of anti-HBs positivity was applied. Compared with the 1-3-year age group, older age categories showed a stepwise decline in the likelihood of anti-HBs seropositivity. Female sex was also independently associated with higher odds of seroprotection than male sex (Table 2).



Age (years)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
n	27	76	123	103	148	114	80	47	43	34	35	35	70	124	78	126	177	255	294	341	393	359

Figure 1. Age-specific distribution of anti-HBs seropositivity and sample size. The proportions of anti-HBs–positive and anti-HBs-negative individuals are shown for each year of age, along with the number of individuals tested in each age group. HB, hepatitis B.

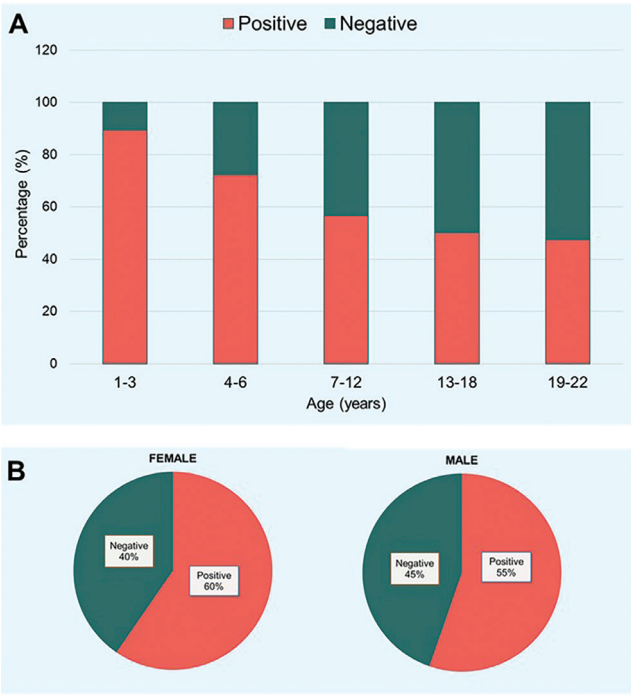


Figure 2. Distribution of anti-HBs serostatus by age group and sex.

(A) Proportions of anti-HBs-positive and anti-HBs–negative individuals according to age group. (B) Distribution of anti-HBs serostatus according to biological sex. HB, hepatitis B.

Discussion

Our findings provide epidemiological insight into age-related patterns of detectable anti-HBs levels among individuals eligible for the national childhood hepatitis B vaccination program. Specifically, they describe the distribution of protective antibodies in a population presumed to have benefited from this childhood immunization program. Moreover, although a substantial proportion of the target population exhibited positive anti-HBs levels during early childhood, seropositivity declined with increasing age. This pattern raises important public health and clinical questions.

One of the key observations among individuals presumed to have been vaccinated under the national immunization program was that the median age of those without protective anti-HBs levels was higher than that of those with protective levels. This finding raises questions about the persistence of measurable antibody levels following childhood vaccination while acknowledging that immunological memory may persist despite declining antibody titers. McMahon et al.[7] investigated individuals aged 6 months and older who received three doses of the hepatitis B vaccine and reported that, after a 22-year follow-up, 60% retained protective antibody levels. A study conducted in Tunisia reported that the overall anti-HBs seropositivity rate among vaccinated students aged 12-17

Table 1. Mann-Whitney U test of median age by initial anti-HBs result.

	Median age (IQR) (years)	Mean rank	Sum of ranks	U	p
Anti-HBs negative (n = 1316)	18.5 (14-21)	1.683.2	2.215.082.5	975.559.5	<0.001
Anti-HBs positive (n = 1766)	17 (6-20)	1.435.9	2.535.820.5		

Anti-HBs, Hepatitis B surface antibody; IQR, Interquartile range.

Table 2. Multivariable logistic regression analysis of factors associated with anti-HBs seropositivity.

Variable	OR	95% CI	p
Age group (years)*			
4-6	0.31	0.19-0.50	<0.001
7-12	0.15	0.10-0.25	<0.001
13-18	0.12	0.08-0.19	<0.001
19-22	0.13	0.08-0.20	<0.001
Sex (female)†	1.26	1.09-1.47	0.002

OR, odds ratio; CI, confidence interval. Nagelkerke R^2=0.079; Hosmer-Lemeshow goodness-of-fit test p=0.877. *Compared with 1-3 years. †Compared with male sex.

years was 68.9%, with seroprotection decreasing significantly with age[8]. Another study found that only 40% of children who received three doses of the hepatitis B vaccine had detectable protective antibodies by week 35, and only 10% remained antibody-positive at 15 years of age[9]. Notably, we observed high antibody response rates in the younger age groups in our study. Once protective antibody levels are achieved following hepatitis B vaccination, long-term immune protection may persist even if measurable antibody levels decline over time[10]. This finding underscores the importance of confirming antibody responses at an early stage after vaccination.

Another factor that may explain the lower anti-HBs seropositivity observed in some older age groups is variation in vaccination coverage over time. In our country, the three-dose hepatitis B vaccination coverage rate was approximately 72% during the early years of the program but increased substantially to 96%-99.3% after 2019[11]. Therefore, the lower seropositivity observed, particularly among individuals aged 19–22 years, may not solely reflect waning detectable antibody levels over time but may also be attributable to lower vaccination coverage during the early years of program implementation. Because individual vaccination records were unavailable, the relative contributions of incomplete vaccination and declining antibody levels could not be distinguished in this cohort. Nevertheless, this finding underscores the importance of maintaining high vaccination coverage to ensure sustained population-level protection across age groups.

Biological sex has an important influence on the immune response[12]. Consistent with our findings, Anticoli et al.[13] reported that female healthcare workers had higher anti-HBs levels than their male counterparts. Another study conducted among vaccinated children reported a higher prevalence of HBV infection in boys than in girls, although the difference was not statistically significant[14]. These findings may be explained by inherent biological differences in immune responses between the sexes.

Based on our findings, we believe that assessing an individual's immune status is more accurate when information from an early post-vaccination antibody test, such as one performed at approximately 1 year of age, is available. However, evidence indicates that antibody levels may decline over time, and many vaccinated individuals lose detectable protective antibody levels after several years[8,15-17]. Bruce et al.[18] reported that a very high seroprotection rate was achieved with a booster dose administered 35 years after completion of the hepatitis B vaccination series. A review suggested that a single booster dose may be beneficial for individuals who fail to develop a protective antibody response following the primary vaccination series[19]. Another review emphasized the importance of incorporating a booster dose into national immunization schedules worldwide[20]. Furthermore, although antibody levels may decline over time, this does not necessarily indicate a loss of protection because immunological memory against HBsAg may persist even when antibodies are no longer detectable[3,10]. In the absence of information on the early antibody response, individuals may be misclassified as non-immune over time. This could result in unnecessary revaccination, increased healthcare costs, and additional workload for healthcare providers. Early confirmation of anti-HBs positivity may help optimize resource utilization and prevent unnecessary interventions by demonstrating vaccine-induced immunity. Establishing national frameworks for the early assessment of antibody responses, particularly in settings with ongoing hepatitis B transmission or intermediate-to-high endemicity, could provide a stronger foundation for developing vaccination strategies. Such an approach may contribute to more efficient resource allocation, reduced healthcare costs, and fewer unnecessary revaccinations, particularly in resource-limited settings.

Study Limitations

Our study has several limitations. First, its retrospective design limited our ability to control for unmeasured confounding factors. Second, the lack of confirmed vaccination history represents an important limitation and prevents direct interpretation of anti-HBs status as evidence of vaccine-induced immunity. Consequently, definitive conclusions regarding the long-term effectiveness of hepatitis B vaccination cannot be drawn, although outcomes were evaluated across age groups targeted by the national vaccination program. Another limitation is that the study was conducted at a single institution, which may limit the generalizability of the findings. However, we believe that our results provide a useful foundation for future studies and may be applicable to other healthcare systems in similar settings.

Conclusion

The high apparent effectiveness observed during early childhood confirms the success of the national hepatitis B vaccination program and underscores the need for more refined post-vaccination monitoring strategies. Although routine post-vaccination testing is not generally recommended for healthy individuals, anti-HBs testing performed between 1 and 3 years of age may be considered in selected high-burden settings to support immunization monitoring, help avoid unnecessary revaccination, and reduce the burden on healthcare systems. However, the cost-effectiveness of such an approach should be evaluated in future studies. These findings highlight the importance of continued evaluation of post-vaccination immunity and support further research to determine optimal follow-up strategies. By informing evidence-based policies and

vaccination strategies, this study provides a useful framework for addressing similar public health challenges globally and promoting a more sustainable and effective approach to hepatitis B vaccination.

Acknowledgment

We express our sincere gratitude to all the physicians whose expertise and efforts contributed to the evaluation of the individuals included in this retrospective study.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Recep Tayyip Erdoğan University (decision no: 2023/237, date: 12.10.2023).

Informed Consent: Informed consent was waived because of the retrospective nature of the study.

Footnotes

Author Contributions

Concept: A.Ö., İ.E.Y., U.K., Y.Y., A.E., Design: A.Ö., İ.E.Y., U.K., Y.Y., A.E., Data Collection or Processing: T.İ., A.Ö., S.M.Ç., Analysis or Interpretation: T.İ., A.Ö., S.M.Ç., M.G.G., Literature Search: T.İ., A.Ö., S.M.Ç., M.G.G., Writing: T.İ., A.Ö., S.M.Ç., İ.E.Y., U.K., Y.Y., M.G.G., A.E.

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CASE REPORT / OLGU SUNUMU

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Invasive Fusariosis with Disseminated Skin Lesions in an Allogeneic Hematopoietic Stem Cell Transplant Recipient: A Case Report and Literature Review

Allojenik Hematopoietik Kök Hücre Nakli Alıcısında Dissemine Deri Lezyonlarıyla Seyreden İnvaziv Fusariozis: Olgu Sunumu ve Literatür Derlemesi

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Abstract

Infections are a major cause of mortality in immunocompromised patients. Invasive fusariosis is a rare opportunistic fungal infection associated with a poor prognosis. We present a rare and challenging case of a 46-year-old man who underwent allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia (M4) and subsequently developed disseminated skin lesions. This case highlights the importance of maintaining a high index of clinical suspicion for invasive fusariosis in immunocompromised patients, particularly because empirical treatment can be challenging owing to the high minimum inhibitory concentration values for amphotericin B. Early microbiological diagnosis and prompt initiation of appropriate antifungal therapy are essential for improving treatment outcomes. Despite receiving antifungal therapy, the patient died of thrombotic complications. Further research is needed to develop effective strategies for the prevention and treatment of invasive fusariosis.

Keywords: Allogeneic hematopoietic stem cell transplantation, antifungal susceptibility, fungal infection, opportunistic infections

Öz

Enfeksiyonlar, immünkompromize hastalarda önemli bir mortalite nedeni olmaya devam etmektedir. İnvaziv fusariozis, nadir görülen, kötü prognostik fırsatçı bir mantar enfeksiyonudur. Akut miyeloid lösemi (M4) nedeniyle allojenik hematopoietik kök hücre nakli uygulanan ve daha sonra dissemine deri lezyonları gelişen 46 yaşındaki erkek hastaya ait nadir ve zorlu bir olguyu sunuyoruz. Bu olgu, immünkompromize hastalarda, özellikle amfoterisin B'ye karşı yüksek minimum inhibitör konsantrasyon değerleri göstermesi nedeniyle ampirik tedavinin zor olduğu durumlarda, invaziv fusariozis için klinik şüphe duyulmasının önemini vurgulamaktadır. Erken mikrobiyolojik tanı ve antifungal tedavi çok önemlidir. İnvaziv fusariozis için önleme ve tedavi stratejileri konusunda daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Allojenik hematopoietik kök hücre nakli, antifungal duyarlılık, fungal enfeksiyon, fırsatçı enfeksiyonlar

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Introduction

Fusarium spp. are recognized as soil saprophytes and important plant pathogens. Certain species can also cause infections in humans. The most common infections caused by *Fusarium* spp. are onychomycosis and keratitis. However, in immunocompromised patients, *Fusarium* spp. can also cause disseminated, life-threatening systemic infections[1].

With the increasing number of solid organ and hematopoietic stem cell transplant (HSCT) recipients, as well as the widespread use of immunomodulatory therapies such as tumor necrosis factor antagonists, the incidence of invasive fungal infections has been increasing worldwide[2]. Although *Fusarium* spp. are relatively rare fungal pathogens, they have been increasingly reported in patients with hematologic malignancies and HSCT recipients, particularly those with profound and prolonged neutropenia[3]. Skin lesions associated with invasive fusariosis typically occur as part of disseminated disease and commonly present as papules or nodules, with or without necrosis. Disseminated fusariosis may also be accompanied by fungemia and characteristic skin lesions, and pulmonary or sinus involvement has been reported. Despite appropriate treatment, the mortality rate remains greater than 50%[4].

In this report, we present a case of invasive fusariosis in a severely immunosuppressed patient who developed the infection after undergoing HSCT for acute myeloid leukemia (AML) and subsequently received chemotherapy for disease relapse.



Figure 1. Erythematous, slightly raised nodular lesions with central necrosis on the extremities and head.

Case Report

A 46-year-old man who had undergone allogeneic HSCT 1 year earlier for AML was hospitalized after a bone marrow smear performed because of abnormal blood counts revealed blastic infiltration. Following the diagnosis of post-HSCT relapse of AML, intensive induction chemotherapy was reinitiated. After receiving the FLAG-IDA (fludarabine, cytarabine, idarubicin, and granulocyte colony-stimulating factor) regimen, the patient achieved complete remission, and a second transplant was planned. Following consolidation chemotherapy, a second allogeneic HSCT was performed using a 9/10 HLA-matched unrelated donor. On post-transplant day 18, while profoundly neutropenic and receiving posaconazole prophylaxis, the patient developed fever and painless, non-pruritic, erythematous, slightly raised nodular lesions measuring less than 1 cm on the extremities and head (Figure 1).

On physical examination, the patient's Glasgow Coma Scale score was 15. Vital signs were as follows: temperature, 38.8 °C; pulse rate, 110 beats/min; respiratory rate, 22 breaths/min; and blood pressure, 110/60 mmHg. Laboratory findings showed normal liver and kidney function, a C-reactive protein level of 95 mg/L (reference range, 0–5 mg/L), a leukocyte count of $0.04 \times 10^3/\mu L$, a neutrophil count of $0 \times 10^3/\mu L$, a hemoglobin level of 8.2 g/dL, and a platelet count of $13 \times 10^3/\mu L$. Blood cultures were obtained, and chest computed tomography and a skin biopsy were performed. Antifungal therapy was changed to liposomal amphotericin B (10 mg/kg/day). Chest computed tomography revealed a nodular lesion in the left lung (Figure 2).

Pathological examination of the skin biopsy revealed concurrent leukemic infiltration and fungal hyphae (Figure 3), and *Fusarium* spp. was isolated from blood cultures (Figure 4).

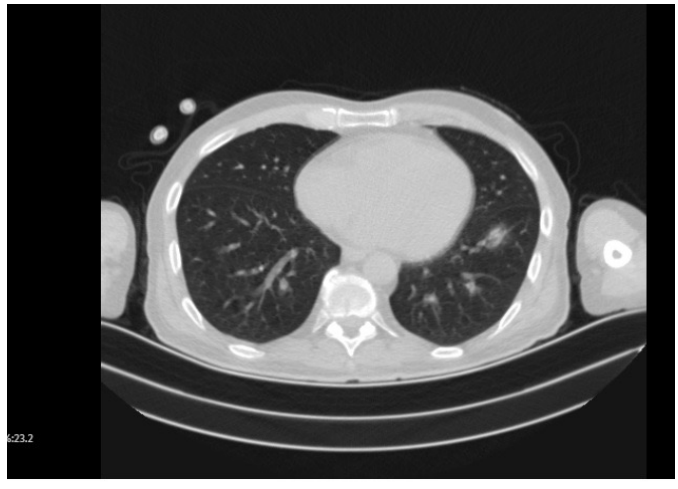

Figure 2. Chest computed tomography showing a nodular infiltrate in the left lung.

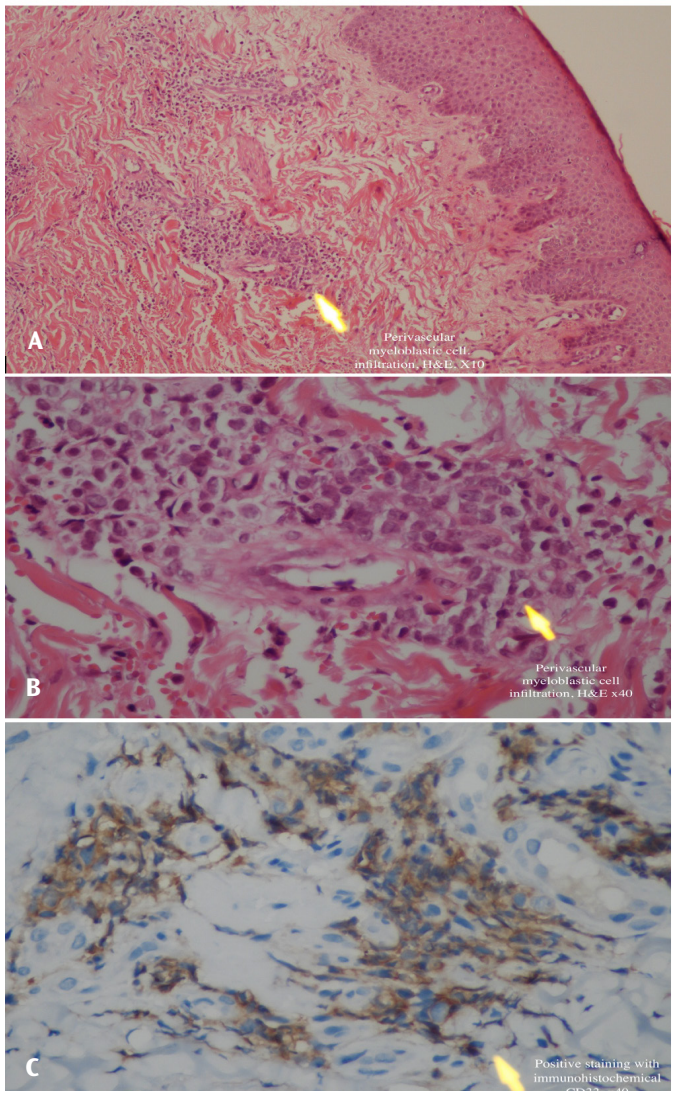

Figure 3. Pathological examination of the skin biopsy. (A) Perivascular myeloblastic cell infiltration (H&E, ×10). (B) Higher-magnification view of myeloblastic infiltration (H&E, ×40). (C) Positive CD33 immunohistochemical staining (×40). H&E, hematoxylin and eosin.

Antifungal susceptibility testing (AFST), performed according to the Clinical and Laboratory Standards Institute M38 guideline, demonstrated a minimum inhibitory concentration (MIC) of >32 µg/mL for amphotericin B, 4 µg/mL for voriconazole, and 32 µg/mL for echinocandins. Voriconazole was subsequently added to the treatment regimen. Although the patient's fever subsided, the central venous catheter was removed because blood cultures remained positive after 72 hours of treatment. Following catheter removal, the patient became afebrile, and subsequent blood cultures showed no microbial growth. Despite antifungal therapy, the patient died on day 18 of treatment due to thrombotic complications. Written informed consent for publication of the clinical information and accompanying images was obtained from the patient.

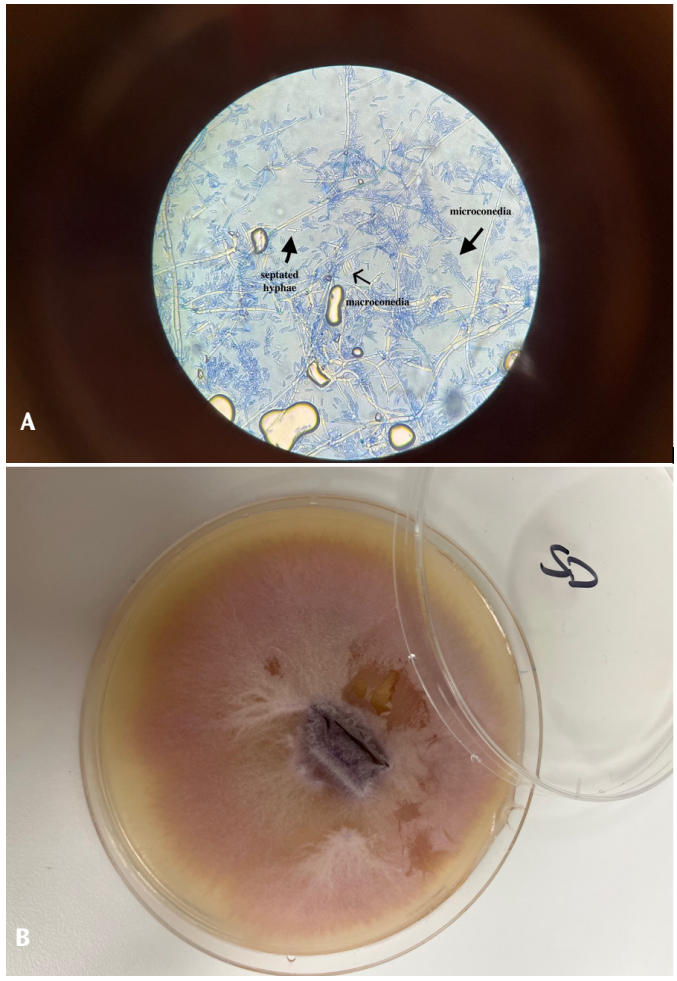

Figure 4. Microbiological identification of *Fusarium* spp. (A) Macroconidia, microconidia, and septate hyphae observed in a lactophenol cotton blue preparation. (B) Pinkish-purple, cottony colony growth on Sabouraud dextrose agar.

Discussion

We report a case of invasive fusariosis in a severely immunocompromised patient who underwent two allogeneic HSCT procedures. Advances in the treatment of hematologic malignancies have improved patient outcomes but have also increased the risk of opportunistic infections[5]. Invasive infections caused by rare fungal pathogens are being reported with increasing frequency in patients with profound immunosuppression. Among these, invasive fusariosis has become increasingly recognized in patients with hematologic malignancies. Individuals with impaired immune function, particularly those with prolonged and profound neutropenia or severe T-cell immunodeficiency, as well as those receiving immunosuppressive therapies such as systemic corticosteroids or chemotherapy, are at particularly high risk[6]. Cases of invasive fusariosis in patients with hematologic malignancies reported during the past 3 years are summarized in Table 1[3,7-17].

Table 1. Reported cases of *Fusarium* infection in immunosuppressed patients.

References	Year	Symptom	Organ involvement	Underlying disease	Treatment	Etiological agent	MIC amphotericin B	Voriconazole
Samborska et al.[3]	2023	Nodular lesion on skin		Relapse ALL	Liposomal amphotericin B + voriconazole	*Fusarium oxysporum*	-	-
Furaijat et al.[7]	2023	Defect of vision	Keratitis + aortic valve involvement	T-cell leukemia and allogeneic bone marrow transplantation	Liposomal amphotericin B + natamycin	*Fusarium petroliphilum*	1 µg/mL	
Kao et al.[8]	2023	Visual impairment + hypoxia	Keratitis + pulmonary nodule	Myelodysplasia + allogeneic bone marrow transplantation	Liposomal amphotericin B + voriconazole	*Fusarium* spp.	>8 µg/mL	>16 µg/mL
Marino et al.[9]	2023	Ulcerated necrotic lesion on the nose		AML + COVID-19	Liposomal amphotericin B + posaconazole	*Fusarium solani* + *Mucorales*		>8 µg/mL
Hannemann et al.[10]	2022	Nodular lesion on skin + fever +	Hepatic renal splenic nodules + left ventricular thrombus	Aplastic anemia Allogeneic stem cell transplantation	Liposomal amphotericin B + voriconazole	*Fusarium solani*	R.	S
Zhong et al.[11]	2022	Necrotic nodular lesion on the skin		ALL	Voriconazole + terbinafine	*Fusarium fujikuroi*	>32 µg/mL	0.25 µg/mL
Fei et al.[12]	2022	Nodular lesion on the skin	Liver-spleen hypodense lesions	ALL + CAR-T + allogeneic bone marrow transplantation	Liposomal amphotericin B + posaconazole-voriconazole	*Fusarium* spp.	-	-
De la Espriella et al.[13]	2022	Necrotic nodular lesion on the skin		Relapse ALL	Liposomal amphotericin B + voriconazole	*Fusarium solani*	8 µg/mL	3 µg/mL
De la Espriella et al. [13]	2022	Generalized erythematous maculopapular skin lesions with a violaceous center + fever	Disseminated osteomyelitis, myositis, and septic arthritis	AML	Liposomal amphotericin B + voriconazole + caspofungin	*Fusarium solani*	3 µg/mL	>32 µg/mL
Brent et al.[14]	2022	Necrotic nodular lesion on the skin + hypoxia	Pulmonary nodule	B-ALL	Liposomal amphotericin B + voriconazole-flucytosine	*Bisifusarium delphinoides*	1 µg/mL	4 µg/mL
Asiimwe et al.[15]	2022	Nodular lesion on the skin + headache sinus pain	Pulmonary nodule Sphenoidal occlusion	Relapsed AML	Liposomal amphotericin B + voriconazole-terbinafine	-	1 µg/mL	8 µg/mL
Al-Farsi et al.[16]	2022	Nodular lesion on the skin		ALL	Liposomal amphotericin B + voriconazole	*Fusarium solani*	1 µg/mL	16 µg/mL
Risum et al.[17]	2022	Headache pain in sinuses	Maxillary cranial involvement	AML	Liposomal amphotericin B + voriconazole-terbinafine	*Fusarium dimerum*	0.5 µg/mL	8 µg/mL

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia; CAR-T, chimeric antigen receptor T-cell therapy; COVID-19, coronavirus disease 2019; MIC, minimum inhibitory concentration.

Our case highlights that prolonged and profound neutropenia during both the AML treatment and HSCT periods was the predominant risk factor for the development of invasive fusariosis.

Fusarium spp. typically cause superficial or localized infections in immunocompetent individuals, whereas in immunocompromised patients, they can cause life-threatening invasive systemic infections. Skin lesions are reported in approximately 70%–75% of cases of invasive fusariosis. In our patient, extensive skin involvement was accompanied by pulmonary involvement and severe immunosuppression[18]. The thrombotic complications observed in our patient may have been associated with the angioinvasive nature of *Fusarium* spp. Angioinvasion can lead to endothelial damage, thrombosis, and possible fungal embolization, all of which may contribute to poor clinical outcomes in patients with invasive fungal disease.

Although the mortality rate exceeds 50%, no gold standard treatment for invasive fusariosis has been established. The limited number of clinical studies on *Fusarium* spp., together with insufficient data on molecular resistance mechanisms, has hindered the establishment of species-specific MIC breakpoints[19]. Species- or species complex-level identification could not be performed because molecular diagnostic methods were unavailable at our center. This represents an important limitation, as identification of the infecting species or species complex (e.g., *Fusarium solani* species complex or *Fusarium oxysporum* species complex) may significantly influence antifungal susceptibility patterns and treatment decisions. Among the available antifungal agents, amphotericin B generally demonstrates the lowest MIC values against *Fusarium* spp., although susceptibility varies considerably among isolates. Voriconazole, posaconazole, and isavuconazole also exhibit antifungal activity, whereas echinocandins are generally considered intrinsically ineffective against *Fusarium* spp.[20]. In six of the cases with available numerical amphotericin B MIC data, the amphotericin B MIC was <4 µg/mL. In contrast, the MIC observed in our case was markedly higher than those reported in the literature (Table 1). This finding highlights the substantial variability in antifungal susceptibility among *Fusarium* isolates and emphasizes the importance of performing AFST to guide individualized therapy. In our case, invasive fusariosis developed despite posaconazole prophylaxis, and the isolate demonstrated voriconazole and amphotericin B MIC values of 4 µg/mL and >32 µg/mL, respectively. The markedly elevated amphotericin B MIC suggests intrinsic or potentially acquired resistance. *Fusarium* species are known to exhibit variable, and often elevated, MIC values for commonly used antifungal agents, which can substantially limit treatment options. Reduced susceptibility to amphotericin B has been associated with differences in cell membrane composition, biofilm formation, and species-specific resistance mechanisms. The elevated MIC observed in our isolate may explain the poor clinical response despite early initiation of liposomal amphotericin B. These findings underscore the importance of AFST in guiding individualized treatment strategies, particularly in immunocompromised patients with disseminated infection[19,20]. However, not all institutions have access to specialized laboratory testing such as AFST or molecular species identification. In resource-limited settings or remote regions, collaboration with reference laboratories should be considered whenever possible. As illustrated by the cases summarized in Table 1, AFST can provide valuable information for optimizing the management of invasive *Fusarium* infections.

Conclusion

Fusarium spp. can cause invasive fungal infections with high mortality rates in immunocompromised patients and remain challenging to diagnose and treat. Early diagnosis and individualized patient evaluation are essential because well-established, evidence-based treatment recommendations are lacking.

Ethics

Informed Consent: Written informed consent for publication of the clinical information and accompanying images was obtained from the patient.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.K., H.S., A.E.T., Concept: A.K., E.E.E., S.K., Design: A.K., E.E.E., H.S., A.E.T., S.K., Data Collection or Processing: A.K., E.E.E., H.S., A.E.T., S.K., Analysis or Interpretation: A.K., Literature Search: A.K., E.E.E., Writing: A.K., E.E.E.

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RESEARCH ARTICLE / ARAŞTIRMA

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Seroprevalence of HPV Infection in Healthcare Workers with Molecular Confirmation of High-Risk HPV

Sağlık Çalışanlarında HPV Enfeksiyonunun Seroprevalansı ve Yüksek Riskli HPV'nin Moleküler Doğrulaması

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Abstract

Introduction: High-risk human papillomavirus (HR-HPV) genotypes, particularly HPV-16 and HPV-18, are the primary etiologic agents of various carcinomas. Healthcare workers face a significant risk of non-sexual occupational exposure to oncogenic HPV through the inhalation of surgical smoke and aerosols generated during medical procedures. This study aimed to determine the seroprevalence of anti-HPV IgG and IgM antibodies and detect circulating HR-HPV deoxyribonucleic acid (DNA) among healthcare workers to assess potential occupational cancer risks.

Materials and Methods: A cross-sectional study evaluated 170 healthcare workers from general hospitals in Al-Shatra General Hospital and Al-Haboubi Teaching Hospital in Dhi Qar Governorate, Iraq. Serum concentrations of anti-HPV IgG and IgM were quantified using an enzyme-linked immunosorbent assay. To investigate transient HPV DNAemia indicative of intense occupational exposure, conventional polymerase chain reaction (PCR) was performed on a subset of 100 seropositive samples (IgM >0.25 nmol/L) to detect circulating cell-free DNA of HPV-16 and HPV-18.

Results: A remarkably high seroprevalence was observed, with 95.9% and 90.0% of participants testing positive for anti-HPV IgG and IgM, respectively. Despite this, molecular analysis revealed a low rate of active viral shedding; HPV-16 DNA was detected in only 7.0% of the subset, whereas HPV-18 was not detected. Participants who tested positive for HPV-16 by PCR exhibited significantly higher anti-HPV IgM levels than PCR-negative individuals ($p < 0.0001$).

Conclusion: The substantial discrepancy between the widespread serological prevalence and the low rate of molecular detection may indicate a potential association between occupational exposure to HR-HPV and transient viremia. This unrecognized exposure underscores a potential latent oncogenic hazard in clinical settings. These findings highlight the urgent need for stringent personal protective measures, improved ventilation during procedures, and targeted prophylactic vaccination strategies for healthcare personnel to mitigate the risk of HPV-driven cancers.

Keywords: Human papillomavirus 16, human papillomavirus 18, ELISA, PCR

Öz

Giriş: Yüksek riskli insan papillomavirüsü (HR-HPV) genotipleri, özellikle HPV-16 ve HPV-18, çeşitli karsinomların birincil etiyolojik ajanlarıdır. Sağlık çalışanları, cerrahi duman ve tıbbi işlemler sırasında oluşan aerosollerin solunması yoluyla onkojenik HPV›ye cinsel olmayan mesleki maruz kalma riskiyle karşı karşıyadır. Bu çalışma, potansiyel mesleki kanser risklerini değerlendirmek amacıyla sağlık çalışanları arasında anti-HPV IgG ve IgM antikorlarının seroprevalansını belirlemeyi ve dolaşımdaki HR-HPV DNA›sını tespit etmeyi amaçlamıştır.

Gereç ve Yöntem: Kesitsel bir çalışmada, Al-Shatra Genel Hastanesi ve Al-Haboubi Eğitim Hastanesi bölgesindeki genel hastanelerden 170 sağlık çalışanı değerlendirilmiştir. Anti-HPV IgG ve IgM'nin serum konsantrasyonları, enzim bağlantılı immünosorbent testi kullanılarak ölçülmüştür. Yoğun mesleki maruziyete işaret eden geçici HPV DNAemisini araştırmak için, dolaşımdaki hücre dışı HPV-16 ve HPV-18 DNA'sını tespit etmek amacıyla 100 seropozitif örnekten oluşan bir alt kümede (IgM > 0,25 nmol/L) geleneksel polimeraz zincir reaksiyonu (PCR) uygulandı.

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Bulgular: Katılımcıların %95,9'unda ve %90,0'ında sırasıyla anti-HPV IgG ve IgM için pozitif test sonucu elde edilmesiyle oldukça yüksek bir seroprevalans gözlemlendi. Buna rağmen, moleküler analiz aktif viral yayılım oranının düşük olduğunu ortaya koydu; HPV-16 DNA'sı alt kümenin sadece %7,0'ında tespit edilirken, HPV-18 tespit edilmedi. PCR ile HPV-16 için pozitif test sonucu veren katılımcılar, PCR negatif bireylere göre anlamlı derecede daha yüksek anti-HPV IgM seviyeleri sergiledi ($P < 0,0001$).

Sonuç: Yaygın serolojik prevalans ile düşük moleküler tespit oranı arasındaki önemli tutarsızlık, mesleki olarak yüksek riskli HPV'ye maruz kalma ile geçici viremi arasında potansiyel bir ilişkiye işaret edebilir. Bu fark edilmeyen maruziyet, klinik ortamlarda potansiyel bir latent onkojenik tehlikeyi vurgulamaktadır. Bu bulgular, HPV kaynaklı kanser riskini azaltmak için sağlık personeline yönelik sıkı kişisel koruyucu önlemlere, işlemler sırasında iyileştirilmiş havalandırmaya ve hedefli profilaktik aşılama stratejilerine acil ihtiyaç olduğunu vurgulamaktadır.

Anahtar Kelimeler: İnsan Papillomavirüsü 16; İnsan Papillomavirüsü 18; ELISA; PCR

Introduction

Millions of new HPV infections occur annually worldwide, highlighting the remarkable transmissibility and widespread distribution of the virus. It has been estimated that approximately 6.2 million people acquire a new HPV infection each year[1]. Human papillomavirus (HPV) is one of the most prevalent sexually transmitted infections worldwide and imposes a substantial public health burden[2]. Taxonomically classified within the Papillomaviridae family, HPV is a small, non-enveloped, double-stranded deoxyribonucleic acid (DNA) virus with an approximate diameter of 55 nm[3]. The viral genome encodes eight proteins, among which the E6 and E7 oncoproteins are of paramount clinical significance. These oncoproteins play a critical role in cellular transformation and oncogenesis by disrupting essential cell cycle regulatory mechanisms. Specifically, E6 facilitates the degradation of the tumor suppressor protein p53, thereby enabling evasion of apoptosis, whereas E7 binds to and inactivates the retinoblastoma protein (pRb), leading to uncontrolled cellular proliferation[4]. The intricate molecular interplay between these viral oncoproteins and host cellular machinery underpins the pathogenesis of HPV-induced malignancies.

To date, more than 200 distinct HPV genotypes have been identified and characterized. Based on their oncogenic potential and epidemiological association with cancer, approximately 13-15 genotypes are classified as high-risk HPV (HR-HPV)[5]. These high-risk variants, including HPV-16, HPV-18, HPV-31, HPV-33, and HPV-35, exhibit a strong oncogenic potential and are closely associated with the development of various anogenital and oropharyngeal carcinomas. Conversely, low-risk genotypes are predominantly associated with benign verruciform lesions[6]. Persistent infection with HR-HPV is the primary causative factor in the development of precancerous and malignant lesions of the cervix, vulva, vagina, penis, and anus. Globally, HPV-16 and HPV-18 are the most prevalent oncogenic genotypes, collectively accounting for more than 70% of all invasive cervical cancer cases[7,8]. The substantial oncogenic potential of these genotypes necessitates rigorous surveillance and molecular profiling in susceptible populations. The transmission dynamics of HPV are predominantly characterized by direct skin-to-skin sexual contact. However, accumulating epidemiological and clinical evidence indicates that non-sexual routes of transmission also contribute to viral spread. These alternative routes include vertical transmission from mother to infant during childbirth, direct contact with contaminated fomites, and horizontal transmission among children through non-sexual physical contact with infected cutaneous lesions[9]. Furthermore, the persistence of the virus in environmental reservoirs and its resistance to desiccation highlight the potential for fomite-mediated transmission in specific settings. In the context of occupational health, healthcare workers are increasingly recognized as a population at risk of non-sexual HPV exposure. Medical personnel, particularly those specializing in gynecology, dermatology, otolaryngology, and urology, frequently encounter HPV-infected tissues. A significant occupational hazard arises from the generation of surgical smoke and aerosols during electrosurgical ablation, laser vaporization, or excision of HPV-associated lesions[10]. Viral DNA and intact virions have been detected in surgical plumes, posing a risk of inhalation-mediated inoculation of the nasal and oropharyngeal mucosa. Consequently, the occurrence of HPV-associated oropharyngeal disease among healthcare providers has prompted investigations into occupational transmission risks. Although prophylactic vaccination, the consistent use of personal protective equipment (PPE), and effective local exhaust ventilation are recommended to mitigate these hazards, comprehensive data on the actual serological and molecular prevalence of HPV among healthcare workers remain limited[11].

Given the potential for unrecognized occupational exposure and the subsequent risk of acquiring high-risk oncogenic strains, it is imperative to evaluate the immunological and virological status of healthcare personnel. Although HPV is strictly an epitheliotropic virus, emerging evidence suggests that circulating cell-free HPV DNA (HPV DNAemia) can be detected in the bloodstream, primarily in patients with invasive HPV-associated carcinomas. However, it remains unknown whether intense occupational exposure, such as the chronic inhalation

of intact virions or viral DNA fragments from surgical smoke, could lead to the transient translocation of these fragments into the systemic circulation. Therefore, exploring the presence of circulating HPV DNA in serum may serve as a novel biomarker of substantial systemic occupational exposure rather than an indicator of localized active infection.

This study aimed to determine the seroprevalence of anti-HPV IgG and IgM antibodies among healthcare workers in Al-Shatra General Hospital and Al-Haboubi Teaching Hospital in Dhi Qar Governorate, Iraq and to investigate the presence of circulating high-risk HPV DNA fragments (HPV-16 and HPV-18) in serum using conventional polymerase chain reaction (PCR) assays as an indicator of transient DNAemia.

Materials and Methods

Study Design and Participants

A descriptive cross-sectional study was conducted between November 2025 and December 2025 across multiple general hospitals in Al-Shatra General Hospital and Al-Haboubi Teaching Hospital in Dhi Qar Governorate, Iraq. The study cohort comprised 170 healthcare workers, including physicians, nurses, and allied healthcare staff from various clinical departments. Specifically, the cohort included surgical staff and gynecologists who were frequently exposed to surgical plumes, laboratory technologists handling clinical specimens, and ward nurses engaged in routine patient care. In accordance with the principles of the Declaration of Helsinki, the research protocol was reviewed and approved by the University of University of Thi-Qar Institutional Scientific Research Ethics Committee (approval no: B-2025-41630, date: 03.11.2025). Written informed consent was obtained from all participants before enrollment and subsequent data collection.

Inclusion and Exclusion Criteria

The target population consisted of male and female healthcare workers aged 20-59 years. The inclusion criteria required participants to have no prior history of prophylactic HPV vaccination, no clinical symptoms indicative of sexually transmitted infections (e.g., genital warts), and a willingness to provide informed consent. Individuals with chronic systemic diseases, pregnant women, and older adults (≥60 years) were excluded from the study to minimize confounding immunological variables. Vaccination status was determined by self-report; although this approach may introduce slight recall bias, the risk is considered minimal because the HPV vaccine is not currently part of the routine national immunization program in the region.

Sample Collection and Preparation

Peripheral venous blood samples (5 mL) were collected from each participant using standard aseptic venipuncture techniques. Whole blood was allowed to clot at room temperature for 30 min, followed by centrifugation at 3,000 × *g* for 10 min. The separated serum was carefully aliquoted into sterile microcentrifuge tubes and stored at -20 °C for a maximum of four weeks before subsequent serological and molecular analyses to preserve nucleic acid integrity.

Serological Assay Procedure

The quantitative determination of anti-HPV IgG and IgM antibodies in serum samples was performed using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit (Sunlong Biotech, China) according to the manufacturer's instructions. Optical density was measured immediately at 450 nm using a microplate reader. Samples with IgG levels >1 nmol/L and IgM levels >0.25 nmol/L were classified as seropositive. These seropositivity thresholds were established strictly according to the manufacturer's instructions without additional modifications by the local laboratory.

Exploratory Detection of Circulating Cell-Free HPV DNA

To investigate the possibility of transient HPV DNAemia resulting from occupational exposure, viral nucleic acids (cell-free DNA) were extracted from 200 µL of serum using the Viral Nucleic Acid Extraction Kit III (Geneaid, Taiwan). For this molecular analysis, a subset of 100 seropositive samples was used. This subset was purposively selected based on the highest anti-HPV IgM concentrations to maximize the probability of detecting transient DNAemia. This kit is specifically optimized for cell-free samples. This system employs a glass fiber spin column optimized for cell-free samples, yielding purified viral DNA within 60 min. The detection of HPV-16 and HPV-18 was performed using conventional PCR with specific primers designed by Macrogen (Seoul, South Korea). The primer sequences were as follows: HPV-16 (5'-CACAGTTATGCACAGAGCTGC-3' and 5'-CATATATTCATGCAATGTAGGTGTA-3') and HPV-18 (5'-CACTTCACTGCAAGACATAGA-3' and 5'-GTTGTGAAATCGTCGTTTTTCA-3')[12]. PCR amplification was conducted in a total reaction volume of 25 µL, comprising 10 µL of master mix, 1 µL each of the forward and reverse primers (10 pmol/µL), 5 µL of the extracted DNA template, and 8 µL of nuclease-free water. The thermal cycling conditions included an initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 45 s. A final extension step was performed at 72 °C for 4 min. The amplified products were resolved by electrophoresis on a 1.8% agarose gel stained with 0.3 mg/mL

safe dye at 80 V for 30 min. Visualization under ultraviolet light identified positive samples based on the presence of a 457-bp amplicon for HPV-16 and a 322-bp amplicon for HPV-18.

Statistical Analysis

Due to the exploratory nature of this study and the specific occupational demographic targeted, a non-probability convenience sampling method was used. The final sample size of 170 healthcare workers was determined based on the availability of eligible participants who met the predefined inclusion and exclusion criteria and provided informed consent during the two-month study period.

Data management and statistical analyses were performed using (insert software, e.g., IBM SPSS Statistics version 26.0). Categorical variables were presented as frequencies and percentages, whereas continuous variables were expressed as means ± standard deviation (SD) or medians (ranges), depending on the normality of the data distribution, which was assessed using the Shapiro-Wilk test and confirmed to be non-normal. Group comparisons were performed using the non-parametric Mann-Whitney U test for continuous variables and the chi-square test for categorical variables. A two-sided p-value < 0.05 was considered statistically significant. Due to the limited number of PCR-positive cases (n = 7), advanced predictive modeling was deferred to prevent statistical overfitting and ensure the robustness of the descriptive findings.

Results

The study cohort consisted of 170 healthcare workers. The age distribution showed that the majority of participants were younger than 30 years (n = 95, 55.9%), followed by those aged 30-39 years (n = 61, 35.9%) and those aged ≥40 years (n = 14, 8.2%). The sex distribution was nearly equal, with 86 males (50.6%) and 84 females (49.4%). Regarding marital status, 121 participants (71.2%) were married, 44 (25.9%) were single, and 5 (2.9%) were divorced (Table 1).

Serological evaluation demonstrated a remarkably high prevalence of HPV-specific antibodies among healthcare workers. Specifically, 95.9% (n = 163) of the participants tested positive for anti-HPV IgG antibodies. Most seropositive individuals (78.2%) exhibited moderate IgG concentrations ranging from 1 to 4 nmol/L. Only a small proportion (6.5%) demonstrated high IgG titers exceeding 12 nmol/L. The overall median IgG concentration across the cohort was 2.04 nmol/L (range, 1.77-2.15 nmol/L). Similarly, a high seroprevalence of anti-HPV IgM antibodies was observed, with 90.0% (n = 153) of the participants testing positive. Among the seropositive participants, 77.1% had IgM concentrations between 0.25 and 1 nmol/L, whereas 2.9% displayed elevated levels greater than 3 nmol/L. The median IgM concentration was 0.39 nmol/L (range, 0.35-0.44 nmol/L). Statistical analysis revealed highly significant differences in the distribution of both IgG and IgM concentrations across the study population ($p < 0.001$) (Table 2).

To validate the presence of active high-risk HPV infections, conventional PCR targeting HPV-16 and HPV-18 was performed on a subset of 100 samples exhibiting moderate-to-high anti-HPV IgM concentrations (>0.25 nmol/L). Despite the high seroprevalence, molecular analysis indicated a low frequency of active viral shedding. Of the 100 samples tested, 93 (93.0%) were negative for both genotypes. Only seven samples (7.0%) tested positive for HPV-16 DNA, whereas all samples were negative for HPV-18.

A comparative analysis of IgM concentrations between PCR-positive and PCR-negative individuals revealed significant differences. Individuals who tested positive for HPV-16 by PCR exhibited significantly higher IgM levels (mean ± SD, 2.89 ± 0.19 nmol/L; range, 2.55-3.00 nmol/L) than PCR-negative individuals (mean ± SD, 0.73 ± 0.59 nmol/L; range, 0.338-2.492 nmol/L). A non-parametric Mann-Whitney U test confirmed that the elevation in anti-HPV IgM concentrations in the PCR-positive group was highly statistically significant compared with that in the PCR-negative group ($U = 651.0$, $p < 0.0001$) (Table 3). Given the limited number of PCR-positive cases (n =

Table 1. Demographic characteristics of the study population

	Occupation	Employee (n = 170)
Age groups	<30 years	95
	30–39 years	61
	≥40 years	14
Sex	Female	84
	Male	86
Marital status	Single Married Divorced	44 121 5
Total	170	

7), further predictive modeling (such as logistic regression or receiver operating characteristic curve analysis) was deferred to avoid statistical overfitting and maintain the robustness of the descriptive findings.

Discussion

Particular attention was given to the healthcare workers group to assess the prevalence of HPV exposure among individuals working in occupational settings. The present study evaluated the immunological response, represented by anti-HPV IgG and IgM antibodies, and confirmed active infection with high-risk genotypes through the detection of viral DNA in blood samples. The findings reveal a complex interplay between high seroprevalence and low molecular detection rates, underscoring the unique occupational risks faced by healthcare workers.

A central finding of this study is the striking discrepancy between serological and molecular detection rates. Serological analysis revealed an exceptionally high prevalence of HPV-specific antibodies, with 90.0% (153/170) testing positive for IgM and 95.9% (163/170) testing positive for IgG. This indicates widespread recent or ongoing exposure (IgM), as well as previous exposure and a long-term immune response (IgG). Despite this high seroprevalence, molecular detection using conventional PCR revealed a comparatively low frequency of active viral shedding, with only 7.0% of participants testing positive for HPV-16 and no cases detected for HPV-18.

This discrepancy between the serological and molecular findings reflects the natural history of HPV infection, in which viral clearance often occurs while specific antibodies remain detectable for prolonged periods[13]. Furthermore, recent studies have corroborated this phenomenon in different populations. For instance, Irakli et al.[14] investigated asymptomatic individuals in Georgia and reported a similar diagnostic divergence in the opposite direction, highlighting that serological and molecular assays measure fundamentally different aspects of HPV infection: historical immunological exposure vs. active viral replication. The serological assay used in the present study detects antibodies against a broad range of antigenically diverse HPV genotypes, whereas the molecular analysis specifically targeted only two high-risk genotypes (HPV-16 and HPV-18).

The high seroprevalence observed in this cohort raises significant concerns regarding occupational exposure among healthcare workers. Although sexual contact remains the primary route of HPV transmission, non-sexual occupational transmission is increasingly recognized as an important public health concern. Recent systematic reviews have highlighted that healthcare providers, particularly those treating HPV-associated lesions, face substantial occupational risks because of exposure to surgical smoke and aerosols generated during electrosurgical ablation, laser vaporization, or excision procedures[11].

Table 2. Serological detection and distribution of healthcare workers according to anti-HPV IgG and IgM concentrations.

Serological markers	Category (nmol/L)	Frequency	Percentage (%)	Median (range)	p-value
ELISA IgG	<1	6	3.50%	2.04 (1.77-2.15)	<0.001
	1-4	133	78.20%		
	4-8	9	5.30%		
	8-12	11	6.50%		
	>12	11	6.50%		
ELISA IgM	<0.25 0.25-1	17 131	10.00 77.10	0.39 (0.35-0.44)	<0.001
	1-2	9	5.30		
Total	2-3 >3	8 5 170	4.70 2.90		

ELISA, enzyme-linked immunosorbent assay; IgM, immunoglobulin M.

Table 3. Descriptive statistics of anti-HPV IgM distribution with PCR results.

PCR results	n	Anti-HPV IgM mean	SD	Median	Min.	Max.
Negative	93	0.73	0.59	0.48	0.338	2.492
Positive	7	2.89	0.19	3.00	2.55	3.00

The results of the Mann-Whitney U test indicated a highly significant difference in IgM concentrations between PCR-positive and PCR-negative participants ($U=651.0$, $p<0.0001$).

anti-HPV, anti–human papillomavirus; IgM, immunoglobulin M; PCR, polymerase chain reaction; SD, standard deviation.

Fox-Lewis et al.[15] demonstrated that HPV DNA is frequently detected in surgical smoke and on PPE, as well as in the oral mucosa and nasal cavities of exposed healthcare workers. Furthermore, procedures such as endotracheal aspiration, intubation, and bronchoscopy have been identified as aerosol-generating procedures that may facilitate viral transmission[11]. Despite these established risks, awareness and preventive practices remain suboptimal. A study by Wu et al. [10] evaluating the knowledge, attitudes, and practices of healthcare providers revealed that fewer than 50% possessed adequate knowledge regarding occupational HPV exposure, and only half had been vaccinated against HPV despite strong recommendations from professional societies. The exceptionally high seroprevalence observed in our study cohort (95.9% for IgG and 90.0% for IgM) strongly suggests continuous environmental exposure within the hospital setting. In occupational environments, the chronic inhalation of aerosolized viral particles may serve as a persistent antigenic stimulus, potentially maintaining elevated IgM levels for longer than those observed in typical mucosal infections. However, this high positivity rate must be interpreted with an important methodological caveat. The commercial ELISA used in this study detects generic anti-HPV antibodies and may not strictly differentiate between mucosal high-risk genotypes and common cutaneous low-risk HPV types (e.g., those causing common skin warts). Consequently, immunological cross-reactivity with other highly prevalent Papillomaviridae strains cannot be entirely ruled out, which may have contributed to the elevated seropositivity rates. Importantly, because this study did not include a non-healthcare control group, community-acquired transmission cannot be definitively excluded and remains a significant confounding factor when interpreting the high seroprevalence. Despite this limitation, the detection of such robust immune responses underscores the need for strict adherence to PPE protocols and the implementation of effective local exhaust ventilation systems.

A demographic analysis of our cohort revealed significant associations between age, sex, marital status, and HPV seroprevalence. Most participants (55.9%) were younger than 30 years, and a statistically significant association was observed between younger age groups and a higher prevalence of HPV antibodies ($p < 0.001$). This finding is consistent with the results reported by Adoga et al.[16], who observed a higher prevalence of HPV antibodies among individuals younger than 30 years than among older cohorts. Similarly, Mujuni et al.[17] reported a steady decline in HPV seroprevalence with increasing age, from 46% among individuals aged 15-25 years to 16.3% among those aged 36-45 years. This pattern is largely attributed to higher levels of sexual activity among younger individuals and the immune system's ability to clear approximately 90% of HPV infections within 2 years, resulting in partial protective immunity that may reduce the risk of reinfection in older individuals[18].

It is important to note that this trend among younger cohorts may primarily reflect behavioral and lifestyle factors rather than being exclusively associated with occupational hazards. Regarding sex distribution, a statistically significant difference was observed ($P = 0.008$) between male (50.6%) and female (49.4%) healthcare workers. The slight predominance of men in the present study may reflect the occupational structure of the study population. These findings are partially consistent with those of a study conducted in China by Xiang et al.[19], which reported nearly identical prevalence rates among women (50.64%) and men (50.79%).[18] However, they contrast with the findings of I. Rezaee Azhar et al.[20] in Iran, where the prevalence was considerably higher among women (87%) than among men (13%). Marital status also appeared to influence exposure, as 71.2% of the healthcare workers in this study were married. Given that HPV is transmitted predominantly through sexual contact, marriage may cumulatively increase the likelihood of HPV exposure over time[21].

The detection of HPV-16 in 7.0% of the molecularly tested subset is clinically significant because of its substantial oncogenic potential and strong association with multiple anogenital and oropharyngeal malignancies[22]. The prevalence of HPV-16 observed in the present study is comparable to that reported by Bakir et al.[23], who found a prevalence of 8.0%. However, it is markedly lower than the 33.3% prevalence reported among women in Saudi Arabia by El-Daly et al.[24], suggesting substantial geographic and epidemiological variation in genotype distribution.

Notably, no cases of HPV-18 were detected in the present study. This absence may be attributed to the limited sample size or regional differences in genotype distribution. This finding is consistent with recent epidemiological data from Thailand (0.62% prevalence)[18] and the Philippines (0.92% prevalence) [25], which indicate a very low prevalence of HPV-18 compared with HPV-16. This observation is generally consistent with the regional epidemiological profile, in which HPV-16 has historically been far more prevalent than HPV-18 in the general population.

Study Limitations

A notable limitation of this study is the scarcity of published literature addressing the serological prevalence of HPV specifically among healthcare workers in the Middle East, making direct regional comparisons challenging. Additionally, the cross-sectional design precludes the establishment of causal relationships between specific occupational tasks and HPV acquisition. Furthermore, the broad-spectrum ELISA kit used in this study may have detected cross-reactive antibodies

against benign, non-oncogenic cutaneous HPV types, limiting our ability to attribute the serological findings exclusively to high-risk strains, because cross-reactivity with ubiquitous, non-oncogenic cutaneous HPV variants is highly probable. Future longitudinal studies with larger sample sizes, detailed occupational exposure histories (e.g., hours spent in operating rooms and specific procedures performed), and comprehensive genotyping of both high-risk and low-risk HPV strains are warranted. Moreover, the extraction of cell-free viral DNA was limited to a volume of 200 µL of serum; given the typically low abundance of circulating cell-free DNA, this relatively small volume may have resulted in false-negative findings, thereby underestimating the true prevalence of DNAemia. Additionally, the use of conventional PCR, which inherently has lower sensitivity and a higher limit of detection than quantitative real-timePCR, may have resulted in false-negative results in individuals with very low-copy transient DNAemia.

Conclusion

The findings of this study highlight a widespread history of prior and ongoing HPV exposure among healthcare workers in Al-Shatra General Hospital and Al-Haboubi Teaching Hospital in Dhi Qar Governorate, Iraq, characterized by high seroprevalence but a low rate of active molecular infection. The elevated risk profile, compounded by occupational hazards such as exposure to surgical smoke, underscores an urgent public health concern. It is crucial to enhance routine molecular and serological screening, strengthen occupational health education, and consider targeted prophylactic vaccination strategies for healthcare personnel in accordance with local health guidelines to mitigate the risk of HPV-driven cancers.

Ethics

Ethics Committee Approval: Approved by the University of University of Thi-Qar Institutional Scientific Research Ethics Committee (approval no: B-2025-41630, date: 03.11.2025).

Informed Consent: Written informed consent was obtained from all participants before enrollment and subsequent data collection.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.Q.R., A.H.M., Concept: A.Q.R., A.H.M., Design: A.Q.R., A.H.M., Data Collection or Processing: A.Q.R., A.H.M., Analysis or Interpretation: A.Q.R., A.H.M., Literature Search: A.Q.R., A.H.M., Writing: A.Q.R., A.H.M.

Conflict of Interest: No conflict of interest was declared by the authors.

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Intestinal Microbiota Changes in Individuals Living with HIV and the Effect of Antiretroviral Therapy

HIV ile Yaşayan Bireylerde Bağırsak Mikrobiyotası Değişiklikleri ve Antiretroviral Tedavinin Etkisi

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Abstract

Introduction: Human immunodeficiency virus (HIV) infection is a chronic infectious disease that can be effectively managed with highly active antiretroviral therapy (HAART). HIV infection is closely associated with gastrointestinal disorders, alterations in the intestinal microbiota, and chronic inflammation. This study aimed to investigate changes in the gut microbiota of HIV-infected and uninfected individuals living in Türkiye.

Materials and Methods: The study included 15 people living with HIV (PLWH) and 10 healthy controls. PLWH were evaluated at baseline and again at least three months after treatment initiation, using a paired longitudinal study design. Blood and fecal samples were collected from all participants. Intestinal microbiota composition was analyzed using 16S ribosomal ribonucleic acid gene sequencing.

Results: Significant differences in intestinal microbiota composition were observed between PLWH and healthy controls. PLWH exhibited significantly lower alpha diversity, along with alterations in the composition of Proteobacteria and Firmicutes. Differences in the relative abundance of several genera, including *Faecalibacterium, Prevotella,* and *Lactobacillus*, were also observed compared with healthy controls. Following HAART, partial changes in microbiota composition were noted, with *Phascolarctobacterium* and *Blautia* among the taxa showing differences relative to the pretreatment period.

Conclusion: HAART was associated with partial restoration of the gut microbiota composition in PLWH. However, microbiota alterations persisted despite treatment.

Keywords: Microbiota, human immunodeficiency virus, highly active antiretroviral therapy, dysbiosis

Öz

Giriş: İnsan immün yetmezlik virüsü (HIV) enfeksiyonu, yüksek etkili antiretroviral tedavi (HAART) ile etkin şekilde kontrol altına alınabilen kronik bir enfeksiyon hastalığıdır. HIV enfeksiyonu gastrointestinal hastalıklar, bağırsak mikrobiyotası değişiklikleri ve kronik enflamasyon ile yakından ilişkilidir. Bu çalışmada, Türkiye'de yaşayan HIV enfeksiyonu olan ve olmayan bireylerde bağırsak mikrobiyotasındaki değişikliklerin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya 15 HIV ile yaşayan birey ve 10 sağlıklı gönüllü dahil edildi. HIV ile yaşayan bireyler başlangıçta ve tedavi başlandıktan en az üç ay sonra tekrar değerlendirildi. Tedavi öncesi ve sonrası örnekler aynı bireylerden alınmış olup eşleştirilmiş boylamsal bir tasarım benimsenmiştir. Tüm katılımcılardan kan ve dışkı örnekleri alındı. Bağırsak mikrobiyotası analizi için 16S ribozomal ribonükleik asit gen dizilemesi yöntemi kullanıldı.

Bulgular: HIV ile yaşayan bireyler ile sağlıklı kontroller arasında bağırsak mikrobiyotası açısından anlamlı farklılıklar saptandı. HIV ile yaşayan bireylerde alfa çeşitlilikte anlamlı azalma gözlenirken; Proteobacteria düzeylerinde değişimler ve Firmicutes kompozisyonunda farklılıklar saptandı.

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Ayrıca, *Faecalibacterium*, *Prevotella* ve *Lactobacillus* cinslerinin göreceli bolluğunda farklılıklar tespit edildi. HAART tedavisi sonrasında, tedavi öncesi döneme kıyasla *Phascolarctobacterium* ve *Blautia* cinslerinde farklılıklar ile karakterize kısmi mikrobiyota değişimleri gözlendi.

Sonuç: HAART tedavisi, HIV ile yaşayan bireylerde bağırsak mikrobiyotasının kısmen düzelmesi ile ilişkilidir. Bununla birlikte, tedaviye rağmen mikrobiyota değişikliklerinin tamamen normale dönmediği görülmüştür.

Anahtar Kelimeler: Mikrobiyota, insan immün yetmezlik virüsü, yüksek etkili antiretroviral tedavi, disbiyozis

Introduction

The gut microbiota is thought to play an important role in maintaining host immune homeostasis. Recent studies have shown that alterations in microbial diversity are associated with numerous health conditions, including inflammatory bowel disease, cardiovascular diseases, and metabolic disorders[1,2]. Human immunodeficiency virus (HIV) has evolved from a fatal disease into a chronic infectious disease that can be effectively managed with highly active antiretroviral therapy (HAART). However, despite HAART, persistent inflammation in people living with HIV (PLWH) has remained an important determinant of morbidity and mortality[3,4]. Despite viral load suppression and an increase in CD4+ T-lymphocyte counts, the literature indicates that chronic inflammation persists because of immune damage in the mucosal tissue[5].

The gut is one of the primary immunologic organs, harboring approximately 70% of the body's immune cells. HIV disrupts the integrity of the epithelial barrier by damaging the gut-associated lymphoid tissue during the early stages of infection, leading to the translocation of microorganisms and microbial products into the circulation. This condition, referred to as a "leaky gut," is considered one of the major causes of HIV-related systemic inflammation[6,7]. In particular, the loss of Th17 cells and the resulting impairment of barrier integrity exacerbate chronic inflammation by facilitating the translocation of microbial products such as lipopolysaccharides[8]. These immunologic changes associated with HIV directly affect the composition and distribution of the gut microbiota. Numerous studies have reported reduced bacterial diversity, particularly changes in the *Prevotella/Bacteroides* ratio, and an increase in facultative anaerobes belonging to the Proteobacteria phylum in PLWH[9,10]. These alterations are thought to trigger inflammatory responses and contribute to metabolic complications. For example, a reduction in *Bacteroides* species has been suggested to impair immune regulation[11].

In patients with HIV, despite virologic suppression achieved with antiretroviral therapy, these alterations in the gut microbiota have been reported to persist and contribute to ongoing inflammatory processes. The persistence of dysbiosis in the gut microbial ecosystem of HIV-positive individuals receiving HAART is considered one of the major contributors to immune activation and chronic inflammation[12,13]. In this context, inflammation in PLWH is thought to result not only from viral replication but also from microbial translocation and disruptions in the gut microbiota. Findings reported in the literature suggest that these alterations in the gut microbiota may play a central role in HIV pathogenesis and the persistence of inflammatory responses[14,15].

The aim of this study was to investigate changes in the intestinal microbiota and the effects of antiretroviral therapy on these changes in PLWH and to determine the relationship between microbiota composition and HIV ribonucleic acid (RNA) levels and CD4+/CD8+ lymphocyte counts, which are markers of inflammation.

Material and Methods

Case Selection

After applying the inclusion and exclusion criteria (Supplementary Material), a total of 25 participants were enrolled between December 2021 and May 2022 from the outpatient clinics of a tertiary care university hospital. The study comprised three groups: 10 healthy controls, 15 treatment-naive PLWH at baseline (untreated), and the same 15 PLWH re-evaluated at their 3 months follow-up visit after initiating antiretroviral therapy, representing a paired longitudinal design. The treated and untreated samples were therefore obtained from the same 15 individuals before and after HAART initiation, and this paired design was accounted for in the statistical analyses. The clinical characteristics of the participants are presented in Table 1.

The study population resided in a southeastern region of Türkiye with distinct socioeconomic and lifestyle characteristics. Although dietary habits were not formally assessed, these environmental factors may have influenced gut microbiota composition. Written informed consent was obtained from all participants. The study protocol was approved by the Dicle University Non-Interventional Research Ethics Committee (approval number: 251, dated: 22.04.2021).

Sample Collection

Two tubes of blood (approximately 5 mL each) were collected from PLWH at baseline and again from the same participants at the 3 months follow-up visit, whereas one tube of blood

(approximately 2.5 mL) was collected from healthy controls at a single time point. Fresh stool samples (approximately 5 g) were collected from all participants and transported to the laboratory under controlled conditions at 4 °C. Stool samples were mixed with phosphate-buffered saline containing 20% glycerol, frozen at −80 °C under nitrogen, and stored until analysis.

Measurement Methods

Bacterial genomic deoxyribonucleic acid (DNA) was isolated from fecal samples using commercially available kits with enzymatic lysis (lysozyme and achromopeptidase). Sample quality was confirmed by gel electrophoresis, spectrophotometry, and fluorometric methods. DNA samples for polymerase chain reaction (PCR) analysis were stored at −20 °C. The V3–V4 region of the 16S ribosomal RNA (rRNA) gene was amplified by amplicon PCR using universal primers. The resulting products were purified, and DNA concentrations were measured using fluorometric methods. The purified DNA libraries were pooled and sequenced using the Illumina MiSeq system. Taxonomic classification was performed using the SILVA 138 reference database, and sequences were denoised using the DADA2 plugin within QIIME2. Chimera removal was performed during the denoising step. Samples with fewer than 10,000 reads were excluded from downstream analyses. Data were normalized by rarefaction to the minimum sample depth before diversity analyses.

Statistical Analysis

Demographic and clinical data were evaluated based on variables including age, sex, HIV RNA levels, CD4+ and CD8+ lymphocyte counts, and antiretroviral therapy use. Mean, median, standard deviation, minimum, and maximum values were calculated for numerical variables. Frequency and percentage distributions were reported for categorical variables. Paired comparisons between untreated and treated samples were performed using the Wilcoxon signed-rank test or paired Student's t-test, as appropriate, based on the within-subject longitudinal study design. Comparisons between independent groups were performed using the Mann–Whitney U test or Student's t-test, as appropriate. The chi-square test or Fisher's exact test was used for categorical variables. Various bioinformatics methods were applied to analyze microbial diversity and composition. The Shannon, Simpson, and Chao1 diversity indices were calculated to assess microbial alpha diversity. Principal coordinates analysis (PCoA) and principal component analysis (PCA) were used for beta diversity analyses to visualize similarities and differences among the groups. Beta diversity was assessed using Bray–Curtis and Jaccard distance metrics. Krona plots, relative abundance analysis, and taxonomic assignment analyses were performed to characterize the microbial community composition. Differences in beta diversity between groups were statistically evaluated using PERMANOVA (999 permutations) based on Bray–Curtis and Jaccard distance metrics, and the results are reported as R^2 values with corresponding p values. Linear discriminant analysis effect size (LEfSe) analysis was used to identify taxa that discriminated between groups based on linear discriminant analysis (LDA) scores. All statistical analyses were performed using QIIME2, R, and SPSS version 26, and a p-value <0.05 was considered statistically significant.

Table 1. Demographic and clinical characteristics of the study groups.

Clinical data	Healthy controls (n = 10)	Untreated (n = 15)	Treated (n = 15)	p-value
Age (years)	32.3 ± 8.9	35.4 ± 9.1	35.7 ± 9.1	0.456
Gender (male/female)	8/2	13/2	13/2	0.376[a]
BMI (kg/m^2)	24.8 ± 2.6	25.1 ± 3.1	25.1 ± 3.1	0.346
Smoking	3 (30%)	5 (33.3%)	5 (33.3%)	0.247[a]
Alcohol consumption	2 (20%)	26.7%	26.7%	0.534[a]
Transmission route				N/A
Heterosexual	-	12 (80%)	12 (80%)	
Homosexual	-	2 (13.3%)	2 (13.3%)	
IV drug use	-	6.7%	1 (6.6%)	
HIV RNA (copies/mL)	-	588,754 ± 1,124,846	578 ± 1,418	p < 0.01
CD4+ T-cell (cells/µL)	-	359 ± 158	655 ± 296	p < 0.001
CD8+ T-cell (cells/µL)	-	1001 ± 179	882 ± 116	p < 0.001
CD4/CD8 ratio	-	0.36 ± 0.14	0.76 ± 0.29	p < 0.001

Data are presented as mean ± SD or n (%).

[a]χ^2 or Fisher's exact test (untreated vs. treated HIV patients).

SD, standard deviation; HIV, human immunodeficiency virus; BMI, body mass index; RNA, ribonucleic acid.

PCR Amplification and Illumina Sequencing

Variable regions of the 16S rRNA gene were amplified by PCR to determine the composition of the intestinal microbiota. In this study, the V3–V4 region of the 16S rRNA gene was targeted to achieve high-resolution characterization of bacterial diversity. Commonly used primer pairs were selected for PCR amplification: forward primer 341F (CCTACGGGNGGCWGCAG) and reverse primer 805R (GACTACHVGGGTATCTAATCC). PCR products were prepared into libraries according to the manufacturer's instructions (Illumina, San Diego, CA, USA) and sequenced on the Illumina MiSeq platform using paired-end sequencing with a read length of 2 × 300 bp. The raw sequences underwent quality filtering, trimming, and chimera removal before downstream analysis and were subsequently processed using QIIME2 (Quantitative Insights Into Microbial Ecology, version 2022.2).

Results

Demographic Characteristics

A total of 15 PLWH and 10 healthy controls were included in the study. Among the participants, 84% (n = 21) were male and 16% (n = 4) were female. The mean age was 35.4 ± 9.1 years, with an age range of 21–50 years. Patients received bictegravir/emtricitabine/tenofovir alafenamide, dolutegravir/emtricitabine/tenofovir disoproxil fumarate, or dolutegravir/lamivudine regimens. Treatment adherence was 100%, and no long-term drug-related adverse effects were observed. Untreated PLWH had significantly higher viral loads than the HAART-treated group, in which complete virologic suppression was achieved in all patients ($p < 0.01$). HAART significantly increased $CD4^+$ T-cell counts and improved the CD4/CD8 ratio ($p < 0.001$ for both), with no significant differences observed among the three HAART regimens. Demographic data are summarized in Table 1.

Fecal Bacterial Diversity in Patients with HIV

Alpha diversity measures microbial richness and evenness within a sample, whereas beta diversity assesses differences in microbial community composition between samples. The Shannon index was used to assess alpha diversity. In this study, alpha diversity was significantly lower in the HIV group than in the healthy control group ($p = 0.034$). Two ordination methods, PCA and PCoA, were used to compare beta diversity. PCoA based on Bray–Curtis dissimilarity revealed partial separation between the groups, although considerable overlap was observed. PERMANOVA did not demonstrate a statistically significant difference in overall microbial community composition according to HIV status ($R^2 = 0.054$, $p = 0.35$) or antiretroviral treatment ($R^2 = 0.131$, $p = 0.055$), suggesting that community-level differences may be subtle and that the current sample size may have limited the statistical power to detect them. A comparative analysis of the three groups is shown in Figures 1 and 2.

Fecal Bacterial Composition in Patients with HIV

LEfSe analysis was used to identify the taxa that best discriminated between groups. LEfSe identified *Clostridium* as the taxon with the highest discriminatory score in the healthy control group, whereas *Veillonellaceae*, *Phascolarctobacterium*, *Alphaproteobacteria*, and *Spirochaetes* showed the highest LDA scores in the untreated HIV group. These findings reflect the taxa that best discriminated between groups and should not be interpreted as direct measures of directional changes in abundance. The genus-level composition and LEfSe results are presented in Figures 3 and 4.

We compared microbial community composition between patients with HIV and healthy controls. *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* constituted the majority of the phylum-level microbiota. At the phylum level, the relative abundance of *Bacteroidetes* appeared higher in untreated PLWH than in healthy controls and treated patients, whereas *Firmicutes* exhibited the opposite pattern, with lower representation in the untreated group. The phylum-level composition is presented in Figure 5.

Microbiota Changes Before and After Treatment

A total of 15 patients with HIV were re-sampled and evaluated at least 3 months after HAART initiation. This group was

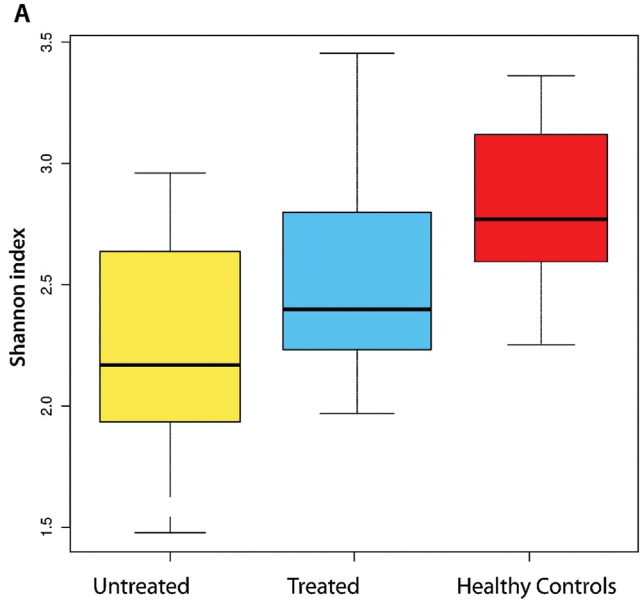

Figure 1. Alpha diversity of the gut microbiota in the study groups. Box-and-whisker plots showing the Shannon index in untreated HIV patients, treated HIV patients, and healthy controls.

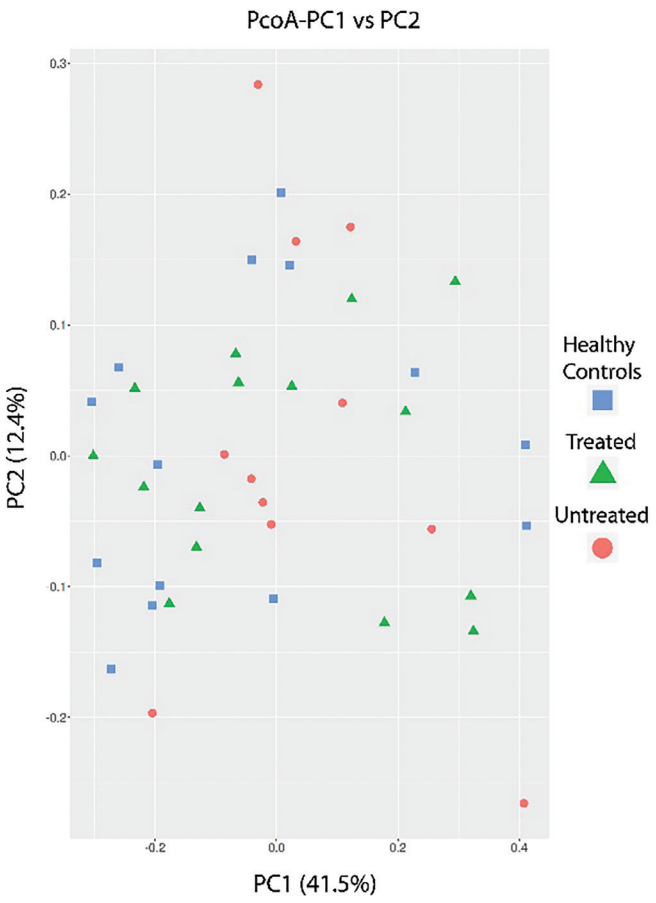

Figure 2. Beta diversity of the gut microbiota in the study groups. PCoA based on Bray–Curtis dissimilarity (PC1 vs. PC2). PC1 explained 41.5% and PC2 explained 12.4% of the total variance. PERMANOVA did not reveal statistically significant differences between groups according to HIV status (R2 = 0.054, p = 0.35) or antiretroviral treatment (R2 = 0.131, p = 0.055). PCoA, principal coordinates analysis.

compared with healthy controls and the treatment-naive HIV group. Posttreatment alpha diversity showed an increasing trend compared with the treatment-naive group, although this difference did not reach statistical significance, likely owing to the limited sample size. Changes were particularly observed in taxa such as *Firmicutes* and *Elusimicrobia*. At the taxonomic level, *Ruminococcaceae* was among the taxa showing differences between the groups. LEfSe analysis further identified *Ruminococcaceae*, *Lachnospiraceae*, and *Oscillospira* as the taxa with the highest discriminatory scores in the treated group compared with untreated PLWH, suggesting compositional shifts following HAART initiation. Despite effective HAART, the fecal microbiota did not appear to fully return to a profile comparable to that of healthy controls. Details are shown in Figures 3 and 5.

Discussion

To our knowledge, this is one of the first studies in Türkiye to compare the intestinal microbiota of PLWH and healthy controls. This study showed that microbial diversity was reduced in the HIV group. At the same time, although diversity partially improved following the initiation of HAART, the improvement appeared to be incomplete.

Thanks to HAART, HIV infection is now considered a manageable chronic disease, and the life expectancy of PLWH is approaching that of the general population. However, quality of life is influenced by many factors, including age, sex, socioeconomic status, $CD4^+$ T-lymphocyte count, and HIV RNA level[16,17]. In recent years, chronic inflammation has been recognized as one of the most important determinants of morbidity and mortality. In this process, the gut microbiota plays a critical role. HIV-induced immune activation and inflammation accelerate immune aging, leading to the earlier onset of comorbidities. Damage to the intestinal epithelial barrier, especially during the early stage of infection, increases intestinal permeability, promotes microbial translocation, and contributes to disease progression by exacerbating systemic inflammation[18,19].

Many studies have reported that alpha diversity decreases in treatment-naive PLWH. Some studies have also shown an increase in microbial diversity after HAART. In our study, a decrease in alpha diversity was observed, consistent with previous reports, and partial improvement was observed after treatment. Unexpectedly, some taxa generally considered beneficial were less represented across all groups. One possible explanation for this observation is the regional dietary and environmental context of our study population; however, because dietary intake was not formally assessed, this interpretation remains speculative and warrants further investigation. In addition, LEfSe analysis was used to identify taxa that discriminated between groups; however, these findings do not directly indicate the magnitude or direction of changes in taxonomic abundance and should therefore be interpreted cautiously[20].

In our study, changes in the intestinal microbiota of PLWH before and after treatment were evaluated, and the effects of treatment on microbial diversity and composition were analyzed. Changes in the relative representation of Firmicutes were observed after treatment, suggesting that the effects of antiretroviral therapy on the gut microbiota may be limited. Previous studies have suggested that dysbiosis of the gut microbiota in PLWH is associated with changes in Actinobacteria and that an increase in this group may be linked to proinflammatory responses[21,22]. These findings suggest that

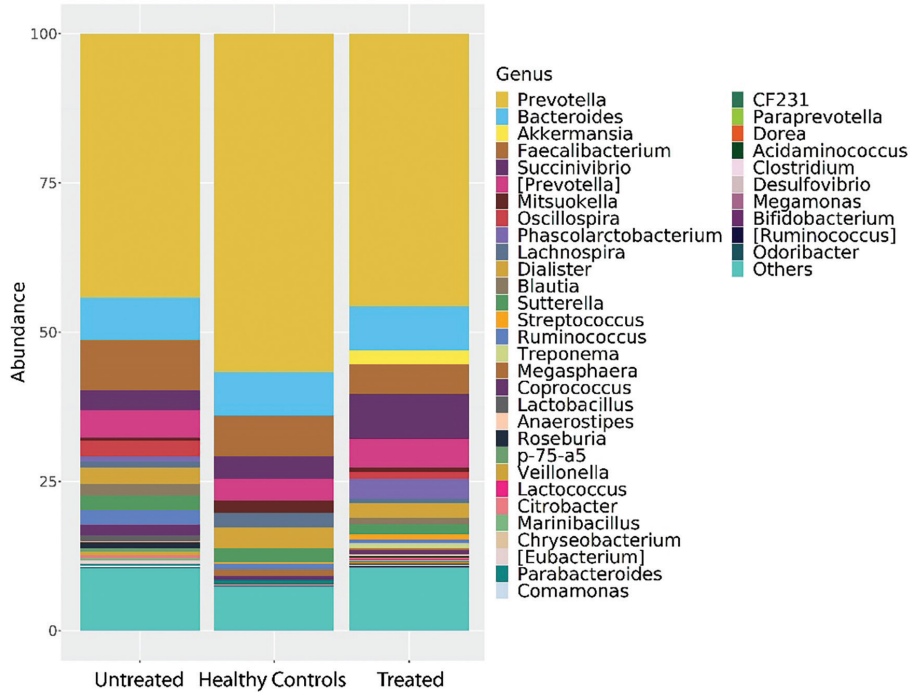

Figure 3. Relative abundance of the gut microbiota at the genus level.

Stacked bar plot showing the most abundant genera in untreated HIV patients, healthy controls, and treated HIV patients.

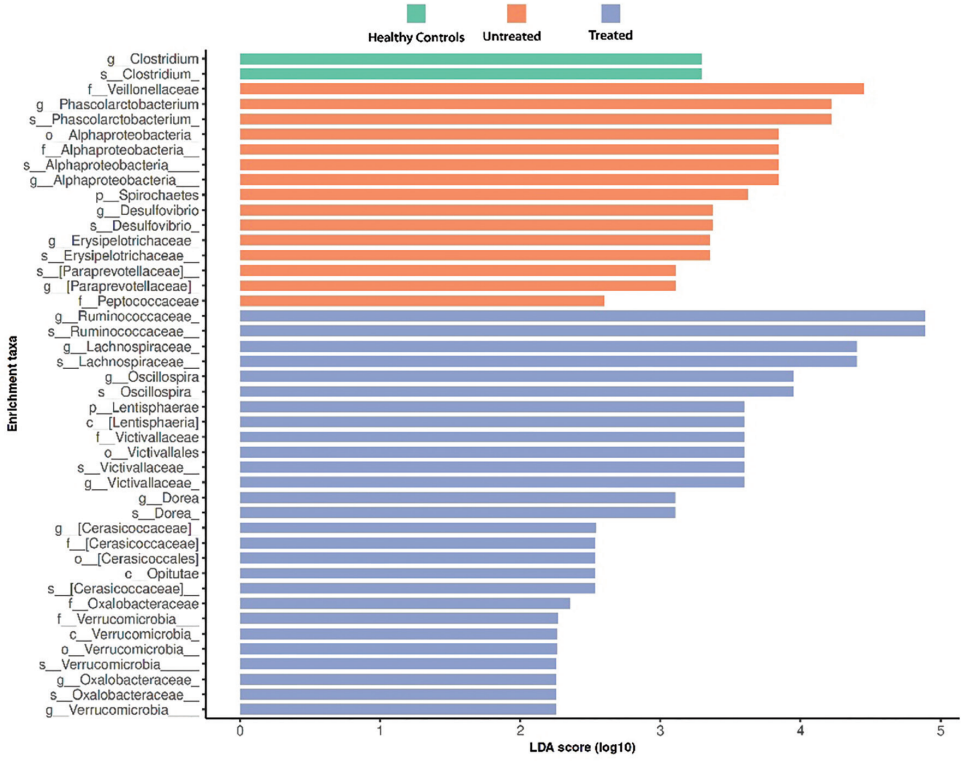

Figure 4. Differentially abundant taxa identified by LEfSe. Linear discriminant analysis (LDA) scores (>2.0, log10). The taxa shown exhibited the highest discriminatory power between groups based on LDA scores; however, these findings do not necessarily reflect the direction or magnitude of differences in abundance.

LEfSe, linear discriminant analysis effect size.

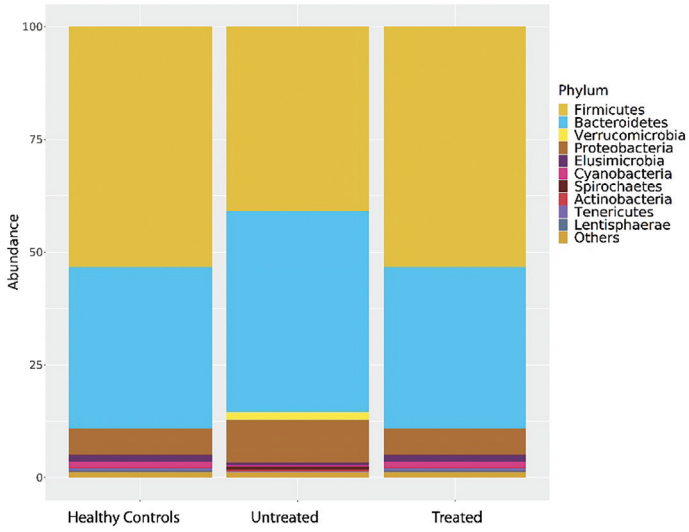

Figure 5. Relative abundance of the major bacterial phyla. Stacked bar plot showing phylum-level composition in healthy controls, untreated HIV patients, and treated HIV patients.

although antiretroviral therapy may have a partially beneficial effect on gut microbiota composition, complete restoration within a short follow-up period appears unlikely. Future long-term follow-up studies will be important for understanding how antiretroviral therapy promotes recovery of the gut microbiota and how different treatment regimens affect the microbiota[23].

Study Limitations

Several limitations of this study warrant consideration. The relatively small sample size is the primary limitation, as it restricts both the statistical power and the generalizability of the findings. This limitation is particularly relevant in microbiome studies, where substantial interindividual variability typically requires larger cohorts to detect significant differences. Consistent with this, PERMANOVA analysis did not reveal statistically significant differences in beta diversity according to HIV status ($R^2 = 0.054$, $p = 0.35$), which likely reflects insufficient statistical power rather than the true absence of community-level differences. The single-center design may also limit the representativeness of the findings, as the study population was drawn from a specific socioeconomic and geographic setting. The three-month follow-up period, although sufficient to assess early changes in the microbiota following HAART initiation, does not permit conclusions regarding long-term microbiota recovery. Potential confounding factors, including dietary habits, lifestyle, and environmental exposures, were not systematically assessed, and their contribution to the observed microbiota patterns cannot be excluded. In addition, data on HIV transmission routes were insufficient to evaluate route-specific differences in the microbiota. These limitations should be carefully considered when interpreting the findings, and larger multicenter studies with longer follow-up periods are warranted.

Conclusion

In conclusion, HIV infection is associated with alterations in the gut microbiota, including reduced microbial diversity, which may contribute to inflammation, immune dysregulation, and disease progression. Although antiretroviral therapy can partially correct dysbiosis, the microbial structure and function may not be fully restored. Therefore, the gut microbiota may play an important role not only in immune regulation but also in metabolic processes and long-term health outcomes. Future long-term, multicenter studies, particularly those involving regional cohorts with distinct environmental characteristics, are needed to better understand gut microbiota dynamics in PLWH and to evaluate whether microbiota-targeted interventions may provide additional benefits for this population.

Ethics

Ethics Committee Approval: The study protocol was approved by the Dicle University Non-Interventional Research Ethics Committee (approval number: 251, dated: 22.04.2021).

Informed Consent: Written informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Y.D., Concept: M.K.Ç., Design: Y.D., Data Collection or Processing: Y.D., Analysis or Interpretation: Y.D., E.S.T., Literature Search: Y.D., Writing: Y.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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Supplementary Material: https://d2v96fxpocvxx.cloudfront.net/a7b689d1-7b5b-481e-8a29-9bd45e97266c/content-images/f3e65afd-bf2d-4bf7-bc2b-4b75a78c1890.pdf

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Seroprevalence of Measles, Mumps, Rubella, and Varicella-Zoster Virus Among People Living with HIV in Türkiye

Türkiye'de HIV ile Yaşayan Bireylerde Kızamık, Kabakulak, Kızamıkçık ve Varisella-Zoster Seroprevalansı

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Abstract

Introduction: Infections caused by measles, mumps, rubella (MMR), and varicella-zoster virus (VZV) can lead to severe illness and complications among people living with human immunodeficiency virus (HIV). This study aimed to evaluate the seroprevalence of MMR and VZV among people living with HIV and provide data to support proactive immunization strategies.

Materials and Methods: This retrospective cross-sectional study included adults living with HIV who were followed up at the infectious diseases and clinical microbiology outpatient clinic between October 2020 and December 2025. Demographic characteristics, baseline $CD4^+$ T-cell counts, and MMR and VZV immunoglobulin G serology results were assessed. Vaccination indications were evaluated according to current guideline recommendations.

Results: A total of 206 patients were included in the study. Of these, 91.3% were male, and the median age was 34 years. Seronegativity rates were 16.8% for measles, 12.9% for mumps, 9.1% for rubella, and 3.4% for VZV. An indication for MMR vaccination was identified in 27.2% of patients, whereas 3.4% had an indication for VZV vaccination. Seronegativity increased with age for rubella but decreased with age for the other viruses.

Conclusion: A substantial immunity gap for MMR persists among people living with HIV, whereas VZV seropositivity remains high. Assessment of serological immunity and vaccination of eligible patients should be integral components of HIV care.

Keywords: HIV, measles, mumps, rubella, varicella-zoster virus

Öz

Giriş: Kızamık, kabakulak, kızamıkçık (MMR) ve varisella-zoster virüsü (VZV) enfeksiyonları HIV ile yaşayan bireylerde ciddi hastalık ve komplikasyonlara yol açabilir. Bu çalışma, HIV ile yaşayan kişilerde MMR ve VZV seroprevalansını değerlendirmeyi ve proaktif aşılama stratejilerini destekleyebilecek veriler sağlamayı amaçlamıştır.

Gereç ve Yöntem: Bu retrospektif kesitsel çalışmaya Ekim 2020-Aralık 2025 arasında enfeksiyon hastalıkları ve klinik mikrobiyoloji polikliniğinde takip edilen erişkin HIV pozitif hastalar dahil edildi. Demografik veriler, başlangıç $CD4^+$ T-hücre sayıları ve MMR ile VZV immünoglobulin G sonuçları incelendi. Aşılama endikasyonları güncel kılavuzlara göre belirlendi.

Bulgular: Toplam 206 hasta değerlendirildi; %91,3'ü erkekti ve medyan yaş 34 yıl idi. Seronegatiflik oranları kızamık için %16,8, kabakulak için %12,9, kızamıkçık için %9,1 ve VZV için %3,4 bulundu. Hastaların %27,2'sinde MMR, %3,4'ünde VZV aşı endikasyonu vardı. Kızamıkçık seronegatifliği yaşla artarken, diğer virüslerde azaldı.

Sonuç: HIV ile yaşayan bireylerde MMR için belirgin bağışıklık eksikliği sürmektedir, buna karşın VZV seropozitifliği yüksektir. Bağışıklık durumunun değerlendirilmesi ve uygun hastalarda aşılama HIV bakımının temel bir parçası olmalıdır.

Anahtar Kelimeler: HIV, kızamık, kabakulak, kızamıkçık, suçiçeği-zona virüsü

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Introduction

With the introduction of antiretroviral therapy, the life expectancy and quality of life of people living with human immunodeficiency virus (HIV) have improved significantly[1,2]. However, the immunosuppressive effects of HIV infection may increase susceptibility to vaccine-preventable infections and result in more severe clinical manifestations of these infections[3,4]. Several studies have demonstrated that people living with HIV have lower seropositivity rates for measles, mumps, rubella (MMR), and varicella-zoster virus (VZV) [5,6]. Accordingly, the European AIDS Clinical Society (EACS) guidelines recommend a proactive approach to screening and vaccination among people living with HIV[7].

In Türkiye, data on serological immunity to these vaccine-preventable infections among people living with HIV are limited. Therefore, this study aimed to evaluate the seroprevalence of MMR and VZV among people living with HIV, identify individuals eligible for vaccination, and contribute to the development of proactive immunization strategies.

Materials and Methods

This retrospective cross-sectional study included all adults living with HIV who were followed up at the Infectious Diseases and Clinical Microbiology outpatient clinic of University of Health Sciences Türkiye, Konya City Hospital between October 1, 2020, and December 31, 2025. Demographic data, including age, sex, education level, and route of HIV transmission, were recorded. Clinical and laboratory variables included baseline $CD4^+$ T-cell count, baseline CD4 percentage, and serological markers for measles immunoglobulin G (IgG), mumps IgG, rubella IgG, and VZV IgG, which were obtained from patient records.

Because measles, mumps, and rubella vaccines are administered as a combined MMR vaccine in Türkiye, MMR immunity was assessed collectively. Patients were considered to have an indication for MMR vaccination if seronegativity was detected for at least one available MMR serological marker (measles, mumps, or rubella IgG). Similarly, patients with a negative available VZV IgG result were considered to have an indication for varicella vaccination. Because serological testing was performed as part of routine clinical practice, complete serological data were not available for all patients. Therefore, vaccination indications were determined based on the available serological results, and unavailable serological results were not considered when determining vaccination indications.

The serological markers were analyzed using enzyme-based immunoassay methods. HIV infection was defined as reactivity in an HIV type 1/2 (HIV-1/2) antigen/antibody assay and was subsequently confirmed using a rapid supplemental test performed at a central public health laboratory (Geenius HIV-1/2 Supplemental Assay, Bio-Rad Laboratories, Redmond, WA, USA).

This study was approved by the University of Health Sciences Türkiye, Konya City Hospital Clinical Research Ethics Committee (approval number: 2026/89; April 13, 2026) and was conducted in accordance with the Declaration of Helsinki. Given the retrospective cross-sectional design of the study, written informed consent was not obtained from the patients.

Statistical Analysis

Continuous variables were presented as means ± standard deviations or medians with interquartile ranges (IQRs; 25th-75th percentiles). As the continuous variables did not follow a normal distribution according to the Shapiro-Wilk test, nonparametric methods were used. Categorical variables were presented as frequencies and percentages. For between-group comparisons, the Mann-Whitney U test was used for non-normally distributed continuous variables, and the chi-square test was used for categorical variables. Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Chicago, IL, USA), and a p value <0.05 was considered statistically significant.

Results

During the study period, 206 patients with HIV infection were identified from the outpatient clinic records. Of these patients, 188 (91.3%) were male, and the median age was 34 years (IQR, 27-44 years). The distribution of HIV transmission routes in the study population was as follows: heterosexual contact, 107 (51.9%); men who have sex with men (MSM), 29 (14.1%); intravenous drug use, 3 (1.5%); and unknown, 67 (32.5%). The median CD4+ T-cell count was 447 cells/mm^3 (IQR, 301-627 cells/mm^3), and the median CD4 percentage was 19.6% (IQR, 14.6%-27.0%) (Table 1).

Measles serology was available for 190 patients (92.2%), mumps serology for 124 patients (60.2%), rubella serology for 198 patients (96.1%), and VZV serology for 179 patients (86.9%). Seropositivity rates were 83.2% for measles, 87.1% for mumps, 90.9% for rubella, and 96.6% for VZV (Table 1). Accordingly, seronegativity was observed in 16.8% of patients for measles, 12.9% for mumps, 9.1% for rubella, and 3.4% for VZV. Regarding the combined MMR vaccine, seronegativity to at least one available MMR component was detected in 27.2% of patients (56/206) based on the available serological results.

Table 1. Demographic characteristics, HIV transmission routes, serological results, and laboratory parameters of people living with HIV included in the study (n = 206).

Variables	
Age (year)	
Mean ± SD	36.6 ± 11.5
Median (IQR)	34 (27–44)
Male, n (%)	188 (91.3%)
Educational status, n (%)	
Primary school	18 (20.5%)
Secondary (middle) school	22 (25%)
High school	22 (25%)
University	26 (29.5%)
HIV transmission route, n (%)	
Heterosexual contact	107 (51.9%)
MSM	29 (14.1%)
IVDU	3 (1.5%)
Unknown	67 (32.5%)
Seropositivity, n (%)	
Measles IgG	158/190 (83.2%)
Mumps IgG	108/124 (87.1%)
Rubella IgG	180/198 (90.9%)
Varicella IgG	173/179 (96.6%)
Laboratory parameters, median (IQR)	
CD4 count (cells/mm^3)	447 (301–627)
CD4 percentage, median (%)	19.6 (14.6–27)

Educational status information was available for 88 patients. HIV, human immunodeficiency virus; IgG, immunoglobulin G; IQR, interquartile range; IVDU, intravenous drug use; MSM, men who have sex with men; SD, standard deviation.

These patients were considered to have an indication for MMR vaccination. The distribution of these patients is shown in Figure 1.

Among patients with an indication for MMR vaccination, 89.3% (50 of 56) had a baseline CD4+ T-cell count ≥200 cells/μL, and all patients with an indication for VZV vaccination (6 patients) had a CD4+ T-cell count ≥200 cells/μL, indicating immunological eligibility for vaccination at the time of presentation.

Among the 14 women of reproductive age (18-49 years), rubella IgG antibodies were negative in 4 (28.6%); 3 of these 4 patients had adequate baseline CD4+ T-cell counts (CD4+ ≥200 cells/μL) for vaccination.

In addition, MMR and VZV serostatuses were analyzed according to the patients' sociodemographic and clinical characteristics. Bivariate analysis showed no significant differences between groups in terms of sex, education level, MSM status, CD4+ T-cell count, or CD4 percentage. Rubella seronegativity increased with advancing age, whereas seronegativity for the other viruses decreased with age. These associations were statistically significant for all viruses except mumps ($p < 0.05$) (Table 2). The distribution of MMR and VZV IgG antibody positivity and negativity according to patients' birth years is shown in Figure 2.

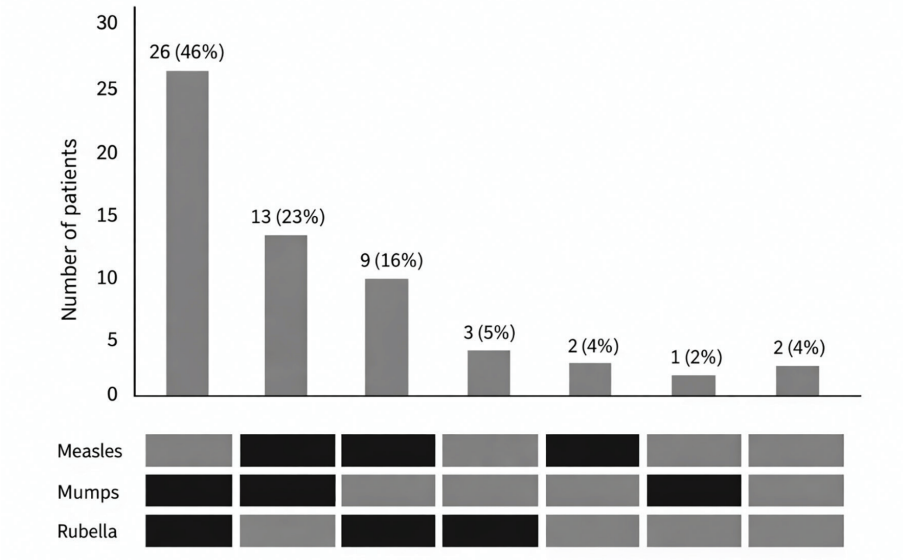

Figure 1. Distribution of the 56 patients (27.2%) identified as having an indication for measles, mumps, and rubella (MMR) vaccination based on the available serological results. Gray squares indicate IgG seronegativity for the corresponding MMR component. IgG, immunoglobulin G.

Table 2. Comparison of demographic and clinical characteristics according to MMR and varicella IgG serostatus in people living with HIV.

Variables	Measles IgG (–) (n = 32)	Measles IgG (+) (n = 158)	p	Mumps IgG (–) (n = 16)	Mumps IgG (+) (n = 108)	p	Rubella IgG (–) (n = 18)	Rubella IgG (+) (n = 180)	p	Varicella IgG (–) (n = 6)	Varicella IgG (+) (n = 173)	p
Male n (%)	28 (87.5)	148 (93.7)	0.260	16 (100)	100 (92.6)	0.595	15 (83.3)	167 (92.8)	0.166	5 (83.3)	160 (92.5)	0.391
Age (year) (median, IQR)	27 (23-29.8)	38 (27.8-46)	**<0.001**	29 (26-42.5)	35.5 (27-44.8)	0.252	43.5 (34.5-52.5)	33 (27-43)	**0.008**	25 (22.3-31.8)	34 (27-44)	**0.026**
MSM, n (%)	5/16 (31.3)	22/92 (23.9)	0.540	3/12 (25)	16/57 (28.1)	1.000	1/7 (14.3)	26/104 (25)	1.000	1/3 (33.3)	24/73 (24.7)	1.000
CD4 count (cells/mm^3)	446 (288-638)	447 (301-626)	0.973	319 (263-703)	419 (279-562)	0.635	340 (180–604)	462 (323-636)	0.173	334 (274-566)	448 (311-622)	0.438
CD4 percentage (%)	21.1 (13.6-30.8)	18.4 (14.6-25.9)	0.227	18.7 (9.8-26.4)	18.4 (14.6-25.9)	0.465	17 (8.8–29.2)	19.8 (14.8-27.2)	0.703	19.7 (16.3-21.5)	19.1 (14.5-27)	0.874
Educational status, n (%)			0.744			0.595			0.782			0.629
Primary school	2 (14.3)	14 (20.6)		1 (10)	8 (19.1)		2 (25)	16 (20.5)		0 (0)	16 (20.8)	
Secondary school	3 (21.4)	17 (25)		3 (30)	10 (23.8)		1 (12.5)	19 (24.4)		0 (0)	20 (26)	
High school	5 (35.7)	15 (22.1)		2 (20)	14 (33.3)		3 (37.5)	19 (24.4)		1 (50)	19 (24.7)	
University	4 (28.6)	22 (32.4)		4 (40)	10 (23.8)		2 (25)	24 (30.8)		1 (50)	22 (28.6)	

HIV, human immunodeficiency virus; IgG, immunoglobulin G; IQR, interquartile range; SD, standard deviation; MSM, men who have sex with men; MMR, measles, mumps, and rubella.

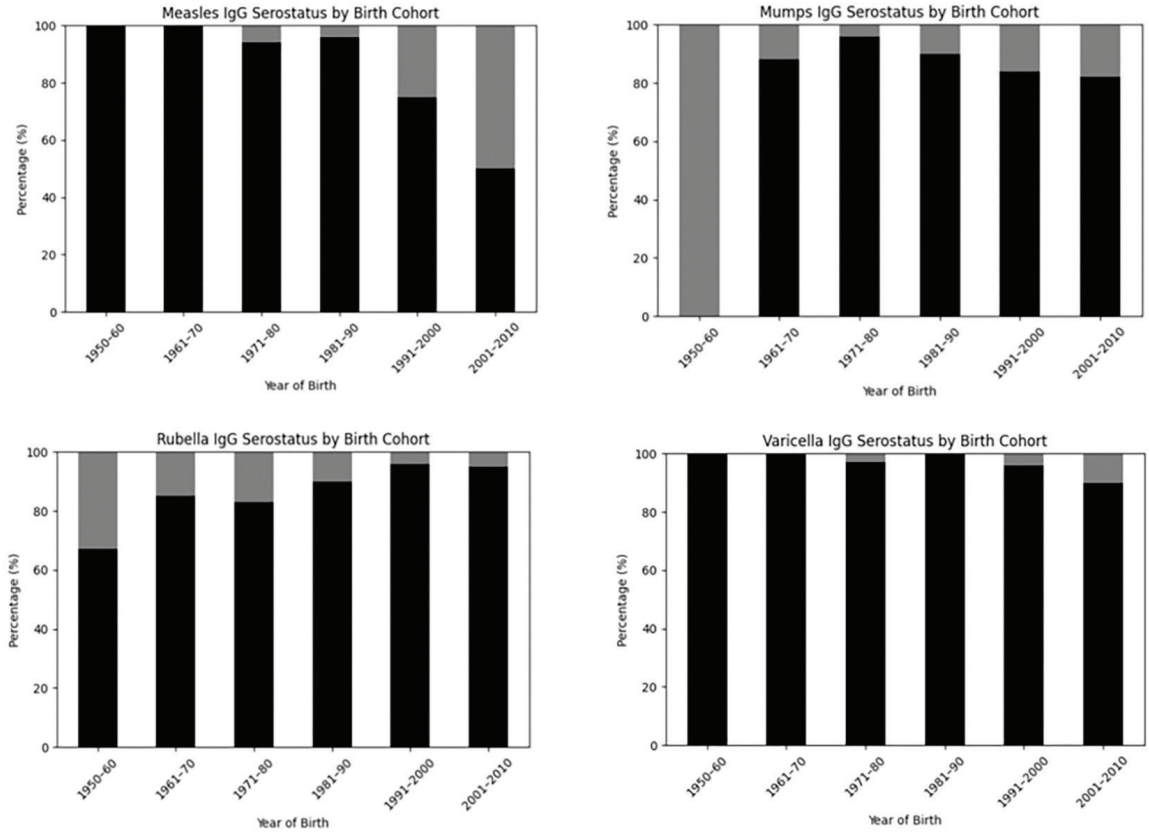

Figure 2. Distribution of MMR and varicella IgG serostatus according to birth cohorts among people living with HIV. Black bars represent IgG-positive individuals, whereas gray bars indicate IgG-negative individuals. MMR, measles, mumps, and rubella; IgG, immunoglobulin G; HIV, human immunodeficiency virus.

Discussion

In this study, the serostatus of MMR and varicella was evaluated in an adult cohort of people living with HIV in Türkiye. We found that 27.2% of patients were susceptible to at least one infection preventable by the MMR vaccine, and 3.4% were susceptible to VZV. The EACS guidelines and the Advisory Committee on Immunization Practices recommend serological assessment of immunity to MMR and VZV in people living with HIV and vaccination of patients without evidence of immunity who have a CD4+ T-cell count ≥200 cells/mm^3 or a CD4 percentage ≥15%[7,8]. In our study, approximately 90% of patients with an indication for MMR vaccination and all patients with an indication for VZV vaccination had CD4$^+$ T-cell counts appropriate for vaccination at the time of presentation, indicating that immunization strategies are generally clinically feasible.

In Türkiye, measles vaccination was included in the national immunization program in 1970; a second dose was introduced for first-grade schoolchildren in 1998, and in 2006, the combined MMR vaccine was incorporated into routine immunization following the addition of mumps and rubella vaccines to the national immunization program[9]. Nevertheless, following migration movements associated with the civil war in Syria in 2011, increases in measles cases and periodic outbreaks have been reported in Türkiye, as in many European countries[3,6,9,10]. This epidemiological change makes the assessment of measles susceptibility even more important, particularly in populations with limited immune protection. In our study, measles seronegativity among people living with HIV was 16.8%. This rate is considerably higher than those reported in HIV cohorts from European countries such as Germany (3.2%)[6], France (4.6%)[11], and the United Kingdom (7%)[3]. However, it is similar to the rate reported in a multicenter HIV cohort from Türkiye (18%)[12]. Considering that measles is associated with a more severe clinical course and increased mortality in individuals with T-cell immunodeficiency[13], this high susceptibility to measles among people living with HIV in our country represents an important public health risk.

Our study also demonstrated a statistically significant association between age and measles serostatus. According to the birth cohort analysis, measles seronegativity was 0% among patients born between 1950 and 1960, whereas this rate progressively increased in subsequent birth cohorts, reaching 50% among those born between 2001 and 2010. This finding may be explained by stronger and more durable immunity among individuals in older birth cohorts, who were more likely to have acquired immunity through natural infection, whereas in younger cohorts, vaccine-induced immunity may wane over time or the primary vaccine response may be insufficient[14,15]. Similarly, previous studies have reported higher measles seropositivity among older age groups and increased susceptibility among younger adults living with HIV[3,6,9-11].

Although there is no strong evidence that mumps or rubella infections have a more severe clinical course in people living with HIV, rubella infection during pregnancy may result in serious fetal and neonatal complications; therefore, maintaining rubella immunity remains clinically and epidemiologically important for women living with HIV[4]. In our study, 12.9% of adults living with HIV were seronegative for mumps, and 9.1% were seronegative for rubella. Furthermore, 28.6% of women of reproductive age were seronegative for rubella, which is noteworthy. This rate is higher than those reported in cohorts of HIV-positive women of reproductive age from Iran (17.3%)[4], Austria (18.2%)[16], and Germany (9%)[6]. However, because the number of female patients in our study was limited, these comparisons should be interpreted with caution. In our cohort, unlike the other three viruses, rubella seronegativity increased with advancing age. A similar trend was observed in another HIV cohort from Türkiye, although the association did not reach statistical significance[12]. In contrast, a cohort from Germany showed decreasing seronegativity with increasing age[6]. These differences may be attributable to historical variations in vaccination schedules, vaccination coverage rates, and the circulation of natural infection between countries.

It is well known that VZV infections, related complications, and mortality occur more frequently among people living with HIV[17,18]. In our study, VZV seropositivity was detected in 96.6% of patients. Since the varicella vaccine was incorporated into the national immunization program in Türkiye in 2013[19], the high seropositivity observed in our adult cohort is likely attributable primarily to a history of natural infection rather than vaccination. Nevertheless, although seropositivity indicates protection against varicella infection, VZV reactivation and its associated clinical manifestations cannot be completely excluded in people living with HIV because of impaired cellular immunity[20,21].

Study Limitations

This study has some limitations. First, the single-center, retrospective design may limit the generalizability of the findings. Furthermore, because serological tests were performed as part of routine clinical practice, complete MMR serological data were not available for all patients. Therefore, indications for MMR vaccination were determined based on the available serological results, and the reported prevalence may have underestimated the true prevalence. In addition, reliable data on the vaccination history of most patients were unavailable. Therefore, seronegativity cannot be attributed

solely to a lack of vaccination; it may also reflect waning vaccine-induced immunity, primary vaccine failure, or HIV-related immune dysfunction. However, this study was not designed to determine the underlying causes of seronegativity but rather to evaluate serological immunity to MMR and VZV among people living with HIV.

Despite these limitations, this study has important strengths. First, it is one of the few studies evaluating both MMR and VZV immunity among people living with HIV in Türkiye. Second, it includes a large adult cohort spanning multiple birth cohorts, providing valuable epidemiological insights into the national context.

Conclusion

In conclusion, our study demonstrates an immunity gap for MMR components among adults living with HIV, whereas VZV seropositivity remains high. These findings support the need for systematic assessment of immunity to vaccine-preventable infections as part of HIV care and vaccination of eligible patients in accordance with current guidelines.

Ethics Committee Approval: This study was approved by the University of Health Sciences Türkiye, Konya City Hospital Clinical Research Ethics Committee (approval number: 2026/89; April 13, 2026) and was conducted in accordance with the Declaration of Helsinki.

Informed Consent: Given the retrospective cross-sectional design of the study, written informed consent was not obtained from the patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.R.T., Y.G., Concept: M.R.T., Y.G., Design: M.R.T., Y.G., Data Collection or Processing: M.R.T., Y.G., Analysis or Interpretation: M.R.T., Y.G., Literature Search: M.R.T., Y.G., Writing: M.R.T., Y.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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Seronegative Disseminated Visceral Leishmaniasis with Secondary Hemophagocytic Lymphohistiocytosis in Advanced HIV Infection: A Case Report

İleri Evre HIV Enfeksiyonunda Seronegatif Yaygın Visseral Leishmaniasis ve Sekonder Hemofagositik Lenfohistiyositoz: Bir Olgu Sunumu

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Abstract

Visceral leishmaniasis (VL) is a life-threatening infection endemic to the Indian subcontinent and may be associated with atypical and severe manifestations in patients with human immunodeficiency virus (HIV) infection. Secondary hemophagocytic lymphohistiocytosis (HLH) is a rare but serious complication of VL. We report a 51-year-old man with advanced HIV infection due to poor adherence to antiretroviral therapy (ART) who presented with prolonged fever, melena, hepatosplenomegaly, and pancytopenia. A series of laboratory investigations ruled out common opportunistic infections. VL with secondary HLH was diagnosed based on the identification of *Leishmania donovani* and hemophagocytosis on bone marrow examination, despite a negative rK39 antigen test. Upper gastrointestinal endoscopy with subsequent biopsy revealed *Leishmania* infiltration, confirming disseminated involvement. The patient was treated with conventional amphotericin B deoxycholate, continuation of ART, and a short course of corticosteroids, resulting in clinical and hematological recovery. This case highlights the diagnostic challenges of seronegative VL in advanced HIV infection and underscores the importance of early tissue diagnosis and targeted therapy in patients presenting with fever, cytopenias, and organomegaly in endemic regions.

Keywords: Visceral leishmaniasis, hemophagocytic lymphohistiocytosis, HIV/AIDS, rK39 antigen test, amphotericin B deoxycholate

Öz

Visseral leishmaniasis (VL), Hint alt kıtasına özgü, yaşamı tehdit eden bir enfeksiyondur ve insan immün yetmezlik virüsü (HIV) enfeksiyonu olan hastalarda atipik ve şiddetli belirtilerle ilişkili olabilir. Sekonder hemofagositik lenfohistiyositoz (HLH), VL'nin nadir ancak ciddi bir komplikasyonudur. Antiretroviral tedaviye (ART) uyumsuzluğu ve ileri evre HIV enfeksiyonu olan ve uzun süreli ateş, melena, hepatosplenomegali ve pansitopeni ile başvuran 51 yaşında bir erkek hastayı bildiriyoruz. Bir dizi laboratuvar incelemesi yaygın fırsatçı enfeksiyonları ekarte etti. Negatif rK39 antijen testine rağmen, kemik iliği incelemesinde *Leishmania donovani* ve hemofagositoz saptanması temelinde sekonder HLH ile birlikte VL tanısı kondu. Üst gastrointestinal endoskopi ve ardından yapılan biyopsi, yaygın tutulumu doğrulayan *Leishmania* infiltrasyonunu ortaya çıkardı. Hasta, konvansiyonel amfoterisin B deoksikolat, ART'nin devamı ve kısa süreli kortikosteroid tedavisi ile tedavi edildi ve klinik ve hematolojik iyileşme sağlandı. Bu vaka, ileri HIV enfeksiyonunda seronegatif VL'nin tanısal zorluklarını ve endemik bölgelerde ateş, sitopeni ve organomegali ile başvuran hastalarda erken doku tanısı ve hedefe yönelik tedavinin önemini vurgulamaktadır.

Anahtar Kelimeler: Viseral leishmaniasis, hemofagositik lenfohistiyositoz, HIV/AIDS, rK39 antijen testi, amfoterisin B deoksikolat

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Introduction

Visceral leishmaniasis (VL) is an endemic and potentially fatal protozoal infection in the Indian subcontinent, classically presenting with prolonged fever, hepatosplenomegaly, and pancytopenia[1,2].

Coinfection with HIV alters the natural history of VL, leading to a higher parasite burden, atypical organ involvement, poor serological responses, and frequent relapses[3,4].

Gastrointestinal dissemination of leishmaniasis, which may manifest as ulcerative lesions and bleeding, is uncommon in immunocompetent individuals but is increasingly recognized in patients with advanced immunosuppression[5,6].

One of the severe and potentially fatal complications of VL is a hyperinflammatory state known as hemophagocytic lymphohistiocytosis (HLH), characterized by persistent fever, cytopenias, hyperferritinemia, and multiorgan dysfunction[7,8]. Infection-triggered HLH is well described, and VL is an important but often underdiagnosed precipitating factor[9,10].

The coexistence of advanced human immunodeficiency virus (HIV) infection, disseminated VL, and secondary HLH presents a significant diagnostic and therapeutic challenge because of overlapping clinical features. We describe a case of disseminated VL presenting with gastric ulcers and secondary HLH in a patient with advanced HIV infection.

Case Report

A 51-year-old migrant worker from Bihar presented to the emergency department with complaints of high-grade fever (>101 °F) associated with chills for three weeks. During this period, he also reported 1-2 episodes of vomiting and melena daily. He complained of diffuse, cramping-type abdominal pain and noted progressive abdominal distension. In addition, he experienced dysphagia, severe generalized weakness, and myalgia.

The patient was a known case of HIV infection and had been receiving antiretroviral therapy (ART) consisting of tenofovir, lamivudine, and dolutegravir (300 mg/300 mg/50 mg) once daily for the past 10 years. He had been under regular follow-up at an ART center in his hometown; however, for the past year, he had poor adherence to ART and irregular follow-up because of migration to our city. His last CD4 count, measured one year earlier, was 482 cells/µL (reference range: 500-1,500 cells/µL).

On presentation to the emergency department, the patient was febrile, with a temperature of 102 °F. He appeared severely undernourished and cachectic, with a body mass index of 16.4 kg/m^2. On examination, he had a systolic blood pressure of 90 mmHg and a heart rate of 116 beats/min. He had severe pallor and mild bilateral pitting pedal edema. Abdominal examination revealed grade 2 splenomegaly and hepatomegaly, with a liver span of 18 cm. The remainder of the systemic examination was unremarkable. The patient was admitted to the medicine ward, and initial laboratory investigations were performed.

A complete hemogram revealed pancytopenia, and the peripheral blood smear showed normocytic, normochromic anemia with leukopenia and thrombocytopenia. No hemoparasites were detected.

Initial laboratory investigations demonstrated severe hypoalbuminemia, elevated transaminases, with aspartate aminotransferase levels higher than alanine aminotransferase levels, euvolemic hypotonic hyponatremia, severe hypocalcemia, and severe vitamin D deficiency. The laboratory findings are summarized in Table 1.

The CD4 count was 157 cells/µL, and the HIV viral load was elevated at 30,000 copies/mL.

Given the advanced immunosuppression, investigations to rule out opportunistic infections were performed. Cryptococcal antigen, cytomegalovirus (CMV) polymerase chain reaction (PCR), and urinary histoplasma antigen tests were negative. Fundus examination showed no evidence of CMV retinitis. The rK39 antigen test for leishmaniasis was also negative.

Routine stool microscopy was positive for occult blood, with no ova or cysts identified. Stool examination using modified acid-fast staining for *Cryptosporidium*, *Isospora*, and *Cyclospora* was negative. Stool culture showed no growth.

Blood cultures for aerobic and anaerobic bacteria, as well as fungal cultures, were also negative.

The patient was started on empirical intravenous ceftriaxone and antipyretics. He received transfusions of two units of packed red blood cells and platelet concentrates. Intravenous calcium gluconate was administered along with vitamin D_3 supplementation.

Despite treatment, the patient continued to experience persistent fever spikes, pancytopenia, hyponatremia, and worsening liver function tests. Serum ferritin, fibrinogen, and triglyceride levels were measured because of suspected HLH and were markedly abnormal. The findings are shown in Table 2.

Under aseptic precautions, bone marrow aspiration and biopsy were performed because of persistent pancytopenia. Samples were sent for microscopic examination, Ziehl-Neelsen (ZN) staining, histopathological examination, and cartridge-based nucleic acid amplification testing (CBNAAT).

Table 1. Laboratory investigations.

Investigation	Result	Reference	Investigation	Result	Reference
Haemoglobin	7.30 gm/dL	13.2-16.6 gm/dL	Total bilirubin	0.80 mg/dL	0.22-1.20 mg/dL
Haematocrit	30.4%	38.0-48.60%	Direct bilirubin	0.50 mg/dL	Upto 0.5 mg/dL
Mean corpuscular volume	77.8 fL	78.2-97.9 fL	Indirect bilirubin	0.30 mg/dL	0.1-1.0 mg/dL
TLC[1]	770/µL	4,000–10,000 /µL	AST[2]	207 U/Lt	8-48 U/Lt
Neutrophil	70%	40-80%	ALT[3]	76 U/Lt	7-55 U/Lt
Lymphocyte	22%	20-40%	ALP[4]	313 U/Lt	40-129 U/Lt
Eosinophils	0%	0-6%	Serum protein	5.10 g/dL	6.4-8.30 g/dL
Monocyte	9%	2-10%	Serum albumin	1.80 g/dL	3.5-5.2 g/dL
PLT[5]	31,000 /µL	150,000–410,000 /µL	Sodium	122 mmol/Lt	136-145 mmol/Lt
Vitamin B12	194 pg/mL	187-883 pg/mL	Potassium	3.92 mmol/Lt	3.50-5.10 mmol/Lt
Lactate dehydrogenase	972 U/Lt	120-246 U/Lt	Serum osmolality	259.2 mOsmol/kg	275-300 mOsmol/kg
Dengue NS1 Ag/IgM/IgG	Negative		Ionized calcium	0.8 mmol/Lt	1.12-1.32 mmol/Lt
Rapid malaria antigen test	Negative		Vitamin D	9.30 ng/mL	20-50 ng/mL
HBsAg	Non-reactive		Urea	42 mg/dL	17-49 mg/dL
Anti-HCV	Non-reactive		Creatinine	0.77 mg/dL	0.6-1.35 mg/dL

TLC[1], total leukocyte count; AST[2], aspartate aminotransferase; ALT[3], alanine aminotransferase; ALP[4], alkaline phosphatase; PLT[5], platelet.

NS1, non-structural protein 1; Ag, antigen; IgM, immunoglobulin M; IgG, immunoglobulin G; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.

Table 2. Laboratory findings.

Investigation	Result	Investigation	Result
Hemoglobin	7.10 gm/dL	Total bilirubin	3.09 mg/dL
TLC	600/µL	Direct bilirubin	2.58 mg/dL
PLT	24,000/µL	Indirect bilirubin	0.5 mg/dL
Sodium	123 mmol/L	AST	599 U/L
Ferritin	9,491 ng/mL (21.8-274.6 ng/mL)	ALT	227 U/L
Triglycerides	282 mg/dL (<150 mg/dL)	ALP	1,579 U/L
Fibrinogen	240 mg/dL (238-498 mg/dL)		

TLC, total leukocyte count; PLT, platelet count; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

Samples were also sent for bacterial, fungal, and mycobacterial cultures, including culture on Lowenstein-Jensen medium.

Bone marrow examination revealed a normocellular marrow with trilineage hematopoiesis and evidence of hemophagocytosis. Numerous intracellular kinetoplast-containing organisms consistent with *Leishmania donovani* bodies (LD bodies) were identified within macrophages, as well as within erythroid and myeloid precursors, confirming the diagnosis of VL (Figures 1 and 2).

Bone marrow aspirate samples were negative on ZN staining and CBNAAT, as well as on bacterial, fungal, and mycobacterial cultures, ruling out tuberculosis and other infectious etiologies.

The patient fulfilled six of the eight HLH-2004 diagnostic criteria: (1) fever, (2) splenomegaly, (3) cytopenias involving all three hematopoietic lineages, (4) hypertriglyceridemia, (5) hyperferritinemia, and (6) evidence of hemophagocytosis in the bone marrow. The remaining criteria included hypofibrinogenemia, which was not present because the serum fibrinogen level was within the normal range, and low or absent natural killer cell activity and elevated soluble interleukin-2 receptor (sCD25) levels, which were not assessed because these tests were unavailable at our institution. Thus, a diagnosis of secondary HLH due to VL was made.

The patient was initiated on intravenous amphotericin B deoxycholate at a dose of 1 mg/kg body weight (50 kg), according to the National Institutes of Health Guidelines for the Prevention and Treatment of Opportunistic Infections in Adults and Adolescents with HIV, because liposomal amphotericin B was unaffordable. ART was reinitiated, and cotrimoxazole (trimethoprim-sulfamethoxazole) 160/800 mg once daily was started for prophylaxis.

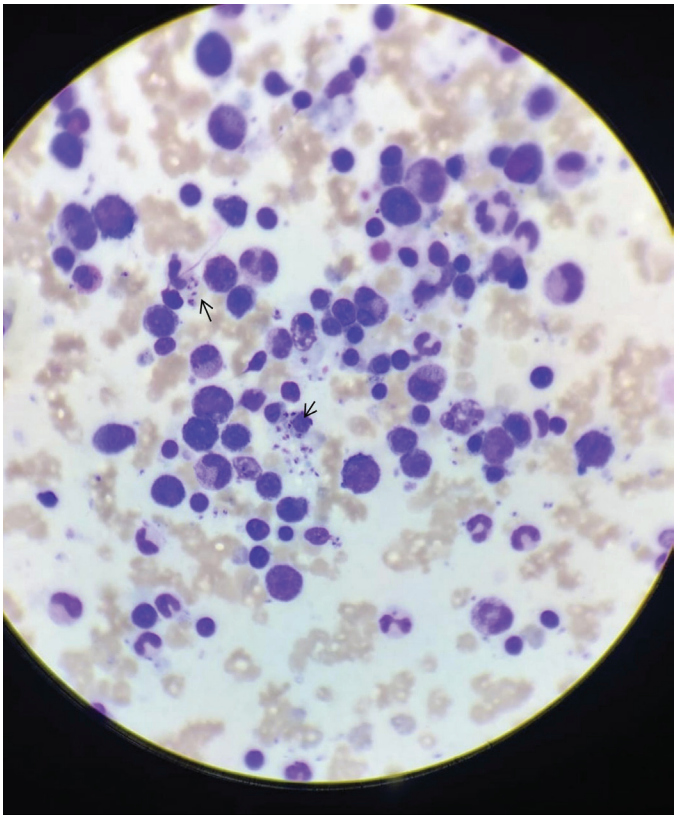

Figure 1. Bone marrow aspirate smears stained with Leishman stain (× 40, oil immersion) showing numerous intracellular and extracellular Leishmania donovani amastigotes (arrows).

Low-dose corticosteroids were administered for 5 days (intravenous dexamethasone, 8 mg once daily), followed by gradual tapering, as adjunctive therapy for HLH management.

In view of the melena and dysphagia, upper gastrointestinal endoscopy was performed. Endoscopy revealed a large excavated ulcer measuring approximately 4 cm, with regular margins and slough, in the prepyloric region. Another ulcer measuring approximately 2 cm and covered with an adherent blood clot was also noted in the prepyloric region (Figures 3-5).

Histopathological examination of biopsy samples from the gastric ulcers demonstrated benign gastric ulcers with multiple intracellular and extracellular *Leishmania donovani* bodies, further supporting the diagnosis of VL.

Intravenous amphotericin B deoxycholate was continued for 30 days with close monitoring of renal function and electrolyte levels, along with appropriate premedication to prevent infusion-related reactions. From the sixth dose onward, the patient developed persistent hypokalemia, requiring daily potassium supplementation; however, renal function remained normal throughout therapy.

The patient showed significant clinical improvement after 10 days of treatment, with defervescence and improved appetite.

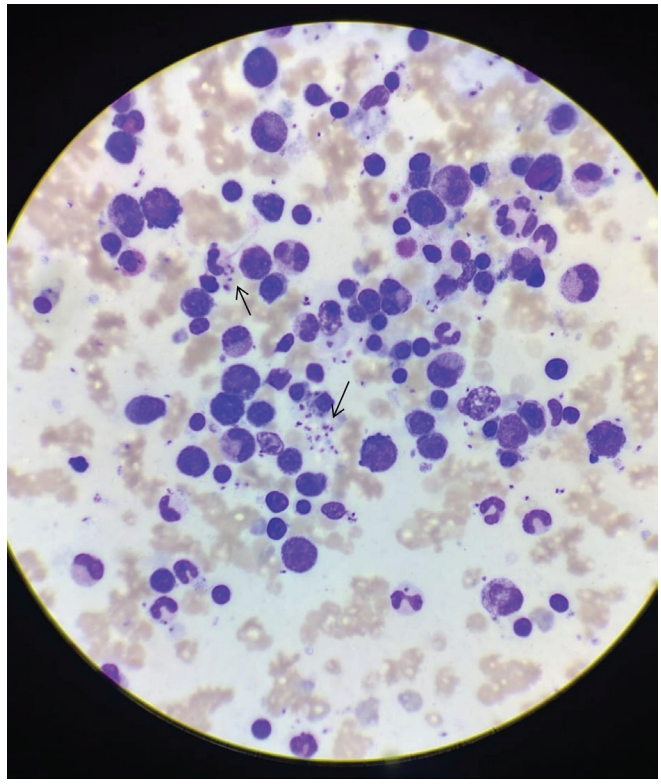

Figure 2. Bone marrow aspirate smears stained with Leishman stain (× 40, oil immersion) showing numerous intracellular and extracellular Leishmania donovani amastigotes (arrows).

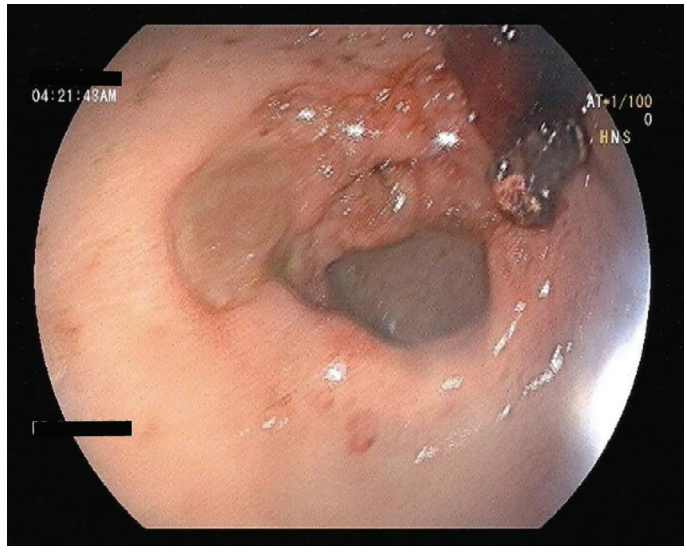

Figure 3. Upper gastrointestinal endoscopy showing gastric ulcers.

Symptoms, including vomiting, dysphagia, melena, and abdominal pain, resolved. There was marked regression of hepatosplenomegaly, and repeat ultrasonography on day 20 showed a normally sized liver and spleen.

Pancytopenia resolved by day 20, with progressive improvement in the hemoglobin level, leukocyte count, and platelet count.

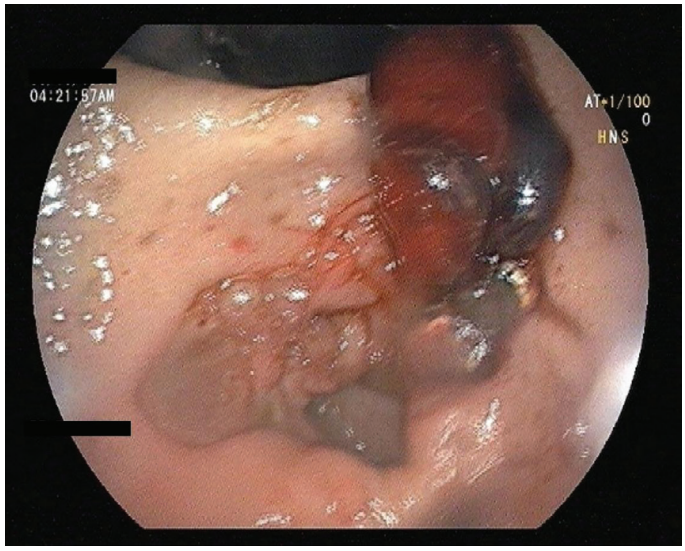

Figure 4. Upper gastrointestinal endoscopy showing gastric ulcers. Liver function tests normalized.

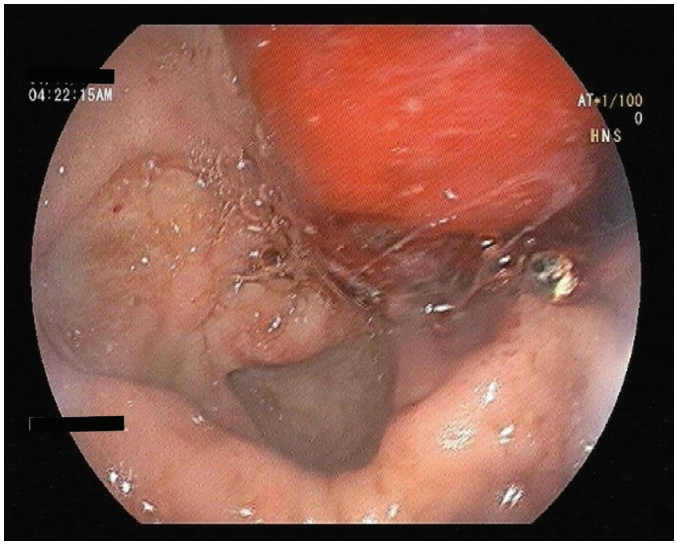

Figure 5. Upper gastrointestinal endoscopy showing gastric ulcers.

After completion of therapy, the patient was discharged on ART and advised to receive monthly amphotericin B deoxycholate at a dose of 1 mg/kg as secondary prophylaxis.

At the 6-month follow-up, there was no evidence of recurrent leishmaniasis, and the CD4 count had improved to 366 cells/µL. Subsequently, the patient was lost to follow-up.

Discussion

VL, or "kala-azar," is the most severe form of leishmaniasis and remains a major public health problem in endemic regions such as India. It is caused by intracellular protozoa of the *Leishmania* genus, which survive and replicate within macrophages and other mononuclear phagocytic cells. More than 20 *Leishmania* species cause human disease, with *Leishmania donovani* being the predominant cause of VL in the Indian subcontinent. Globally, the incidence of leishmaniasis is estimated at 700,000-1 million new cases annually, with nearly 90% of VL cases reported from a limited number of countries, including India, Bangladesh, Brazil, Ethiopia, South Sudan, and Sudan[1,2,11].

In India, VL is primarily an anthroponotic disease transmitted by *Phlebotomus argentipes* and disproportionately affects socioeconomically disadvantaged populations, particularly migrant workers residing in or originating from endemic areas, including Bihar, Jharkhand, West Bengal, and eastern Uttar Pradesh[1,2]. In addition to vector-borne transmission, leishmaniasis may rarely be acquired through needle sharing among people who inject drugs, transfusion of infected blood, or vertical transmission from mother to child[3].

Most *Leishmania* infections in immunocompetent hosts remain asymptomatic, with parasites persisting in a latent state for prolonged periods. However, progression to active disease may occur months to years later when host cell-mediated immunity is compromised[12].

Classical VL manifests with prolonged fever, weight loss, hepatosplenomegaly, hypoalbuminemia, and pancytopenia due to infiltration of the reticuloendothelial system, bone marrow suppression, and chronic immune activation. Without timely treatment, VL is almost uniformly fatal, with death resulting from complications such as secondary infections, severe cytopenias, hepatic dysfunction, or immune-mediated syndromes such as HLH[2,9].

HIV and VL are mutually reinforcing infections with synergistic pathogenic effects. Since the first reports of HIV-VL coinfection in the late 1980s, cases have been documented in more than 45 countries worldwide[3,13]. Although the incidence declined substantially in high-income countries following the introduction of combination ART, HIV-VL coinfection remains a significant concern in parts of Asia, Africa, and Latin America[3,13,14].

In India, the burden of VL–HIV coinfection has increased over time, with recent estimates suggesting that 5%-7% of patients with VL are coinfected with HIV, particularly in endemic states such as Bihar[15-17].

HIV infection increases the risk of developing clinical VL by more than 100-fold, while VL accelerates HIV disease progression by enhancing viral replication and worsening $CD4^+$ T-cell depletion[5]. Both pathogens target macrophages and

dendritic cells, resulting in profound immune dysregulation, increased parasite loads, and impaired immune recovery despite virologic suppression[18,19].

Clinically, the most common presentation of leishmaniasis in people living with HIV (PLHIV) is severe systemic visceral disease, characterized by prolonged fever, weight loss, hepatosplenomegaly, and pancytopenia.[4,20,21]. However, splenomegaly may be less prominent than in immunocompetent individuals, and atypical manifestations are frequent in patients with advanced immunosuppression ($CD4^+$ T-cell count <200 cells/mm^3)[20]. These manifestations include gastrointestinal, mucosal, pulmonary, serosal, and cutaneous involvement[22]. Gastrointestinal leishmaniasis may manifest as abdominal pain, diarrhea, malabsorption, gastrointestinal bleeding, dysphagia, or ulcerative lesions[6,22]. High parasite burdens, poor serological responses, and relapses are also frequent in immunosuppressed hosts[19,23].

In the present case, the patient exhibited classical features of VL along with atypical manifestations—notably melena, dysphagia, and gastric ulcers—which were subsequently confirmed to result from gastrointestinal involvement by *Leishmania donovani*. Our patient had poor ART adherence for nearly a year, resulting in virologic failure and significant immunosuppression (CD4 count, 157 cells/µL), which most likely predisposed him to severe disseminated leishmaniasis.

The diagnosis of VL relies on a combination of clinical suspicion, serological testing, and parasitological confirmation. Serological tests such as the rK39 immunochromatographic assay detect host antibodies directed against a recombinant kinesin-related antigen of *Leishmania* species and are widely used as rapid diagnostic tests for VL in endemic areas because of their ease of use and high sensitivity in immunocompetent individuals, with a reported sensitivity of 97% in the Indian subcontinent. However, in advanced HIV infection, rK39 sensitivity is significantly reduced because of an impaired humoral immune response, leading to false-negative results. Consequently, a negative rK39 test should not exclude the diagnosis when clinical suspicion remains high, and a tissue-based diagnosis may be required for confirmation[24,25].

Parasitological confirmation through the demonstration of *Leishmania donovani* bodies remains the diagnostic gold standard. Bone marrow aspiration, splenic aspiration, lymph node biopsy, and tissue biopsies from involved organs can demonstrate intracellular amastigotes, with splenic aspiration having the highest sensitivity but also an increased procedural risk[16,13]. Bone marrow examination, although less sensitive than splenic aspiration, is safer and particularly valuable in patients with pancytopenia and suspected HLH because it allows simultaneous evaluation for hemophagocytosis and opportunistic infections[13,10].

Molecular methods such as PCR offer high sensitivity but are not universally available in many resource-limited endemic regions[5].

In our patient, the initial evaluation for common opportunistic infections was negative. The rK39 serological test for leishmaniasis was also negative, consistent with the reported reduced sensitivity of this test in individuals with HIV infection. Persistent fever, worsening cytopenias, hepatosplenomegaly, and biochemical evidence of hyperinflammation prompted further evaluation.

Bone marrow aspiration and biopsy revealed hemophagocytosis along with intracellular *Leishmania donovani* bodies, establishing the diagnosis of VL with secondary HLH. Additionally, upper gastrointestinal endoscopy with biopsy of the gastric ulcers demonstrated intracellular and extracellular *Leishmania donovani* bodies, confirming disseminated VL with gastrointestinal involvement—an uncommon but well-documented manifestation in VL-HIV coinfection. It is also noteworthy that *Leishmania* amastigotes were identified on routine histopathological examination of the gastric biopsy specimens rather than through targeted testing for leishmaniasis. This finding highlights the importance of meticulous pathological evaluation in immunocompromised patients presenting with atypical gastrointestinal lesions, as careful examination of routinely processed biopsy specimens may provide the first clue to an otherwise unsuspected diagnosis.

HLH is a rare but life-threatening hyperinflammatory syndrome driven by uncontrolled activation of cytotoxic T lymphocytes and macrophages, resulting in excessive cytokine release and multiorgan dysfunction. HLH may be primary (genetic) or secondary, with infections being the most common triggers in adults[7,9,26].

HLH is diagnosed using the HLH-2004 criteria proposed by the Histiocyte Society, which require either a pathogenic HLH-associated genetic mutation or fulfillment of at least five of eight criteria: fever, splenomegaly, cytopenias involving ≥2 blood cell lineages, hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis in tissue samples, low or absent natural killer cell activity, hyperferritinemia, and elevated sCD25 levels[7].

Clinically, HLH presents with persistent high-grade fever, hepatosplenomegaly, lymphadenopathy, and rapid systemic deterioration, while laboratory abnormalities typically include pancytopenia, markedly elevated serum ferritin

levels, hypertriglyceridemia, coagulopathy, transaminitis, and cholestatic liver dysfunction with rising bilirubin levels. Cholestasis in HLH is attributed to cytokine-mediated hepatocellular injury, sinusoidal infiltration by activated macrophages, and hepatic hemophagocytosis and may be a marker of severe disease[8]. In PLHIV, HLH often follows a more fulminant course because of immune dysregulation and delayed pathogen clearance[27].

VL is a recognized but uncommon infectious trigger of secondary HLH in adults.

Diagnosis is particularly challenging in VL–HIV coinfection because of overlapping clinical and laboratory features with HLH. This overlap may lead to delayed recognition of VL and the inappropriate initiation of aggressive immunosuppressive therapy without antiparasitic treatment, potentially resulting in fatal outcomes[9].

In our patient, the diagnosis of secondary HLH was established based on the fulfillment of six HLH-2004 criteria: persistent fever, splenomegaly, pancytopenia, hypertriglyceridemia, marked hyperferritinemia, and hemophagocytosis on bone marrow examination. The precipitating trigger was VL in the setting of advanced HIV infection. The patient also developed progressive conjugated hyperbilirubinemia with a cholestatic pattern, consistent with hepatic involvement in HLH.

Management of VL in PLHIV requires a combined approach addressing both infections. International guidelines recommend initiating or optimizing ART as soon as patients can tolerate therapy, as immune reconstitution improves survival and reduces relapse rates[16,13].

Reinitiation of ART in patients with VL-HIV coinfection may occasionally be associated with immune reconstitution inflammatory syndrome (IRIS), manifesting as paradoxical worsening of previously diagnosed leishmaniasis or unmasking of subclinical disease. Although IRIS was not observed in our patient, awareness of this phenomenon is important when restarting ART in severely immunosuppressed individuals with VL[4,13].

Among antileishmanial agents, liposomal amphotericin B is preferred because of its superior efficacy and lower nephrotoxicity compared with amphotericin B deoxycholate. In accordance with current guidelines, amphotericin B deoxycholate remains an acceptable alternative when liposomal formulations are unaffordable, provided that close monitoring is ensured. Other alternatives include miltefosine and pentavalent antimonial agents such as meglumine antimoniate[13].

In cases of VL-associated HLH, prompt initiation of targeted antileishmanial therapy is the cornerstone of management. Adjunctive low-dose corticosteroids may be beneficial in controlling the cytokine storm and preventing organ failure in severe cases[28].

More aggressive HLH-directed regimens are generally reserved for refractory disease because of concerns that they may worsen the underlying infection[28].

Secondary prophylaxis is recommended for all patients with CD4 counts <200 cells/μL because of the high risk of relapse. Discontinuation should be considered only after sustained immune recovery, as evidenced by a CD4 count exceeding 350 cells/μL or an undetectable viral load for at least 6 months[3,13].

Our patient was successfully treated with amphotericin B deoxycholate (1 mg/kg/day for 30 days), along with continuation of ART and a short course of low-dose corticosteroids for HLH. Despite electrolyte disturbances, notably hypokalemia, treatment was completed successfully with careful monitoring. The patient demonstrated rapid clinical and laboratory improvement, with resolution of fever, cytopenias, hepatosplenomegaly, and liver dysfunction, supporting previous reports that successful treatment of VL may lead to the resolution of secondary HLH without the need for intensive HLH-directed immunochemotherapy in many cases[9,10].

Secondary prophylaxis with monthly amphotericin B deoxycholate (1 mg/kg) was instituted as recommended by the World Health Organization and National Institutes of Health guidelines[16,13].

This case highlights the complex diagnostic and therapeutic challenges posed by the coexistence of advanced HIV infection, disseminated VL, and secondary HLH. In endemic settings, VL and HLH should be considered in PLHIV who present with prolonged fever, pancytopenia, and organomegaly, even when serological tests such as rK39 are negative. Atypical manifestations, including gastrointestinal involvement, and the substantial clinical overlap among HIV infection, VL, and HLH can delay diagnosis, underscoring the importance of maintaining a high index of suspicion and pursuing early tissue-based diagnostic confirmation when indicated. Prompt initiation of antileishmanial therapy is critical and can be lifesaving. This case also illustrates the challenges associated with limited access to liposomal amphotericin B, which may necessitate the use of amphotericin B deoxycholate and increase the risk of treatment-related adverse effects, such as persistent hypokalemia. Furthermore, poor adherence to ART because of migration and socioeconomic barriers can exacerbate immunosuppression, increase disease severity, and predispose patients to relapse. Strengthening access to diagnostic facilities, affordable treatment options, and uninterrupted HIV care is essential to improving outcomes in this vulnerable population.

Ethics

Informed Consent: Written informed consent for publication was obtained from the patient and his son.

Footnotes

Authorship Contributions

Concept: G.S.S., U.S., Design: U.S., S.K., Data Collection or Processing: U.S., Analysis or Interpretation: G.S.S., S.K., Literature Search: U.S., N.O., Writing: U.S., N.O.

Conflict of Interest: The authors declare no conflicts of interest.

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Stigma Faced by Patients with Hepatitis in Türkiye: A Cross-sectional Study

Türkiye'de Hepatit Hastalarının Maruz Kaldığı Damgalama: Kesitsel Bir Çalışma

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Abstract

Introduction: Structural stigma (SS) is one of the main types of stigma and refers to stigmatizing processes that occur above the individual level. SS among patients with hepatitis in Türkiye and worldwide has rarely been studied. We aimed to determine the experiences of SS among patients with chronic hepatitis B (CHB) or chronic hepatitis C (CHC) and identify factors that may be associated with SS.

Materials and Methods: A cross-sectional survey was conducted among patients with CHB or CHC infection. The survey included questions about experiences of SS in five different domains: the workplace, marriage procedures, healthcare settings, military service, and procedures for traveling abroad. Experiencing stigma in at least one domain or being required to undergo hepatitis testing and obtain physician approval in any domain was defined as overall SS experience.

Results: The study included 221 patients with CHB and 15 with CHC. When all SS domains were evaluated, 113 (47.9%) patients were found to have experienced SS in at least one domain. Univariate analysis showed that the experience of SS was significantly more common among younger patients ($p < 0.001$). Overall SS was most common among unemployed patients. A significant association was observed between educational level and stigma ($p = 0.010$). In the multivariable analysis, several factors were significantly associated with overall stigma. These included younger age [adjusted odds ratio (aOR): 0.95, 95% confidence interval (CI): 0.92-0.98, $p = 0.001$)], a diagnosis of hepatitis C (aOR: 3.6, 95% CI: 1.0-12.3, $p = 0.045$), and belonging to the laborer group (aOR: 6.1, 95% CI: 1.6-24.0, $p = 0.010$).

Conclusion: Patients with hepatitis experience SS in multiple domains. Addressing SS should be a key focus of multilevel interventions.

Keywords: Hepatitis, structural stigma, institutional stigma, discrimination

Öz

Giriş: Yapısal damgalanma (YD), damgalanmanın temel türlerinden biri olup, birey düzeyinin üzerinde gerçekleşen damgalama süreçlerini ifade eder. Ülkemizde ve dünyada hepatit hastalarında YD nadiren incelenmiştir. Bu çalışmada, viral hepatit hastalarının YD deneyimlerinin ve bununla ilişkili olabilecek durumların belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Kronik hepatit B (HBV) ve Kronik hepatit C (HCV) hastaları arasında kesitsel bir anket çalışması yürütülmüştür. Ankette, iş yeri, evlilik işlemleri, sağlık hizmetleri, askerlik hizmeti ve yurt dışına çıkış işlemleri olmak üzere beş farklı alanda YD deneyimlerini sorgulayan sorular yer almıştır. En az bir alanda damgalanma yaşanması veya herhangi bir alanda hepatit testi yaptırılması ve hekim onayı istenmesi, genel YD olarak tanımlanmıştır.

Bulgular: Çalışmaya 221 HBV ve 15 HCV hastası dahil edilmiştir. Tüm YD alanları birlikte değerlendirildiğinde, 113 (%47,9) hastanın en az bir alanda YD'a maruz kaldığı saptanmıştır. Tek değişkenli analizde, YD deneyiminin genç yaşlarda istatistiksel olarak daha yüksek olduğu görülmüştür ($p < 0,001$). Genel YD deneyiminin en sık işsiz grupta görüldüğü belirlenmiştir. Eğitim düzeyi ile damgalanma arasında anlamlı bir ilişki gözlenmiştir

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p = 0,010). Çok değişkenli analizde, genel damgalanma deneyimi üzerinde anlamlı etkisi olan bazı faktörler belirlenmiştir. Bunlar; genç yaş [düzeltilmiş olasılık oranı (aOR): 0,95, %95 güven aralığı (GA): 0,92-0,98, p = 0,001), hepatit C tanısı alma (aOR: 3,6; %95 GA: 1,0-12,3; p = 0,045) ve işçi grubuna dahil olma (aOR: 6,1; %95 GA: 1,6-24,0; p = 0,010) olarak bulunmuştur.

Sonuç: Hepatit hastaları birçok alanda YD'a maruz kalmaktadır. YD ile mücadele, çok düzeyli müdahalelerin temel odak noktalarından biri olabilir.

Anahtar Kelimeler: Hepatit, yapısal damgalanma, kurumsal damgalanma, ayrımcılık

Introduction

Globally, an estimated 254 million people live with hepatitis B virus (HBV) and 50 million with hepatitis C virus (HCV)[1]. There have been many important developments in the fight against viral hepatitis, including the development of vaccines and antiviral drugs. Despite these achievements, stigma remains a significant challenge that needs to be addressed.

The classification of stigma varies across diseases and among authors. Some classify stigma into three major subtypes, whereas others distinguish up to five subtypes[2,3]. Structural stigma (SS) is one of the most significant forms of stigma. It encompasses processes that operate beyond the individual and interpersonal levels and is best defined as "societal-level conditions, cultural norms, and institutional policies that impinge upon the opportunities, resources, and well-being of stigmatized individuals"[4].

Stigma can have negative consequences that may hinder global hepatitis strategies by reducing the uptake of diagnostic screening and clinical care and lowering medication adherence[5,6]. The World Health Organization 2022-2030 viral hepatitis strategy includes addressing stigma and discrimination, as well as social and structural barriers[7]. Beyond the treatment process and disease elimination, stigma also affects quality of life[8,9]. People with hepatitis face inequalities in education, employment, and career development[5]. Many employers and institutions refuse to accept individuals with a positive HBV test result[10,11]. Indeed, once SS becomes established, institutional policies and practices can become normalized, and stigma can have negative consequences beyond the context of interpersonal interactions[12].

Although they vary between countries and institutions, practices and laws are in place to protect the rights of patients with hepatitis[13]. However, these laws and policies may not be sufficient to overcome SS because healthcare practitioners and even patients are often unaware of SS and may normalize stigmatizing practices.

In some diseases, such as HIV infection, stigma has been more extensively addressed and studied in relation to its causes, effects, and potential solutions[5]. There are few quantitative or qualitative studies addressing SS among patients with hepatitis. Most stigma studies have been conducted in urban areas. However, understanding experiences and practices in rural areas, where community dynamics, perceptions, and attitudes may differ, is important for future improvements.

In this study, we aimed to determine the SS experiences of patients with chronic hepatitis B or C in a rural area and identify situations that may be associated with SS.

Materials and Methods

Study Design and Participants

A cross-sectional survey was conducted to explore experiences and perceptions of SS among patients with HBV and HCV infection. The study was conducted among adults (≥18 years of age) with HBV or HCV infection who presented for care at the outpatient infectious diseases clinic of Fatsa State Hospital between November 20, 2022, and May 20, 2023. All patients with HBV or HCV infection who presented to the outpatient clinic during the study period were assessed for eligibility. Consecutive sampling was used, and all eligible patients presenting during the study period were invited to participate.

Inclusion criteria:

Hepatitis B surface antigen (HBsAg) or HBV DNA positivity, with or without treatment

Anti-HCV or HCV RNA positivity, with or without treatment

Exclusion criteria:

Individuals with communication difficulties that prevented them from sharing their experiences of stigma, such as those with intellectual disabilities

Patients with false-positive HBsAg or anti-HCV results

Patients first diagnosed with hepatitis during the outpatient visit

Patients with HIV coinfection

Instruments

A custom-built questionnaire was administered to patients by two infectious diseases specialists. The questionnaire was developed based on previously published studies on stigma and was reviewed by two infectious diseases specialists for content validity. The survey included demographic information

and questions regarding experiences of SS in five different domains (Supplementary Table 1). Verbal informed consent was obtained from all participants before the interview.

The primary outcome was overall SS experience, defined as reporting at least one episode of perceived discrimination or being subjected to hepatitis-specific institutional requirements, such as additional medical testing or physician approval, in any of the five predefined domains. Although some of these requirements may reflect legal or administrative procedures, they were included because they may function as structural barriers and contribute to stigmatizing experiences from the patient's perspective. All questions were answered using a yes/no response format. In cases of discrimination, additional details regarding the discriminatory experience were collected.

Educational attainment was classified according to International Standard Classification of Education 1997 (ISCED-97)[14]. Levels 4-6 of ISCED-97 were grouped under a single category, tertiary education, because of the small number of patients in these levels. Occupations were categorized according to an article-specific occupational classification based on the field in which participants worked. Data were collected between November 20, 2022, and May 20, 2023.

Table 1. Characteristics of the participants.

Demographic characteristics	n (236)	(%)
Age		
Median (IQR)	47 (38-56)	
Sex		
Female	121	51.3
Male	115	48.7
Diagnosis		
CHB	221	93.6
CHC	15	6.4
Treatment		
Yes	150	63.6
No	86	36.4
Marital Status		
Married	209	88.6
Single	22	9.3
Divorced	5	2.1
Occupation		
Unemployed	98	41.5
Laborer	67	28.4
Office worker	30	12.7
Farmer	20	8.5
Other[a]	21	8.9
Education		
Illiterate/primary education	109	46.2
Lower secondary education	81	34.3
Upper secondary education	24	10.2
Tertiary education	22	9.3

a, furniture craftsman, civil engineer, tradesman, masseur, and marketer. IQR, interquartile range; CHB, chronic hepatitis B; CHC, chronic hepatitis C.

This study was conducted in accordance with the principles of the Declaration of Helsinki (2000). Ethical approval was obtained from the Ordu University Ethics Committee (approval number: 2022-234) on October 27, 2022.

Data Analysis

SPSS Statistics for Windows, version 25 (IBM Corp.), was used for the statistical analyses. Descriptive statistics, including medians and percentages, were used to summarize patient characteristics. The normality of continuous variables was assessed using both visual methods (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). All continuous variables were non-normally distributed and were therefore analyzed using non-parametric methods. The Pearson chi-square test and Fisher exact test were used to evaluate categorical variables. The Mann-Whitney U test was used to compare the distributions of continuous variables between two independent groups. Results were evaluated using a 95% confidence interval, and statistical significance was defined as $p < 0.05$. A multivariable logistic regression model was used to assess independent predictors of SS. This analysis was limited to the factors evaluated in the questionnaire. Variables associated with SS at $p < 0.20$ in univariate analysis, as well as variables identified as predictors in similar studies in the literature, were included in the multivariable models. Final models were calculated using the enter method. Model fit was evaluated using the Hosmer–Lemeshow goodness-of-fit test. Model discrimination was assessed using Nagelkerke's pseudo-R^2. Odds ratios (ORs) were used to report statistical associations.

Results

The study included a total of 236 patients, including 221 with CHB, and 15 with CHC. The median age was 47 years; 51.3% of the patients were female and 48.7% were male. The demographic characteristics of the patients and their experiences of SS across different domains are shown in Table 1.

When all SS domains were evaluated together, 113 (47.9%) patients were found to have experienced SS in at least one domain. The ways in which patients experienced SS are summarized in the Figure 1.

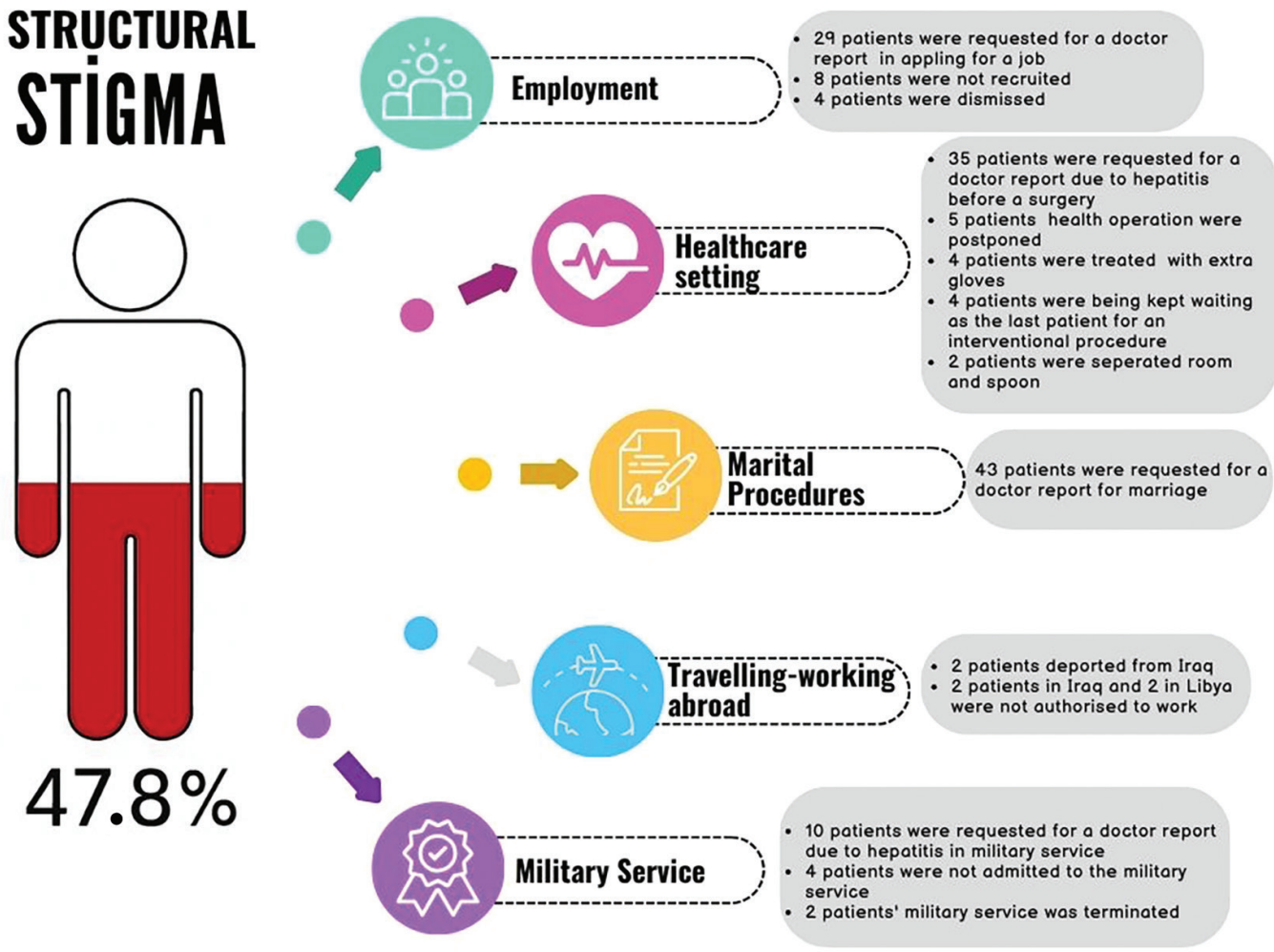

Figure 1. Details of SS experiences evaluated across five domains. SS, structural stigma.

Overall, 39.0% of participants reported being asked to provide a physician's report because of their hepatitis status. The distribution of variables according to SS is shown in Table 2.

Univariate analysis showed that SS was significantly more common among younger patients ($p < 0.001$). Overall SS experience was most common among unemployed patients. A significant association was observed between educational level and stigma ($p = 0.010$). Factors associated with overall SS experience are shown in Table 3.

Table 4 presents the results of the multivariable regression analyses of overall SS, employment-related stigma, and healthcare-setting stigma. Several factors were identified as significantly associated with overall SS experience. These included younger age [adjusted OR (aOR): 0.95, 95% confidence interval (CI): 0.92-0.98, p = 0.001), a diagnosis of hepatitis C (aOR: 3.6, 95% CI: 1.0-12.3, $p = 0.045$**), and belonging to the laborer group (aOR: 6.1, 95% CI: 1.6-24.0, $p = 0.010$). The goodness-of-fit of the multivariable logistic regression model was acceptable, as demonstrated by the Hosmer-Lemeshow goodness-of-fit test (χ^2: 6.771, df: 8, $p = 0.562$). The model explained approximately 20.3% of the variance in overall SS according to Nagelkerke R^2 (Cox and Snell R^2: 0.152).

Discussion

This study demonstrated that SS, which has not been widely studied in patients with chronic hepatitis, is relatively common and warrants greater attention. Nearly half (48%) of our patients with chronic hepatitis had experienced SS at least once in their lifetime.

The prevalence of stigma among patients with chronic hepatitis has varied across studies because stigma encompasses multiple subtypes and its measurement has not been standardized worldwide. Although comprehensive stigma scales are available, the lack of validation and reliability studies across all countries, substantial differences in the perception of stigma among societies, and the presence of antistigma legislation

Table 2. Distribution of patients with hepatitis according to different SS domains and overall SS experience.

Structural stigma domain	n (236)	%
Employment-related stigma[a]	33	14.0
Stigma in healthcare settings	60	25.4
Stigma in military service[b]	12	25.5
Stigma related to traveling abroad[c]	6	11.3
Request for a physician's report before applying for a job[a]	29	16.3
Request for a physician's report before a surgery[d]	35	24.5
Request for a physician's report in military service[b]	10	21.7
Request for a physician's report in marital procedures[e]	43	33.1
Request for a physician's report from any institution	92	39.0
Overall SS experience	113	47.9

a, Persons who had applied for a job at least once and had been diagnosed with chronic hepatitis at the time of application were evaluated. b, Persons who had applied for military service and had been diagnosed with chronic hepatitis at the time of application were evaluated. c, Persons who had traveled abroad at least once and had been diagnosed with chronic hepatitis during this period were evaluated. d, Persons who had at least one planned surgery and had been diagnosed with chronic hepatitis during this period were evaluated. e, Persons who had applied for marriage at least once and had been diagnosed with chronic hepatitis at that time were evaluated. SS, structural stigma.

Table 3. Factors associated with overall SS experience.

	Experienced stigma n (%)	Did not experience SS n (%)	p	OR (95% CI)
Median age (min-max)	42 (20–67)	50 (20–69)	**<0.001***	
Sex				
Female	56 (49.6)	65 (52.8)	0.614	1
Male	57 (50.4)	58 (47.2)		1.1 (0.7–1.9)
Diagnosis				
CHB	104 (92.0)	117 (95.1)	0.332	1
CHC	9 (8.0)	6 (4.9)		1.7 (0.6–4.9)
Treatment				
No	46 (40.7)	40 (32.5)	0.192	1
Yes	67 (59.3)	83 (67.5)		0.7 (0.4–1.2)
Occupation				
Farmer	3 (2.7)	17 (13.8)	**0.020****	1
Unemployed	46 (40.7)	52 (42.3)		5 (1.4–18)
Other	10 (8.8)	11 (8.9)		5.2 (1.2–23)
Office worker	15 (13.3)	15 (12.2)		5.7 (1.4–24)
Laborer	39 (34.5)	28 (22.8)		7.9 (2.1–29)
Education				
Tertiary education	13 (11.5)	9 (7.3)	**0.010*****	1
Upper secondary education	18 (15.9)	6 (4.9)		2.1 (0.6–7.3)
Lower secondary education	39 (34.5)	42 (34.1)		0.6 (0.3–1.7)
Illiterate/primary education	43 (38.1)	66 (53.7)		0.5 (0.2–1.2)
Total	113	123		

*Mann-Whitney U test was applied. **The groups that accounted for the difference were the farmer and laborer groups, respectively. ***The illiterate/primary education group was combined and analyzed. The groups that accounted for the difference were the upper secondary education and illiterate/primary education groups, respectively. OR, odds ratio; CI, confidence interval; min-max, minimum-maximum; CHB, chronic hepatitis B; CHC, chronic hepatitis C.

Table 4. Evaluation of factors associated with employment-related, healthcare-setting, and overall stigma using multivariable logistic regression models.

	Employment-related stigma		Healthcare setting stigma		Overall stigma	
	p	**OR_{adj} (95% CI)**	**p**	**OR_{adj} (95% CI)**	**p**	**OR_{adj} (95% CI)**
Age	**0.022**	0.95 (0.91–0.99)	0.961	1 (0.97-1.04)	**0.001**	0.95 (0.92-0.98)
Diagnosis						
Hepatitis B	0.443	1		1	**0.045**	1
Hepatitis C		2 (0.3-12)	0.154	2.4 (0.7-7.7)		3.6 (1.0-12.3)
Treatment						
No				1		1
Yes	0.880	1.1 (0.5-2.5)	0.309	1.5 (0.7–2.8)	0.718	0.9 (0.5-1.6)
Occupation						
Farmer		1[a]		1		1
Unemployed		-	**0.044**	4.8 (1.04-22.6)	0.065	3.5 (0.9-13.4)
Other		-	0.527	1.8 (0.3-12)	0.166	3 (0.6-14.9)
Office worker		-	0.714	1.4 (0.2-9.4)	0.370	2.1 (0.4-10.1)
Laborer		-	0.293	2.4 (0.5-12)	**0.010**	6.1 (1.6-24)
Education						
Tertiary education		1		1		1
Upper secondary education	0.645	1.5 (0.3-7.9)	0.666	1.4 (0.3-5.7)	0.931	1.1 (0.3-4.2)
Lower secondary education	0.460	0.6 (0.1-2.7)	0.503	0.6 (0.2-2.4)	0.072	0.3 (0.1-1.1)
Illiterate/primary education	0.682	0.7 (0.1–3.7)	0.570	0.7 (0.2-2.5)	0.091	0.4 (0.1-12.2)

a, Adjusted odds ratio (OR_{adj}) and confidence intervals (CIs) could not be calculated since the number of people experiencing employment-related stigma in the farmer group was 0.

in various countries pose significant challenges to assessing stigma using a universal scale.

Since, most studies have evaluated knowledge and attitudes among different populations and the number of studies specifically addressing SS is limited, it is not possible to determine the prevalence of SS among patients with hepatitis from the existing scientific literature. However, some studies have shown that patients with hepatitis experience stigma in various institutional settings, consistent with our findings[11,15-17]. A study comparing patients with hepatitis B and non-hepatitis B controls found that the majority of controls believed that HBV carriers should not be employed in the restaurant industry. Similarly, 44.0% of controls stated that HBV carriers should not work with children[18]. The same study reported that 40.0% of patients with HBV had been required to undergo preemployment HBV testing, and 29.0% of these individuals believed that they had lost job opportunities because of their disease status[18]. In another study on barriers faced by people with hepatitis B, Na et al.[19] reported that institutional discrimination was the most frequently identified environmental barrier. In a study conducted among patients with hepatitis B in Sierra Leone, 73.6% of respondents reported at least one instance of enacted stigma[20].

In our study, a high proportion of patients diagnosed with hepatitis reported being asked to provide a medical report because of their hepatitis status. There may be several reasons why an institution might request a medical report from a physician stating that "there are no medical reasons why the individual is unable to work." Fear and misconceptions regarding the transmission of hepatitis B and C viruses through bodily fluids may be leading causes of SS[11].

Many unnecessary precautions, such as the inappropriate use of gloves and isolation of patients in separate rooms, are implemented when caring for patients diagnosed with hepatitis in healthcare settings[21,22]. In our study, patients reported frequent discrimination in healthcare settings, including practices such as refusing to perform dental procedures, keeping patients waiting until they were the last to undergo interventional procedures, requesting unnecessary preoperative infectious disease consultations, and wearing additional gloves. In our opinion, stigma reproduced through healthcare practices and settings may reinforce social stigma because healthcare professionals are expected to possess evidence-based knowledge and avoid misconceptions about viral hepatitis. Similarly, the perceptions of institutional managers, legislators, and other leaders can influence public

attitudes and behaviors and contribute to both formal and informal discrimination.

In a review evaluating factors associated with stigma, the factors varied across studies[5]. Among studies of stigma in patients with hepatitis, the associations between educational level, age, and stigma have varied, as most studies were cross-sectional and conducted in different regions[23]. Because these studies investigated not only SS but also other types of stigma, their findings may differ from those of our study, in which younger age was associated with greater SS experience. Our study was conducted in a rural area, where older people may tend to live more isolated lives. However, increased hepatitis testing and greater awareness of hepatitis in society and institutions compared with the past may have contributed to lower levels of stigma toward older people.

Although hepatitis C infection emerged as an independent predictor of overall SS in the multivariable analysis, this finding should be interpreted with caution. Only 15 participants in our cohort had hepatitis C infection, resulting in wide confidence intervals and limiting the precision and stability of the estimated effect. Therefore, the observed association may have been influenced by sparse-data bias and should be considered hypothesis-generating rather than conclusive. Nevertheless, the finding is biologically and socially plausible. In Türkiye, hepatitis B is moderately endemic and is predominantly acquired through perinatal or early childhood transmission, making it a more familiar condition to the general population than hepatitis C. Consequently, misconceptions and limited public awareness regarding hepatitis C may contribute to higher levels of SS. Furthermore, hepatitis C has historically been associated with injection drug use, a behavior that is itself highly stigmatized, which may further reinforce the SS experienced by affected individuals. Further studies involving larger numbers of patients with hepatitis C are needed to confirm this association.

When stigma was analyzed according to occupation, a heterogeneous distribution was observed. The lower prevalence of stigma among farmers may be explained by their less frequent interaction with institutions. The higher levels of stigmatization observed among laborers and unemployed individuals suggest that these groups may have fewer opportunities or resources to protect their rights than other occupational groups.

SS should also be interpreted within the context of national legal and institutional regulations. In Türkiye, certain administrative procedures, such as requests for physician reports or hepatitis-related medical evaluations, may be implemented for legal, occupational, or public health purposes rather than with discriminatory intent. Nevertheless, when such requirements are applied exclusively to individuals with viral hepatitis without clear evidence-based justification, or when they exceed existing recommendations, they may function as structural barriers and contribute to patients' experiences of stigma. From the patient's perspective, repeated hepatitis-specific administrative requirements may reinforce perceptions of exclusion or unequal treatment, regardless of the original intent of the policy.

Study Limitations

The present study has several limitations. First, it was conducted at a single center and was not designed to provide national-level estimates; therefore, the findings may not be generalizable to the broader population. Another limitation is the use of a custom-developed questionnaire. Although the questionnaire was developed based on previously published stigma studies and its content validity was reviewed by two infectious diseases specialists, formal psychometric evaluation, including reliability testing (e.g., Cronbach's alpha), construct validity assessment, and pilot testing, was not performed. Therefore, some degree of measurement error cannot be excluded. Nevertheless, because no validated instrument specifically assessing SS related to hepatitis in the Turkish context was available at the time of the study, a purpose-built questionnaire was considered the most appropriate approach for capturing context-specific experiences. Future studies should focus on developing and validating standardized instruments for measuring SS among people living with viral hepatitis. Despite these limitations, this study is among the few to evaluate SS among patients with hepatitis B and C in Türkiye and globally.

Conclusion

In conclusion, SS is highly prevalent among patients with hepatitis, with nearly half of the participants reporting stigma in at least one institutional domain. Younger age, hepatitis C diagnosis, and being a laborer were independently associated with greater experiences of stigma. These findings suggest that SS is not only a social concern but may also constitute a barrier to equitable access to care, patient well-being, and engagement with health services. Targeted multilevel interventions, including institutional policy review, healthcare worker education and awareness, and patient rights-based approaches, are needed to reduce stigma and improve clinical and public health outcomes.

Supplementary Table 1: https://d2v96fxpocvxx.cloudfront.net/bda9171a-fae8-4995-8276-2138323f1e16/content-images/ed801de8-acd3-431b-a231-2f77ae0ecfa6.pdf

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ordu University Ethics Committee (approval number: 2022-234) on October 27, 2022.

Informed Consent: Verbal informed consent was obtained from all participants before the interview.

Footnotes

Authorship Contributions

Concept: T.K., A.D., Design: T.K., Data Collection or Processing: T.K., A.D., Analysis or Interpretation: T.K., A.D., Literature Search: T.K., Writing: T.K., A.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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Role of the Monocyte-to-Lymphocyte Ratio as a Biomarker for Pulmonary Tuberculosis

Akciğer Tüberkülozunda Biyomarker Olarak Monosit-Lenfosit Oranının Rolü

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Abstract

Introduction: Pulmonary tuberculosis (PTB) is a major global health concern, and rapid and reliable diagnosis in endemic areas is crucial. The monocyte-to-lymphocyte ratio (MLR) represents the relationship between absolute monocyte and lymphocyte counts, and recent studies suggest that an elevated MLR is a promising diagnostic and prognostic biomarker for PTB. It provides insight into the immune response to chronic infection, making it a potential biomarker. This study aimed to evaluate changes in the MLR in patients with active PTB before and after treatment completion.

Materials and Methods: This retrospective study was conducted in the Department of Pathology at Kasturba Medical College, Manipal Academy of Higher Education, Manipal in Karnataka over a 4-year period. The study included 136 patients diagnosed with PTB and 150 healthy controls. The MLR was assessed in patients with PTB before and after treatment completion and compared with that in healthy controls. Data were analyzed using appropriate statistical methods.

Results: Patients with PTB had significantly higher MLR values than the control group ($p < 0.05$). The MLR also significantly decreased following treatment in patients with PTB ($p < 0.0001$), suggesting a significant treatment effect. Receiver operating characteristic analysis showed that an MLR cut-off value of 0.38 yielded a sensitivity of 76.47% and a specificity of 94.67% for detecting active PTB.

Conclusion: This study confirms that the MLR is a valuable biomarker for PTB. It effectively distinguishes patients with PTB from healthy controls and may be useful for monitoring treatment response. The established cut-off value of 0.38 demonstrated moderate sensitivity and high specificity, indicating that the MLR may be a reliable tool for assessing disease progression and treatment efficacy in PTB.

Keywords: Biomarker, tuberculosis, monocytes, predictive, ratio, pulmonary

Öz

Giriş: Akciğer tüberkülozu (PTB) küresel bir sağlık sorunudur ve endemik bölgelerde hızlı ve güvenilir tanı çok önemlidir. Monosit-lenfosit oranı (MLR), mutlak monosit ve lenfosit sayımları arasındaki ilişkiyi temsil eder ve son çalışmalar, yüksek MLR'nin PTB için umut vadeden bir tanısal ve prognostik biyomarker olduğunu göstermektedir. Kronik enfeksiyona karşı bağışıklık yanıtına dair bilgi sağlar ve bu da onu potansiyel bir biyomarker haline getirir. Bu çalışma, aktif PTB'li hastalarda tedavi tamamlanmadan önce ve sonra MLR'deki değişiklikleri değerlendirmeyi amaçlamıştır.

Gereç ve Yöntem: Bu retrospektif çalışma, Karnataka'da bir üçüncü basamak sağlık kuruluşunun Patoloji Bölümü'nde 4 yıllık bir süre boyunca yürütülmüştür. Çalışmaya, tüberküloz tanısı konmuş 136 hasta ve 150 sağlıklı kontrol dahil edildi. Monosit-lenfosit oranı (MLR) değerleri, tüberkülozlu hastalarda tedavi öncesi ve sonrası değerlendirildi ve sağlıklı kontrol grubuyla karşılaştırıldı. Veriler uygun istatistiksel yöntemler kullanılarak analiz edildi.

Bulgular: Tüberkülozlu hastaların MLR değerleri kontrol grubuna göre anlamlı derecede daha yüksekti ($p < 0,05$). MLR ayrıca, tüberkülozlu hastalarda tedavi sonrası anlamlı derecede azaldı ($p < 0,0001$), bu da önemli bir tedavi etkisine işaret etmektedir. Alıcı işletim karakteristik analizi, 0,38'lik bir MLR eşik değerinin, aktif tüberkülozu tespit etmede %76,47 duyarlılık ve %94,67 özgüllük sağladığını gösterdi.

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Sonuç: Bu çalışma, MLR'nin tüberküloz için değerli bir biyobelirteç olduğunu doğrulamaktadır. Tüberkülozlu hastaları sağlıklı kontrol grubundan etkili bir şekilde ayırt eder ve tedavi yanıtının izlenmesinde faydalı olabilir. Belirlenen 0,38 eşik değeri, orta düzeyde duyarlılık ve yüksek özgüllük göstermiş olup, MLR'nin PTB'de hastalık ilerlemesini ve tedavi etkinliğini değerlendirmek için güvenilir bir araç olabileceğini göstermektedir.

Anahtar Kelimeler: Biyobelirteç, tüberküloz, monositler, prediktif, oran, akciğer

Introduction

Tuberculosis (TB) is an infection caused by the bacterium *Mycobacterium tuberculosis*, with the lungs being the most commonly affected organ, accounting for 79%-87% of all cases[1]. Pulmonary TB (PTB) is one of the leading causes of death worldwide. According to the World Health Organization Global Tuberculosis Report 2024, the estimated TB incidence rate in 2023 was 134 per 100,000 population, representing a 0.2% increase compared with 2022. Five countries accounted for 56% of the global total of TB cases, with India being the largest contributor, accounting for 26%. The TB-affected population comprised 55% men, 33% women, and 12% children and young adults[2]. Hematological manifestations of TB infection can provide insights into its pathogenesis. Lymphocytes and monocytes play important roles in the induction of the immune response, and their levels in the peripheral blood may reflect the host immune response[3]. Some studies have suggested that the ratio of monocytes to lymphocytes in peripheral blood correlates with the extent of TB[4]. Other studies have found that an increased monocyte-to-lymphocyte ratio (MLR) is associated with active TB and is affected by antitubercular treatment[5,6]. This study aimed to evaluate changes in the MLR in patients with PTB before and after anti-TB treatment and compare the values with those of healthy controls to establish a significant cut-off value for this biomarker.

Materials and Methods

Study Population

A retrospective study was conducted Kasturba Medical College, Manipal Academy of Higher Education in Manipal, Karnataka, between January 2021 and December 2024. All newly diagnosed patients with PTB who had positive sputum acid-fast bacilli or GeneXpert (Cepheid, Sunnyvale, CA, USA) results, were aged >18 years, and had complete post-treatment follow-up records were included in the study. Pediatric cases; cases of extrapulmonary TB; patients with other comorbidities, such as hepatitis B, hepatitis C, or human immunodeficiency virus (HIV), or other known underlying systemic conditions; and patients who were lost to follow-up after treatment were excluded. Healthy controls were recruited from the routine health checkup center of the hospital and comprised individuals with no prior history of TB, no current active TB or related symptoms, and no known comorbidities. Controls were aged >18 years and were recruited during the same study period. Written informed consent was not obtained because no patient-identifiable data were included in the study. The study was conducted after obtaining ethical clearance from the Kasturba Medical College and Kasturba Hospital Institutional Research Ethics Committee on May 28, 2025 (protocol number: IEC2: 165/2025).

Data Collection

Complete blood counts (CBCs) of peripheral blood samples were performed by Kasturba Medical College, Manipal Academy of Higher Education, Manipal hematology laboratory using five-part differential automated hematology analyzers [SYSMEX (Sysmex Corporation, Kobe, Japan) and DxH 900 (Beckman Coulter, Brea, CA, USA)]. CBC testing was subject to strict quality assurance procedures in the laboratory, which was accredited by the National Accreditation Board for Testing and Calibration Laboratories and operated in accordance with ISO standards. Absolute monocyte and lymphocyte counts were retrieved from the CBC reports of the selected patients. The MLR for each patient was calculated before initiation of and after completion of the treatment regimen. Treatment completion was defined as completion of the standard 6-months antitubercular regimen with clinical and microbiological resolution. Post-treatment blood parameters were obtained from the available laboratory reports following review of the patients' medical records. All data were obtained from the hospital's medical records.

Statistical Analysis

All quantitative data were described using the mean, median, and interquartile range (IQR). A non-parametric Wilcoxon signed-rank test was used to compare MLR values before and after treatment in patients with PTB. The Mann-Whitney U test was used to compare age and MLR values between patients and controls. A p value <0.05 was considered statistically significant. The Wilcoxon signed-rank test was used to compare absolute monocyte and lymphocyte counts before and after treatment completion. Categorical variables, including sex, were compared between groups using Pearson's chi-square test. Additionally, multivariable logistic regression analysis was performed to evaluate the MLR as an independent predictor of active PTB after adjustment for sex, with the odds ratio (OR) scaled to a 0.1-unit increase in the MLR. Furthermore,

receiver operating characteristic (ROC) curve and Youden index analyses were performed to establish the cut-off value for the MLR in patients with TB. The MLR value corresponding to the maximum area under the curve in the ROC analysis was selected as the cut-off value, and its sensitivity and specificity were calculated. All statistical analyses were performed using RStudio software for Windows.

Results

A total of 136 newly diagnosed patients with PTB and 150 healthy controls were included in the study. The median age was 54 years (range, 19-87 years) among the patients and 53 years (range, 23-89 years) among the healthy controls. Of the 136 patients, 101 (74%) were males and 35 (26%) were females. Among the 150 controls, 84 (56%) were males and 66 (44%) were females. Because the data were not normally distributed, the Mann–Whitney U test was performed, and no statistically significant difference in age distribution was identified between the PTB and control groups ($p = 0.3997$). Pearson's chi-square test revealed a statistically significant difference in sex distribution between the two groups ($p = 0.0019$). To account for this demographic imbalance, multivariable logistic regression analysis was performed. MLR remained an independent and statistically significant predictor of active PTB after adjustment for sex [adjusted OR: 4.05 per 0.1-unit increase, 95% confidence interval (CI): 2.79-5.87, $p < 0.001$], indicating that each 0.1-unit increase in MLR was associated with an approximately 4-fold increase in the odds of being a patient with PTB. Sex was not significantly associated with PTB status after adjustment (adjusted OR: 0.58, 95% CI: 0.27-1.26, $p = 0.169$).

Peripheral white blood cell (WBC) counts in patients with PTB before and after treatment were evaluated to assess changes in WBC subpopulations. The total leukocyte count decreased significantly after treatment completion, with median values of 8.8 (IQR: 6.67–11.1) and 6.9 (IQR: 5.7-9.0), respectively. Similarly, the absolute monocyte count showed a statistically significant decrease following treatment ($p < 0.001$), from a median value of 0.75 (IQR: 0.59-1.03) to 0.60 (IQR: 0.48-0.79). In contrast, the absolute lymphocyte count showed a statistically significant increase after treatment ($p < 0.001$), from a median value of 1.34 (IQR: 0.90-1.87) to 1.90 (IQR: 1.39-2.38). The post-treatment values for all parameters studied were similar to those of the healthy controls (Table 1).

The MLR was calculated from the absolute monocyte and lymphocyte counts of patients with PTB before treatment and after completion of treatment. The median MLR before treatment was 0.580 (IQR: 0.39-0.90), whereas the median MLR after treatment was 0.331 (IQR: 0.24-0.50), representing a significant decrease following treatment. The Wilcoxon signed-rank test demonstrated a significant decrease in MLR following treatment ($p < 0.0001$), suggesting that treatment significantly influenced MLR levels (Figure 1).

Data were also obtained from a separate group of healthy controls, and the MLR was calculated from their absolute monocyte and lymphocyte counts. The Mann-Whitney U test was used for statistical analysis. The median MLR among healthy controls was 0.222 (IQR: 0.18-0.27). The MLR of healthy controls was compared with that of patients with PTB before treatment, and the MLR values were significantly higher in patients with PTB than in controls ($p < 0.05$) (Figure 2). A summary of the median MLR values in patients with PTB and healthy controls is presented in Table 2.

To use the MLR as a biomarker for PTB, an appropriate cut-off value must be established. ROC curve analysis was

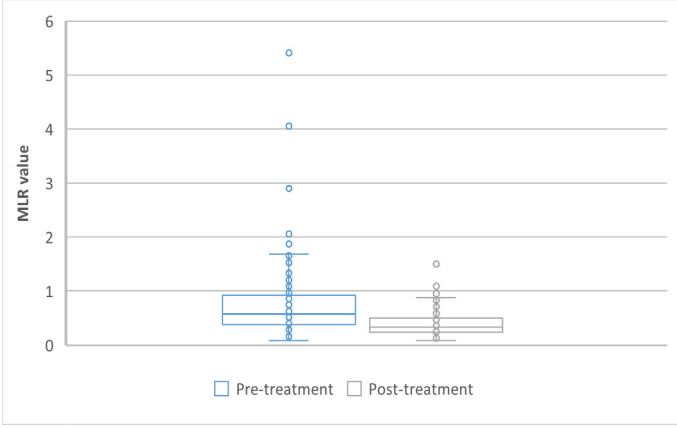

Figure 1. This is a boxplot image depicting that the median monocyte-to-lymphocyte ratio (MLR) value is higher in PTB patients prior treatment and the reduction in the median MLR post-treatment. PTB, pulmonary tuberculosis.

Table 1. The median total leucocyte count, absolute monocyte counts, and absolute lymphocyte counts in pulmonary tuberculosis patient's pre-treatment and post-treatment as compared to healthy controls. The IQR is given within the brackets.

Parameters	Pre-treatment values of PTB patients	Post-treatment values of PTB patients	Healthy controls
Total leucocyte count (n x 10^3µL)	8.8 (6.67-11.1)	6.9 (5.7-9)	6.95 (5.7-8.3)
Absolute monocyte count (n x 10^3µL)	0.75 (0.59-1.03)	0.6 (0.48-0.79)	0.5 (0.42-0.62)
Absolute lymphocyte count (n x 10^3µL)	1.34 (0.9-1.87)	1.9 (1.39-2.38)	2.23 (1.88-2.66)

IQR, interquartile range; PTB, pulmonary tuberculosis.

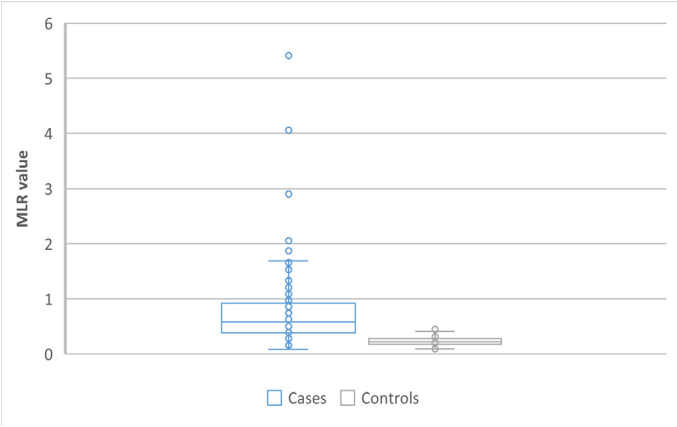

Figure 2. This is a boxplot image depicting that the median monocyte-to-lymphocyte ratio (MLR) value is higher in PTB patients' in comparison to the median MLR in healthy controls which is significantly lower. PTB, pulmonary tuberculosis.

Table 2. Summary showing statistical results of MLR values.

Group	Median MLR	Interquartile range
PTB patients-pre treatment	0.580	0.39 -0.90
PTB patients-post treatment	0.331	0.24-0.50
Healthy controls	0.222	0.18-0.27

MLR, monocyte-to-lymphocyte ratio; PTB, pulmonary tuberculosis.

performed to evaluate the MLR against GeneXpert results, which served as the reference standard and were positive for patients with PTB and negative for healthy controls. The ROC analysis demonstrated that the MLR was a strong predictor of GeneXpert status. At an optimal threshold of 0.3816, the MLR had a sensitivity of 76.47% and specificity of 94.67%, with a Youden index of 0.7114. This optimal threshold was used as the decision boundary, with an MLR >0.3816 indicating a higher likelihood of PTB. In our study population, the negative predictive value of the established MLR cut-off was 99.98%, suggesting that the MLR cut-off may be effective for ruling out TB and could therefore serve as a useful screening tool (Figure 3).

Discussion

PTB remains a global health challenge, particularly in endemic regions where rapid and reliable diagnostic tools are essential. The MLR has increasingly been recognized as a clinically relevant biomarker in TB, representing the balance between innate and adaptive immune responses. An elevated MLR in PTB has been attributed to concurrent monocytosis and lymphopenia, with the latter arising primarily from CD4+ T-cell depletion secondary to chronic immune activation. The shift toward monocyte-driven inflammation is characteristic of active TB[7,8].

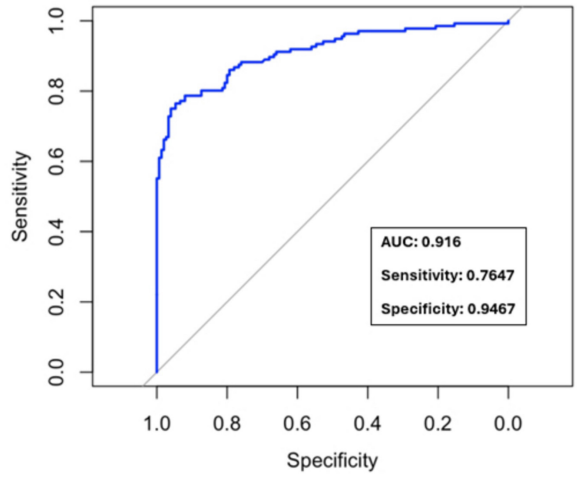

Figure 3. ROC curve analysis, blue line shows the performance of the monocyte to lymphocyte ratio. The curve is significantly above the diagonal, indicating that the ratio has strong predictive power in distinguishing between positive and negative cases. A steeper ascent early in the curve suggests that high sensitivity is achieved even at low false positive rates. ROC, receiver operating characteristic; AUC, area under the curve.

A total of 136 newly diagnosed patients with PTB were included in the study, and 150 individuals were included as controls. Of the 136 patients, 101 (74%) were males and 35 (26%) were females. The median age of patients with PTB was 54 years, which was comparable to that reported in the study by Wang et al.[5], in which the median age was 55 years.

The total leukocyte count was elevated before treatment in patients with PTB and showed a significant decrease following treatment. The absolute lymphocyte count increased significantly, whereas the absolute monocyte count decreased significantly following treatment. These findings were comparable to those reported by Wang et al.[6] The decrease in absolute monocyte and leukocyte counts following treatment was consistent with the findings of Okeke et al.[9]; however, their study also reported a mild decrease in the absolute lymphocyte count following treatment. In a study evaluating various blood parameters in patients with active PTB, Ngahane et al.[10] reported that monocytosis and lymphopenia were the most common hematological abnormalities. Monocytosis in PTB is considered important and may be attributed to the release of chemoattractants following the engulfment of bacilli by macrophages, resulting in increased monocyte production when some organisms overcome host defense mechanisms. However, lymphocyte counts may vary, either increasing or decreasing, and generally normalize following treatment. Peripheral lymphopenia in PTB may be mechanistically explained by the redistribution of lymphocytes toward the site of infection. The variability in lymphocyte counts reported

across different studies highlights the complex and dynamic nature of the immune response in TB[9,11-13].

Monocytosis is a recognized feature of PTB, whereas lymphocytes contribute to infection containment and mycobacterial clearance. Given this immunological interplay, the MLR has been studied as a prognostic marker in PTB, including its potential to predict the risk of active TB in adults, infants, postpartum women, and individuals with HIV infection. An increased MLR in PTB may indicate disease severity and serve as an indicator of treatment effectiveness[9,11]. Naranbhai et al.[14] first established the predictive role of the MLR in patients with PTB for the early detection of disease in 3- to 4-month-old children. In our study, the median MLR was significantly higher in patients with PTB (0.58) than in healthy controls (0.22). The MLR value observed in our study (0.58) was comparable to that reported by La Manna et al.[15], who compared the MLR, absolute monocyte count, and monocytic CD64 expression as biomarkers for discriminating between TB stages. The MLR values reported by La Manna et al.[15] and Choudhary et al.[16], which were 0.50 and 0.48, respectively, were consistent with the value obtained in the present study (0.58). The higher MLR in patients with TB than in controls was consistent with findings from studies by Wang et al.[5], Ngahane et al.[10], and Liana et al.[17], who investigated the MLR in patients with active PTB and assessed its diagnostic value. Van Ginderdeuren et al.[18] reported a considerably lower MLR value of 0.22, which could be attributed to their study population, which included HIV-positive individuals (Table 3).

The increase in MLR observed in PTB reflects underlying immune dysregulation involving type I and type II interferons, which promote monocyte production while reducing lymphocyte counts through apoptosis and sequestration. The increased number of monocytes, accompanied by alterations in their transcriptional and functional profiles, may lead to an impaired antimycobacterial response. Treatment of PTB reduces inflammation and interferon signaling, allowing monocyte function to normalize and resulting in a decline in the MLR. Thus, the MLR may serve as a dynamic marker of disease activity and treatment response[19,20]. In the present study, the median MLR was 0.58 before treatment and 0.33 after treatment. A significant decrease in the MLR was observed following treatment for TB. These findings were consistent with those of studies comparing the MLR before and after treatment completion in various populations of patients with PTB, including those with PTB. Iqbal et al.[11] concluded that the MLR could be considered a prognostic marker in TB and may also be useful for monitoring the response to therapy. Wang et al.[5] analyzed changes in the MLR before and after treatment completion and found that the elevated MLR decreased with therapy and was correlated with treatment effectiveness and phase. Wang et al.[6] also investigated the role of the MLR in monitoring TB therapy and reported a decrease in the MLR following treatment, in addition to significant differences in the MLR across different age groups, sexes, and types of TB. Okeke et al.[9] investigated the impact of TB infection and treatment on the MLR and neutrophil-to-lymphocyte ratio and similarly reported a decrease in the MLR among patients undergoing anti-TB treatment (Table 4).

Using ROC curve analysis, an MLR cut-off value of 0.3816 was established. Patients were more likely to have PTB if the MLR was >0.38. At this threshold, the sensitivity was 76.47% and the specificity was 94.67%, with a Youden index of 0.7114. Notably, the negative predictive value was remarkably high (99.98%), supporting the potential utility of the MLR as a non-invasive biomarker for PTB screening in adults. However, because this was a retrospective case-control study, the extremely high negative predictive value may have been partially influenced by the study design and patient selection. Therefore, this finding should be interpreted cautiously when considering its application to general population screening.

When compared with previous studies, our findings were generally consistent. Choudhary et al.[16] reported that an MLR cut-off value >0.378 demonstrated good overall diagnostic utility for PTB in their cohort of HIV-infected Kenyan children,

Table 3. The median MLR value of different studies comparing active TB cases and healthy controls.

Studies	MLR in cases	MLR in controls	P value
Wang et al.[5]	0.36	0.16	<0.001
La Manna et al.[15]	0.50	0.18	<0.0001
Fayed et al.[19]	0.46	0.15	0.0001
Ngahane et al.[10]	0.39	0.19	<0.001
Liana et al.[17]	0.97	0.57	<0.0001
Choudhary et al.[16]	0.48	0.23	<0.01
Van Ginderdeuren et al.[18]	0.22	0.34	N/A
Present study	0.58	0.22	<0.05

MLR, monocyte-to-lymphocyte ratio; TB, tuberculosis; N/A, not applicable.

Table 4. The median MLR values by different studies done on TB patients before treatment and after anti-tuberculosis treatment.

Studies	MLR before treatment	MLR after treatment	p value
Iqbal et al.[11]	0.24	0.19	0.006
Wang et al.[5]	0.47	0.22	< 0.001
Wang et al.[6]	0.45	0.32	< 0.001
Okeke et al.[9]	0.11	0.08	<0.05
Present study	0.58	0.33	<0.0001

MLR, monocyte-to-lymphocyte ratio; TB, tuberculosis.

in contrast to the adults with PTB without comorbidities included in our cohort. Despite differences in the study populations, the MLR cut-off values established in both studies were comparable.

La Manna et al.[15] established a lower MLR cut-off value of 0.28 when comparing patients with PTB and healthy controls. They reported a higher sensitivity (91.04%) and comparable specificity (93.55%). This difference may be attributed to heterogeneity in the study population, which included patients with latent, active, and treated TB, as well as a relatively small healthy control group. The study also proposed a revised MLR threshold of 0.34 for distinguishing active PTB from TB after treatment, with a sensitivity of 77.61% and specificity of 77.42%, respectively. This threshold more closely aligns with our findings and supports the relevance of the MLR in distinguishing active PTB.

Liana et al.[17] reported an MLR cut-off value of 0.476 in culture-positive TB cases, with higher sensitivity (95.1%), lower specificity (70%), and a comparable negative predictive value (95.45%). The differences in cut-off values and diagnostic performance may be attributed to their inclusion of a broader age range and their comparison of patients with chronic cough with and without TB.

Buttle et al.[21] investigated the prognostic significance of the MLR and reported sex-specific MLR cut-off values for a heterogeneous study population. The study established the optimal diagnostic points based on ROC analysis, with MLR cut-off values of 0.375 in males and 0.33 in females for sputum-positive contacts and patients with active PTB. The minimal difference between the cut-off values established in our study and those reported in their study may be attributed to the inclusion of diverse groups, including PTB contacts, patients initially suspected of having PTB, and HIV-positive individuals.

In our study, an MLR cut-off value of 0.3816 was established, which was consistent with the thresholds reported in similar previous studies. This value aligns with the cut-off threshold proposed by Wang et al.[5], as it is above the 25th percentile (0.24), and is comparable to those reported by Choudhary et al.[16] and Buttle et al.[21], further supporting its potential clinical relevance in PTB. Although MLR values may vary across studies because of differences in population characteristics and study design, our study primarily focused on confirmed cases of active PTB without comorbid conditions or extrapulmonary manifestations, providing a relatively uniform cohort and potentially a more specific MLR threshold. Our threshold remained within the range reported in the literature and demonstrated strong diagnostic utility.

Study Limitations

This retrospective, single-center study had a modest sample size, with many cases excluded because of loss to follow-up. The exclusion of patients with comorbidities may limit the generalizability of the findings. Sex-based variation in the MLR was not assessed. Additionally, because this study relied on CBC parameters, external factors, such as corticosteroid use or the presence of non-active underlying inflammatory conditions that may influence blood cell counts, could not be completely controlled for. Furthermore, there was a statistically significant difference in sex distribution between the study groups, reflecting the epidemiological predominance of males among patients with PTB. Multivariable analysis confirmed that sex did not independently influence the predictive value of the MLR. Nevertheless, future prospective studies with sex-matched cohorts are warranted. Larger, prospective, multicenter studies are needed to validate MLR cut-off values across diverse populations, assess changes in MLR over time in patients with PTB, and evaluate its predictive value for treatment success or failure.

Conclusion

Our study reinforces and builds upon previous research supporting the diagnostic and prognostic value of the MLR in PTB. The established cut-off value of 0.38 demonstrated high specificity and a strong negative predictive value, highlighting its potential utility for screening and early detection of PTB, particularly in TB-endemic areas where rapid and cost-effective diagnostic tools are crucial. Although cut-off values may vary across studies, our findings are consistent with the existing literature and demonstrate strong diagnostic utility. Further validation through multicenter studies is warranted.

Ethics

Ethics Committee Approval: The study was conducted after obtaining ethical clearance from the Kasturba Medical College and Kasturba Hospital Institutional Research Ethics Committee on May 28, 2025 (protocol number: IEC2: 165/2025).

Informed Consent: Written informed consent was not obtained because no patient-identifiable data were included in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: R.K., G.P., Concept: R.K., G.P., Design: R.K., Data Collection or Processing: S.K.N., R.S.S.K. Analysis or Interpretation: S.K.N., R.S.S.K., Literature Search: S.K.N., G.P., Writing: S.K.N., R.K., G.P., R.S.S.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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Immune Reconstitution Inflammatory Syndrome in Patients with Human Immunodeficiency Virus Infection: A Multicenter BUHASDER Study

HIV Enfekte Hastalarda İmmün Rekonstitüsyon İnflamatuvar Sendromu: Çok Merkezli Bir BUHASDER Çalışması

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Abstract

Introduction: The advent of effective antiretroviral therapy (ART) has markedly improved the survival and quality of life of people living with human immunodeficiency virus (HIV). Nevertheless, the rapid restoration of immune function following ART initiation can precipitate immune reconstitution inflammatory syndrome (IRIS), a condition that may lead to clinical deterioration. This study aimed to characterize the clinical features, therapeutic approaches, and outcomes of patients with HIV infection who developed IRIS.

Materials and Methods: This retrospective multicenter study included 13 centers. Data from 31 patients with HIV infection diagnosed with immune IRIS were collected from electronic medical records and analyzed. Demographic characteristics, immunologic and virologic status at ART initiation, IRIS type, associated opportunistic infections, treatment strategies, and clinical outcomes were evaluated. In addition, the clinical and laboratory characteristics of survivors (n = 26) and non-survivors (n = 5) were compared. A p value < 0.05 was considered statistically significant.

Results: The mean age of patients at ART initiation was 42.74 ± 12 years (median, 43 years; range, 18-61 years). Twenty-one patients (67.7%) developed paradoxical IRIS, whereas 10 (32.3%) developed unmasking IRIS. The most common OIs were *Mycobacterium tuberculosis*, *Pneumocystis jirovecii*, and cytomegalovirus disease. At ART initiation, 71% of patients had a CD4+ T-cell count < 100 cells/mm^3, and 74% had an HIV RNA level >100,000 IU/mL. In the exploratory mortality analysis, central nervous system (CNS) involvement (p = 0.001), progressive multifocal leukoencephalopathy (PML) (p = 0.012), lymphoma (p = 0.020), meningismus (p = 0.048), and worsening cerebrospinal fluid findings (p = 0.012) were more frequently observed among non-survivors, as was a higher baseline lymphocyte count at ART initiation (median, 1,640 vs. 870 cells/mm^3; p = 0.036).

Conclusion: Most patients who developed IRIS had advanced immunosuppression and a high baseline viral load at ART initiation. CNS involvement and PML were more frequently observed among non-survivors; however, given the small number of deaths, these findings are hypothesis-generating and require confirmation in larger multicenter studies.

Keywords: HIV infection, paradoxical IRIS, unmasking IRIS

Öz

Giriş: Antiretroviral tedavinin (ART) kullanıma girmesi, insan immün yetmezlik virüsü (HIV) ile yaşayan bireylerin yaşam süresi ve yaşam kalitesini belirgin şekilde artırmıştır. Bununla birlikte, ART başlanmasını takiben immün fonksiyonların hızlı şekilde yeniden kazanılması, klinik kötüleşmeye yol açabilen immün rekonstitüsyon inflamatuvar sendromunu (IRIS) tetikleyebilmektedir. Bu çalışmanın amacı, IRIS gelişen HIV enfekte hastaların klinik özelliklerini, tedavi yaklaşımlarını ve sonuçlarını değerlendirmektir.

Gereç ve Yöntemler: Bu retrospektif çok merkezli çalışmaya 13 merkez katıldı. IRIS tanısı alan 31 HIV ile enfekte hastanın verileri elektronik hasta kayıtlarından elde edilerek analiz edildi. Demografik özellikler, ART başlangıcındaki immünolojik ve virolojik durum, IRIS tipi, eşlik eden fırsatçı enfeksiyonlar, tedavi yaklaşımları ve klinik sonuçlar değerlendirildi. Ayrıca, IRIS geliştikten sonra yaşayan 26 hasta ile ölen 5 hastanın klinik ve laboratuvar özellikleri karşılaştırıldı. İstatistiksel anlamlılık düzeyi p < 0,05 olarak kabul edildi.

Bulgular: IRIS gelişen HIV ile enfekte hastaların ART başlangıcındaki ortalama yaşı 42,74 ± 12 yıl (medyan: 43; minimum-maksimum: 18-61) idi. Olguların 21'inde (%67,7) paradoksal, 10'unda (%32,3) ise maskelenmemiş IRIS gelişti. En sık görülen fırsatçı enfeksiyonlar *Mycobacterium tuberculosis*, *Pneumocystis jirovecii* ve sitomegalovirüs hastalığı idi. Hastaların %71'inde ART başlangıcında CD4+ T lenfosit sayısı <100 hücre/mm^3, %74'ünde ise HIV RNA düzeyi >100,000 IU/mL olarak saptandı. Tanımlayıcı mortalite analizinde santral sinir sistemi (SSS) tutulumu (p = 0,001), progresif multifokal lökoensefalopati (PML) (p = 0,012), lenfoma (p = 0,020), menenjismus (p = 0,048) ve kötüleşen beyin omurilik sıvısı bulguları (p = 0,012) ölen hastalarda daha sık gözlendi; benzer şekilde ART başlangıcındaki lenfosit sayısı da ölen hastalarda daha yüksekti (medyan: 1640'a karşı 870 hücre/mm^3, p = 0,036).

Sonuç: Bu çalışmada IRIS gelişen hastaların büyük çoğunluğunda ART başlangıcında ileri derecede immünsüpresyon ve yüksek viral yük bulunduğu gösterildi. SSS tutulumu ve PML ölen hastalarda daha sık gözlendi; ancak ölüm sayısının azlığı nedeniyle bu bulgular hipotez oluşturucudur ve daha geniş çok merkezli çalışmalarda doğrulanmalıdır.

Anahtar Kelimeler: HIV enfeksiyonu, paradoksal IRIS, maskelenmemiş IRIS

Introduction

The use of antiretroviral therapy (ART) induces both quantitative and qualitative changes in the immune response, significantly improving survival rates and quality of life among individuals living with human immunodeficiency virus (HIV)[1]. A rapid decline in HIV RNA levels and swift immune reconstitution can, however, lead to the development of immune reconstitution inflammatory syndrome (IRIS). IRIS is characterized by an excessive and pathologic inflammatory response to antigens derived from opportunistic infections (OIs)[2].

IRIS may manifest in two distinct clinical forms in individuals with HIV infection. Paradoxical IRIS refers to the clinical deterioration of an OI that initially responded to treatment before ART initiation. In contrast, unmasking IRIS occurs when a previously undiagnosed or subclinical OI becomes clinically apparent after ART initiation, resulting in an exaggerated inflammatory response[3]. The development of IRIS is influenced by the degree of immune restoration following ART, the underlying antigenic burden, and the genetic susceptibility of the host[4].

IRIS can be triggered by various infectious agents, such as *Mycobacterium tuberculosis*, *Mycobacterium avium* complex

(MAC), herpesviruses, hepatitis B (HBV) and C (HCV) viruses, polyomaviruses, cytomegalovirus (CMV), *Cryptococcus neoformans*, *Pneumocystis jirovecii*, *Toxoplasma gondii*, *Leishmania* spp., and *Strongyloides stercoralis*. In addition, to infectious causes, non-infectious conditions such as autoimmune diseases and malignancies may also contribute to the development of IRIS[5-7].

In our country, the occurrence of IRIS among individuals with HIV infection is considered relatively rare[8,9]. This multicenter study was conducted to characterize patients who developed IRIS and to present nationwide data. The aim of this study was to identify both infectious and non-infectious causes of IRIS in patients with HIV infection and to evaluate the clinical characteristics, treatment strategies, and outcomes of these patients.

Materials and methods

Study Design

This study was conducted by the Scientific Research Committees of The Association for Prevention of Infectious Diseases (BUHASDER). This retrospective study included patients with HIV infection aged 18 years and older who developed IRIS and were followed between January 2010 and December 2024 across 13 centers in Türkiye.

Medical data were collected from hospital records. A total of 31 patients were identified as having developed IRIS during this period.

The approval was granted by the Ethics Committee of University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital in accordance with the principles of the Declaration of Helsinki (approval number: 2025/44, date: 12.02.2025). As this was a retrospective study, the requirement for informed consent from participants was waived by the Ethics Committee of University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital.

Inclusion Criteria

To ensure diagnostic standardization across participating centers, all centers were provided with a standardized case report form together with the predefined IRIS diagnostic criteria before data collection. Participating investigators were instructed to include only patients who fulfilled these criteria. After data submission, all case report forms were centrally reviewed to verify compliance with the predefined diagnostic criteria. Only patients meeting all diagnostic criteria were included in the final analysis.

Patients were included in the study if they met the following criteria[10-12]:

- Onset of clinical symptoms within the first 6 months of ART
- A reduction of >1 $\log_{10}$ in plasma HIV RNA level
- Presence of an exaggerated and/or atypical inflammatory response
- An increase in CD4+ T-lymphocyte count
- Exclusion of alternative causes of clinical deterioration

Definitions

Paradoxical IRIS: Clinical worsening of a previously diagnosed OI following initiation of ART, despite an initial favorable response to treatment[3,12].

Unmasking IRIS: Clinical presentation of a previously undiagnosed OI after ART initiation, accompanied by an exaggerated inflammatory response[3,12].

Statistical Analysis

Statistical analysis was performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Because of the small number of deaths, continuous variables were compared between survivors and non-survivors using the Mann-Whitney U test. Categorical variables were compared using the chi-square test or Fisher exact test when the expected cell count was less than 5. Continuous variables are presented as median (25th-75th percentile), whereas categorical variables are expressed as number and percentage (%). A 2-sided p value <0.05 was considered statistically significant.

Results

During the study period, 970 patients with HIV infection were followed, and 31 (3.2%) developed IRIS. Among these, 87.1% were male, and the median age at ART initiation was 43 years [interquartile range (IQR), 34-50) (Table 1). The mean body mass index was 21.4 ± 3.0. Laboratory parameters at ART initiation are shown in Table 2.

Integrase inhibitors were administered to 29 (93.5%) patients. In one case, darunavir/ritonavir was switched to dolutegravir because of a drug–drug interaction following TB diagnosis (Table 3). Corticosteroid dose and treatment duration data were available for 19 of the 21 patients who received corticosteroids. The mean corticosteroid dose was 176.84 ± 291.91 mg (median, 80 mg; range, 20-1,000 mg), and the mean treatment duration was 24.73 ± 22.44 days (median, 20 days; range, 1-90 days). In patients with paradoxical IRIS, the mean corticosteroid dose was 207.14 ± 337.28 mg (median, 80 mg; range, 20-1,000 mg), with a mean treatment duration of 22.57 ± 22.66 days (median, 19 days; range, 1-90 days).

In patients with unmasking IRIS, the mean corticosteroid dose was 92.00 ± 38.98 mg (median, 80 mg; range, 60-160 mg), and the mean treatment duration was 30.80 ± 23.14 days (median, 21 days; range, 10-66 days).

Table 1. Characteristics of patients with IRIS.

Characteristics	Total (n = 31), n (%)	Paradoxical (n = 21), n (%)	Unmasking (n = 10), n (%)
Sex (male)	27 (87.1)	18 (85.7)	9 (90)
Sex (female)	4 (12.9)	3 (14.2)	1 (10)
Mode of HIV transmission			
Heterosexual	11 (35.5)	7 (33.3)	4 (40)
Male-to-male sexual contact*	11 (35.5)	7 (33.3)	4 (40)
Bisexual*	6 (19.3)	4 (19)	2 (20)
Unknown	3 (9.7)	3 (14.2)	-
Substance use			
Cigarettes	23 (74.1)	16 (76.1)	7 (70)
Alcohol	11 (35.5)	6 (28.6)	5 (50)
Recreational drugs	7 (22.5)	4 (19)	3 (30)
Comorbidities			
No comorbidity	27 (87.1)	19 (90.4)	8 (80)
Chronic hepatitis B	2 (6.4)	1 (4.7)	1(10)
Diabetes mellitus	1 (3.2)	1 (4.7)	-
Hypertension	1 (3.2)	-	1 (10)
Symptoms and signs at the start of IRIS			
Fever	22 (70.9)	13 (61.9)	9 (90)
Cough	13 (41.9)	9 (42.8)	4 (40)
Sweating	10 (32.2)	6 (28.5)	4 (40)
Headache	9 (29)	7 (33.3)	2(20)
Shortness of breath	9 (29)	7 (33.3)	2(20)
Increase in lymph nodes	7 (22.5)	5 (23.8)	2 (20)
Meningismus	4 (12.9)	2 (9.5)	2 (20)
Laboratory results at the start of IRIS			
Increased liver tests	3 (9.6)	1 (4.7)	2 (20)
Worsening of CSF findings	3 (9.6)	2 (9.5)	1 (10)
Progression on radiological imaging	13 (41.9)	10 (47.6)	3 (30)

*Self-reported sexual behavior. CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; IRIS, immune reconstitution inflammatory syndrome.

Table 2. Laboratory values at the start of antiretroviral therapy.

Laboratory tests	Mean ± SD	Median (IQR)
Leukocyte count (cells/mm^3)	6271 ± 2962	5790 (3685-7995)
Lymphocyte count (cells/mm^3)	1031 ± 758.3	900 (585-1419)
Hemoglobin (g/dL)	11.1 ± 2.2	11 (9.9-12.6)
Platelet count (cells/mm^3)	219161 ± 111006	186000 (141500-281000)
Creatinine (mg/dL)	0.8 ± 0.2	0.8 (0.7-0.9)

IQR, interquartile range; SD, standard deviation.

Ten different opportunistic pathogens were identified as causes of IRIS, with *Mycobacterium tuberculosis* and *Pneumocystis jirovecii* being the most common (Table 4).

Multiple pathogens were detected in four patients: CMV and *Pneumocystis jirovecii* in two cases; CMV, *Pneumocystis jirovecii*, and *Candida* spp. in one case; and CMV, *Mycobacterium tuberculosis*, and HBV in another. MAC and progressive multifocal leukoencephalopathy (PML) were detected simultaneously in one case.

Paradoxical IRIS was observed in 21 patients (67.7%). The mean time to IRIS development following ART initiation was 47.9 ± 48.6 days (median, 30 days; IQR, 16-60 days). For paradoxical IRIS, the mean duration was 47.7 ± 49.3 days (median, 30 days; IQR, 10–60 days), and for unmasking IRIS, it was 48.4 ± 49.5 days (median, 31.5 days; IQR, 19-56 days).

The mean CD4+/CD8+ T-cell ratio at ART initiation was 0.1. HIV RNA levels exceeded 100,000 IU/mL in 23 patients (74.1%). CD4+ T-cell counts at ART initiation were <100 cells/mm^3 in 22 patients (71%) and 100-300 cells/mm^3 in seven patients (22.5%). One patient with a CD4+ T-cell count >300 cells/mm^3 had coinfection with CMV, TB, and HBV. Another patient with a CD4+ T-cell count of 507 cells/mm^3 was being followed for lymphoma. The mean time to HIV RNA negativity in 19 patients was 177.7 ± 115.9 days (median, 170 days; IQR, 77-250 days). CD4+ T-cell counts, HIV RNA levels, and C-reactive protein values at ART initiation and IRIS diagnosis are presented in Table 5. Figure 1 shows the mean CD4+ T-cell counts at ART initiation according to the condition causing IRIS, whereas Figure 2 shows the median HIV RNA levels at ART initiation according to the condition causing IRIS.

Corticosteroids were used in 21 (67.7%) patients, and non-steroidal anti-inflammatory drugs were used in eight patients (25.8%). One patient with IRIS-associated Kaposi sarcoma received doxorubicin. ART was continued in all patients.

Five of the 31 patients with IRIS died during follow-up, resulting in an all-cause mortality rate of 16.1%, and one additional patient developed permanent sequelae. The underlying IRIS-associated conditions among patients who died were PML-associated IRIS (n = 2), concurrent PML- and MAC-associated IRIS (n = 1), *Cryptococcus neoformans*-associated IRIS (n = 1), and lymphoma-associated IRIS (n = 1). The characteristics of cases with a fatal outcome are shown in Table 6.

An exploratory mortality analysis was performed by comparing survivors (n = 26) and non-survivors (n = 5). Non-survivors had significantly higher baseline lymphocyte counts at ART initiation (median, 1,640 vs. 870 cells/mm^3; p = 0.036). CNS involvement (100% vs. 23.1%; p = 0.001), PML (p = 0.012), lymphoma (p = 0.020), meningismus (p = 0.048), worsening cerebrospinal fluid findings (p = 0.012), and male-to-male sexual contact (p = 0.023) were more frequently observed

among non-survivors. No significant differences were observed in age, sex, CD4+ T-cell count, HIV RNA level, corticosteroid treatment, timing of ART initiation, or time to IRIS development between the 2 groups (Tables 7-11).

Table 3. ART used in patients with IRIS.

ART	Total (n = 31), n (%)	Paradoxical (n = 21), n (%)	Unmasking (n = 10), n (%)
TDF/FTC + DTG	14 (45)	11 (52)	3 (30)
TAF/FTC + BIC	9 (29)	4 (19)	5 (50)
TDF/FTC + RAL	2 (6)	2 (9.5)	—
3TC + DTG	2 (6)	—	2 (20)
TDF/FTC + PI/r	2 (6)	2 (9.5)	—
EVG/COBI/FTC/TDF	1 (3)	1 (4.7)	—
TDF/FTC + DTG/PI/r	1 (3)	1 (4.7)	—
Corticosteroid dose (mg)*	176.84 ± 291.91	207.14 ± 337.28	92 ± 38.98
Corticosteroid duration (days)	24.73 ± 22.44	22.57 ± 22.66	30.8 ± 23.14

*Corticosteroid doses are presented as the initial daily dose, expressed as methylprednisolone equivalents. 3TC, lamivudine; ART, antiretroviral therapy; BIC, bictegravir; COBI, cobicistat; DTG, dolutegravir; EVG, elvitegravir; FTC, emtricitabine; IRIS, immune reconstitution inflammatory syndrome; PI/r, darunavir/ritonavir; RAL, raltegravir; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

Table 4. Conditions associated with IRIS development.

Condition	Total (n = 31), n (%)	Paradoxical (n = 21), n (%)	Unmasking (n = 10), n (%)	CD4+ T-cell count <100 cells/mm³, n (%)	HIV RNA level >100,000 IU/mL, n (%)
Opportunistic infections					
Mycobacterium tuberculosis (n = 7)	7 (22.5)	6 (28.5)	1 (10)	5 (71.4)	6 (85.7)
Hematopoietic	4 (12.9)	4 (19)	-	3 (42.8)	3 (42.8)
Lymphatic	2 (6.4)	1 (4.7)	1 (10)	2 (28.5)	2 (28.5)
Pulmonary	2 (6.4)	2 (9.5)	-	2 (28.5)	2 (28.5)
Gastrointestinal	1 (3.2)	1 (4.7)	-	-	1 (14.2)
Pneumocystis jirovecii (n = 7)	7 (22.5)	5 (23.8)	2 (20)	7 (100)	6 (85.7)
CMV (n = 6)*	6 (19.3)	4 (19)	2 (20)	4 (66.6)	4 (83.3)
Pulmonary	6 (19.3)	4 (19)	2 (20)	4 (66.6)	5 (83.3)
Gastrointestinal	1 (3.2)	1 (4.7)	-	0 (0)	1 (16.6)
Hematopoietic	1 (3.2)	1 (4.7)	1 (10)	1 (16.6)	1 (16.6)
PML (n = 6)	6 (19.3)	6 (28.5)	-	5 (83.3)	2 (33.3)
Cryptococcus neoformans (n = 3)	3 (9.6)	1 (4.7)	2 (20)	1 (66.6)	1 (66.6)
MAC (n = 2)	2 (6.4)	1 (4.7)	1 (10)	2 (100)	2 (100)
Toxoplasmosis (n = 2)	2 (6.4)	1 (4.7)	1 (10)	2 (100)	0 (0)
Hepatitis B (n = 1)	1 (3.2)	1 (4.7)	-	0 (0)	1 (100)
Candida (n = 1)	1 (3.2)	1 (4.7)	-	1 (100)	1 (100)
Kaposi sarcoma (n = 2)	2 (6.4)	1 (4.7)	1 (10)	0 (0)	2 (100)
Malignancy					
Lymphoma (n = 1)	1 (3.2)	-	1 (10)	0 (0)	0 (0)
IRIS start time (days)	5 (16.1) 26 (83.8)	4 (19) 17 (80.9)	1 (10) 9 (90)	3 (60) 19 (73)	3 (60) 20 (73)
>90 days (n = 5)	5 (16.1)	3 (14.2)	2 (20)	3 (60)	2 (40)

*CMV infection was detected in a total of 6 patients. In 1 patient, both gastrointestinal and pulmonary CMV infection were present; in another, both the hematopoietic and pulmonary systems were affected. In 4 patients, only the pulmonary system was affected by CMV.

CMV, cytomegalovirus; IRIS, immune reconstitution inflammatory syndrome; MAC, *Mycobacterium avium* complex; PML, progressive multifocal leukoencephalopathy. HIV, human immunodeficiency virus; RNA, ribonucleic acid; IU, international unit.

Table 5. Laboratory results of patients who developed IRIS.

Laboratory tests	Pre-ART		Onset of IRIS	
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)
CD4+ T-cell count (cells/mm^3)	101.2 ± 108.6	61 (33-117)	198.9 ± 222.1	125 (78.5-230)
CD4+ T-cell count (%)	8.2 ± 5.2	7 (4.7-11.5)	15.4 ± 9.1	13.1 (8.2-20)
HIV RNA level	3305789 ± 11720715	480000 (98250-1371653)	127901.1 ± 490506.8	626.5 (296-2306)
C-reactive protein (mg/dL)	33.3 ± 39	15.6 (7.5-39)	64.5 ± 75.8	31.5 (11.5-105.5)

IQR, interquartile range; IRIS, immune reconstitution inflammatory syndrome; SD, standard deviation; ART, antiretroviral therapy; HIV, human immunodeficiency virus; RNA, ribonucleic acid.

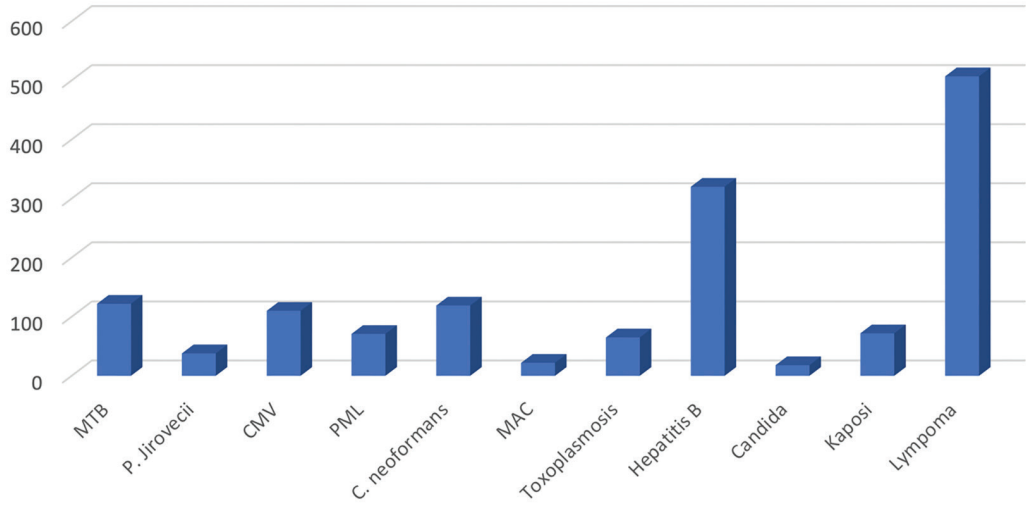

Figure 1. Mean CD4+ T-cell counts at the start of ART. MTB, *Mycobacterium tuberculosis*; CMV, cytomegalovirus; MAC, *Mycobacterium avium* complex; PML, progressive multifocal leukoencephalopathy; ART, antiretroviral therapy.

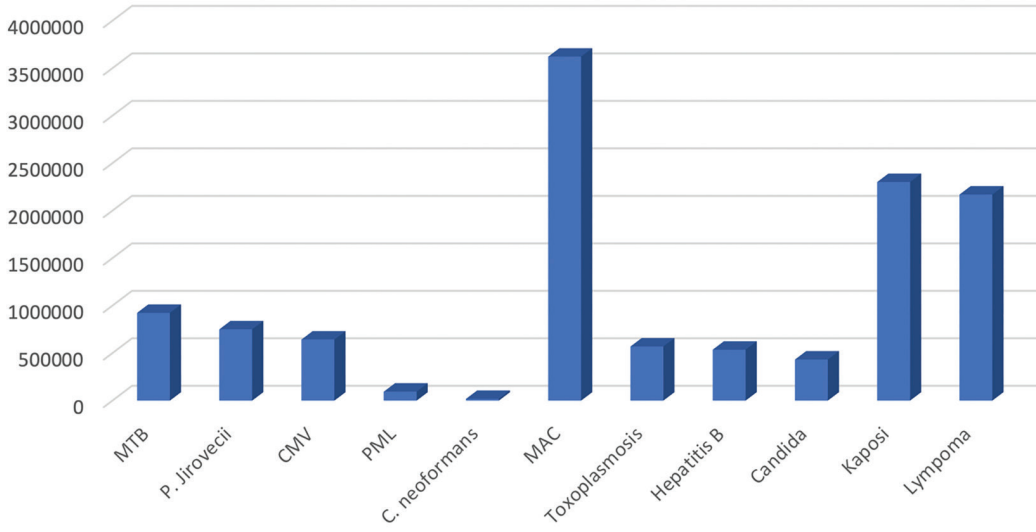

Figure 2. Median HIV RNA levels at the start of ART. MTB, *Mycobacterium tuberculosis*; CMV, cytomegalovirus; MAC, *Mycobacterium avium* complex; PML, progressive multifocal leukoencephalopathy; ART, antiretroviral therapy.

Table 6. Characteristics of patients with fatal outcomes.

Etiologic agent	Site(s) of involvement	Survival after IRIS onset (days)	Treatment	Clinical course
Cryptococcus neoformans	Central nervous system, pulmonary system, dermatologic involvement	4	ART	Cardiopulmonary arrest
Progressive multifocal leukoencephalopathy	Central nervous system	82	ART, corticosteroid (100 mg for 5 days)	Clinical deterioration with progression of MRI findings
Progressive multifocal leukoencephalopathy	CNS	59	ART, corticosteroid (80 mg for 45 days)	Comatose state, decerebrate posturing, loss of brainstem reflexes, bilateral Babinski signs
MAC and progressive multifocal leukoencephalopathy	CNS, lymphatic system	7	ART	Numbness in the hands and feet, inability to walk, decreased Glasgow Coma Scale score
Lymphoma	CNS, lymphatic system, hematopoietic system	90	Chemotherapy	Thrombocytopenia and SAH

ART, antiretroviral therapy; CNS, central nervous system; IRIS, immune reconstitution inflammatory syndrome; MAC, *Mycobacterium avium* complex; MRI, magnetic resonance imaging; SAH, subarachnoid hemorrhage.

Table 7. Comparison of baseline characteristics between survivors and non-survivors among patients with immune reconstitution inflammatory syndrome.

Variable	Total (n = 31)	Non-survivors (n = 5)	Survivors (n = 26)	p value
Age, median (IQR), y	43 (34-50)	44 (40-57)	43 (33.25-49.25)	0.361
Male sex, n (%)	27 (87.1)	5 (100)	22 (84.6)	0.347
Heterosexual transmission, n (%)	11 (35.5)	0 (0)	11 (42.3)	0.07
Male-to-male sexual contact, n (%)	11 (35.5)	4 (80)	7 (26.9)	0.023
Bisexual transmission, n (%)	6 (19.4)	1 (20)	5 (19.2)	0.968
Smoking, n (%)	23 (74.2)	5 (100)	18 (69.2)	0.15
Alcohol use, n (%)	12 (38.7)	3 (60)	9 (34.6)	0.286
Recreational drug use, n (%)	7 (22.6)	2 (40)	5 (19.2)	0.309
Body mass index, kg/m^2	21.5 (20–23.25)	23 (20.0–24.7)	21.4 (19.0–23.5)	0.521
Presence of comorbidity, n (%)	4 (12.9)	2 (40)	2 (7.7)	0.112

IQR, interquartile range.

Table 8. Laboratory parameters at ART initiation according to mortality among patients with immune reconstitution inflammatory syndrome.

Variable	Total (n = 31)	Non-survivors (n = 5)	Survivors (n = 26)	p value
WBC count at ART initiation, cells/mm^3	5790 (3650-8090)	6310 (5030-9285)	5645 (3470-8290)	0.420
Lymphocyte count at ART initiation, cells/mm^3	900 (550-1439)	1640 (880-3095)	870 (487.5-1347.5)	0.036
Hemoglobin at ART initiation, g/dL	11 (9.8-12.7)	12 (10.4-15.35)	10.9 (9.55-12.55)	0.188
Platelet count at ART initiation, cells/mm^3	186000 (140000-287000)	165000 (125500-260500)	196000 (137000-290750)	0.629
Serum creatinine at ART initiation, mg/dL	0.8 (0.7-0.9)	1.0 (0.68-1.325)	0.795 (0.70-0.90)	0.090
CD4+ T-cell count at ART initiation, cells/mm^3	61 (29-126)	126 (31-319)	58 (29-100.5)	0.390
CD4+ T-cell percentage at ART initiation, %	7 (4.5-11.7)	6 (4-10.45)	7.5 (4.3-11.775)	0.628
CD8+ T-cell count at ART initiation, cells/mm^3	529 (312-972)	1180 (250.5-2096.5)	516.5 (343.25-817.5)	0.420
CD8+ T-cell percentage at ART initiation, %	55 (21-70)	52 (23-67)	62 (53-76)	0.514
HIV RNA level at ART initiation, IU/mL	480000 (94500-1543305)	450000 (78357.5-3095000)	508500 (100125-1285826.25)	0.957

ART, antiretroviral therapy; HIV, human immunodeficiency virus; RNA, ribonucleic acid; WBC, white blood cell.

Table 9. Comparison of immune reconstitution inflammatory syndrome-associated conditions between survivors and non-survivors.

Underlying condition associated with IRIS	Total (n = 31)	Non-survivors (n = 5)	Survivors (n = 26)	p value
Toxoplasmosis	2 (6.5)	0 (0)	2 (7.7)	0.521
Progressive multifocal leukoencephalopathy	6 (19.4)	3 (60)	3 (11.5)	0.012
Pneumocystis jirovecii pneumonia	4 (12.9)	0 (0)	4 (15.4)	0.347
Tuberculosis	7 (22.6)	0 (0)	7 (26.9)	0.187
Disseminated *Mycobacterium avium* complex infection	2 (6.5)	1 (20)	1 (3.8)	0.178
Lymphoma	1 (3.2)	1 (20)	0 (0)	0.020
Kaposi sarcoma	2 (6.5)	0 (0)	2 (7.7)	0.521
Cytomegalovirus diseases	6 (19.4)	0 (0)	6 (23.1)	0.232
Cryptococcosis	3 (9.7)	1 (20)	2 (7.7)	0.394

IRIS, immune reconstitution inflammatory syndrome.

Table 10. Clinical characteristics and organ involvement according to mortality among patients with immune reconstitution inflammatory syndrome.

Clinical characteristics	Total (n = 31)	Non-survivors (n = 5)	Survivors (n = 26)	p value
Central nervous system involvement, n (%)	11 (35.5)	5 (100)	6 (23.1)	0.001
Pulmonary involvement, n (%)	16 (51.6)	2 (40)	14 (53.8)	0.570
Hematopoietic involvement, n (%)	4 (12.9)	1 (20)	3 (11.5)	0.605
Gastrointestinal involvement, n (%)	3 (9.7)	1 (20)	2 (7.7)	0.436
Dermatologic involvement, n (%)	3 (9.7)	1 (20)	2 (7.7)	0.394
Fever, n (%)	22 (71.0)	5 (100)	17 (65.4)	0.118
Cough, n (%)	13 (41.9)	1 (20)	12 (46.2)	0.278
Night sweats, n (%)	10 (32.3)	1 (20)	9 (34.6)	0.522
Headache, n (%)	9 (29.0)	3 (60)	6 (23.1)	0.096
Dyspnea, n (%)	9 (29.0)	2 (40)	7 (26.9)	0.555
Lymphadenopathy, n (%)	7 (22.6)	2 (40)	5 (19.2)	0.309
Meningismus, n (%)	4 (12.9)	2 (40)	2 (7.7)	0.048
Elevated liver function tests, n (%)	3 (9.7)	0 (0)	3 (11.5)	0.424
Worsening cerebrospinal fluid (CSF) findings, n (%)	3 (9.7)	2 (40)	1 (3.8)	0.012
Radiological progression, n (%)	13 (41.9)	2 (40)	11 (42.3)	0.924
Paradoxical immune reconstitution inflammatory syndrome, n (%)	21 (67.7)	3 (60)	18 (69.2)	0.686

Table 11. Clinical course and treatment according to mortality among patients with immune reconstitution inflammatory syndrome.

Variable	Total (n = 31)	Non-survivors (n = 5)	Survivors (n = 26)	p value
Time from ART initiation to IRIS onset, days	30 (14-60)	52 (14-60)	29 (13.75-61.25)	0.809
Follow-up duration, days	80 (32.5-260)	109 (41.5-350)	57 (31.25-188.75)	0.470
Non-steroidal anti-inflammatory drug treatment, n (%)	8 (25.8)	0 (0)	8 (30.8)	0.150
Corticosteroid treatment, n (%)	21 (67.7)	2 (40)	19 (73.1)	0.296

ART, antiretroviral therapy; IRIS, immune reconstitution inflammatory syndrome.

Discussion

IRIS has long been recognized as a serious complication in people living with HIV, with reported incidence rates ranging from 3% to 54%, particularly among patients with advanced immunosuppression and a high HIV viral load at ART initiation[13]. In our study, IRIS developed in 3.2% of patients, representing the lower end of the reported range. We believe that this relatively low incidence may be explained by several factors. First, our stringent diagnostic criteria, including the systematic reevaluation of all suspected cases according to predefined diagnostic criteria, may have reduced the number of confirmed IRIS cases. Second, the retrospective design may have resulted in underrecognition of mild or undocumented IRIS episodes. Finally, population-specific or genetic factors may also have contributed to the observed incidence[5,14].

A notable finding of our study was that paradoxical IRIS accounted for 68% of all cases, whereas unmasking IRIS was less common. This distribution may be related to the fact that HIV infection was frequently diagnosed during the evaluation of an OI, with the OI typically being identified before the initiation of ART. In contrast, Pérez-Rueda et al.[13] reported that 59% of cases were unmasking IRIS, whereas Hoyo-Ulloa et al.[1] found unmasking IRIS in 81% of patients. These differences suggest that the relative frequencies of paradoxical and unmasking IRIS may vary according to the timing of HIV diagnosis, the burden of OIs at presentation, and local clinical practice regarding ART initiation.

Both infectious and non-infectious causes may contribute to IRIS development[7]. In our study, the most common OIs associated with IRIS were *Mycobacterium. tuberculosis* and *Pneumocystis jirovecii*. Previous studies have identified *Varicella zoster*, *Mycobacterium tuberculosis*, MAC, *Pneumocystis jirovecii*, and *Cryptococcus neoformans* as the most common opportunistic pathogens associated with IRIS[1]. Similarly, a meta-analysis of 54 studies published between 1998 and 2009, including 1,699 IRIS cases, reported *Mycobacterium tuberculosis*, herpes infections, cryptococcal meningitis (CM), CMV retinitis, and PML as the most frequently associated infections[11]. Despite the success of the National Tuberculosis Control Program in reducing TB incidence in our country from 29.4 per 100,000 in 2005 to 11.4 per 100,000 in 2022[15], tuberculosis (TB) remains a public health concern. Therefore, the predominance of TB-IRIS in our population with HIV infection is unsurprising.

In our study, six of the seven TB-IRIS cases (86%) were classified as paradoxical. Furthermore, CD4+ T-cell counts were below 100 cells/mm^3 in 71.4% of these patients, and 85.7% had HIV RNA levels >100,000 IU/mL. The initiation of ART in patients with TB poses a substantial risk for IRIS, which may cause serious morbidity or even mortality[16]. Paradoxical TB-IRIS has been reported in 18%-57% of patients with HIV infection[17-19]. The high proportion of paradoxical TB-IRIS and the immunologic characteristics observed in our study are consistent with previously reported risk factors, including a short interval between starting TB treatment and ART, low baseline CD4+ T-cell counts, high baseline viral load, rapid viral suppression, and disseminated TB[14].

In our study, unmasking IRIS developed later than paradoxical IRIS. This finding is consistent with previous reports indicating that the timing of IRIS onset varies according to the underlying pathogen and type of IRIS. IRIS typically develops within 12 weeks of ART initiation. Ratnam et al.[20] reported a median onset of 12 weeks, with 65% of cases occurring within the first 3 months of ART. Similarly, Pérez-Rueda et al.[13] observed a longer time to IRIS development in patients with unmasking IRIS and parasite-related infections.

In our study, 71% of patients had baseline CD4+ T-cell counts below 100 cells/mm^3, supporting the well-established association between advanced immunosuppression and the development of IRIS. It is well-established that initiating ART when CD4+ T-cell counts are below 350 cells/mm^3 increases the risk of IRIS[13]. Notably, one patient with a CD4+ T-cell count >300 cells/mm^3 had concurrent *Mycobacterium tuberculosis*, CMV, and HBV infections, while another patient with a CD4+ T-cell count of 507 cells/mm^3 was diagnosed with lymphoma following ART initiation. These findings suggest that although severe CD4+ T-cell depletion is a major risk factor, IRIS may also occur in patients with higher CD4+ T-cell counts when other conditions associated with immune dysregulation are present. This observation is consistent with previous reports indicating that immune reconstitution involves not only quantitative CD4+ T-cell recovery but also functional immune changes that may contribute to IRIS pathogenesis[14].

Integrase strand transfer inhibitor (INSTI)-based ART results in more rapid virologic suppression and immune recovery, leading to the hypothesis that it may precipitate earlier IRIS in susceptible patients. However, current evidence, including a meta-analysis of randomized controlled trials, does not support an overall increase in the risk of IRIS with INSTI-based regimens compared with other ART classes[21]. In our cohort, 29 of the 31 patients were receiving an INSTI-based ART regimen at the time of IRIS diagnosis. This finding most likely reflects the widespread adoption of INSTI-based regimens as preferred first-line therapy because of their favorable efficacy, tolerability, safety profile, and current guideline recommendations, rather than an association between INSTI use and the development of IRIS. Given the limited sample size and absence of a comparison group, no conclusions can

be drawn regarding the relationship between INSTI-based ART and the risk of IRIS in our cohort.

Of the 31 patients, 21 received corticosteroids; however, detailed data on corticosteroid dose and treatment duration were available for only 19 patients. Among these patients, the mean corticosteroid dose was 176.8 ± 291.9 mg (median, 80 mg), and the mean treatment duration was 24.7 ± 22.4 days (median, 20 days). Current EACS guidelines recommend continuation of ART and treatment of the underlying OI in most cases of OI-associated IRIS, while reserving corticosteroids for patients with life-threatening or severe inflammatory manifestations rather than for routine use. The guidelines provide disease-specific corticosteroid regimens for TB-IRIS and severe PML-IRIS, whereas evidence supporting corticosteroid use in CM-associated IRIS remains limited. For most other forms of IRIS, no specific recommendations regarding corticosteroid dosing or treatment duration are available[12]. Therefore, the wide variability in corticosteroid doses and treatment durations observed in our cohort likely reflects the heterogeneous spectrum of underlying OIs and the individualized management of severe IRIS cases.

The reported all-cause mortality among patients with IRIS is approximately 4.5%, although mortality varies substantially according to the underlying OI, access to treatment, diagnostic criteria, degree of immunosuppression, and geographic setting[22]. Central nervous system (CNS) involvement has consistently been associated with poor outcomes, with reported mortality rates of 20.8% for CM-IRIS, 22% for PML-IRIS, and up to 75% for CNS TB-IRIS[7,23]. In the present study, the all-cause mortality rate was 16.1% (5/31). The IRIS-associated conditions observed among patients who died included PML-associated IRIS (n = 2), concurrent PML- and MAC-associated IRIS (n = 1), *Cryptococcus neoformans*-associated IRIS (n = 1), and lymphoma-associated IRIS (n = 1). Consistent with previous reports, CNS involvement and PML were more frequently observed among non-survivors than among survivors. Non-survivors also more frequently presented with meningismus and worsening cerebrospinal fluid findings, suggesting more severe CNS involvement. However, because only 5 deaths occurred, multivariable analysis was not feasible, and these findings should be interpreted as exploratory associations rather than independent predictors of all-cause mortality.

Study Limitations

Our study was multicenter; however, the number of IRIS cases was limited, and the retrospective design and small sample size were the main limitations. In addition, several subgroup analyses were based on very small numbers of patients. Therefore, subgroup-specific percentages and mortality rates should be interpreted with considerable caution and regarded as descriptive rather than conclusive, and no generalized conclusions regarding subgroup-specific outcomes can be drawn. Furthermore, a comparison between patients with and without IRIS could not be performed, precluding the identification of factors associated with IRIS development. Nevertheless, published data on HIV-associated IRIS from Türkiye are scarce, and, to our knowledge, no previous multicenter study has comprehensively evaluated the clinical characteristics and outcomes of patients with IRIS across multiple HIV referral centers in the country. Despite its limitations, our study provides valuable multicenter descriptive data on the clinical spectrum, underlying OIs, and outcomes of HIV-associated IRIS in routine clinical practice. Future prospective multicenter studies including larger cohorts and standardized data collection methods are warranted to better define the epidemiology, clinical characteristics, outcomes, and predictors of IRIS development.

Conclusion

Recognizing the characteristics of patients who develop IRIS and improving awareness of this condition will enable a more informed clinical approach and ensure timely interventions.

Ethics

Ethics Committee Approval: The approval was granted by the Ethics Committee of University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital in accordance with the principles of the Declaration of Helsinki (approval number: 2025/44, date: 12.02.2025).

Informed Consent: As this was a retrospective study, the requirement for informed consent from participants was waived by the Ethics Committee of University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.A., F.Y.K., M.T.D., S.A., M.T., Y.Ç., Concept: E.A., D.Ö.E., E.S.A., A.B., M.T.D., S.A., S.Ş., N.T., T.D.Ç., A.Ş.Ç., S.A.N., Ş.K., Design: D.Ö.E., H.Ö.Ö., A.B., T.Ö.K., Y.Ç., S.Ş., T.D.Ç., A.Ş., A.Ş.Ç., Ş.K., Data Collection or Processing: E.A., H.Ö.Ö., F.Y.K., E.S.A., M.T.D., S.A., T.Ö.K., M.T., Y.Ç., N.T., A.Ş., A.Ş.Ç., S.A.N., Analysis or Interpretation: H.Ö.Ö., E.S.A., T.Ö.K., M.T., N.T., T.D.Ç., A.Ş., Literature Search: E.A., D.Ö.E., H.Ö.Ö., F.Y.K., A.B., Ş.K., Writing: E.A., D.Ö.E., F.Y.K., S.A., S.Ş.

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Diagnostic Performance of Combined Routine Inflammatory Biomarkers for the Identification of Active Tuberculosis

Aktif Tüberkülozun Belirlenmesinde Kombine Rutin Enflamatuvar Biyobelirteçlerin Tanısal Performansı

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Abstract

Introduction: Conventional diagnostic approaches for tuberculosis (TB) often require prolonged periods to yield definitive results, which may delay timely clinical decision-making. Host-derived inflammatory biomarkers reflecting innate immune responses have gained increasing attention; however, the diagnostic value of integrating the long pentraxin 3 (PTX3) with routinely available acute-phase proteins and hematological parameters remains insufficiently explored. We hypothesized that a multiparametric inflammatory panel reflecting systemic inflammation and innate immune responses could improve the diagnostic performance for TB. Therefore, this study aimed to evaluate the diagnostic performance of infection-related biomarkers, including PTX3, C-reactive protein (CRP), and serum amyloid A (SAA), together with hematological parameters (lymphocytes, monocytes, neutrophils, eosinophils, basophils, and platelets), and to investigate whether different biomarker combinations improve the identification of active TB.

Materials and Methods: In this cross-sectional study, peripheral blood samples were analyzed using a Sysmex XN-1000 hematology analyzer. CRP and SAA levels were measured using chemiluminescence, and PTX3 levels were measured using enzyme-linked immunosorbent assay. Diagnostic performance was assessed using receiver operating characteristic curve analysis.

Results: In the TB group, CRP, SAA, WBC, monocyte count (MONO), and NEU levels were significantly higher, whereas LYM and BASO levels were significantly lower than those in the control group ($p < 0.05$). Among the evaluated biomarker combinations, CRP–MONO yielded the numerically highest area under the curve (AUC; 0.943), followed by CRP-LYM (AUC, 0.934).

Conclusion: Our findings suggest that combining routinely available inflammatory biomarkers with hematological parameters may have potential utility in distinguishing patients with active TB from healthy controls. These exploratory findings require validation in larger, independent, multicenter cohorts before clinical application.

Keywords: C-reactive protein, infection, pentraxin 3, serum amyloid A, white blood cells, tuberculosis

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Öz

Giriş: Tüberküloz (TB) için kullanılan geleneksel tanı yaklaşımları çoğu zaman kesin sonuç elde etmek için uzun süre gerektirmekte ve bu durum zamanında klinik karar verilmesini geciktirebilmektedir. Doğal bağışıklık yanıtını yansıtan konak kaynaklı inflamatuvar biyobelirteçler son yıllarda giderek daha fazla ilgi görmektedir. Ancak uzun pentraxin ailesinden olan PTX3'ün rutin olarak kullanılan akut faz proteinleri ve hematolojik parametrelerle birlikte değerlendirilmesinin tanısal değeri henüz yeterince araştırılmamıştır. Bu çalışmada, sistemik inflamasyonu ve doğal bağışıklık dinamiklerini yansıtan çok parametreli bir inflamatuvar panelin TB tanısındaki performansı artırabileceği hipotez edilmiştir. Bu nedenle çalışmanın amacı, PTX3, C-reaktif protein (CRP) ve serum amiloid A (SAA) gibi enfeksiyon ilişkili biyobelirteçlerin; lenfosit, monosit, nötrofil, eozinofil, bazofil ve trombosit gibi hematolojik parametrelerle birlikte tanısal performansını değerlendirmek ve farklı biyobelirteç kombinasyonlarının TB hastalarının belirlenmesine katkısını araştırmaktır.

Gereç ve Yöntem: Bu kesitsel çalışmada periferik kan örnekleri Sysmex XN-1000 hematoloji analizörü kullanılarak analiz edilmiştir. CRP ve SAA düzeyleri kemilüminesans yöntemiyle, PTX3 düzeyleri ise ELISA yöntemi ile ölçülmüştür. Tanısal performans, alıcı işletim karakteristiği eğrisi analizi kullanılarak değerlendirilmiştir.

Bulgular: TB grubunda CRP, SAA, WBC, MONO ve NEU düzeyleri kontrol grubuna göre anlamlı derecede yüksek, LYM ve BASO düzeyleri ise anlamlı derecede düşük bulunmuştur ($p < 0,05$). Değerlendirilen biyobelirteç kombinasyonları arasında sayısal olarak en yüksek tanısal performans CRP–MONO modelinde (AUC: 0.943), ardından CRP-LYM modelinde (AUC: 0.934) elde edilmiştir.

Sonuç: Bulgularımız, rutin olarak elde edilebilen inflamatuar biyobelirteçlerin hematolojik parametrelerle birleştirilmesinin, aktif TB'li hastaları sağlıklı kontrollerden ayırt etmede potansiyel bir fayda sağlayabileceğini düşündürmektedir. Bu keşif niteliğindeki bulguların klinik uygulamaya geçmeden önce daha büyük, bağımsız, çok merkezli kohortlarda doğrulanması gerekmektedir.

Anahtar Kelimeler: C-reaktif protein, enfeksiyon, pentraxin 3, serum amiloid A, beyaz kan hücreleri, tüberküloz

Introduction

Tuberculosis (TB) is one of the oldest known chronic infectious diseases in human history and remains a major cause of morbidity and mortality caused by *Mycobacterium tuberculosis*[1]. Despite being treatable, it has remained a major global health problem for decades and was the leading cause of death from a single infectious pathogen until 2019[2,3]. According to the World Health Organization (WHO), approximately one-quarter of the global population has been infected with *Mycobacterium tuberculosis* and is estimated to have latent TB infection[4].

Inflammation plays an important role in the pathogenesis of TB[5]. C-reactive protein (CRP), serum amyloid A (SAA), and pentraxin 3 (PTX3) are acute-phase reactants released in response to cytokines, particularly interleukin-6, during infectious diseases. These proteins contribute to the host response to infection and inflammation[6,7]. PTX3 is a key component of innate immunity and functions as a soluble pattern recognition molecule. CRP is released from the liver, indicating a systemic response to local inflammation, whereas PTX3 is directly released by damaged tissues, reflecting the local inflammatory status[8,9]. SAA also plays a significant role in acute and chronic inflammation and is used as an indicator of inflammation in clinical laboratories[10]. Additionally, severe TB cases frequently exhibit hematological abnormalities[11]. In TB, complete blood count indices, including neutrophil (NEU) count, platelet (PLT) count, and CRP levels, may be useful for determining disease severity and monitoring treatment response[5]. Various studies have suggested that CRP and PLT distribution width could serve as potential biomarkers of TB severity[12-14].

Despite advances in diagnostic technologies, current tools for the detection of *Mycobacterium tuberculosis* infection remain limited by delayed turnaround times and suboptimal sensitivity, leading to missed or delayed diagnoses worldwide[15,16]. Although culture remains the gold standard for TB diagnosis, the time required to obtain results often extends over several weeks[17]. Consequently, increasing attention has been directed toward host-derived inflammatory biomarkers that reflect the innate immune response during infection. While acute-phase proteins have been widely investigated, the diagnostic value of integrating the long pentraxin PTX3 with routinely available inflammatory and hematological parameters has not been sufficiently explored. We hypothesized that a multiparametric inflammatory panel reflecting both systemic inflammation and innate immune dynamics could improve diagnostic performance in TB. The novelty of the present study lies in the simultaneous evaluation of PTX3, conventional acute-phase reactants, and routinely available hematological parameters, as well as their two- and three-marker combinations, within the same cohort. Rather than focusing on a single biomarker class, this exploratory approach integrates markers reflecting different components of the inflammatory response and evaluates their diagnostic performance both individually and in combination for discriminating active TB from healthy controls. Therefore, the present study aimed to evaluate PTX3, CRP, and SAA together with peripheral blood cell counts and to investigate whether specific biomarker combinations enhance the identification of patients with active TB using a cost-effective and clinically applicable diagnostic strategy. Given the relatively small sample size and exploratory design, this study should be considered a pilot investigation intended to assess the potential diagnostic utility of combined inflammatory biomarkers in active TB.

Materials and Methods

This study was conducted in accordance with the ethical principles outlined in the 1975 Declaration of Helsinki. Ethical approval was obtained from the Mersin University Clinical Research Ethics Committee (decision number: 2023/359, dated May 24, 2023). Because the analyses were performed using blood samples collected as part of routine clinical testing, the Ethics Committee determined that additional written informed consent was not required. This was a single-center, cross-sectional study. Participants were consecutively enrolled during the study period, and blood samples collected during routine clinical evaluation were analyzed for the study biomarkers.

Study Groups

The target sample size was determined pragmatically based on the estimated number of eligible patients presenting to the Chest Diseases Outpatient Clinic. Hospital records from the preceding 12-month period indicated that approximately 50 patients who met the eligibility criteria had been diagnosed with TB. Considering the anticipated number of eligible patients during the study period, recruitment feasibility, and the potential for data loss, a minimum target of 20 patients with active TB and 20 healthy controls was established. No formal a priori sample-size or power calculation was performed. Participants were consecutively selected from individuals presenting to the Chest Diseases Outpatient Clinic between March 1, 2022, and March 1, 2023. TB was diagnosed based on clinical evaluation and confirmed by microbiological culture positivity for *Mycobacterium tuberculosis*. Patients with culture-confirmed active pulmonary TB (PTB) were included in the study. The patient group comprised 24 individuals with newly diagnosed active TB who had not previously received TB treatment and had no concomitant infectious or chronic diseases. The control group comprised 24 healthy volunteers recruited from individuals presenting to the hospital laboratory for routine laboratory testing during the same study period. Controls were not recruited from the Chest Diseases Outpatient Clinic. Individuals with a history of infectious or chronic disease or clinical symptoms suggestive of active infection were excluded from the control group.

Sample Collection

For PTX3, CRP, and SAA analyses, 7–10 mL of peripheral blood was collected into vacuum gel tubes without anticoagulant (Vacusera, Türkiye). For WBC, LYM, monocyte count (MONO), NEU, eosinophil (EOS), basophil (BASO), and PLT counts, 3-5 mL of peripheral blood was collected into hemogram tubes containing Na_2EDTA (Vacusera, Türkiye). Blood samples collected in vacuum gel tubes without anticoagulant were centrifuged at 4,000 rpm for 10 minutes to obtain serum. Subsequently, approximately 1-1.5 mL of serum was carefully transferred to a sterile Eppendorf tube and stored at −20°C until analysis. All samples were analyzed within 6 months of storage at −20°C to maintain analytical integrity. Blood samples collected in Na_2EDTA tubes were analyzed within a maximum of 2 hours after collection.

Analysis Process

WBC, LYM, MONO, NEU, EOS, BASO, and PLT were analyzed within 1-2 hours after blood collection using an automated hematology analyzer (XN-1000, Sysmex Corp., Japan) based on electrical impedance and flow cytometry. Serum samples stored at −20°C until the day of analysis were thawed at room temperature before CRP, PTX3, and SAA measurements. Quantitative CRP and SAA measurements were performed using the chemiluminescence-based Maglumi X3 Automated System (Snibe Co. Ltd., Shenzhen, China), whereas PTX3 was measured using the Human Ptx3 Enzyme-Linked Immunosorbent Assay (ELISA) kit (Lot No. 201609, SunRedBio, Shanghai) according to the manufacturer's instructions.

Statistical Analysis

Quantitative data were analyzed using SPSS version 20.0 (IBM Corporation, Armonk, New York, USA). The normality of continuous variables was assessed using the Shapiro-Wilk test. Comparisons of continuous variables between the 2 groups were performed using the Mann-Whitney U test. Spearman rank correlation coefficients were calculated to assess correlations between the measured variables. Receiver operating characteristic (ROC) curve analysis was performed to evaluate diagnostic performance, and the area under the curve (AUC) was calculated. Optimal cut-off values were determined using Youden's index, defined as sensitivity + specificity − 1, with the threshold that maximized the Youden index selected as the optimal cut-off value. The AUCs of CRP alone and the CRP-MONO combination were compared using DeLong's test for correlated ROC curves. Binary logistic regression analysis was used to evaluate the combined diagnostic performance of the biomarkers, with TB status as the dependent variable. Regression coefficients (β), odds ratios (ORs), 95% confidence intervals (CIs), and p values were estimated for each model. The predicted probability of active TB was derived from the logistic regression equation, where $\text{logit}(p) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k$ and $p = 1/[1 + \exp(-\text{logit}(p))]$. Detailed regression coefficients and model-specific equations are provided in the Supplementary Material. For the CRP–MONO threshold-based analysis, the two biomarker thresholds were jointly evaluated using an OR classification rule. A participant was classified as positive when CRP was ≥5.44 mg/L or MONO was $\geq 1.04 \times 10^3/\mu L$. Sensitivity, specificity, and the Youden index

were calculated for this combined binary classification rule. This threshold-based analysis was distinct from the continuous combined biomarker analysis. Analyses with a p value <0.05 were considered statistically significant.

Results

A total of 48 individuals, including 24 patients with active TB and 24 healthy controls, were included in the study. In the TB group, 16 participants (66.7%) were male and 8 (33.3%) were female, whereas the control group consisted of 19 males (79.2%) and 5 females (20.8%). The mean age was 47.3 ± 20.8 years in the TB group and 41.7 ± 9.0 years in the control group, with no statistically significant difference between the groups (p = 0.240). The median (IQR) ages were 51 (28-61) years and 43 (37.8-48) years in the TB and control groups, respectively. All patients in the TB group had PTB. Nine patients (37.5%) with PTB had cavitary lesions on radiologic imaging. The acid-fast bacilli (AFB) smear positivity rate among TB patients was 83.3% (20/24), and at least one of the three *Mycobacterium tuberculosis* strains (12.5%) was resistant to a first-line anti-TB drug.

In the TB group, CRP, SAA, WBC, MONO, and NEU levels were significantly higher, whereas LYM and BASO levels were significantly lower than those in the control group (p < 0.05). No significant differences were observed between the groups in PTX3, PLT, or EOS levels (p = 0.853, p = 0.170, and p = 0.116, respectively) (Table 1). When the patient group was evaluated according to AFB smear positivity, only CRP levels differed significantly between AFB smear-positive and AFB smear-negative patients (p = 0.042).

When correlations between the parameters were analyzed, moderate positive correlations were observed between CRP and SAA, WBC, MONO, and NEU (r = 0.597, 0.308, 0.395, and 0.530, respectively); between MONO and WBC, NEU, and EOS (r = 0.674, 0.608, and 0.315, respectively); and between LYM and WBC and BASO (r = 0.315 and 0.398, respectively). A strong positive correlation was observed between NEU and WBC (r = 0.894) (p < 0.05). In addition, moderate negative correlations were observed between LYM and CRP (r = −0.511), LYM and SAA (r = −0.443), and CRP and BASO (r = −0.407) (Table 2).

The diagnostic performance analysis of different combinations of parameters revealed that the CRP-LYM (AUC, 0.934), CRP-NEU (AUC, 0.932), and PTX3-CRP (AUC, 0.905) pairs, as well as the CRP-MONO–LYM (AUC, 0.939), CRP-MONO-NEU (AUC, 0.934), CRP-WBC-NEU (AUC, 0.929), and CRP-PTX3-MONO (AUC, 0.917) triple combinations, yielded relatively high AUC values. However, given the non-significant diagnostic performance of PTX3 alone, the AUC values observed for PTX3-containing combinations should not be interpreted as evidence of an incremental diagnostic contribution from PTX3. Among the evaluated combinations, CRP–MONO yielded the numerically highest AUC (0.943) (Table 3, Figure 1). However, the difference between the AUC of CRP alone (0.936) and that of the CRP–MONO combination (0.943) was not statistically significant (DeLong test, p = 0.307).

When the diagnostic performance of the parameters was analyzed individually, CRP (AUC, 0.936), MONO (AUC, 0.828), and NEU (AUC, 0.795) demonstrated the best diagnostic performance as individual parameters for TB diagnosis. For differentiating patients with active TB from healthy controls, the optimal cut-off value for CRP was 4.97 mg/L, with a Youden index of 0.875, sensitivity of 91%, and specificity of 95%. The

Table 1. Median, minimum-maximum(min-max), and p values of PTX3, CRP, SAA, WBC, LYM, MONO, NEU, EOS, BASO, and PLT parameters in the tuberculosis and control groups.

Laboratory parameters	Tuberculosis group (n = 24) Median (min-max)	Control group (n = 24) Median (min-max)	p value
PTX3 ng/mL	1.509 (0.432–19.02)	1.451 (1.106–6.868)	0.853
CRP mg/L	47.470 (0.27–242.83)	2.100 (0.20–15)	< 0.001
SAA µg/mL	235.000 (0.57–300)	12.400 (0.10–300)	0.004
WBC × 10^3/µL	9.595 (4.41–14.88)	7.780 (5.35–9.42)	0.030
LYM × 10^3/µL	1.835 (0.58–3.12)	2.580 (1.15–3.49)	0.004
MONO × 10^3/µL	0.805 (0.48–1.86)	0.570 (0.41–0.83)	< 0.001
NEU × 10^3/µL	6.045 (2.82–10.78)	4.415 (2.46–5.88)	< 0.001
EOS × 10^3/µL	0.150 (0.0–0.76)	0.060 (0.02–0.34)	0.116
BASO × 10^3/µL	0.055 (0.010–0.55)	0.105 (0.020–1.26)	0.004
PLT × 10^3/µL	293.500 (10.5–463)	263.000 (195–380)	0.170

n, Number of samples; p, values of significance with difference for each group. PTX3, pentraxin 3; CRP, C-reactive protein; SAA, serum amyloid A; WBC, white blood cell count; LYM, lymphocyte count; MONO, monocyte count; NEU, neutrophil count; EOS, eosinophil; BASO, basophils; PLT, platelet.

optimal cut-off value for MONO was 0.630 × 10^3/μL, with a Youden index of 0.542, sensitivity of 83%, and specificity of 71%. The optimal cut-off value for NEU was 5.35 × 10^3/μL, with a Youden index of 0.667, sensitivity of 75%, and specificity of 91% (Table 4). Furthermore, the CRP–MONO combination yielded the numerically highest AUC (0.943), with cut-off values of 5.44 mg/L for CRP and 1.04 × 10^3/μL for MONO. The Youden index was 0.875, with a sensitivity of 91% and specificity of 95% (Table 4).

Table 2. Spearman correlation coefficients (r values) among PTX3, CRP, SAA, WBC, LYM, MONO, NEU, EOS, BASO, and PLT.

	PTX3	CRP	SAA	WBC	LYM	MONO	NEU	EOS	BASO	PLT
PTX3	1.000	0.155	0.004	−0.064	0.038	−0.033	−0.097	0.127	0.039	−0.120
CRP	0.155	1.000	**0.597****	**0.308***	**−0.511****	**0.395****	**0.530****	0.038	**−0.407****	0.057
SAA	0.004	**0.597****	1.000	0.078	**−0.443****	0.275	0.244	−0.057	−0.219	0.109
WBC	−0.064	**0.308***	0.078	1.000	**0.315***	**0.674****	**0.894****	0.137	0.025	0.162
LYM	0.038	**−0.511****	**−0.443****	**0.315***	1.000	0.150	−0.064	0.125	**0.398****	−0.163
MONO	−0.033	**0.395****	0.275	**0.674****	0.150	1.000	**0.608****	**0.315***	−0.185	0.237
NEU	−0.097	**0.530****	0.244	**0.894****	−0.064	**0.608****	1.000	0.101	−0.198	0.210
EOS	0.127	0.038	−0.057	0.137	0.125	**0.315***	0.101	1.000	−0.198	0.014
BASO	0.039	**−0.407****	−0.219	0.025	**0.398****	−0.185	−0.198	−0.198	1.000	0.023
PLT	−0.120	0.057	0.109	0.162	−0.163	0.237	0.210	0.014	0.023	1.000

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). Correlation coefficients were interpreted as follows: $|r| \leq 0.30$, weak; $0.30 < |r| \leq 0.70$, moderate; and $|r| > 0.70$, strong. PTX3, pentraxin 3; CRP, C-reactive protein; SAA, serum amyloid A; WBC, white blood cell count; LYM, lymphocyte count; MONO, monocyte count; NEU, neutrophil count; EOS, eosinophil; BASO, basophils; PLT, platelet.

Table 3. PTX3, CRP, SAA, WBC, LYM, MONO, NEU, EOS, BASO, PLT, and the diagnostic performance values (AUC: Area Under the Curve).

Tests	AUC	95 % CI	p value
PTX3	0.516	0.346–0.686	0.853
CRP	**0.936**	**0.851–1.000**	**<0.001**
SAA	0.736	0.592–0.880	0.005
WBC	0.683	0.521–0.846	0.030
LYM	0.746	0.604–0.887	0.004
MONO	0.828	0.711–0.945	<0.001
NEU	0.795	0.651–0.939	<0.001
EOS	0.632	0.458–0.806	0.117
BASO	0.739	0.598–0.879	0.005
PLT	0.615	0.454–0.777	0.170
CRP–MONO	**0.943**	**0.862–1.000**	**<0.001**
CRP–LYM	0.934	0.849–1.000	<0.001
CRP–NEU	0.932	0.842–1.000	<0.001
PTX3-CRP	0.905	0.807–1.000	<0.001
CRP–MONO–LYM	0.939	0.857–1.000	<0.001
CRP–MONO–NEU	0.934	0.846–1.000	<0.001
CRP–WBC–NEU	0.929	0.835–1.000	<0.001
CRP–MONO-PTX3	0.917	0.824–1.000	<0.001

p, values of significance with difference in each group; CI, confidence interval, AUC, area under the curve; PTX3, pentraxin 3; CRP, C-reactive protein; SAA, serum amyloid A; WBC, white blood cell count; LYM, lymphocyte count; MONO, monocyte count; NEU, neutrophil count; EOS, eosinophil; BASO, basophils; PLT, platelet.

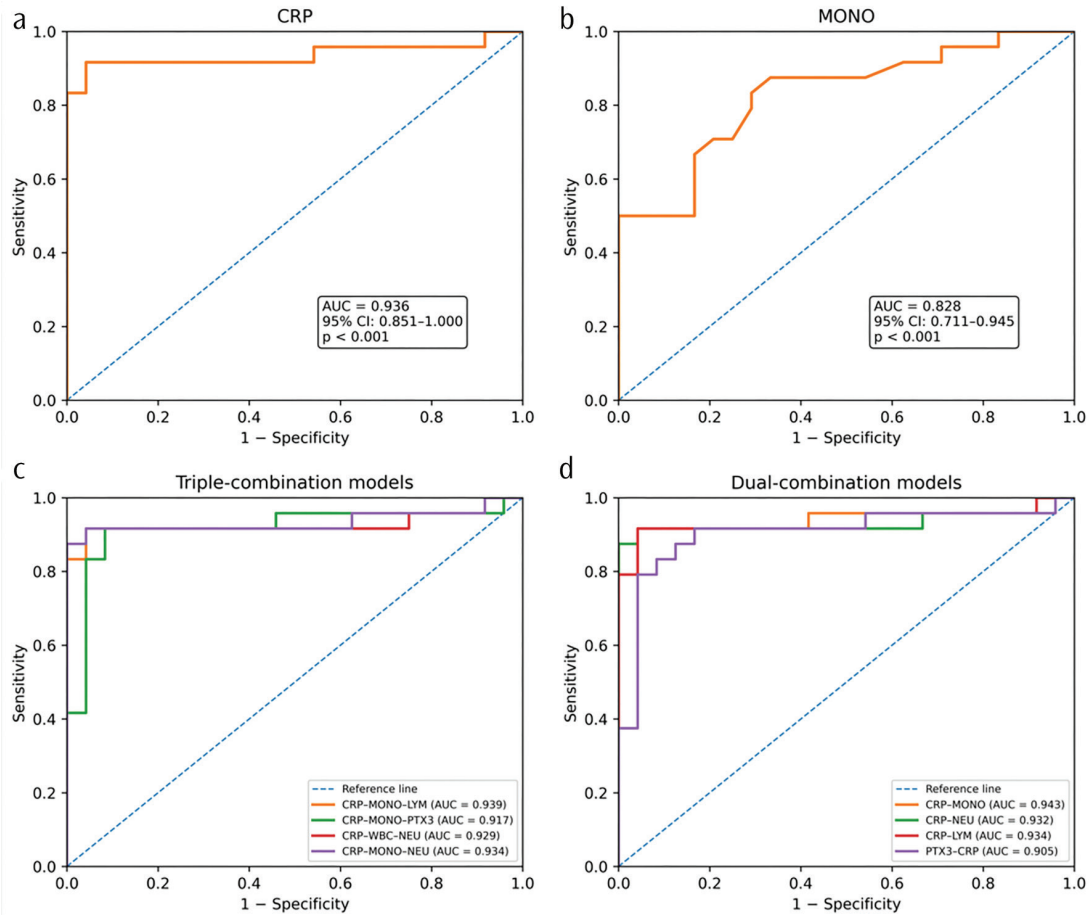

Figure 1. Receiver operating characteristic (ROC) curve analyses of inflammatory biomarkers and combined biomarker scores for the diagnosis of active TB. (A) ROC curve for CRP. (B) ROC curve for MONO. (C) ROC curves for selected triple-marker combinations. (D) ROC curves for selected dual-marker combinations. AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; LYM, lymphocyte count; MONO, monocyte count; NEU, neutrophil count; PTX3, pentraxin 3; WBC, white blood cell count.

Table 4. The cut-off values, sensitivity, specificity, and Youden indices for CRP, SAA, WBC, MONO, NEU, LYM, and BASO parameters, which exhibit significant differences between the TB and control groups.

	Cut-off point	Sensitivity	Specificity	Youden index
CRP	4.97 mg/L	0.917	0.958	0.875
SAA	41.75 µg/mL	0.667	0.833	0.5
WBC	9.415 × 10^3/µL	0.542	0.958	0.5
MONO	0.63 × 10^3/µL	0.833	0.708	0.542
NEU	5.355 × 10^3/µL	0.75	0.917	0.667
LYM	2.165 × 10^3/µL	0.667	0.833	0.5
BASO	0.11 × 10^3/µL	0.875	0.5	0.375
CRP;MONO	5.44 mg/L; 1.04 × 10^3/µL	0.917	0.958	0.875

For the CRP–MONO combination, positivity was defined as CRP ≥5.44 mg/L or MONO ≥1.04 × 10^3/µL. Sensitivity, specificity, and the Youden index were calculated according to this combined binary classification rule. CRP, C-reactive protein; SAA, serum amyloid A; WBC, white blood cell count; LYM, lymphocyte count; MONO, monocyte count; NEU, neutrophil count; BASO, basophils; TB, tuberculosis.

Discussion

Rapid and accurate differentiation of patients with TB from healthy controls is crucial for effective disease management. However, traditional TB diagnostic methods can be costly and may require considerable time to yield definitive results. There remains an ongoing need for low-cost, accurate, and rapid methods, including triage tests, that are easily accessible and practical for use in clinical settings[18,19]. Blood tests and biochemical analyses routinely performed in clinical settings can provide rapidly accessible indicators of disease[20].

The importance of hematological parameters has been emphasized for predicting the risk of TB development, identifying active TB infection, and monitoring the response to antimicrobial therapy. Complete blood count parameters have been proposed as potential discriminatory biomarkers for inflammatory diseases[21]. Acute-phase proteins such as PTX3, CRP, and SAA are routinely measurable and have been recommended as supportive discriminatory biomarkers in infectious diseases[6,7]. The combined use of acute-phase proteins in bacterial infections has also been suggested to provide additional information and improve sensitivity for evaluating inflammatory status and supporting diagnosis[10]. Therefore, the present study evaluated the diagnostic performance of whole blood indices (WBC, LYM, MONO, NEU, EOS, BASO, and PLT) and blood proteins (PTX3, CRP, and SAA), both individually and in combination, which can be readily analyzed from blood and serum samples in patients with active TB.

CRP is an acute-phase protein and a member of the pentraxin family that is produced in response to proinflammatory stimuli. CRP, which has been extensively studied in TB, has emerged as a promising marker for both diagnosis and monitoring of treatment response[22,23]. Similarly, SAA, another acute-phase protein, has multiple immunological functions[6]. SAA has been identified as a potential marker of TB and may play an important immunological role in reinforcing granulomatous responses to mycobacterial antigens[24]. Several studies have reported significantly elevated levels of SAA and CRP in patients with TB[25-27]. In the present study, both SAA and CRP levels were significantly higher in the TB group than in healthy controls ($p = 0.004$ and $p < 0.001$, respectively).

Despite being classified as a non-specific marker of infection, CRP has been shown in systematic reviews and meta-analyses to be superior to symptom-based approaches for identifying TB. Because of its sensitivity and specificity, the WHO has recommended including CRP in diagnostic algorithms for HIV-infected individuals[28]. In a study by Samuels et al. involving 765 samples, a CRP cut-off value of 8 mg/L yielded a sensitivity of 79.8% and a specificity of 62.8% for differentiating patients with TB from healthy controls[22]. Recent WHO guidance highlights the importance of systematic screening and triage approaches for TB and emphasizes the need for tools that can identify individuals who require confirmatory diagnostic testing[29]. In the present study, a CRP cut-off value of 4.97 mg/L yielded a sensitivity of 91% and a specificity of 95%. Although these sensitivity and specificity values were encouraging, they should be interpreted cautiously because the study compared patients with active TB with healthy controls rather than clinically relevant disease-control groups. Although CRP is a widely available and routinely measured inflammatory marker, the CRP cut-off value identified in the present study (4.97 mg/L) should also be interpreted cautiously, as biomarker thresholds may vary according to laboratory methods, assay characteristics, patient populations, and clinical settings. Therefore, this cut-off should not be considered a universally applicable diagnostic threshold and requires validation in independent and clinically representative cohorts before routine clinical use.

PTX3 plays a role in the acute-phase response to injury, trauma, and infection[30]. PTX3, like CRP, is a member of the pentraxin family but is synthesized extrahepatically[31]. Whereas CRP is produced almost exclusively in the liver, PTX3 is locally expressed by various cells at sites of inflammation and is stored in NEU-specific granules[32,33]. In this study, although serum PTX3 levels were higher in patients with TB than in the control group, the difference was not statistically significant. Azzurri et al.[31] reported that plasma PTX3 levels were significantly higher in patients with TB than in healthy controls. The discrepancy between their findings and those of the present study may be attributable to differences in the local synthesis of PTX3 or sample size. Although PTX3 alone did not demonstrate significant discriminatory performance, some PTX3-containing combinations yielded relatively high AUC values. However, these findings do not establish an incremental diagnostic contribution of PTX3 and should therefore be considered exploratory and validated in larger, independent cohorts.

In some diseases, specific types of WBCs increase in response to immune system activation, which may provide insight into the underlying cause of inflammation. WBC differentials (NEU, LYM, MONO, EOS, and BASO) are commonly used in the diagnosis of diseases affecting specific WBC subtypes[34]. Hematological abnormalities have been reported to occur commonly in patients with TB, in whom the immune response plays a significant role in disease pathogenesis[11]. Monocytes play a critical role in host immunity during *Mycobacterium tuberculosis* infection. NEUs also have multifaceted roles in host defense and are activated in response to bacterial

exposure during the early stages of infection. Both monocyte and NEU counts increase in TB and decrease significantly after treatment[21]. Consistent with these findings, the present study observed elevated monocyte and NEU counts in patients with TB. Moreover, moderate correlations were observed between monocytes, NEUs, and CRP. Among the individual parameters, CRP, MONO, and NEU demonstrated the highest diagnostic performance, with AUCs of 0.936, 0.828, and 0.795, respectively. A formal comparison of the ROC curves was performed using DeLong's test. Although the CRP-MONO combination yielded a numerically higher AUC than CRP alone, the difference between the 2 ROC curves was not statistically significant (DeLong test, $p = 0.307$).

Specifically, lymphopenia has been observed in 46% of untreated patients with PTB and 76.92% of patients with miliary TB[35]. Kang et al.[36] demonstrated that lymphocyte counts were significantly lower in patients with active TB than in healthy controls. Similarly, in the present study, lymphocyte count was positively correlated with WBC and basophil counts and negatively correlated with CRP and SAA levels ($p = 0.004$).

PLT values have been associated with inflammatory reactions and immune responses. Research indicates that PLTs play an important role in the immune system[21]. Tozkoparan et al.[12] reported that PLT counts were significantly higher in patients with active TB. However, in the present study, no significant difference in PLT counts was observed between patients with active TB and controls. Conversely, other studies have associated thrombocytopenia with TB. For example, thrombocytopenia was reported in 37.5% of 128 Indian patients[37]. Therefore, differences in sample size and participant selection may have contributed to the variation in findings.

Few studies have examined the combined diagnostic performance of multiple biomarkers in TB[38]. This study comprehensively evaluated the diagnostic performance of combinations of PTX3, CRP, SAA, WBC, LYM, MONO, NEU, EOS, BASO, and PLT for distinguishing patients with active TB from healthy controls. An important feature of the present study was the integrated evaluation of biomarkers representing different components of the inflammatory response within the same exploratory framework. Specifically, PTX3 and conventional acute-phase reactants were evaluated together with routinely available hematological parameters, both individually and in multiple 2- and 3-marker combinations. This multiparametric approach extends the assessment beyond a single biomarker class and enables comparative evaluation of different inflammatory marker combinations within the same cohort.

Given the relatively small sample size and the evaluation of multiple biomarker combinations, the models may be susceptible to overfitting. Therefore, the reported diagnostic performance should be considered exploratory rather than definitive, and these findings should be regarded as hypothesis-generating until validated in larger, independent cohorts.

Although PTX3 levels were not significantly different between groups in this study, combinations containing PTX3 yielded relatively high AUC values. However, no incremental diagnostic contribution of PTX3 beyond CRP-based models was demonstrated. Therefore, the findings regarding PTX3 should be interpreted cautiously and considered exploratory. These findings suggest that selected biomarker combinations may warrant further investigation as practical and accessible diagnostic approaches, particularly in resource-limited settings. Larger, multicenter studies that include clinically relevant disease-control groups are needed to validate these findings before their clinical applicability can be established.

Study Limitations

The relatively small sample size and single-center design may restrict the generalizability of the findings. Furthermore, the study was limited to the pretreatment phase of TB, precluding assessment of biomarker changes during or after therapy. Internal validation techniques, such as bootstrap resampling or cross-validation, were not performed in this exploratory pilot study. Consequently, the reported model performance may be optimistic, and the robustness and generalizability of the prediction models remain uncertain. Therefore, these findings require validation in larger, independent, preferably multicenter cohorts. Future studies should also incorporate longitudinal follow-up to validate and extend these results for broader clinical application. Another limitation is the lack of detailed evaluation of potential confounding factors, such as smoking status, comorbid diseases, and subclinical inflammatory conditions, all of which may influence inflammatory biomarker levels. Therefore, the observed biomarker differences cannot be considered entirely specific to TB.

Another important limitation is the absence of clinically relevant disease-control groups. Consequently, the diagnostic performance reported in this study reflects discrimination between active TB and healthy individuals rather than the differential diagnosis encountered in routine clinical practice. Future studies should include patients with pneumonia, sarcoidosis, malignancy, and other inflammatory lung diseases. Because the control group consisted of healthy individuals, the specificity values observed in this study may be overestimated compared with those in real-world clinical settings, where patients with TB-like symptoms are evaluated.

Conclusion

This exploratory pilot study suggests that combinations of routinely available inflammatory and hematological parameters may have potential utility for distinguishing patients with active TB from healthy controls. Among the evaluated combinations, CRP–MONO yielded the numerically highest AUC. PTX3 alone showed limited discriminatory performance, and its incremental diagnostic contribution within combined models was not demonstrated. These findings should be considered hypothesis-generating rather than confirmatory and should not be interpreted as establishing clinically applicable diagnostic models. Larger, independent, multicenter studies that include clinically relevant disease-control groups are required to validate these findings before their potential clinical application can be established.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Mersin University Clinical Research Ethics Committee (decision number: 2023/359, dated May 24, 2023).

Informed Consent: Because the analyses were performed using blood samples collected as part of routine clinical testing, the Ethics Committee determined that additional written informed consent was not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices: K.E., E.S.Ö., Concept: K.E., T.B., E.S.Ö., L.T., Design: K.E., T.B., N.C., Data Collection or Processing: K.E., N.C., G.A., Analysis or Interpretation: K.E., T.B., L.T., G.A., Literature Search: K.E., T.B., L.T., N.C., E.S.Ö., G.A., Writing: K.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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REVIEW / DERLEME

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Antimicrobial Adverse Events in Periprosthetic Joint Infections: A Narrative Review of Incidence, Drug Class-Specific Toxicities, and Risk Factors

Periprostetik Eklem Enfeksiyonlarında Antimikrobiyal Yan Etkiler: Sıklık, İlaç Sınıfına Özgü Toksisiteler ve Risk Faktörleri Üzerine Anlatısal Bir İnceleme

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Abstract

Periprosthetic joint infection (PJI) is a severe complication of arthroplasty, frequently requiring prolonged antimicrobial therapy. Despite being essential for infection control, extended antibiotic exposure predisposes patients to adverse drug events (ADEs), which may compromise treatment efficacy and patient safety. This review aims to synthesize current evidence on the incidence, spectrum, and risk factors of antimicrobial-related ADEs in PJI, with an emphasis on drug class-specific toxicities and their therapeutic implications. A narrative literature search was conducted in PubMed, Embase, and the Cochrane Library from January 2010 to September 2025. Eligible studies included randomized trials, prospective and retrospective cohort studies, and systematic reviews evaluating ADEs associated with antimicrobial therapy for PJI. Data were extracted on incidence, severity, drug classes, treatment modifications, and identified risk factors. A narrative synthesis was performed because of methodological heterogeneity. Across the analyzed literature, the reported incidence of ADEs ranged from 7.8% to 46%, with approximately 7.8% to 20.4% classified as severe events. Vancomycin and piperacillin–tazobactam combinations showed the highest rates of nephrotoxicity, whereas rifampin and linezolid were commonly associated with hepatotoxicity and hematologic toxicity, respectively. Prolonged treatment duration, intravenous administration, advanced age, obesity, and comorbidities significantly increased the risk of ADEs. Emerging evidence suggests that oral step-down therapy and shorter antimicrobial regimens may provide comparable efficacy while offering improved safety profiles. Overall, antimicrobial-related ADEs are common and clinically significant in PJI management, often leading to treatment modification or discontinuation. Optimizing regimen selection, treatment duration, and monitoring, along with adopting standardized ADE reporting frameworks, remains essential to enhancing safety and therapeutic outcomes in PJI care.

Keywords: Periprosthetic joint infection, antimicrobial toxicity, adverse drug events, antimicrobial safety, risk factors

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Öz

Periprostetik eklem enfeksiyonu (PJI), sıklıkla uzun süreli antimikrobiyal tedavi gerektiren, artroplastinin ciddi bir komplikasyonudur. Enfeksiyon kontrolü için gerekli olmasına rağmen, uzun süreli antibiyotik maruziyeti hastaları, etkinliği ve hasta güvenliğini tehlikeye atabilecek advers ilaç olaylarına (ADE) yatkın hale getirir. Bu derleme, PJI'da antimikrobiyal ilaçlara bağlı ADE görülme sıklığı, yelpazesi ve risk faktörlerine ilişkin güncel kanıtları özetlemeyi amaçlamakta olup, ilaç sınıfına özgü toksik etkilere ve bunların terapötik sonuçlarına vurgu yapmaktadır. Ocak 2010 ile Eylül 2025 tarihleri arasında PubMed, Embase ve Cochrane Kütüphanesi'nde anlatısal bir literatür taraması yapılmıştır. PJI'ya yönelik antimikrobiyal tedaviyle ilişkili ADE'leri değerlendiren randomize çalışmalar, prospektif ve retrospektif kohortlar ve sistematik incelemeler çalışmaya dahil edilmiştir. İnsidans, şiddet, ilaç sınıfları, tedavi değişiklikleri ve belirlenen risk faktörlerine dair veriler çıkarılmıştır. Metodolojik heterojenlik nedeniyle anlatısal bir sentez yapılmıştır. Analiz edilen literatürde, bildirilen ADE'lerin insidansı %7,8 ile %46 arasında değişmekte olup, yaklaşık %7,8 ile %20,4'ü şiddetli olaylar olarak sınıflandırılmıştır. Vankomisin ve piperasilin-tazobaktam kombinasyonları en yüksek nefrotoksisite oranlarını gösterirken, rifampin ve linezolid sırasıyla hepatotoksisite ve hematolojik toksisite ile ilişkilendirilmiştir. Uzun süreli tedavi, intravenöz uygulama, ileri yaş, obezite ve eşlik eden hastalıklar ADE riskini önemli ölçüde artırmıştır. Ortaya çıkan kanıtlar, oral kademeli tedavi ve daha kısa antimikrobiyal rejimlerin, daha iyi güvenlik profilleri sunarken, karşılaştırılabilir etkinlik sağlayabileceğini göstermektedir. Antimikrobiyal ilişkili ADE'ler, PJI yönetiminde sık olarak ortaya çıkar ve klinik olarak önemlidir ve genellikle tedavi değişikliğine veya tedavinin sonlandırılmasına yol açar. Periprostetik eklem enfeksiyonu tedavisinde güvenliği ve terapötik sonuçları iyileştirmek için rejim seçiminin, süresinin ve takibinin optimize edilmesi ve standartlaştırılmış ADE raporlama çerçevelerinin benimsenmesi esastır.

Anahtar Kelimeler: Periprostetik eklem enfeksiyonu, antimikrobiyal toksisite, advers ilaç reaksiyonları, antimikrobiyal güvenlik, risk faktörleri

Introduction

Periprosthetic joint infection (PJI) is one of the most serious complications following arthroplasty, with reported incidence rates of approximately 1%-2% after primary arthroplasty and higher rates after revision procedures[1,2]. These infections are associated with substantial morbidity, functional impairment, repeated hospitalizations, and increased healthcare expenditures[3,4]. Management is complex and typically requires a multidisciplinary approach that combines surgical intervention with prolonged courses of pathogen-directed antimicrobial therapy[5-7].

Antimicrobial therapy remains a cornerstone of PJI management, particularly the use of biofilm-active agents such as rifampin for staphylococcal infections[8,9]. However, several characteristics specific to PJI management substantially increase the risk and clinical consequences of antimicrobial-associated adverse drug events (ADEs). Treatment duration is considerably longer than that for most infectious diseases, typically extending for 6-12 weeks and, among patients receiving suppressive antibiotic therapy (SAT), for months or even years[5-7]. In addition, effective management of biofilm-associated infections often requires combination antimicrobial regimens, increasing the likelihood of drug-drug interactions and cumulative or overlapping toxicities[2,10,11]. The use of agents with narrow therapeutic indices further increases the risk of treatment-related toxicity[11]. These treatment-related factors are compounded by the demographic and clinical characteristics of patients with PJI, who are typically older and frequently have chronic kidney disease, obesity, and polypharmacy, all of which independently increase susceptibility to antimicrobial toxicity[1,10,12]. Collectively, these disease- and treatment-specific characteristics distinguish antimicrobial-related ADEs in PJI from the broader spectrum of antimicrobial toxicities and support their recognition as a distinct clinical challenge.

Class-specific toxicities are well established, including vancomycin-associated nephrotoxicity[9], rifampin-related hepatotoxicity and drug-drug interactions[13], linezolid-induced myelosuppression and neuropathy[14], and *Clostridioides difficile* infection associated with broad-spectrum agents[15]. Such toxicities can compromise patient safety and necessitate antimicrobial treatment modification or discontinuation, potentially jeopardizing infection control[4]. Increasing evidence from prospective and retrospective studies has underscored the clinical significance of antimicrobial-associated ADEs in PJI. Prolonged antibiotic use beyond 1 year does not confer additional benefit but increases the risk of ADEs[16]. Similarly, the choice between intravenous therapy and highly bioavailable oral agents is often influenced by concerns regarding tolerability[4]. SAT is also increasingly used when curative surgical management is not feasible; however, it carries inherent risks, including cumulative toxicity and the lack of standardized monitoring protocols[3,6]. Emerging evidence further suggests that discontinuation of chronic suppressive therapy following debridement, antibiotics, and implant retention (DAIR) may achieve outcomes comparable to those of continued suppression. This finding challenges assumptions regarding the necessity of prolonged therapy and raises additional concerns about cumulative antimicrobial toxicity[16].

Despite increasing recognition of antimicrobial-related ADEs in PJI, the available evidence remains fragmented. Many studies inadequately report adverse events, use heterogeneous definitions and monitoring strategies, or focus primarily on

treatment efficacy rather than safety. These limitations hinder comprehensive assessment of the incidence, spectrum, and determinants of antimicrobial toxicity in this population.

Given the pivotal role of antimicrobial therapy in PJI management, a comprehensive synthesis of evidence regarding associated ADEs is warranted. This review aims to provide an updated narrative overview of the incidence, spectrum, and therapeutic implications of antimicrobial-related ADEs in PJI, with particular emphasis on associated risk factors.

Materials and Methods

Literature Search Strategy

A literature search was conducted to identify relevant studies evaluating antimicrobial-associated adverse events in the management of PJIs. PubMed/MEDLINE, Embase, and the Cochrane Library were searched for studies published from January 2010 through September 2025. The search strategy combined Medical Subject Headings terms and free-text terms, including *"prosthetic joint infection," "periprosthetic joint infection," "antimicrobial adverse events," "antibiotic toxicity," "antibiotic-related complications,"* and *"side effects."* Boolean operators ("AND" and "OR") were used to optimize the sensitivity and specificity of the search.

The reference lists of included studies and recent systematic reviews were also manually screened to identify additional potentially eligible publications. The search was limited to English-language articles involving human subjects.

Eligibility Criteria

Studies were eligible for inclusion if they met the following criteria:

- Population: Adult patients with PJI confirmed according to standard diagnostic criteria.
- Intervention: Any antimicrobial therapy administered for the treatment or suppression of PJI, including intravenous, oral, or chronic suppressive antibiotic regimens.
- Outcomes: Studies reporting the incidence, type, or therapeutic consequences of ADEs attributable to antimicrobial therapy.
- Study design: Randomized controlled trials (RCTs), prospective or retrospective cohort studies, case-control studies, and systematic reviews.

Exclusion criteria were as follows: (1) studies that did not involve PJI; (2) case reports; (3) studies that did not explicitly report ADEs; and (4) non-English-language publications.

Study Selection and Data Extraction

Two independent reviewers screened the titles and abstracts of identified studies for eligibility, followed by full-text evaluation of potentially relevant studies. Discrepancies were resolved through discussion and, when necessary, consultation with a third reviewer.

A standardized data extraction form was used to collect the following variables: study characteristics; antimicrobial regimens, including drug class, route of administration, and treatment duration; incidence and type of ADEs; severity grading, when available; and therapeutic consequences, including treatment modification or discontinuation.

Data Synthesis, Quality Assessment and Risk of Bias

Findings were synthesized according to antimicrobial class, with emphasis on the incidence, spectrum, risk factors, and

Table 1. Incidence, severity, and treatment modifications associated with antimicrobial-related adverse events in PJI.

Antimicrobials	Incidence of ADEs[†]	Severity	Treatment modification rate
Vancomycin ± Piperacillin–tazobactam[11]	12.6 % overall; highest with combination	20.4 % severe (CTCAE[‡] grade ≥3)	12.6 % required modification
Daptomycin[35]	7.8 %	Not reported	Not reported
Fluoroquinolones[27]	Not reported	7.8 % severe ADEs	35.6 % discontinued
Rifampin ± co-trimoxazole[10]	46 %	Predominantly mild to moderate; gastrointestinal tract most affected	6 % discontinued; 29 % dose adjustment
Clindamycin ± rifampin[36]	Not reported	Mild; diarrhea most frequently reported	14 % discontinued
Linezolid + rifampin + clindamycin[37]	40.9 %	22.7 % Grade 3 neutropenia, anemia, hyponatremia	Discontinuation of linezolid in severe ADEs (22.7 %)
Linezolid[28]	33 %	Not reported	9 % discontinued
β-lactams[3]	33.8 %	Not reported	18 % discontinued or switched
Suppressive therapy (TMP–SMX[§] and combinations)[30]	11.1–16.3 %	Not reported	Not specified

[†]**ADE,** adverse drug event; [‡]CTCAE, Common Terminology Criteria for Adverse Events; [§]TMP–SMX, trimethoprim–sulfamethoxazole.

therapeutic implications of antimicrobial-associated ADEs. Where appropriate, evidence from RCTs and prospective cohort studies was prioritized over that from retrospective observational studies to strengthen the interpretation of findings, while observational studies were retained to capture real-world safety data.

Methodological quality and potential sources of bias were considered during data interpretation rather than evaluated using a formal risk of bias scoring system. Particular attention was given to study design, sample size, completeness of ADE reporting, and the methods used to attribute adverse events to antimicrobial therapy. Studies that employed standardized causality assessment or toxicity grading systems, such as the World Health Organization–Uppsala Monitoring Centre (WHO–UMC) system criteria or the Common Terminology Criteria for Adverse Events (CTCAE), were considered methodologically more robust.

Rationale for Narrative Review Design

Although several methodological features commonly associated with systematic reviews were incorporated, this study was intentionally conducted as a narrative review. This approach was selected for three main reasons. First, the objective was to provide an integrative, drug class-oriented synthesis of ADEs, their risk factors, and clinical implications rather than to generate pooled estimates of incidence. Substantial heterogeneity among the eligible studies in ADE definitions, severity grading, follow-up duration, patient populations, and antimicrobial regimens precluded meaningful quantitative synthesis. Second, attempting to derive aggregated incidence estimates under these conditions could have overstated the precision and comparability of the available evidence. A narrative synthesis therefore allowed qualitative integration and critical contextualization of findings across antimicrobial classes while preserving clinically relevant differences among studies. Third, the review incorporated complementary sources of evidence, including pharmacological reviews, pharmacovigilance reports, and clinical practice guidelines, which were essential for contextualizing drug class-specific toxicity profiles but would not typically meet the eligibility criteria for a formal systematic review. Nevertheless, methodological safeguards traditionally used in systematic reviews were retained to enhance the transparency and methodological rigor of the review process.

Classes of Antimicrobials and Their Adverse Events

Beta-lactams

Beta-lactams remain foundational agents for susceptible PJI cases. Adverse effects include hypersensitivity reactions, ranging from mild rash to life-threatening anaphylaxis[10,17]. With prolonged therapy, hematologic toxicities, including neutropenia and thrombocytopenia, may occur. High-dose cephalosporins and carbapenems can cause neurotoxicity, particularly in patients with renal dysfunction[10,11,17]. The use of broad-spectrum agents also significantly increases the risk of *Clostridioides difficile* infection, particularly among older patients[18,19].

Glycopeptides

Vancomycin is widely used to treat PJIs caused by methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci but carries a significant risk of nephrotoxicity, reported in 5%-15% of patients, particularly with prolonged therapy or concomitant use of nephrotoxic agents[10,11,16,20]. Infusion-related reactions, such as "red man syndrome," are common but can generally be prevented through slow infusion and appropriate premedication[16,21]. Hematologic toxicity, including neutropenia, is uncommon but has been reported during extended treatment courses[11]. Teicoplanin is considered less nephrotoxic; however, robust comparative data in patients with PJI remain limited[16,22].

Lipopeptides

Daptomycin is an alternative for patients who are intolerant of vancomycin and has demonstrated efficacy in PJI management. The most clinically relevant adverse event is myopathy, with creatine kinase (CK) elevation occurring in 2%-5% of cases and warranting weekly CK monitoring[4,23]. Rarely, eosinophilic pneumonia can develop after 2-3 weeks of therapy, necessitating immediate discontinuation[4,24,25]. Unlike rifampin, daptomycin has no major drug-drug interactions, making it a suitable option for patients with multiple comorbidities and concomitant medications[4,25].

Rifamycins

Rifampin is an important component of therapy for staphylococcal PJIs because of its biofilm activity, but hepatotoxicity is a major limitation. Up to 20% of patients develop asymptomatic elevations in transaminase levels, whereas clinically significant hepatitis, although rare, may necessitate treatment discontinuation[13,26]. Rifampin also strongly induces cytochrome P450 enzymes, resulting in clinically important interactions with anticoagulants, antiplatelet agents, and immunosuppressants[4,13]. Gastrointestinal intolerance and rash may also contribute to treatment discontinuation, potentially compromising biofilm-active therapy[6,10].

Fluoroquinolones

Fluoroquinolones, which are commonly combined with rifampin, are valued for their high oral bioavailability but

are associated with a broad range of toxicities. Reported neurologic toxicities include peripheral neuropathy and seizures, whereas musculoskeletal complications include tendinopathy and tendon rupture, particularly among older adults and patients receiving concomitant corticosteroid therapy[16,27]. Additional concerns include dysglycemia and QT interval prolongation, the latter of which may limit their use in patients with underlying cardiac conduction abnormalities or a predisposition to arrhythmias[16,27].

Oxazolidinones

Linezolid is used for multidrug-resistant gram-positive PJIs but is limited by hematologic toxicity, with thrombocytopenia occurring in up to 30% of patients after 14 days of therapy[17,28,29]. Other adverse events include anemia and leukopenia, consistent with bone marrow suppression[17,28,29]. Prolonged treatment is associated with peripheral and optic neuropathy, which may be related to mitochondrial toxicity; cases of lactic acidosis have also been reported[17,28,29]. Tedizolid, which has a lower risk of hematologic toxicity, may offer a safer alternative; however, clinical evidence supporting its use in PJI remains limited[17,28].

Trimethoprim–Sulfamethoxazole (TMP-SMX)

TMP-SMX is frequently used for long-term suppressive therapy. Its adverse effects include bone marrow suppression, manifested as leukopenia and thrombocytopenia, particularly with prolonged treatment courses[30]. Severe cutaneous hypersensitivity reactions, including Stevens-Johnson syndrome, are rare but have been reported in orthopedic cohorts[31]. Electrolyte abnormalities, particularly hyperkalemia, are also common among patients with renal impairment[31].

Clindamycin

Clindamycin is primarily reserved for patients with beta-lactam allergies and susceptible infections. The most serious adverse event is *Clostridioides difficile* colitis, which is relatively common and potentially life-threatening[18,32]. Other toxicities include hepatotoxicity and hypersensitivity syndromes, such as drug reaction with eosinophilia and systemic symptoms[32,33].

Fosfomycin

Fosfomycin may be considered as part of salvage regimens for infections caused by multidrug-resistant organisms and is associated with electrolyte disturbances, including hypokalemia and hypernatremia, as well as gastrointestinal intolerance. Evidence supporting its use in PJIs remains limited and warrants further controlled evaluation[34].

Incidence, Severity, and Treatment Modifications Associated with Antimicrobial-Related Adverse Events in PJIs

Antimicrobial therapy for PJI is associated with a substantial burden of ADEs. Across prospective and retrospective cohort studies, the reported overall incidence of ADEs ranges from 7.8% to 46%, with severe events accounting for 7.8%-20.4% of cases, depending on the antimicrobial regimen and treatment duration[3,10,11,27,28,30,35-37].

Adverse event severity is commonly graded according to the CTCAE, version 5.0. Under this system, Grade 1-2 events are considered mild to moderate, Grade 3 events are severe and typically require treatment modification or discontinuation, and Grade 4-5 events are considered life-threatening or fatal, respectively[11].

Table 1 summarizes the reported incidence, severity, and therapeutic consequences of antimicrobial-associated ADEs across antimicrobial agents used to treat PJI[3,10,11,27,28,30,35-37]. Marked variability was observed among antimicrobial classes, with rifampin-based combination regimens consistently associated with the highest overall ADE rates, whereas daptomycin demonstrated the lowest reported rates[10,35]. Treatment discontinuation or dose modification was most frequent with agents requiring closer safety monitoring, with dose adjustment required in up to 29% of patients receiving rifampin-containing regimens and treatment discontinuation reported in 22.7% of patients receiving a linezolid–rifampin–clindamycin combination because of severe hematological toxicity[10,37]. Likewise, discontinuation rates reached 35.6% for fluoroquinolones and 18% for β-lactams, indicating that treatment-limiting toxicity is not restricted to a single antimicrobial class[3,27].

Risk Factors for Adverse Events in PJI Therapy

The development of adverse events during antimicrobial therapy for PJI is influenced by a combination of patient-, treatment-, and infection-related factors. Identifying these risk factors is critical for individualizing antimicrobial regimens and improving patient safety.

Patient-Related Risk Factors

Advanced age and chronic comorbidities substantially increase the risk of antimicrobial toxicity. Older patients and those with underlying conditions such as hypertension, hepatic dysfunction, congestive heart failure, and coronary artery disease are particularly susceptible to nephrotoxicity, especially when treated with agents that undergo renal elimination. The increased susceptibility to acute kidney injury (AKI) in these populations likely reflects both reduced renal

reserve and altered pharmacokinetics[10,12,38]. Obesity [body mass index (BMI) ≥ 30 kg/m^2] is an independent predictor of antibiotic-associated ADEs and treatment failure. Alterations in pharmacokinetic and pharmacodynamic parameters in individuals with obesity, including an increased volume of distribution and variable drug clearance, may contribute to drug accumulation and toxicity[10,12].

Treatment-Related Risk Factors

Drug-Related Risk Factors

The pharmacokinetic and pharmacodynamic properties of antimicrobial agents are important determinants of their toxicity risk. Vancomycin administration, particularly in combination with other antimicrobial agents such as piperacillin-tazobactam, is associated with an increased incidence of nephrotoxicity, particularly AKI. Concurrent use of these agents has been associated with synergistic nephrotoxic effects[11,39]. Prolonged courses of rifampin therapy, frequently used as part of combination regimens, have been associated with gastrointestinal adverse effects, including nausea and diarrhea, as well as cutaneous manifestations such as rash. These toxicities may necessitate treatment modification, including dose adjustment or discontinuation[10].

Treatment Duration and Adverse Events

The duration of antibiotic therapy in PJI management remains a critical determinant of both treatment efficacy and safety. Although prolonged regimens are intended to improve infection control, they may increase the risk of adverse events without providing additional clinical benefit.

Extending antibiotic treatment beyond 1 year has not demonstrated an incremental benefit over standard 6-week regimens following DAIR, nor has prolonged therapy been associated with a reduction in adverse events[16]. Similarly, oral therapy extending beyond 12 months has not been shown to improve outcomes and may promote antimicrobial resistance[40,41]. A systematic review and meta-analysis likewise found no significant difference in infection recurrence between short (4-12 weeks) and prolonged treatment courses, further highlighting the limited clinical utility of extended therapy[41].

Shorter intravenous courses (<4 weeks) have demonstrated noninferiority to longer regimens for infection control, while being associated with fewer adverse effects and a reduced healthcare burden[42,43]. Conversely, intravenous therapy exceeding 6 weeks for *Cutibacterium acnes* shoulder PJI was associated with a significantly increased complication rate, particularly among older adults[44]. Prolonged rifampin-based regimens are also frequently associated with gastrointestinal intolerance and treatment discontinuation[10].

SAT, often reserved for patients who are unsuitable for curative surgery, raises additional concerns regarding long-term tolerability. Evidence suggests that SAT is associated with higher adverse event rates than curative treatment approaches. In a systematic review of long-term SAT combined with DAIR, 15.4% of patients developed adverse effects, leading to premature treatment discontinuation in several cases[45].

Administration Route Impact

The route of antibiotic administration is a key determinant of both efficacy and safety in the management of PJI. Emerging evidence indicates that oral antibiotic therapy is non-inferior to intravenous (IV) therapy for infection control while offering advantages in terms of safety and tolerability.

Multiple studies have demonstrated comparable treatment efficacy between oral and IV regimens, with oral therapy associated with fewer adverse events and avoidance of complications related to intravenous access[46,47]. Intravenous vancomycin has consistently been associated with higher rates of nephrotoxicity and treatment discontinuation than oral agents or alternative IV options such as daptomycin[11,23].

In revision shoulder arthroplasty, IV antibiotic use was associated with a higher incidence of adverse events than oral therapy[48]. Likewise, a multicenter study of PJI following total knee arthroplasty found no significant difference in adverse event rates between prolonged oral therapy and initial IV treatment, supporting oral antibiotics as a safe and effective alternative[41].

Knowledge Gaps and Future Directions

Despite the recognized importance of ADEs, their reporting in PJI studies remains limited. Clinical trials in this field primarily focus on efficacy outcomes, such as reinfection rates and implant survival, whereas ADEs are often treated as secondary or exploratory endpoints. Moreover, the lack of standardized definitions and reporting frameworks for antimicrobial-related ADEs makes comparisons across studies challenging. This limited availability of comprehensive safety data restricts clinicians' ability to balance therapeutic efficacy and tolerability when selecting antimicrobial regimens. Consequently, current clinical guidelines rely more heavily on microbiological rationale and limited efficacy data than on systematic evaluations of treatment safety. Developing a standardized consensus framework for reporting ADEs in PJI research is therefore a critical step toward optimizing treatment and improving patient safety.

A second major knowledge gap concerns the clinical consequences of ADEs. Adverse events frequently necessitate dose reductions, antimicrobial substitutions, or premature discontinuation of otherwise effective therapies[11]. Such

modifications may increase the risk of infection relapse and reduce the likelihood of successful eradication. For example, penicillin-associated ADEs have been associated with higher rates of hospital readmission, postoperative anemia, blood transfusion, and secondary PJI among patients with antibiotic intolerance[49].

Furthermore, the economic and healthcare impacts of ADEs remain insufficiently explored. Toxicity-related complications often require additional laboratory monitoring, specialist consultations, and unplanned readmissions, all of which contribute to increased healthcare utilization and costs.

Several strategies show promise for reducing the impact of ADEs in PJI management. First, oral step-down therapy can reduce complications associated with intravenous access and shorten hospital stays. The OVIVA trial demonstrated that oral antimicrobial therapy for complex bone and joint infections was non-inferior to prolonged intravenous treatment while significantly reducing catheter-associated complications and treatment burden[50]. Second, accumulating evidence indicates that shorter antibiotic regimens may be sufficient in selected PJI cases, potentially reducing cumulative toxicity without compromising efficacy[41]. Third, long-acting antimicrobials, such as dalbavancin, which has an extended half-life and favorable safety profile, have been successfully used off-label for bone and joint infections. These agents may improve adherence and reduce treatment discontinuation related to ADEs[51,52].

Finally, individualized therapeutic approaches that integrate therapeutic drug monitoring, assessment of host-related factors, and precision dosing may enable clinicians to maximize treatment efficacy while minimizing toxicity.

Study Limitations

This review has several limitations that warrant consideration. First, despite the implementation of methodological safeguards to enhance transparency and reproducibility, the narrative design precluded formal quantitative synthesis, including pooled estimates of ADE incidence and quantitative benefit-risk analyses, and did not eliminate the potential for selection and reporting bias. Second, substantial heterogeneity in ADE definitions, severity grading systems, patient populations, antimicrobial regimens, follow-up durations, and reporting practices limited direct comparisons across studies and likely contributed to the wide range of reported overall ADE incidence. Third, the available evidence was derived predominantly from retrospective, single-center observational studies with variable sample sizes and methodological quality. Furthermore, relatively few studies applied standardized causality assessment tools, such as the WHO-UMC system or the Naranjo algorithm, to establish the relationship between antimicrobial exposure and reported ADEs, increasing the potential for misclassification of adverse events. Fourth, publication and reporting biases may have resulted in underreporting of mild or subclinical ADEs, whereas clinically significant events requiring treatment modification or discontinuation were more likely to be documented. RCTs, systematic reviews, and meta-analyses using standardized outcome definitions are therefore needed to generate more precise estimates of ADE incidence and quantify the benefit–risk profile of individual antimicrobial regimens.

Conclusion

Antimicrobial-related ADEs represent a significant yet underrecognized challenge in the management of PJIs. Across clinical studies, ADEs affect up to 46% of patients and frequently necessitate treatment modification or discontinuation. Vancomycin–piperacillin–tazobactam combinations are associated with the highest risk of nephrotoxicity, whereas rifampin and linezolid are major contributors to hepatotoxic and hematologic toxicity, respectively. Patient-specific factors, including advanced age, renal impairment, obesity, and polypharmacy, further increase susceptibility to severe ADEs. Increasing evidence supports shorter and orally administered antimicrobial regimens that can maintain infection control while minimizing toxicity and healthcare burden.

Future clinical research should prioritize standardized ADE reporting frameworks and incorporate safety endpoints into PJI treatment trials. The adoption of precision dosing, therapeutic drug monitoring, and novel long-acting antimicrobials may further improve treatment safety. Overall, a patient-centered, evidence-based approach that balances antimicrobial efficacy with tolerability is critical for optimizing clinical outcomes while minimizing treatment-related harm in patients with PJIs.

Footnotes

Authorship Contributions

Concept: O.S., H.E., Design: O.S., H.E., Analysis or Interpretation: O.S., H.E., H.L., I.K., M.M., M.M., L.A., Literature Search: O.S., H.E., Writing: O.S., H.E., H.L., I.K., M.M., M.M., L.A.

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Corynebacterium striatum Bacteremia: Contamination or a Missed Cause of Infective Endocarditis? A Case Series and Proposed Clinical Algorithm

Corynebacterium striatum Bakteriyemisi: Kontaminasyon mu, Yoksa İnfektif Endokarditin Gözden Kaçan Bir Nedeni mi? Olgu Serisi ve Klinik Algoritma Önerisi

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Abstract

Corynebacterium striatum is often regarded as a contaminant, potentially delaying the diagnosis of infective endocarditis (IE). We evaluated the clinical characteristics of IE associated with *Corynebacterium striatum* bacteremia, highlighted diagnostic challenges, and proposed a diagnostic algorithm. Five patients with *Corynebacterium striatum* bacteremia who fulfilled the 2023 Duke-ISCVID criteria for IE were retrospectively analyzed. Clinical, echocardiographic, microbiological, treatment, and outcome data were reviewed. Native valve involvement predominated, and persistent bacteremia was the most consistent finding. Vegetations mainly involved the aortic and mitral valves. In one patient, the initial interpretation of *Corynebacterium striatum* as a contaminant delayed the diagnosis of IE. Severe complications were common, and three of the five patients died. Persistent or recurrent *Corynebacterium striatum* bacteremia should not be dismissed as contamination, particularly in patients with compatible clinical findings. Early echocardiographic evaluation should be considered when IE is suspected. We propose a diagnostic algorithm integrating microbiological and clinical findings to support the timely diagnosis of IE.

Keywords: *Corynebacterium striatum*, infective endocarditis, bacteremia

Öz

Corynebacterium striatum, klinik uygulamada sıklıkla kontaminant olarak değerlendirilen bir mikroorganizmadır ve bu durum infektif endokardit (İE) tanısında gecikmeye yol açabilmektedir. Bu çalışmada, *Corynebacterium striatum* bakteriyemisine bağlı İE olgularının klinik özelliklerini değerlendirmeyi, tanısal güçlükleri ortaya koymayı ve pratik bir tanısal algoritma önermeyi amaçladık. 2023 Duke-ISCVID kriterlerini karşılayan beş *Corynebacterium striatum* bakteriyemisi olgusu retrospektif olarak değerlendirildi. Klinik, ekokardiyografik, mikrobiyolojik, tedavi ve sonuç verileri analiz edildi. Natif kapak tutulumu baskındı ve persistan bakteriyemi en belirgin ortak bulguydu. Vejetasyonlar en sık aort ve mitral kapakta saptandı. Bir olguda *Corynebacterium striatum* üremesinin başlangıçta kontaminasyon olarak değerlendirilmesi İE tanısında gecikmeye yol açtı. Ciddi komplikasyonlar sık görüldü ve beş hastanın üçü yaşamını kaybetti. Persistan veya tekrarlayan *Corynebacterium striatum* bakteriyemisi, özellikle klinik olarak uyumlu olgularda kontaminasyon olarak değerlendirilmemelidir. İE şüphesi bulunan hastalarda erken ekokardiyografik değerlendirme önem taşımaktadır. Bulgularımız doğrultusunda, mikrobiyolojik ve klinik bulguları bütünleştiren pratik bir tanısal algoritma öneriyoruz.

Anahtar Kelimeler: *Corynebacterium striatum*, infektif endokardit, bakteriyemi

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Introduction

Corynebacterium striatum is a Gram-positive bacillus that is part of the normal flora of human skin and nasal mucosa[1]. When isolated from clinical specimens, it is often regarded as a contaminant and may be overlooked without further investigation. However, recent studies have demonstrated that this microorganism can cause bacteremia and various invasive infections[2,3].

Corynebacterium striatum is known to exhibit nosocomial transmission, particularly in intensive care units, in association with prolonged hospitalization, invasive procedures, and broad-spectrum antibiotic use[4-6]. In addition, *Corynebacterium striatum* has been reported to cause invasive infections, including bacteremia, catheter-related infections, and infective endocarditis (IE)[2,3,8].

IE is an infection of the endocardial surface of the heart and is diagnosed according to the modified Duke criteria. In the updated Duke-ISCVID criteria published in 2023, *Corynebacterium striatum* is recognized as a typical microorganism in the presence of intracardiac prosthetic material, further emphasizing its clinical significance in IE[7].

In this context, we aimed to highlight the clinical significance of *Corynebacterium striatum* bacteremia, which is frequently considered as contamination, through five cases illustrating the diagnostic challenges associated with IE. The cases were retrospectively identified from our institutional records. In all patients, *Corynebacterium striatum* was isolated from at least one blood culture set, and species identification was routinely performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as part of the standard microbiology laboratory workflow.

Case Report

Case 1

A 75-year-old woman with a history of hypertension and atrial fibrillation presented to the emergency department with severe abdominal pain. Imaging revealed a thrombus occluding the lumen of the superior mesenteric artery, and she underwent emergency surgery for mesenteric ischemia. On postoperative day 5, while being monitored in the intensive care unit, she developed fever (38.1°C), dyspnea, and desaturation, requiring intubation. With preliminary diagnoses of nosocomial pneumonia and intra-abdominal sepsis, antimicrobial therapy was changed to meropenem, teicoplanin, and fluconazole. Peripheral blood, catheter blood, and tracheal aspirate cultures were obtained.

On the day antimicrobial therapy was initiated and again 3 days later, two sets of peripheral blood cultures and catheter blood cultures were obtained while the patient was receiving teicoplanin and meropenem. These cultures grew *Corynebacterium striatum*, indicating persistent bacteremia despite glycopeptide therapy.

Because of the persistent bacteremia, transthoracic echocardiography (TTE) was performed because IE was suspected. The ejection fraction (EF) was 60%, and moderate-to-severe aortic and mitral regurgitation with suspected mitral valve vegetation was observed. Transesophageal echocardiography (TEE) subsequently demonstrated a thrombus in the left atrial appendage, a 3 × 3 mm vegetation on the non-coronary cusp of the aortic valve, an 8 × 5 mm mobile vegetation on the posterior leaflet of the mitral valve, and a 5 × 4 mm vegetation in the left ventricular outflow tract.

Based on *Corynebacterium striatum* bacteremia, left-sided vegetations, fever, valvular insufficiency, and arterial embolic event, the patient was diagnosed with definite IE according to the modified 2023 Duke-ISCVID criteria. Antimicrobial therapy was revised to vancomycin, and cardiovascular surgery consultation was planned. However, the patient died of cardiac arrest on day 4 of vancomycin therapy.

Case 2

A 72-year-old man with chronic obstructive pulmonary disease (COPD) and a history of mechanical mitral valve replacement 13 years earlier was hospitalized because of melena. Initial evaluation showed atrial fibrillation on electrocardiography and a functioning prosthetic mitral valve with an EF of 55% on TTE.

On the second day of hospitalization, he developed fever (38.2°C) and dyspnea. He was admitted to the intensive care unit with a preliminary diagnosis of pneumosepsis, and meropenem therapy was initiated. Two sets of blood cultures obtained during the febrile episode grew *Corynebacterium striatum*. Given the coexistence of prosthetic valve material and bacteremia, IE was considered more likely than contamination. TEE demonstrated a 6 × 8 mm vegetation on the mechanical mitral valve without obstruction, and the patient was diagnosed with prosthetic valve endocarditis. Vancomycin and rifampin were added to the treatment regimen. Although clinical stabilization was achieved, the patient refused further treatment and was discharged at his own request.

Case 3

A 39-year-old man with no known chronic disease presented with approximately 1 month of fever, night sweats, and fatigue. On physical examination, his temperature was 39°C

and his heart rate was 110/min. Because no obvious infectious focus was identified and chest tomography revealed a 5-cm pleural effusion, TTE was performed for further evaluation. It showed an EF of 60%, severe aortic regurgitation, and a 13 × 11 mm mobile vegetation on the aortic valve. With a preliminary diagnosis of IE, three sets of blood cultures were obtained, and empirical therapy with gentamicin and ampicillin-sulbactam was initiated.

All blood cultures grew *Corynebacterium striatum*, and the patient, who also had a positive rheumatoid factor, was diagnosed with definite IE. The aggressive course, characterized by a large vegetation and severe valvular destruction in an immunocompetent patient, was particularly notable.

Because of severe aortic regurgitation and the large vegetation, the patient underwent a Bentall procedure with mechanical aortic valve replacement. Perioperative valve cultures and follow-up blood cultures showed no growth. Given his stable postoperative course, parenteral gentamicin was completed for 2 weeks and ampicillin-sulbactam for 4 weeks. The patient was discharged in good condition with an additional 2-week course of oral amoxicillin-clavulanate.

Case 4

A 60-year-old man with hypertension, type 2 diabetes mellitus, and a history of cerebrovascular disease was admitted to the intensive care unit after cardiopulmonary arrest. On the third day of hospitalization, increased secretions and elevated acute-phase reactants were observed. Because chest tomography showed findings suggestive of aspiration, piperacillin-tazobactam was initiated for presumed aspiration pneumonia and continued for 7 days.

Ten days after completing therapy, the patient developed fever, elevated inflammatory markers, tonic-clonic seizures, and subsequently respiratory arrest. Empirical meropenem therapy was initiated. Because blood and tracheal aspirate cultures did not yield a pathogen and clinical stabilization was achieved, meropenem was discontinued on day 14. However, after treatment discontinuation, inflammatory markers increased again, and blood cultures grew *Corynebacterium striatum*, prompting the initiation of vancomycin. All catheters were exchanged; however, blood cultures obtained on days 4 and 6 of vancomycin therapy remained positive for *Corynebacterium striatum*. Persistent bacteremia despite glycopeptide therapy suggested an underlying intravascular focus.

TTE was therefore performed and demonstrated an approximately 20 × 12 mm vegetation on the mitral valve. These findings were considered compatible with possible IE. Notably, the diagnosis of IE was considered only after bacteremia persisted despite treatment. Although advanced diagnostic workup and surgical evaluation were planned, the patient died 4 days after TTE.

Case 5

An 82-year-old man with hypertension, COPD, and a grade 1 meningioma in the left parietal lobe had previously presented to another center with severe headache. Cranial computed tomography revealed a cerebral hemorrhage, and computed tomography angiography demonstrated an arteriovenous fistula. He was admitted to the neurosurgery department and underwent surgery for a carotid-cavernous arteriovenous fistula. The patient was intubated and monitored in the neurosurgical intensive care unit for 3 days and was discharged after a total hospital stay of 6 days.

Retrospective review of the investigations from this first hospitalization showed that one set of blood cultures had grown *Corynebacterium striatum*. However, TTE at that time showed no findings suggestive of IE, and the growth was interpreted as contamination because it was present in only one set and the patient's neurosurgical condition was considered the dominant clinical problem. Consequently, no further evaluation for IE was performed, and the patient was discharged.

Eight days after discharge, he was readmitted with a 2-day history of fever, palpitations, cough, sputum production, and wheezing. Chest tomography showed an active infiltrate in the right upper lobe, and he was hospitalized in the infectious diseases department with a preliminary diagnosis of aspiration pneumonia. Given the recent hospitalization and the previous blood culture positivity for *Corynebacterium striatum*, the earlier result was reconsidered as potentially clinically significant rather than as simple contamination. Empirical vancomycin and meropenem therapy was initiated, and two sets of blood cultures and sputum cultures were obtained.

When two sets of blood cultures again grew *Corynebacterium striatum*, the bacteremia was considered clinically significant, and further evaluation for IE was planned. TEE demonstrated severe mitral regurgitation, rupture of the chordae tendineae, and mild aortic regurgitation. Funduscopic examination showed no Roth spots, and the rheumatoid factor was negative. Because of recurrent *Corynebacterium striatum* bacteremia and rupture of the chordae tendineae, the patient was considered to have possible IE, and vancomycin therapy was continued for 6 weeks. Meropenem therapy was completed for 2 weeks for pneumonia, and the patient was subsequently discharged.

Approximately 1 month later, he presented again with fever, fatigue, redness of the left leg, and general deterioration. Doppler ultrasonography of the left lower extremity showed findings consistent with thrombophlebitis. Follow-up TTE

demonstrated severe mitral regurgitation, rupture of the chordae tendineae, and a 7 × 7 mm mass prolapsing into the left ventricle. Three new sets of blood cultures grew *Corynebacterium striatum* that was resistant to penicillin G but susceptible to vancomycin, teicoplanin, and linezolid. Cranial magnetic resonance imaging revealed findings compatible with new cardioembolic subacute cortical infarctions. Repeat TEE showed severe mitral regurgitation and rupture of the posterior chordae tendineae.

Based on persistent/recurrent *Corynebacterium striatum* bacteremia, valvular pathology, and embolic complications, the patient was diagnosed with IE. Cardiovascular surgery recommended surgical intervention, but the patient declined the procedure. An additional 6-week course of vancomycin therapy was planned. During follow-up, he developed intraventricular hemorrhage and intracranial shift, underwent neurosurgical intervention, and died during the postoperative period.

This case represents an important missed diagnostic opportunity because the *Corynebacterium striatum* growth identified during the first hospitalization was initially interpreted as contamination.

The clinical and microbiological characteristics of the cases are summarized in Table 1. The 2023 Duke-ISCVID classification, the major and minor diagnostic criteria fulfilled by each patient, and the key clinical implications derived from each case are presented in Table 2.

Discussion

Although *Corynebacterium striatum* has long been regarded as a contaminant in clinical specimens, it has increasingly been recognized as a cause of invasive infections in recent years[2,9]. Its increasing frequency, particularly in bacteremia and device-related infections, suggests that the clinical significance of this organism should be reconsidered.

In the literature, IE caused by *Corynebacterium* species has been reported predominantly in older patients and in association with prosthetic valves[8]. In contrast, only one patient in our series had a prosthetic valve, whereas the majority had native valve involvement. This finding suggests that *Corynebacterium striatum* should not be considered exclusively a prosthetic valve pathogen. Recent case reports also support the occurrence of native valve IE[10,11] in patients without predisposing structural heart disease.

A comparison of our findings with those of previously published studies is presented in Table 3[8-11,14].

As shown in Table 3, most previously reported cases involved prosthetic valves or isolated cases of native valve IE with

Table 1. Clinical and Microbiological Characteristics of Patients with *Corynebacterium striatum* IE.

Case	Age/ sex	Comorbidities	Valve type	Valve involved	Vegetation size, mm	Persistent/ recurrent bacteremia	Echocardiographic findings	Treatment	Complications	Outcome
1	75/F	HT, AF, mesenteric ischemia	Native	Aortic + mitral	3 x 3 (aortic), 8 x 5 (mitral), 5x4 (LVOT)	Yes	TTE/TEE: multiple vegetations, LAA thrombus	Vancomycin	Arterial embolic events, cardiac arrest	Death
2	72/M	COPD, prosthetic MV	Prosthetic	Mitral	6 x 8	No	TEE: vegetation on mechanical MV	Vancomycin + rifampin	None	Discharged
3	39/M	None	Native	Aortic	13 x 11	No	TTE: large mobile vegetation, severe aortic regurgitation	Ampicillin-sulbactam + gentamicin → Bentall procedure with mechanical aortic valve replacement	Severe aortic regurgitation	Recovered
4	60/M	HT, type 2 DM, cerebrovascular disease	Native	Mitral	20 x 12	Yes	TTE: large vegetation	Vancomycin	Sepsis	Death
5	82/M	HT, COPD, grade 1 meningioma	Native	Mitral	7 × 7	Yes	TTE/TEE: severe MR, rupture of the chordae tendineae, and a mass prolapsing into the left ventricle	Vancomycin	Cardioembolic cerebral infarctions, intraventricular hemorrhage	Death

AF, atrial fibrillation; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HT, hypertension; IE, infective endocarditis; LAA, left atrial appendage; LVOT, left ventricular outflow tract; MV, mitral valve; TEE, transesophageal echocardiography; TTE, transthoracic echocardiography; F, female; M, male.

Table 2. 2023 Duke–ISCVID classification and clinical implications of the five patients.

Case	2023 Duke–ISCVID classification	Major criteria fulfilled	Minor criteria fulfilled	Clinical implication
1	**Definite IE**	Positive blood cultures for *Corynebacterium striatum;* echocardiographic evidence of vegetations	Fever; predisposing condition (moderate-to-severe aortic and mitral regurgitation); vascular phenomenon (mesenteric arterial embolism)	Persistent bacteremia despite appropriate antimicrobial therapy should prompt immediate evaluation for IE.
2	**Definite IE**	Positive blood cultures for *Corynebacterium striatum;* prosthetic mitral valve vegetation	Fever; predisposing condition (prosthetic valve)	*Corynebacterium striatum* bacteremia in patients with prosthetic valves should be considered highly suggestive of IE rather than contamination.
3	**Definite IE**	Positive blood cultures for *Corynebacterium striatum;* aortic valve vegetation	Fever; predisposing condition (severe aortic regurgitation); immunologic phenomenon (positive rheumatoid factor)	Native valve IE may occur even in immunocompetent patients and may follow an aggressive clinical course.
4	**Definite IE***	Persistent positive blood cultures for *Corynebacterium striatum;* mitral valve vegetation	Fever	Persistent bacteremia despite glycopeptide therapy strongly suggests an uncontrolled endovascular focus and warrants prompt echocardiographic assessment.
5	**Definite IE**	Recurrent positive blood cultures for *Corynebacterium striatum;* echocardiographic evidence of valvular destruction (rupture of the posterior chordae tendineae with severe mitral regurgitation)	Predisposing condition (severe mitral regurgitation); vascular phenomenon (cardioembolic cerebral infarctions)	A single positive blood culture should not automatically be dismissed as contamination in patients with compatible clinical findings; recurrent bacteremia warrants reassessment for IE.

*Case 4 was classified as definite IE according to the 2023 Duke–ISCVID criteria based on persistent positive blood cultures, echocardiographic evidence of vegetation, and compatible clinical findings. IE, infective endocarditis.

Table 3. Comparison of *Corynebacterium striatum* IE with published reports.

Study	Number of cases	Valve type	Persistent bacteremia	Affected valve	Complications	Mortality
Bläckberg et al.[8]	30	Mostly prosthetic	Frequent	Mitral and aortic	Embolic events and heart failure	Moderate to high
Dilmen et al.[9]	1	Native	Present	Mitral	Valve destruction	None
Usuda et al.[10]	1	Native	Absent	Aortic	None	None
Biscarini et al.[11]	1	Native	Absent	Mitral	None	None
Marull and Casares[14]	1	Prosthetic	Present	Mitral	Sepsis	Present
Present study	**5**	**Mostly native**	**Frequent (persistent/ recurrent)**	**Mitral + aortic**	**Embolic events, valvular damage, neurological complications**	**High (3/5)**

IE, infective endocarditis.

relatively favorable outcomes. In contrast, our series was distinguished by a predominance of native valve involvement, frequent persistent or recurrent bacteremia, and a markedly higher rate of severe complications and mortality. These findings suggest that *Corynebacterium striatum* may cause a more aggressive form of disease than previously appreciated.

One of the most striking findings of our study was the diagnostic difficulty encountered during the clinical course. In one patient, *Corynebacterium striatum* isolated from a single blood culture set was initially interpreted as contamination, leading to a delay in diagnosis. However, subsequent recurrent bacteremia, valvular pathology, and embolic complications demonstrated that this initial interpretation was incorrect. These findings emphasize that even a single positive blood culture should not be automatically dismissed as contamination, particularly in patients with compatible clinical findings.

The clinical significance of *Corynebacterium striatum* should be reconsidered, especially in the presence of persistent or recurrent bacteremia. In our series, persistent bacteremia was observed in multiple patients despite appropriate antimicrobial therapy, suggesting an underlying intravascular focus. This finding highlights the need for further investigation, including echocardiographic evaluation. To facilitate clinical decision-making, we developed a practical, stepwise diagnostic and management algorithm that integrates clinical and microbiological findings and provides a structured framework for distinguishing contamination from true infection and supporting the early diagnosis of IE (Figure 1).

This flowchart summarizes a stepwise clinical approach incorporating the evaluation of the clinical context, the number and persistence of positive blood cultures, and patient-related risk factors. Persistent or recurrent bacteremia or multiple positive blood cultures should prompt further investigation, including echocardiographic evaluation for IE. Early recognition and appropriate management may reduce complications and improve clinical outcomes.

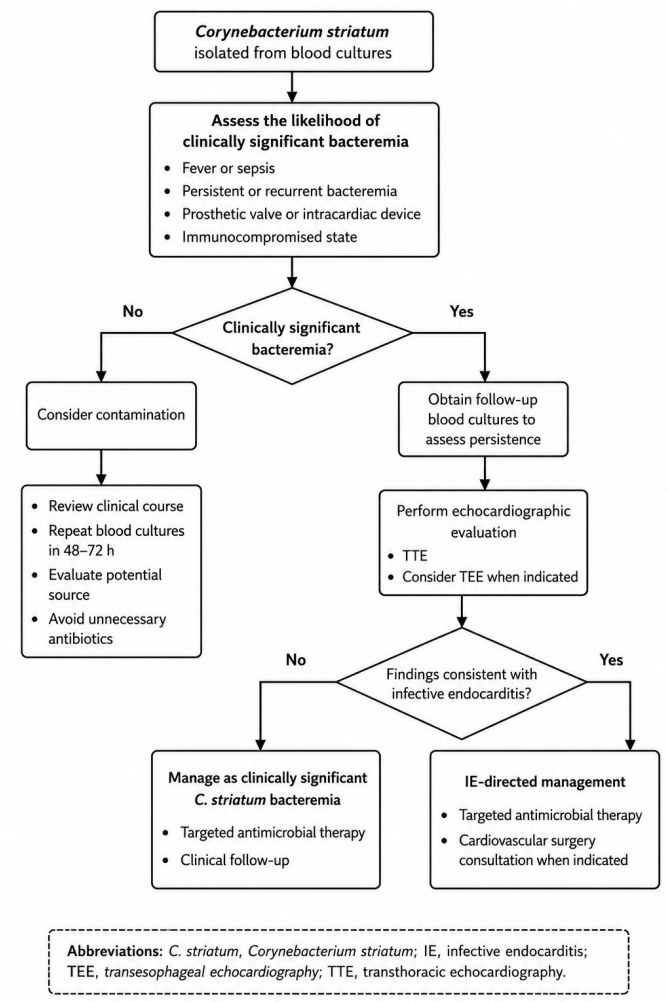

Figure 1. Proposed diagnostic and management algorithm for *Corynebacterium striatum* bacteremia. The algorithm provides a stepwise approach that integrates the clinical context, blood culture patterns, and bacteremia persistence to distinguish contamination from true infection and guide the evaluation for IE and appropriate management.

The algorithm integrates key clinical variables, including the number and persistence of positive blood cultures, the presence of intravascular devices, and patient-specific risk factors. It emphasizes that even a single positive blood culture should not be automatically dismissed as contamination, particularly in patients with compatible clinical findings. Furthermore, it highlights the importance of early echocardiographic evaluation in cases of suspected deep-seated infection, particularly IE.

Nosocomial transmission represents another important issue, particularly among critically ill patients[4-6,12]. In our series, bacteremia developed in patients with prolonged hospitalization and a history of invasive procedures, supporting the role of *Corynebacterium striatum* as a nosocomial pathogen.

Antimicrobial resistance also poses a major challenge in the management of *Corynebacterium striatum* infections. Previous studies have reported resistance to multiple antimicrobial classes, including beta-lactams and quinolones[2,8,15,16]. In our series, glycopeptides were the most commonly used agents, highlighting their importance in the treatment of severe infections.

The high mortality observed in our study is noteworthy. A considerable proportion of patients developed severe complications, including embolic events, valvular destruction, and clinical deterioration. The prognosis appeared to be worse in cases with delayed diagnosis and persistent bacteremia. These findings indicate that *Corynebacterium striatum* is not a benign organism and may lead to serious outcomes when not recognized and managed appropriately.

The main limitations of this study include its retrospective design, incomplete antimicrobial susceptibility data in some cases, and the relatively small sample size. Nevertheless, given the rarity of *Corynebacterium striatum* IE, such case series can provide valuable clinical insights.

Although *Corynebacterium striatum* is frequently considered a contaminant in clinical practice, our findings demonstrate that it may represent a clinically significant and potentially fatal cause of IE.

The predominance of native valve involvement, frequent persistent or recurrent bacteremia, and high mortality observed in our series underscore the need for increased

clinical awareness. Misclassification as contamination may lead to delayed diagnosis and worse clinical outcomes.

Clinicians should maintain a high index of suspicion when *Corynebacterium striatum* is isolated from blood cultures, particularly in the presence of persistent or recurrent bacteremia or repeated positive blood cultures. Early echocardiographic evaluation should be strongly considered to facilitate the timely diagnosis of IE and appropriate management.

Ethics

Informed Consent: Because of the retrospective design of the study and the anonymization of patient data, the requirement for informed consent was waived.

Footnotes

Authorship Contributions

Concept: D.K.Ö., H.T., Design: D.K.Ö., H.T., Data Collection or Processing: D.K.Ö., H.T., Analysis or Interpretation: D.K.Ö., Literature Search: D.K.Ö., Writing: D.K.Ö.

Conflict of Interest: The authors declare no conflicts of interest.

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Clinical Characteristics, Microbiological Profile, and Treatment Outcomes of Necrotizing Fasciitis and Fournier's Gangrene: A 10-Year Single-Center Experience

Nekrotizan Fasiit ve Fournier Gangreninin Klinik Özellikleri, Mikrobiyolojik Profili ve Tedavi Sonuçları: On Yıllık Tek Merkez Deneyimi

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Abstract

Introduction: To evaluate the clinical characteristics, microbiological profile, treatment strategies, and outcomes of adult patients with necrotizing fasciitis and Fournier's gangrene managed at İnönü University Turgut Özal Medical Center, Malatya, Türkiye over a 10-year period.

Materials and Methods: This retrospective, single-center cohort study included adult patients diagnosed with necrotizing fasciitis and/or Fournier's gangrene between January 2016 and December 2025. Demographic characteristics, clinical manifestations, laboratory findings, microbiological results, treatment approaches, and clinical outcomes were analyzed.

Results: Twenty-two patients were included. The median age was 56.5 years, and 63.6% were male. Necrotizing fasciitis accounted for 68.2% of cases, whereas Fournier's gangrene accounted for 31.8%. Diabetes mellitus was the most common comorbidity (36.4%). Swelling and erythema were present in all patients, whereas bullae and/or necrosis occurred in 72.7%. Microbiological cultures were positive in 68.2% of patients, with Gram-negative microorganisms being the most frequently isolated organisms; polymicrobial infection was identified in 18.2%. Surgical debridement was performed in 95.5% of patients, with a median time to the first debridement of 24 hours. Intensive care unit admission was required in 68.2% of patients, and vacuum-assisted closure therapy was applied in 40.9%. A complete clinical response was achieved in 54.5% of patients, whereas the in-hospital mortality rate was 9.1%.

Conclusion: Despite the high frequency of advanced disease requiring surgical intervention and intensive care support, favorable outcomes were achieved in most patients. Early surgical debridement, appropriate empirical antimicrobial therapy guided by local microbiological epidemiology, and multidisciplinary management remain essential components of optimal care for necrotizing soft tissue infections.

Keywords: Necrotizing fasciitis; Fournier's gangrene; necrotizing soft tissue infection; surgical debridement; polymicrobial infection

Öz

Giriş: Bu çalışmada, üçüncü basamak bir referans merkezinde 10 yıllık dönemde izlenen nekrotizan fasiit ve Fournier gangrenli erişkin hastaların klinik özellikleri, mikrobiyolojik profilleri, tedavi yaklaşımları ve klinik sonuçlarının değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Ocak 2016-Aralık 2025 tarihleri arasında nekrotizan fasiit ve/veya Fournier gangreni tanısıyla takip edilen erişkin hastalar retrospektif olarak incelendi. Demografik özellikler, klinik bulgular, laboratuvar verileri, mikrobiyolojik sonuçlar, tedavi yaklaşımları ve klinik sonuçlar değerlendirildi.

Bulgular: Çalışmaya 22 hasta dahil edildi. Ortanca yaş 56,5 yıl olup hastaların %63,6'sı erkekti. Olguların %68,2'si nekrotizan fasiit, %31,8'i Fournier gangreni tanısı aldı. En sık komorbidite diabetes mellitus (%36,4) idi. Şişlik ve eritem tüm hastalarda, bül ve/veya nekroz ise %72,7'sinde saptandı.

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Mikrobiyolojik kültürler hastaların %68,2'sinde pozitif olup etkenler arasında Gram-negatif mikroorganizmalar baskındı; polimikrobiyal enfeksiyon oranı %18,2 idi. Hastaların %95,5'ine cerrahi debridman uygulandı ve ilk debridmana kadar geçen ortanca süre 24 saatti. Yoğun bakım yatışı %68,2 hastada gerekli olurken, vakum yardımlı kapama tedavisi %40,9 hastada uygulandı. Kültür pozitif hastaların %73,3'ünde başlangıç ampirik antimikrobiyal tedavisinin uygun olduğu saptanırken, olguların %40,0'ında kültür ve duyarlılık sonuçlarına göre tedavi modifikasyonu yapıldı; bunlar de-eskalasyon, eskalasyon veya antifungal tedavi eklenmesini içeriyordu. Hastaların %54,5'inde tam klinik yanıt elde edilirken, hastane içi mortalite oranı %9,1 olarak bulundu.

Sonuç: İleri evre hastalık ve yüksek yoğun bakım gereksinimine rağmen olguların büyük kısmında olumlu klinik sonuçlar elde edildi. Erken cerrahi debridman, uygun ampirik antimikrobiyal tedavi, yerel mikrobiyolojik epidemiyolojinin dikkate alınması ve multidisipliner yaklaşım nekrotizan yumuşak doku enfeksiyonlarının başarılı yönetiminde temel unsurlar olmaya devam etmektedir.

Anahtar Kelimeler: Nekrotizan fasiit, Fournier gangreni, nekrotizan yumuşak doku enfeksiyonları, cerrahi debridman, polimikrobiyal enfeksiyon

Introduction

Necrotizing soft tissue infections are rapidly progressive infections involving the skin, subcutaneous tissue, fascia, and, occasionally, muscle, and are associated with substantial morbidity and mortality. Among these infections, necrotizing fasciitis and Fournier's gangrene represent the most severe clinical forms, frequently necessitating extensive surgical debridement, intensive care support, and prolonged hospitalization. Despite advances in surgical techniques, antimicrobial therapy, and critical care management, these infections remain significant causes of adverse clinical outcomes worldwide[1-4].

Early diagnosis remains challenging because the initial clinical presentation may resemble cellulitis, abscesses, or other uncomplicated soft tissue infections. Delayed recognition and treatment are associated with an increased risk of sepsis, organ failure, and death[5,6]. Diabetes mellitus, chronic kidney disease, malignancy, immunosuppression, trauma, and perineal or urogenital infections have been identified as major predisposing factors[7]. Characterization of the microbiological profile is essential for identifying polymicrobial infections, antimicrobial-resistant pathogens, and appropriate empirical antimicrobial coverage[8,9].

The cornerstone of management includes prompt surgical debridement, early initiation of broad-spectrum antimicrobial therapy, intensive care support when necessary, and multidisciplinary collaboration[10,11]. Nevertheless, mortality rates remain considerable, and data regarding factors associated with clinical outcomes remain limited[12,13]. Furthermore, long-term studies comprehensively evaluating the clinical characteristics, microbiological findings, surgical management, and treatment outcomes of necrotizing fasciitis and Fournier's gangrene remain relatively scarce in Türkiye[14].

This study aimed to evaluate the clinical characteristics, microbiological profiles, and treatment outcomes of patients with necrotizing fasciitis and Fournier's gangrene managed over a 10-year period at İnönü University Turgut Özal Medical Center, Malatya, Türkiye and to provide real-world data regarding the management of these severe infections.

Materials and Methods

Study Design

This retrospective, single-center cohort study was conducted at İnönü University Turgut Özal Medical Center, Malatya, Türkiye. Adult patients diagnosed with necrotizing fasciitis and/or Fournier's gangrene between January 1, 2016, and December 31, 2025, were retrospectively evaluated.

Study Population

Patients aged ≥18 years with a confirmed diagnosis of necrotizing fasciitis or Fournier's gangrene and available clinical, laboratory, microbiological, and treatment data were included. Patients with incomplete medical records, unconfirmed diagnoses, or non-infectious soft tissue disorders were excluded. The study cohort comprised patients who received multidisciplinary care during the study period.

Ethics Approval

The study was approved by the İnönü University Ethics Committee (decision number: 2026/9970, date: 21.04.2026) and was conducted in accordance with the principles of the Declaration of Helsinki. Due to the retrospective and anonymized nature of the study, informed consent was waived.

Definitions

The diagnosis of necrotizing fasciitis and Fournier's gangrene was established based on the clinical presentation, radiological findings when available, intraoperative findings, laboratory parameters, and microbiological investigations. Histopathological confirmation was available for 13 of the 22 patients (59.1%). In the remaining nine patients (40.9%), tissue specimens were not submitted for histopathological examination, and the diagnosis was established based on compatible clinical and radiological findings, together with

characteristic intraoperative findings documented during surgical debridement. Fournier's gangrene is defined as a necrotizing soft tissue infection involving the perineal and genital regions[12]. Septic shock was defined according to the Sepsis-3 criteria[13].

Clinical Outcomes

Clinical outcomes were categorized as complete response, partial response, or treatment failure. Complete response was defined as complete resolution of the clinical signs and symptoms of infection, accompanied by normalization of inflammatory markers and no further requirement for antimicrobial or surgical treatment. Partial response was defined as evident clinical improvement without complete recovery, accompanied by persistent laboratory evidence of inflammation and/or microbiological evidence of ongoing infection requiring continued antimicrobial therapy and/or additional surgical management. Treatment failure was defined as clinical progression, persistent evidence of infection despite appropriate medical and/or surgical treatment, or death. Mortality was defined as in-hospital death.

Data Collection

Data were retrospectively obtained from electronic medical records, patient files, and institutional laboratory databases. Demographic characteristics, comorbidities, clinical manifestations, vital signs, laboratory findings, microbiological results, anatomical sites of infection, intensive care requirements, surgical interventions, adjunctive treatment modalities, length of hospital stay, complications, and clinical outcomes were recorded.

Microbiological Assessment

Available wound, tissue, surgical specimen, and blood culture results were reviewed. Isolated microorganisms were classified as Gram-positive, Gram-negative, or anaerobic bacteria, and the presence of polymicrobial infection was recorded[11,12]. Because anaerobic cultures were not routinely performed during the study period, they were obtained only in selected cases based on clinical indications.

Treatment and Surgical Management

Data regarding the timing and number of surgical debridements, additional surgical procedures, antimicrobial therapy, intensive care support, hyperbaric oxygen therapy, and vacuum-assisted closure (VAC) applications were collected. Early surgical debridement was defined as an intervention performed as soon as possible following diagnosis[12]. Empirical antimicrobial therapy consisted of a carbapenem, glycopeptide, and intravenous clindamycin in accordance with the institutional treatment protocol for necrotizing soft tissue infections. The appropriateness of empirical antimicrobial therapy was evaluated among culture-positive patients based on whether the initial regimen included at least one antimicrobial agent active against the identified pathogen according to antimicrobial susceptibility testing. Antimicrobial therapy was subsequently continued unchanged or modified through deescalation, escalation, or addition of antifungal therapy according to microbiological findings and the clinical course.

Outcome Measures

The primary outcome was in-hospital mortality. Secondary outcomes included clinical response, intensive care requirements, duration of hospitalization, number of surgical interventions, and development of complications.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics software (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro-Wilk test and are presented as medians with interquartile ranges. Comparisons between groups were performed using the Mann–Whitney U test. Categorical variables are presented as frequencies and percentages and were compared using Fisher's exact test. Owing to the limited number of mortality events, multivariable analyses were not performed. A two-sided p value <0.05 was considered statistically significant.

Results

Baseline Characteristics

A total of 22 patients were included in the study. The median age was 56.5 years, and 14 patients (63.6%) were male. Based on the clinical diagnosis, 15 patients (68.2%) were classified as having necrotizing fasciitis and 7 (31.8%) as having Fournier's gangrene. Diabetes mellitus was the most common comorbidity, occurring in 36.4% of patients.

At presentation, swelling and erythema were observed in all patients, whereas bullae and/or necrosis were detected in 72.7%, severe or disproportionate pain in 50.0%, and hypotension/shock in 18.2% of patients. The median duration of symptoms before diagnosis was 7 days. Laboratory evaluation demonstrated a median leukocyte count of 18.58 $\times 10^9$/L, a median C-reactive protein level of 19.5 mg/dL, and a median procalcitonin level of 2.34 ng/mL. Demographic, clinical, and laboratory characteristics of the study population are summarized in Table 1.

Table 1. Demographic, clinical, and laboratory characteristics of patients with necrotizing fasciitis and Fournier's gangrene.

Variable	n (%) or median (IQR)
Number of patients	22
Age, years	56.5 (45-68)
Male sex	14 (63.6)
Necrotizing fasciitis	15 (68.2)
Fournier's gangrene	7 (31.8)
Diabetes mellitus	8 (36.4)
Malignancy	2 (9.1)
Immunosuppression	3 (13.6)
History of trauma or wound	9 (40.9)
Recent surgical history	6 (27.3)
History of perineal/urogenital infection	7 (31.8)
Symptom duration, days	7 (3-10)
Severe/disproportionate pain	11 (50.0)
Swelling/erythema	22 (100)
Bullae/necrosis	16 (72.7)
Hypotension/shock	4 (18.2)
Leukocyte count ($\times 10^9$/L)	18.58 (11.71-27.10)
CRP (mg/dL)	19.5 (13.25-24.4)
Procalcitonin (ng/mL)	2.34 (0.73-4.19)
Creatinine (mg/dL)	1.10 (0.83-1.58)

Data are presented as n (%) or median (IQR). IQR, interquartile range; CRP, C-reactive protein.

Microbiological Findings, Treatment Characteristics, and Clinical Outcomes

Wound or tissue cultures were obtained from 19 patients, with microbiological growth detected in 13 of 19 patients (68.4%). Gram-negative microorganisms predominated among the culture-positive isolates, whereas polymicrobial infection was identified in 18.2% of patients. Deep tissue involvement was observed in all patients, muscle involvement or myonecrosis in 72.7%, and perineal/genital involvement in 40.9%.

Among the 22 patients, 21 (95.5%) received empirical broad-spectrum antimicrobial therapy consisting of a carbapenem, glycopeptide, and intravenous clindamycin at diagnosis. Overall, positive microbiological cultures were identified in 15 patients, including 13 positive wound/tissue cultures and two blood cultures yielding *Candida parapsilosis*. The initial empirical antimicrobial regimen provided adequate coverage in 11 of the 15 culture-positive patients (73.3%). Culture-directed antimicrobial modification was required in six patients: therapy was de-escalated in two patients according to antimicrobial susceptibility results, escalated to colistin in two patients because of resistant Gram-negative pathogens, and supplemented with antifungal therapy in two patients following the isolation of *Candida parapsilosis* from blood cultures. In the remaining patients, the initial empirical antimicrobial regimen was continued without modification for at least 14 days.

Surgical debridement was performed in 21 of 22 patients (95.5%). Surgical data for one patient transferred from an outside hospital were unavailable because the operative records could not be retrieved. The median number of debridement procedures was one. Intensive care admission was required in 15 patients (68.2%), and VAC therapy was applied in 9 patients (40.9%).

Complete clinical response was achieved in 54.5% of patients, whereas 36.4% demonstrated a partial response. Treatment failure occurred in 9.1% of patients despite appropriate medical and surgical management. The median length of hospital stay was 22 days, and complications developed in 36.4% of patients. Overall, in-hospital mortality was 9.1%. Because only two mortality events occurred during the study period, further comparative analyses of mortality were not performed. Detailed data are presented in Table 2.

Discussion

Necrotizing fasciitis and Fournier's gangrene remain among the most severe bacterial infections encountered in clinical practice, frequently resulting in extensive tissue destruction, septic shock, intensive care admission, and death despite advances in modern medical care. In the present 10-year cohort, most patients presented with advanced disease characterized by deep tissue involvement, myonecrosis, and a high requirement for intensive care support. Nevertheless, the overall in-hospital mortality rate remained relatively low at 9.1%. This finding is noteworthy because the study population predominantly consisted of patients with advanced disease managed at İnönü University Turgut Özal Medical Center, Malatya, Türkiye using a multidisciplinary treatment approach. Taken together, our findings highlight the importance of early recognition, prompt surgical intervention, appropriate empirical antimicrobial therapy, and close multidisciplinary collaboration in the management of severe necrotizing soft tissue infections.

Mortality remains the most clinically relevant outcome in necrotizing soft tissue infections and continues to be reported at considerable rates worldwide. In a recent systematic review and meta-analysis, El-Qushayri et al.[10] reported mortality rates exceeding 20% among patients with Fournier's gangrene. Similarly, Hakkarainen et al. [14] reported mortality rates of approximately 16% across different forms of necrotizing soft tissue infections whereas Yilmazlar et al.[15] reported mortality approaching 28% among patients with severe Fourniers

Table 2. Microbiological findings, treatment characteristics, and clinical outcomes.

Variable	n (%) or median (IQR)
Microbiological findings	
Wound/tissue culture obtained	19 (86.4)
Positive microbiological culture	15 (68.2)
Gram-negative pathogen	11 (50.0)
Gram-positive pathogen	3 (13.6)
Blood cultures positive for *Candida parapsilosis*	2 (9.1)
Polymicrobial infection	4 (18.2)
Extent of infection	
Deep tissue involvement	22 (100)
Muscle involvement or myonecrosis	16 (72.7)
Perineal/genital involvement	9 (40.9)
Treatment characteristics	
Surgical debridement†	21 (95.5)
Time to first debridement, hours	24 (15–24)
Number of debridements	1 (1–2)
VAC therapy	9 (40.9)
Broad-spectrum empirical antimicrobial therapy initiated	21 (95.5)
Appropriate empirical antimicrobial therapy*	11 (73.3)
Culture-guided antimicrobial modification	6 (40.0)
• Deescalation*	2 (13.3)
• Escalation to colistin*	2 (13.3)
• Addition of antifungal therapy*	2 (13.3)
Intensive care admission	15 (68.2)
Clinical outcomes	
Length of hospital stay, days	22 (13–30)
Complete clinical response	12 (54.5)
Partial clinical response	8 (36.4)
Treatment failure	2 (9.1)
Complication	8 (36.4)
Mortality	2 (9.1)

Data are presented as n (%) or median (IQR). *Percentages for appropriate empirical antimicrobial therapy, culture-guided antimicrobial modification, deescalation, escalation, and addition of antifungal therapy were calculated using the 15 patients with positive microbiological cultures as the denominator. †One patient was referred from an outside hospital, and the operative records could not be retrieved; therefore, the surgical procedure performed before referral could not be verified. IQR, interquartile range; VAC, vacuum-assisted closure.

gangrene. In comparison, the in-hospital mortality rate in our cohort was 9.1%, despite the high frequency of deep tissue involvement, myonecrosis, and intensive care requirements. Although direct comparisons should be interpreted cautiously because of differences in patient populations, disease severity, and study design, several characteristics of our management strategy may have contributed to these favorable outcomes. Surgical debridement was performed in nearly all patients, with a median time to first debridement of 24 hours after diagnosis, and empirical broad-spectrum antimicrobial therapy was initiated promptly in nearly all patients. Furthermore, empirical therapy provided adequate initial antimicrobial coverage in nearly three-quarters of culture-positive patients, with subsequent culture-guided optimization when indicated. Together with multidisciplinary management involving infectious diseases specialists, surgeons, and intensive care physicians, these factors may have facilitated timely therapeutic decision-making and close clinical monitoring throughout the disease course.

Diabetes mellitus was the most common comorbidity in our cohort, affecting 36.4% of patients. This finding is consistent with previous studies that have identified diabetes mellitus as a major predisposing factor for both necrotizing fasciitis and Fournier's gangrene[16]. Hyperglycemia-related impairment of neutrophil function, microvascular dysfunction, and reduced tissue perfusion may promote rapid bacterial proliferation and contribute to extensive soft tissue destruction. Benjelloun et al. similarly reported a more severe clinical course and higher complication rates among diabetic patients with Fournier's gangrene[17]. Likewise, Temoçin et al.[9] identified diabetes mellitus as the most common comorbidity in a contemporary Turkish cohort. Collectively, these findings emphasize the role of diabetes mellitus as an important host-related risk factor for the development and progression of necrotizing soft tissue infections. They also underscore the need for heightened clinical vigilance and early intervention in patients with diabetes mellitus who present with suspected necrotizing soft tissue infections.

The clinical manifestations observed in our study were largely consistent with the classical presentation of necrotizing soft tissue infections. Swelling, erythema, and necrotic skin changes were the predominant clinical findings, whereas severe or disproportionate pain was reported in half of the patients. This finding is clinically relevant because pain out of proportion to physical findings is widely recognized as a hallmark of necrotizing fasciitis; however, its absence does not exclude the diagnosis. Goh et al. emphasized that early-stage disease can closely mimic cellulitis or other uncomplicated soft tissue infections, potentially resulting in delayed diagnosis and treatment[18]. Consistent with this observation, the median duration of symptoms before diagnosis in our cohort was 7 days, suggesting that delayed presentation, delayed clinical recognition, or both may still occur despite increasing awareness of these infections. In conjunction with the markedly elevated inflammatory markers observed at presentation, these findings highlight the importance of maintaining a high index

of clinical suspicion and obtaining early surgical evaluation in patients with compatible risk factors and rapidly progressive soft tissue infections, even when classical clinical features are absent or incomplete.

One of the most notable microbiological findings of the present study was the predominance of Gram-negative microorganisms among culture-positive cases. Misiakos et al.[19] reported that Enterobacterales species constitute a substantial proportion of pathogens isolated from patients with necrotizing soft tissue infections, highlighting the importance of broad Gram-negative antimicrobial coverage during the initial management of these infections. Similarly, Cipriani et al.[20] found that Gram-negative organisms were frequently isolated from patients with Fournier's gangrene, although polymicrobial infections remained common in their multicenter cohort. In our cohort, Gram-negative pathogens constituted the largest microbiological group, whereas polymicrobial infections were less frequent than those reported in previous studies. This difference should be interpreted cautiously, as anaerobic cultures were not routinely performed during the study period, and prior antimicrobial exposure before microbiological sampling may have reduced the microbiological yield. In addition, the relatively high proportion of perineal and genital infections in our cohort may have contributed to the predominance of Gram-negative pathogens. From a therapeutic perspective, empirical broad-spectrum antimicrobial therapy provided adequate initial coverage in 73.3% of culture-positive patients, while culture-guided treatment modification was required in the remaining patients. These findings support current recommendations for prompt initiation of broad-spectrum empirical antimicrobial therapy, followed by timely optimization based on microbiological culture and antimicrobial susceptibility results.

The requirement for intensive care support was remarkably high in our cohort, affecting more than two-thirds of patients and reflecting the substantial physiological burden associated with extensive tissue destruction, systemic inflammation, and sepsis. Lin et al.[21] demonstrated that disease severity scoring systems may help identify patients at increased risk of adverse outcomes and facilitate early risk stratification in Fournier's gangrene. Similarly, Iacovelli et al. reported that adjunctive wound management strategies, such as VAC therapy, may provide additional benefits in selected patients after adequate surgical debridement[22]. Current international guidelines further emphasize that successful management of severe necrotizing soft tissue infections depends on early recognition, prompt source control, broad-spectrum empirical antimicrobial therapy, and appropriate intensive care support when indicated[23]. In our cohort, despite the high rate of intensive care admission, the overall in-hospital mortality rate remained relatively low. Although the limited number of mortality events precludes definitive conclusions, these findings suggest that timely multidisciplinary management incorporating early surgical intervention, appropriate antimicrobial therapy, and intensive care support may contribute to favorable clinical outcomes. Nevertheless, the high rate of intensive care utilization also underscores the substantial healthcare resources required to manage these life-threatening infections.

Strengths and Clinical Implications

This study provides a 10-year real-world overview of the clinical characteristics, microbiological profile, treatment strategies, and outcomes of patients with necrotizing fasciitis and Fournier's gangrene managed at a tertiary referral center. The inclusion of detailed microbiological data, empirical antimicrobial treatment patterns, and culture-guided therapeutic modifications provides clinically relevant insights into the management of these life-threatening infections. Our findings underscore the importance of prompt surgical intervention, appropriate empirical antimicrobial therapy informed by local microbiological epidemiology, and multidisciplinary management to optimize patient care.

Study Limitations

This study has several limitations. First, its retrospective, single-center design may have introduced selection bias and limited the generalizability of the findings. Second, the relatively small sample size reduced the statistical power to identify independent predictors of adverse outcomes and precluded multivariable analyses. Third, because anaerobic cultures were not routinely performed, the frequency of polymicrobial and anaerobic infections may have been underestimated. Nevertheless, the 10-year study period and the availability of detailed clinical, microbiological, and treatment data provide valuable real-world insights into the management of these uncommon but life-threatening infections.

Conclusion

Necrotizing fasciitis and Fournier's gangrene remain life-threatening infections that require prompt recognition and multidisciplinary management. Our findings underscore the importance of early surgical debridement, appropriate empirical antimicrobial therapy informed by local microbiological epidemiology, and timely optimization of treatment based on culture results. Larger, multicenter studies are warranted to validate these findings and further improve the management of necrotizing soft tissue infections.

Ethics

Ethics Committee Approval: The study was approved by the İnönü University Ethics Committee (decision number: 2026/9970, date: 21.04.2026) and was conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Due to the retrospective and anonymized nature of the study, informed consent was waived.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.K., A.H.K., F.M., S.A.T., Z.B.Y., Z.Ç., Concept: A.K., Design: A.K., A.H.K., S.A.T., Data Collection or Processing: A.H.K., Z.B.Y., Z.Ç., Analysis or Interpretation: A.K., A.H.K., F.M., S.A.T., Z.B.Y., Z.Ç., Literature Search: A.K., A.H.K., S.A.T., Writing: A.K., A.H.K., S.A.T.

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REVIEW / DERLEME

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Ebola Disease: An Updated Review in the Context of the 2026 Bundibugyo Virus Outbreak

Ebola Hastalığı: 2026 Bundibugyo virüsü Salgını Eşliğinde Güncel Bir Değerlendirme

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Abstract

Ebola disease (EBOD) is a severe viral illness with a high mortality rate caused by viruses of the genus *Orthoebolavirus* within the family *Filoviridae*. The emergence of a new outbreak caused by Bundibugyo virus (BDBV) in Ituri Province of the Democratic Republic of the Congo on May 15, 2026, along with the detection of cross-border cases in Uganda, has once again underscored the global public health significance of EBOD. This review provides a comprehensive overview of the current terminology and classification, pathogenesis, modes of transmission, clinical and laboratory findings, diagnostic methods, case definitions, treatment approaches, Infection prevention and control (IPC) measures, contact tracing, vaccination strategies, and long-term sequelae associated with EBOD. Currently licensed vaccines and monoclonal antibody therapies are approved only for *Orthoebolavirus zairense*, limiting treatment options during BDBV outbreaks. Accelerating the development of pan-filovirus vaccines and therapeutics, strengthening early diagnostic capacity, and improving IPC infrastructure remain key priorities.

Keywords: Ebola disease, Bundibugyo virus, *Orthoebolavirus*, filovirus, outbreak

Öz

Ebola hastalığı (EBOD), *Filoviridae* ailesine ait *Orthoebolavirus* türlerinin neden olduğu, yüksek mortaliteli ağır seyirli viral bir hastalıktır. 15 Mayıs 2026'da Demokratik Kongo Cumhuriyeti'nin Ituri Eyaleti'nde Bundibugyo virüsüne (BDBV) bağlı yeni bir salgının başlaması ve Uganda'da sınır ötesi olgular saptanması, EBOD'un küresel halk sağlığı güncelliğini yeniden ön plana taşımıştır. Bu derleme; EBOD'un güncel terminoloji ve sınıflandırması, patogenezi, bulaş yolları, klinik ve laboratuvar bulguları, tanı testleri, vaka tanımları, tedavi yaklaşımları, enfeksiyon önlem ve kontrolü, temaslı takibi, aşılama stratejileri ile uzun dönem sekeller konularını kapsamlı biçimde ele almaktadır. Mevcut lisanslı aşı ve monoklonal antikor tedavilerinin yalnızca *Orthoebolavirus zairense* için onaylı olması, BDBV kaynaklı salgınlarda tedavi seçeneklerini kısıtlamaktadır. Pan-filovirüs tedavi ve aşı geliştirme çalışmalarının hızlandırılması, erken tanı kapasitesinin güçlendirilmesi ve enfeksiyon kontrol altyapısının iyileştirilmesi öncelikli hedefler olmaya devam etmektedir.

Anahtar Kelimeler: Ebola hastalığı, Bundibugyo virüsü, *Orthoebolavirus*, filovirüs, salgın

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Introduction

Definition, Classification, and Current Terminology

Ebola disease (EBOD) is a severe viral illness caused by viruses of the genus *Orthoebolavirus* within the family *Filoviridae*. Under the current classification, the principal *Orthoebolavirus* species known to cause human disease include Ebola virus (*Orthoebolavirus zairense*, EBOV), Sudan virus (*Orthoebolavirus sudanense*, SUDV), Bundibugyo virus (*Orthoebolavirus bundibugyoense*, BDBV), and Taï Forest virus (*Orthoebolavirus taiense*, TAFV). The diseases caused by these viruses are designated Ebola virus disease (EVD), Sudan virus disease (SVD), Bundibugyo virus disease (BVD), and Taï Forest virus disease, respectively. Thus, the term "Ebola disease" is used as a broad designation for diseases caused by different *Orthoebolavirus* species[1,2].

History and Epidemiology

EBOD was first recognized in 1976 during simultaneous outbreaks in present-day South Sudan and the Democratic Republic of the Congo (DRC), caused by SUDV and EBOV, respectively. Subsequent outbreaks occurred predominantly in Central and East Africa, particularly in the DRC, Uganda, Gabon, and Sudan. Although the magnitude and mortality of outbreaks have varied according to viral species and epidemiological context, EBOV outbreaks have generally been associated with higher case-fatality rates.

The 2014-2016 West African outbreak, initially reported in Guinea and subsequently spreading to Liberia and Sierra Leone, remains the largest EVD outbreak recorded to date, with more than 28,000 cases and 11,308 deaths. Limited healthcare capacity, inadequate surveillance systems, deficiencies in infection prevention and control (IPC), urban transmission, and extensive population mobility contributed to its rapid expansion. Travel-associated cases identified in Africa, Europe, and North America further underscored the potential for international dissemination of the disease.

Recurrent outbreaks have continued, particularly in the DRC, including the 2018-2020 outbreak, which resulted in 3,470 cases and 2,287 deaths. The 2022 outbreak in Uganda further underscored the persistent public health significance of SUDV[3-5].

Mortality varies according to the causative viral species, access to healthcare, and the epidemiological and clinical characteristics of individual outbreaks. Comparative epidemiological characteristics, pooled case-fatality rates, and currently available vaccines and therapeutic options for EBOV, SUDV, and BDBV are summarized in Table 1.

Overall mortality was 61.3% among patients treated in sub-Saharan Africa, compared with 24.5% among those treated outside sub-Saharan Africa[6]. Epidemiological risk is also shaped by the social context in which exposure occurs. Differences in exposure risk by sex and age appear to reflect social roles and caregiving responsibilities rather than established biological differences in susceptibility[20].

Environmental Drivers and One Health Perspective

Environmental and social changes may influence both the risk of zoonotic spillover and the subsequent geographic spread of EBOD outbreaks. Changes in land use, including deforestation and other forms of forest conversion, may modify human-wildlife interfaces and increase opportunities for contact with potential animal reservoirs. A spatial analysis of 27 Ebola outbreak sites and 280 control locations in Central and West Africa found that outbreak occurrence near the margins of the rainforest biome was associated with dense forest loss during the preceding 2 years. However, this observational association does not demonstrate that forest loss alone causes EBOD outbreaks, and the mechanisms connecting environmental change, human-wildlife contact, and zoonotic spillover remain incompletely understood[21].

Following a spillover event, transport connectivity and population mobility may facilitate geographic dissemination by connecting remote outbreak locations with other settlements and population centers. An analysis of EBOV outbreaks found that the extent of road and river networks surrounding spillover sites was significantly associated with the number of cases recorded during the first 100 days of an outbreak[22]. The 2014-2016 West African epidemic further demonstrated how introduction into capital cities, extensive population mobility, and cross-border movement, coupled with weak surveillance, inadequate IPC, and strained healthcare systems, can facilitate sustained transmission and international spread[3-5]. However, the infrastructure analysis did not incorporate indicators of population density, settlement size, or urban-rural status and therefore did not directly assess urbanization as an independent determinant of outbreak spread[22]. Moreover, because the analysis included only EBOV outbreaks, these findings should be extrapolated cautiously to outbreaks caused by other *Orthoebolavirus* species.

Taken together, these findings support a One Health approach that integrates human disease surveillance with animal and environmental monitoring while fostering coordination among public health, veterinary, wildlife, and environmental authorities. Incorporating land-use and population-connectivity data into this multisectoral framework may improve assessment of spillover risk, facilitate early outbreak detection, and strengthen preparedness. Nevertheless,

Table 1. Comparative epidemiological characteristics and preventive and therapeutic options for EBOV, SUDV, and BDBV.

Characteristic	EBOV	SUDV	BDBV
Taxonomic designation and associated disease	Ebola virus *(Orthoebolavirus zairense);* Ebola virus disease (EVD)	Sudan virus *(Orthoebolavirus sudanense);* Sudan virus disease (SVD)	Bundibugyo virus *(Orthoebolavirus bundibugyoense);* Bundibugyo virus disease (BVD)
First recognized outbreak	1976, Yambuku, present-day DRC	1976, Nzara, present-day South Sudan	2007, Bundibugyo District, Uganda
Principal epidemiological pattern	Recurrent outbreaks mainly in Central Africa; responsible for the 2014-2016 West African epidemic and the 2018-2020 DRC outbreak	Outbreaks reported predominantly in Uganda and present-day South Sudan, including major outbreaks in Uganda in 2000 and 2022	Historically associated with outbreaks in Uganda and the DRC; responsible for the evolving 2026 outbreak in the DRC with cross-border cases detected in Uganda
Pooled case-fatality rate, 1976-2022[6]	66.6%	48.5%	32.8%
Licensed or authorized vaccine availability	ERVEBO® is the only currently licensed and World Health Organization (WHO)-prequalified vaccine for EVD caused by EBOV. The former EU marketing authorization for the Ad26.ZEBOV/MVA-BN-Filo regimen (Zabdeno/Mvabea) was withdrawn in May 2026 for commercial reasons[7,8]	No licensed vaccine; candidate vaccines have undergone clinical evaluation	No licensed vaccine; candidate vaccine platforms are undergoing accelerated development and have been prioritized for clinical evaluation, but human efficacy has not been established
Licensed specific therapeutic options	Ansuvimab (mAb114; Ebanga™) and REGN-EB3 (Inmazeb™), supported by randomized human clinical trial evidence[9-11]	No licensed therapy with demonstrated clinical efficacy [12]	No licensed therapy with demonstrated clinical efficacy
Principal investigational therapeutic approaches	Broadly neutralizing and pan-filovirus antibodies and broad-spectrum antiviral combinations remain under investigation	Pan-ebolavirus monoclonal antibody therapy with MBP134/MBP431, alone or in combination with remdesivir, has demonstrated protection in non-human primate models[13,14]	The PARTNERS trial evaluates MBP134 and remdesivir, alone and in combination; maftivimab has also been prioritized for evaluation, and obeldesivir is being studied as post-exposure prophylaxis[12,15,16]
Current level of species-specific therapeutic evidence	Randomized human efficacy data are available for EBOV-specific monoclonal antibodies	Evidence remains predominantly preclinical; human efficacy has not been established	Human clinical trials are ongoing, but efficacy results are not yet available
Reported replication and disease kinetics	Faster *in vitro* replication and higher peak viral titers than BDBV; a shorter interval from symptom onset to death has been reported in available comparative data[17,18]	Direct comparative evidence remains limited	Slower *in vitro* replication, lower peak viral titers, and a longer interval from symptom onset to death than EBOV have been reported; however, species-specific human data remain limited[17,18]
Experimental susceptibility to remdesivir	*In vitro* activity is present, but monotherapy did not improve outcomes in the PALM trial[17,19]	Remdesivir-based combination therapy has shown protection in non-human primate models[14]	Greater *in vitro* inhibitory activity of remdesivir against BDBV than EBOV has been reported in experimental systems; this finding does not establish clinical efficacy[17]

Optimized supportive care remains the cornerstone of management for all three diseases. Pooled case-fatality rates represent estimates across outbreaks and should not be interpreted as fixed measures of intrinsic viral virulence, because outcomes are also influenced by outbreak setting, surveillance capacity, timing of diagnosis, and access to supportive care. Vaccine and therapeutic availability is reported as of July 2026. Investigational interventions should not be considered clinically effective until supported by appropriate human efficacy data. Experimental differences in replication kinetics and antiviral susceptibility should not be interpreted as direct measures of clinical virulence or treatment efficacy. Some comparisons are based on *in vitro,* minigenome, or animal model data, and direct species-to-species human evidence remains limited.

BDBV, Bundibugyo virus; BVD, Bundibugyo virus disease; DRC, Democratic Republic of the Congo; EBOV, Ebola virus; EVD, Ebola virus disease; SUDV, Sudan virus; SVD, Sudan virus disease.

evidence linking specific environmental and social drivers to Ebola emergence remains predominantly observational, and causal relationships have not been definitively established[23].

The 2026 BVD Outbreak: Epidemiological Characteristics, Public Health Response, and Lessons Learned

On May 15, 2026, an outbreak of BVD was declared in Ituri Province in northeastern DRC, and outbreak-associated cases were subsequently identified in Uganda. As of July 21, 2026, the DRC had reported 2,536 laboratory-confirmed cases and 1,033 deaths among confirmed cases across Ituri, North Kivu, South Kivu, Haut-Uele, and Tshopo provinces. Uganda had reported 20 confirmed cases, including 2 deaths, and 1 probable case resulting in death. Fifteen confirmed infections were imported from the DRC, whereas 5 resulted from secondary transmission among contacts and healthcare workers. No sustained community transmission had been documented, and the most recent confirmed case in Uganda was identified on June 21, 2026. An imported case was also reported in France; the patient subsequently recovered, and no secondary transmission was detected. Because case investigations, laboratory confirmation, and data harmonization were ongoing, these reported figures should be considered provisional[24-27].

Reported cases accumulated considerably faster than during previous major EBOD outbreaks. By mid-July, the World Health Organization (WHO) had characterized the event as the third-largest Ebola outbreak on record and reported that approximately 2,000 confirmed cases had been identified within about 2 months, whereas the 2018-2019 outbreak in the DRC required more than 10 months to reach a comparable case count. Nevertheless, the epidemic curve should be interpreted cautiously. The rapid increase in cumulative case counts may not reflect transmission occurring exclusively during the same period, because intensified case finding, expanded access to laboratory testing, and retrospective analysis of previously collected specimens may have incorporated earlier infections into the surveillance dataset. Ituri remained the principal epicenter, although the detection of cases across five provinces demonstrated the potential for continued national and regional spread[15,26].

The response has unfolded in a complex setting in which multiple operational challenges have compounded one another. Armed conflict, population displacement, and restricted access to affected communities have coincided with limitations in healthcare services, laboratory capacity, IPC resources, and referral systems. Community mistrust, misinformation, and security incidents involving healthcare facilities and personnel have further disrupted surveillance and response activities. As of July 15, 2026, 119 confirmed infections, including 36 deaths, had been reported among healthcare workers in the DRC. This substantial occupational burden indicates ongoing exposure in both healthcare and community settings and highlights gaps in the consistent implementation of IPC measures. Collectively, these conditions may delay case recognition, disrupt contact follow-up, compromise safe referral and clinical care, and allow transmission chains to remain undetected[26].

On May 17, 2026, the WHO Director-General determined that the outbreak constituted a Public Health Emergency of International Concern under the International Health Regulations. The decision emphasized the need for coordinated international action in response to rapid geographic expansion, documented cross-border transmission, and continuing epidemiological uncertainty. The absence of licensed BDBV-specific vaccines and therapeutics further heightened the need for research and regulatory preparedness. Vaccine candidates were prioritized for expedited development, and clinical studies evaluating therapeutic and post-exposure prophylactic interventions were initiated during the outbreak. These approaches are discussed in the relevant sections below[15,16,26,28].

The public health response has focused on three complementary objectives: achieving earlier case identification and isolation, reducing transmission associated with healthcare and community exposure, and strengthening coordination across affected provinces and national borders. Measures supporting these objectives have included expanding diagnostic capacity, conducting active contact follow-up, establishing safer referral and clinical care pathways, training and protecting healthcare workers, implementing community-based risk communication, ensuring safe and dignified burial practices, and facilitating more timely exchange of epidemiological information among neighboring countries. Maintaining dedicated isolation and treatment capacity and ensuring reliable access to appropriate personal protective equipment (PPE) have remained particularly important in newly affected areas[26,29].

This experience demonstrates that standard containment measures may become less effective when delayed diagnosis, population mobility, insecurity, healthcare worker exposure, and limited health system capacity occur simultaneously. Preparedness for future outbreaks should therefore include decentralized laboratory networks that can be rapidly expanded, pre-established mechanisms for cross-border surveillance and data sharing, adequately trained and protected healthcare workers, predefined isolation and referral pathways, and community engagement structures led by trusted local stakeholders. Clinical research platforms with pre-approved protocols are also needed so that vaccine,

therapeutic, and prophylactic candidates can be evaluated without avoidable delays during an active outbreak[26,29].

Rationale and Scope of the Review

This review provides a comprehensive evaluation of the terminology, epidemiology, pathogenesis, clinical manifestations, diagnosis, treatment, vaccination, and IPC strategies related to Ebola, drawing on evidence from previous outbreaks and current international guidance. Particular emphasis is placed on the evolving 2026 BDBV outbreak, its epidemiological and public health implications, and persistent species-specific gaps in prevention and treatment. In contrast to previous reviews, which have focused predominantly on EBOV, this review integrates the evolving epidemiology of the 2026 BDBV outbreak with species-specific evidence on diagnosis, prevention, investigational therapeutics, One Health-based surveillance, and practical clinical management.

Review Methodology

Search Strategy

A targeted literature search was conducted in PubMed/MEDLINE and Scopus from database inception through July 2026. The search strategy combined the terms "Ebola disease," "Ebola virus disease," "Bundibugyo virus," "BDBV," "Sudan virus," "SUDV," "*Orthoebolavirus*," and "filovirus" with terms related to epidemiology, outbreaks, pathogenesis, transmission, clinical manifestations, diagnosis, case definitions, treatment, therapeutics, vaccination, IPC, contact tracing, and long-term sequelae. Boolean operators were used, and the reference lists of relevant reviews, guidelines, and key original studies were screened to identify additional publications.

Official guidelines, case definitions, surveillance updates, outbreak reports, and media briefings issued by the WHO and its regional offices, Africa Centres for Disease Control and Prevention (Africa CDC), Centers for Disease Control and Prevention (CDC), European CDC (ECDC), UK Health Security Agency (UKHSA), International Committee on Taxonomy of Viruses, Republic of Türkiye Ministry of Health, and other relevant public health and regulatory authorities were also reviewed. Selected conference abstracts and proceedings were considered only when they provided relevant historical or time-sensitive information unavailable in full peer-reviewed publications and contained sufficient methodological or outcome detail.

Eligibility Criteria

Peer-reviewed original studies, clinical trials, observational studies, systematic reviews, meta-analyses, authoritative guidelines, and official institutional reports relevant to human *Orthoebolavirus* infections were considered. Recent evidence and current international recommendations were prioritized, whereas seminal historical publications were retained when necessary. Preclinical and animal studies were included only when they provided relevant evidence on pathogenesis, vaccine candidates, or investigational therapeutic approaches for which human data were limited.

The peer-reviewed literature search was restricted to articles published in English, although relevant national guidance published in Turkish was also considered. Duplicate records, dissertations, non-peer-reviewed commentaries, publications outside the clinical, epidemiological, diagnostic, preventive, or therapeutic scope of the review, and documents lacking sufficient relevant information were excluded.

Study Selection and Data Synthesis

Retrieved records were independently screened at the title and abstract levels by two authors, and the full texts of potentially relevant publications were independently assessed. Disagreements were resolved through discussion and consensus.

Given the narrative nature of this review, the evidence was synthesized qualitatively and organized into thematic sections. Particular attention was given to distinguishing EBOV-specific human evidence from findings related to other *Orthoebolavirus* species and from preclinical or animal data. No formal protocol registration, standardized risk-of-bias assessment, or quantitative evidence synthesis was performed. The thematic synthesis was designed to provide a comprehensive and critical overview of the current evidence, consistent with the scientific scope of the *Mediterranean Journal of Infection, Microbes and Antimicrobials*.

Pathogenesis

Orthoebolavirus species typically enter the body through mucosal surfaces, disrupted skin, or the parenteral route. Following entry, they initially replicate in immune cells, including monocytes, macrophages, and dendritic cells, before disseminating through the lymphatic system and bloodstream to the liver, spleen, adrenal glands, endothelium, and other tissues. Early suppression of the interferon response compromises host defenses, whereas the robust release of proinflammatory cytokines from infected cells promotes systemic inflammation. These processes contribute to hepatocyte necrosis, adrenal injury, lymphocyte apoptosis, endothelial dysfunction, and coagulation abnormalities. Consequently, increased vascular permeability, fluid loss, hypotension, shock, and multiorgan failure may develop[30-32].

Although experimental studies have identified several mechanisms underlying filovirus immune evasion, species-specific differences in these mechanisms have also been reported[31]. Human evidence regarding pathogenesis is

derived predominantly from EBOV infection, and the direct contribution of viral replication to organ-specific injury remains incompletely understood[30,33]. Accordingly, the clinical relevance of these mechanisms to BDBV and SUDV infections should be interpreted with caution.

Modes of Transmission

Fruit bats of the family *Pteropodidae* are considered the natural reservoirs of *Orthoebolavirus* species. Zoonotic transmission to humans has been reported following close contact with the blood, body secretions, or organs of infected fruit bats, non-human primates, antelopes, and other wild animals[2]. Infection of non-reservoir hosts, including humans, may facilitate cross-species transmission and initiate outbreaks[22].

The primary risk factor for human-to-human transmission is direct contact with the blood and body fluids of symptomatic patients. Although blood, feces, and vomitus are among the principal sources of transmission, viral RNA has also been detected in several other body fluids, including breast milk, saliva, urine, semen, cerebrospinal fluid, and ocular fluid. However, detection of viral RNA by real-time reverse transcription polymerase chain reaction (RT-PCR) does not necessarily indicate the presence of viable, infectious virus. Therefore, the potential for transmission through specific body fluids may vary according to the stage of disease and viral load[2,34]. Patients are generally not considered infectious before symptom onset, with infectiousness beginning when clinical manifestations develop[2,5].

Laboratory studies have demonstrated that *Orthoebolavirus* species can be transmitted through droplet inoculation via the oral and conjunctival routes; however, the relevance of these experimental findings to routine human transmission remains uncertain[35]. The risk of transmission increases substantially during the acute phase of illness, when viral load is highest, particularly among patients with severe symptoms such as vomiting and during the period immediately after death[36]. Mother-to-child transmission of EBOD has been documented, and maternal EVD during the 2014-2016 West African outbreak was associated with extremely high rates of fetal loss and neonatal mortality[37,38]. The detection of EBOV RNA and, in rare cases, viable virus in the semen of male survivors indicates that sexual transmission may occur during the convalescent phase. Accordingly, semen testing and counseling regarding safer sexual practices are recommended as components of survivor programs[39].

Experimental transmission observed under controlled conditions should not be interpreted as evidence that airborne transmission represents a major route of person-to-person spread. Nevertheless, enhanced respiratory protection remains appropriate during aerosol-generating procedures because such procedures may increase the risk of occupational exposure.

Clinical and Laboratory Findings

Clinical Findings

EBOD is a severe systemic filovirus infection that typically presents with sudden-onset, non-specific symptoms following an incubation period of 2-21 days. Because the precise timing and route of exposure are often uncertain, estimates of the interval between exposure and symptom onset remain limited[40]. In an analysis of published human exposure data, the mean incubation period for EBOD was reported as 6.22 $\pm$ 1.57 days across all routes of transmission, 5.85 $\pm$ 1.42 days following percutaneous exposure, and 7.34 $\pm$ 1.35 days following person-to-person or infected animal contact[41]. A meta-analysis reported that the incubation period for BVD caused by BDBV, the virus responsible for the current 2026 outbreak, may range from 5.7 to 11.3 days. Species-stratified random-effects analyses suggested that BDBV infection may be associated with a longer interval from symptom onset to death and a shorter interval from symptom onset to recovery than the corresponding estimates for other *Orthoebolavirus* species; however, BDBV-specific data remain limited[18].

The clinical course generally follows a recognizable but variable pattern. During the early phase of infection, typically within the first 1-3 days after symptom onset, patients present with non-specific manifestations such as fever, loss of appetite, weakness, headache, myalgia, and arthralgia[40]. During the 2013-2016 West African EVD outbreak, fatigue, loss of appetite, abdominal pain, diarrhea, vomiting, fever, and muscle pain were among the most frequently reported clinical manifestations[40,42-44].

In its clinical guidance, the CDC pragmatically categorizes EBOD manifestations as “dry” and “wet” symptoms. During the early stage, non-specific “dry symptoms,” including fever, myalgia, headache, weakness, and fatigue, predominate. Approximately 4-5 days after symptom onset, gastrointestinal involvement becomes more prominent, and “wet symptoms,” such as diarrhea, vomiting, abdominal pain, and unexplained bleeding, may develop[45]. Although this distinction does not represent a formal staging system, it provides a practical clinical framework for field triage, early case recognition, and timely implementation of IPC measures.

As “wet” symptoms emerge, gastrointestinal fluid losses may become profound and may reach 5-10 L/day in some patients[30,45]. The resulting hypovolemia, electrolyte disturbances, hypotension, and shock are major contributors to mortality. Gastrointestinal manifestations are therefore

important not only for case recognition but also as indicators of the need for early and intensive supportive care[30,45].

Maculopapular exanthema is a characteristic but variably reported finding in filovirus infections. In EBOD, a rash usually appears on days 5-7 of illness. It is generally erythematous, non-pruritic, and maculopapular; may involve the trunk, neck, face, and upper extremities; and may resolve with desquamation during recovery[40,45]. The frequency of rash in previous EBOD outbreaks has been reported to range from 25% to 52%[46]. However, its diagnostic value is limited because the rash may be difficult to detect, particularly in individuals with darker skin, and because reporting has been inconsistent across outbreaks. Therefore, rash should be regarded as a supportive clinical finding in EBOD, but its absence does not exclude the disease[30,45,47].

Although bleeding manifestations are considered one of the classic features of EBOD, they do not occur in all cases and are more commonly observed in severe or advanced disease. Petechiae, ecchymoses, epistaxis, oozing from vascular access sites, and mucosal bleeding are among the milder hemorrhagic manifestations, whereas gastrointestinal bleeding, disseminated intravascular coagulation (DIC), and shock occur more frequently in severe cases. During the West African outbreak, blood in the stool was reported in approximately 6% of patients, whereas clinically significant bleeding occurred predominantly during the late phase of illness and in pregnancy[47].

Bleeding manifestations were reported more frequently during the 1976 Yambuku outbreak than during many subsequent EVD outbreaks, including the West African outbreak[30,45,48]. In the EVISTA cohort from the 10th EVD outbreak in the DRC, the prevalence of bleeding at admission was approximately 16%, similar to that observed during the West African outbreak, whereas bleeding complications developed in 39% of patients during follow-up[19]. These findings indicate that, despite the term "hemorrhagic fever," the absence of bleeding does not exclude EBOD and that the reported frequency of bleeding may vary according to disease severity, timing of clinical assessment, outbreak setting, and data collection methods. Whether clinically meaningful differences in bleeding manifestations exist among *Orthoebolavirus* species remains insufficiently defined.

Neurological manifestations are less common but represent an important clinical component of severe disease. They may result from direct central nervous system involvement, metabolic disturbances, shock, hypoglycemia, or multiorgan failure. Confusion, delirium, altered consciousness, seizures, and encephalitis have been reported[30]. In the EVISTA cohort from the DRC, the prevalence of neurological manifestations increased from 12% at admission to 27% during follow-up, and severe manifestations, including agitation and coma, were reported in more than one-quarter of patients[19].

In severe cases, death usually occurs within 6-16 days after symptom onset as a result of septic shock, DIC, and multiorgan failure. During the West African outbreak, the mean interval from symptom onset to death was approximately 7.5 days[30,45]. Among survivors, clinical recovery generally begins toward the end of the first week; however, fatigue, arthralgia, myalgia, uveitis, and other post-acute symptoms may persist for weeks or months[45].

Vaccination may modify the clinical presentation of EVD. Observational studies comparing vaccinated and unvaccinated patients with EBOV infection in the DRC reported lower frequencies of several clinical manifestations, particularly hemorrhagic manifestations, among vaccinated individuals. These findings suggest that the sensitivity of symptom-based case definitions may be reduced in vaccinated populations[49,50]. Most detailed descriptions of clinical progression and prognostic markers are derived from EBOV cohorts. Therefore, the frequency and prognostic significance of individual clinical manifestations should not be assumed to be identical in BDBV or SUDV infections, for which species-specific clinical data remain limited.

Laboratory Findings

Laboratory findings in EBOD varies according to the stage and clinical severity of the disease. Leukopenia and marked lymphopenia are typical findings during the early phase, whereas neutrophilia may develop as the disease progresses. Thrombocytopenia is a common laboratory abnormality, with platelet counts typically ranging from 50,000 to 100,000/mm^3. Elevation of liver enzymes is also common, and transaminase levels may exceed 1,000 IU/L. In severe and fatal cases, the increase in aspartate aminotransferase (AST) is more pronounced than that in alanine aminotransferase (ALT), and AST levels may reach 7-12 times those of ALT.

Elevated amylase levels may indicate pancreatic involvement or systemic inflammation, while increased creatine kinase levels may result from muscle injury. Marked fluid loss due to severe vomiting and diarrhea may lead to electrolyte and acid–base disturbances, including hypokalemia, hyponatremia, hypocalcemia, and metabolic acidosis. In severe cases, the coagulation system is also affected; prolonged prothrombin time and activated partial thromboplastin time, together with increased fibrin degradation products and D-dimer levels, may be consistent with DIC[30,45,51]. These laboratory abnormalities are not specific to EBOD and should therefore be interpreted in the context of the clinical phase, disease severity, and epidemiological setting. Moreover, most detailed laboratory

descriptions are derived from EBOV cohorts, and whether the frequency or prognostic significance of these abnormalities differs in BVD or SVD remains insufficiently defined. The clinical, laboratory, and diagnostic features of EBOD according to disease phase are summarized in Table 2.

Diagnostic Tests

The diagnosis of EBOD is based on a combination of clinical findings, epidemiological risk, and laboratory diagnostic testing. Because the early manifestations of EBOD are non-specific, the differential diagnosis should be guided by the travel itinerary, the interval between travel or exposure and symptom onset, and relevant patient, animal, vector, food, water, and environmental exposures. The principal differential diagnoses and distinguishing clinical features in travelers returning from Ebola-affected regions are summarized in Table 3.

Malaria should be investigated urgently in every febrile traveler returning from a malaria-endemic region affected by Ebola. However, identification of malaria or another infection should not, by itself, preclude further evaluation for EBOD when a compatible epidemiological exposure has been identified[35,58,60]. In some outbreak settings, up to 95% of suspected cases have tested negative for EBOD, underscoring the need to maintain a broad differential diagnosis that includes other endemic febrile illnesses[62].

According to current WHO guidance, nucleic acid amplification test (NAAT)-based methods, particularly RT-PCR, remain the reference standard for laboratory confirmation in individuals who meet the EBOD case definitions. Because viral RNA levels may be below the detection threshold during the early phase of illness, a negative result from a specimen collected within the first 72 hours after symptom onset does not reliably exclude EBOD. Therefore, repeat testing with a second specimen is

Table 2. Clinical, laboratory, and diagnostic features of EBOD according to disease phase[9,10,52,53].

Disease phase[54-56]	Approximate timing	Clinical findings	Laboratory findings	Diagnostic test interpretation/case classification[35]
Incubation period	2-21 days after exposure	No symptoms are present. Infectiousness is generally not expected	No specific laboratory abnormalities are usually present	Routine testing is not recommended for asymptomatic contacts; symptom monitoring should be performed for 21 days
Early phase/dry symptoms	First 1-3 days	Fever, weakness, fatigue, headache, myalgia, arthralgia, loss of appetite, and sore throat	Leukopenia, lymphopenia, early thrombocytopenia, and elevated transaminases may develop	NAAT (+): acute confirmed case. NAAT (−) or not performed + IgM (+): acute confirmed case. If NAAT is negative within the first 72 hours and clinical suspicion persists, repeat testing is required
Gastrointestinal phase/wet symptoms	Usually, after day 4-5	Nausea, vomiting, watery diarrhea, abdominal pain, and marked fluid loss; bleeding may occur in some cases	Electrolyte disturbances, metabolic acidosis, worsening thrombocytopenia, and elevated AST/ALT levels	NAAT (−) >72 hours after symptom onset: discarded case. Ag-RDT may support triage but should be confirmed by NAAT.
Severe disease phase	End of the first week and thereafter	Shock, altered mental status, confusion, seizures, severe bleeding, DIC, and multiorgan failure	Neutrophilia, marked thrombocytopenia, AST-predominant transaminase elevation, renal dysfunction, prolonged PT/aPTT, and elevated D-dimer levels	High viral load; NAAT (+): acute confirmed case. Once the diagnosis is confirmed, isolation, treatment, and contact tracing should be initiated promptly
Post-symptomatic/ convalescent phase	Weeks to months	Fatigue, arthralgia, myalgia, headache, uveitis, visual or hearing problems, and psychosocial effects	Acute-phase abnormalities may resolve; viral RNA persistence may occur in immunologically privileged sites	NAAT (−) or not performed + IgM (+) + IgG (+): confirmed convalescent case. NAAT (−) or not performed + IgM (−) + IgG (+), >3 months, with clinical/ epidemiological compatibility: confirmed convalescent case
Indeterminate serological interpretation	Early post-symptomatic period, especially <3 months	Clinical findings may have resolved; history is important	Acute laboratory abnormalities may have resolved	NAAT (−) or not performed + IgM (−) + IgG (+), <3 months, with clinical/ epidemiological compatibility: indeterminate

NAAT/RT-PCR is the main diagnostic method for acute disease. A negative result within the first 72 hours does not definitively exclude EBOD; testing should be repeated if clinical suspicion persists. The distinction between "dry" and "wet" symptoms is not a formal staging system but a practical clinical approach. Ag-RDT, rapid antigen diagnostic test; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DIC, disseminated intravascular coagulation; EBOD, Ebola disease; IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; PT, prothrombin time; aPTT, activated partial thromboplastin time; RT-PCR, real-time reverse transcription polymerase chain reaction.

Table 3. Differential diagnosis of suspected EBOD in travelers returning from an Ebola-affected region[2,56-61].

Disease	Key epidemiological or exposure clues	Features shared with EBOD	Distinguishing clues	Principal diagnostic tests
EBOD	Travel to or residence in an affected area within 21 days; contact with a symptomatic or deceased case, body fluids, health care settings, unsafe burial practices, or potentially infected wildlife	Fever, profound fatigue, headache, myalgia, vomiting, diarrhea, thrombocytopenia, organ dysfunction, shock, and possible bleeding	Compatible with high-risk exposure; gastrointestinal "wet symptoms" often become prominent as illness progresses. Bleeding may be absent, particularly early in the disease	Species-specific *Orthoebolavirus* RT-PCR/ NAAT
Malaria	Travel to a malaria-endemic area; mosquito exposure; incomplete or absent chemoprophylaxis	Fever, headache, myalgia, fatigue, vomiting, thrombocytopenia, jaundice, altered consciousness, and multiorgan dysfunction	Parasitemia, anemia, and hemolysis may be present. Clinical findings alone may not reliably distinguish malaria from early EBOD	Thick and thin blood films and malaria rapid diagnostic test
Enteric fever	Consumption of contaminated food or water	Fever, headache, fatigue, abdominal pain, vomiting, diarrhea, or constipation	A more gradual onset and sustained febrile illness may occur; gastrointestinal bleeding generally develops later when present	Blood culture, preferably before antimicrobial therapy; stool culture or molecular testing when appropriate
Shigellosis	Contaminated food or water; fecal-oral exposure	Fever, abdominal pain, diarrhea, dehydration, and occasional blood in the stool	Frequent small-volume stools, dysentery, mucus, and tenesmus are more suggestive of shigellosis	Stool culture or gastrointestinal molecular testing
Cholera	Exposure to unsafe water or food, particularly during a local cholera outbreak	Profuse diarrhea, vomiting, severe dehydration, hypotension, and shock	Abrupt, high-volume watery diarrhea with little or no fever; systemic inflammatory and hemorrhagic manifestations are usually absent	Stool culture, molecular testing, or validated rapid diagnostic testing
Leptospirosis	Exposure to floodwater, freshwater, mud, or environments contaminated with animal urine	Fever, headache, myalgia, vomiting, jaundice, acute kidney injury, bleeding, and shock	Conjunctival suffusion, prominent calf or lumbar muscle tenderness, and combined jaundice and renal dysfunction may provide diagnostic clues	PCR during early illness; IgM serology or microscopic agglutination testing according to illness stage
Rickettsiosis	Tick or other arthropod exposure; rural or safari travel	Fever, headache, myalgia, rash, thrombocytopenia, and elevated transaminases	An inoculation eschar, regional lymphadenopathy, or a characteristic rash may be present	PCR from an eschar or appropriate specimen and paired serology
Bacterial sepsis or meningococcemia	Possible respiratory, urinary, intra-abdominal, catheter-related, skin, or central nervous system source	Fever, hypotension, shock, thrombocytopenia, purpura, coagulopathy, and multiorgan failure	A focal source, meningismus, rapidly progressive purpura, or microbiological evidence of bacterial infection may be present	Blood cultures and source-directed cultures or molecular tests; cerebrospinal fluid testing when clinically appropriate and safe
Acute viral hepatitis	Food- or waterborne, bloodborne, sexual, or parenteral exposure depending on the hepatitis virus	Fever, fatigue, nausea, vomiting, abdominal discomfort, jaundice, and elevated transaminases	Jaundice and hepatocellular injury usually dominate the clinical picture; marked ALT-predominant elevations may support acute viral hepatitis	Virus-specific serology and NAAT when indicated

Table 3. Continued

Disease	Key epidemiological or exposure clues	Features shared with EBOD	Distinguishing clues	Principal diagnostic tests
Crimean-Congo hemorrhagic fever	Tick bite; contact with livestock, animal blood, or tissues; slaughterhouse exposure; residence in or travel to an endemic region, including Türkiye	Sudden fever, headache, myalgia, thrombocytopenia, elevated transaminases, bleeding, shock, and multiorgan failure	Clinical overlap with EBOD is substantial; tick- and livestock-related exposures and geographic epidemiology are therefore particularly important	CCHFV RT-PCR during acute illness and virus-specific serology according to illness stage
Other viral hemorrhagic fevers (VHFs)—including Marburg virus disease, Lassa fever, dengue, and yellow fever	Marburg: cave, mine, or bat exposure; Lassa: residence in West Africa or rodent exposure; dengue/yellow fever: mosquito exposure and relevant geography	Fever, headache, myalgia, gastrointestinal symptoms, thrombocytopenia, hepatic dysfunction, bleeding, and shock	Marburg virus disease may be clinically indistinguishable from EBOD; pharyngitis or facial edema may suggest Lassa fever; retro-orbital pain and hemoconcentration may suggest dengue; prominent jaundice may suggest yellow fever	Agent-specific RT-PCR/NAAT, antigen testing, and serology according to the suspected pathogen and illness stage

Clinical findings alone cannot reliably confirm or exclude EBOD. Isolation precautions, public health notification, and Ebola-specific testing should not be delayed in patients meeting the relevant epidemiological and clinical criteria. CCHFV, Crimean–Congo hemorrhagic fever virus; EBOD, Ebola disease; NAAT, nucleic acid amplification test; RT-PCR, reverse transcription polymerase chain reaction; VHF, viral hemorrhagic fever.

recommended when clinical and epidemiological suspicion remains high[57].

In surviving patients, ethylenediaminetetraacetic acid (EDTA) whole blood or plasma is the preferred specimen, whereas oral or buccal swabs are used for deceased individuals. Oral swabs are not recommended for living patients because their sensitivity for detecting viral RNA is substantially lower than that of blood samples. Venous blood should be collected in an EDTA tube, with a recommended minimum volume of 5 mL. Blood specimens may be stored at room temperature for up to 24 hours or at 0°C–5°C for up to 1 week; specimens requiring longer storage should be maintained at -20°C or -70°C[63,64].

Because species identification has direct clinical, therapeutic, and epidemiological implications, current WHO guidance emphasizes diagnosis at the species level. Diagnostic algorithms may therefore incorporate sequential pan-filovirus, genus-specific, and species-specific NAAT assays. Serological tests, including immunoglobulin (Ig)M- and IgG-based assays, are not recommended as stand-alone tests for acute diagnosis but may contribute to the identification of previous infections and epidemiological investigations[57].

Rapid antigen diagnostic tests (Ag-RDTs) may support outbreak triage and postmortem screening, particularly in settings with limited access to centralized molecular testing. However, their diagnostic performance is lower than that of RT-PCR-based testing, and they should not be used as stand-alone tests to confirm or exclude acute EBOD[65,66]. The principal value of Ag-RDTs is operational. They may facilitate rapid initial triage and decentralized screening, but a negative result should be interpreted in the context of symptom duration, specimen type, viral load, and pretest probability.

Whole-genome sequencing is a complementary method that may help identify viral variants, investigate the origin of an outbreak, and establish epidemiological links among cases. However, it does not replace NAAT-based testing for the initial confirmation of acute infection[57].

The processing of specimens from suspected cases requires strict biosafety precautions. WHO recommends a risk-based biosafety approach, with procedures involving non-inactivated specimens performed in appropriately equipped laboratories using validated containment measures. Because viral culture requires high-containment facilities and carries substantial biological risk, it is not recommended for routine diagnostic use[57].

Case Definitions

An analysis based on Ebola treatment data from the DRC showed that each one-day delay in supportive care was associated with an 11% increase in the probability of death, highlighting the critical importance of rapid case detection and timely initiation of patient care[67]. Field data from the 2014-2015 West African EVD outbreak also demonstrated that admission to an Ebola treatment unit within 24 hours of symptom onset was associated with approximately half the mortality risk observed among patients admitted later, further supporting the importance of early isolation and specialized supportive care[68]. Taken together, these findings underscore the need for case definitions that facilitate rapid case identification, isolation, diagnostic testing, and referral while maintaining adequate sensitivity during outbreak response.

The case categories included in the 2014 WHO guideline on Ebola and Marburg case definitions continue to serve as the fundamental case-classification framework in the current WHO outbreak toolbox. These definitions are based on clinical findings, epidemiological exposure, and laboratory confirmation. Accordingly, EBOD cases are classified as suspected, probable, confirmed, or discarded cases.

A suspected case is defined as a person with sudden-onset high fever and a history of contact with an EBOD case or a sick or dead animal; a person with sudden-onset fever and at least three symptoms, including headache, weakness, loss of appetite, muscle or joint pain, abdominal pain, dysphagia, vomiting, dyspnea, diarrhea, or hiccups; or a person with unexplained bleeding or sudden unexplained death.

A probable case is defined as a suspected case evaluated by a clinician or a deceased suspected case with an epidemiological link to a confirmed case for whom a laboratory specimen could not be obtained.

A confirmed case is defined as a case in which viral RNA is detected by RT-PCR or EBOV-specific IgM antibodies are detected.

A discarded case refers to a suspected or probable case with negative laboratory test results[55,69].

Although WHO case definitions are essential surveillance tools for outbreak management, their clinical performance remains limited. A systematic review and meta-analysis reported pooled sensitivity and specificity values of 81.5% and 35.7%, respectively, for WHO EBOD case definitions, indicating the potential for both missed cases and false-positive classifications. In the same study, a history of contact was identified as a strong predictor, whereas severe fatigue was the most sensitive symptom. The diagnostic value of fever varied according to the threshold applied and the stage of disease. These findings highlight the importance of using case definitions in conjunction with laboratory confirmation and epidemiological risk assessment rather than as stand-alone diagnostic tools[30,70,71]. Increasing clinical experience from the 2013-2016 West African EVD outbreak further suggests that more specific combinations of symptoms may improve diagnostic assessment[72].

In the 2025 surveillance case definition, the CDC places EBOD within the broader category of VHFs. A suspected case is defined as a case with clinical findings consistent with acute disease onset and an epidemiological link, or a case in which VHF or a VHF-causing agent is identified on the death record. A confirmed case requires laboratory evidence, including VHF-specific NAAT, genome sequencing, IgM positivity, a fourfold rise in IgG titer, or viral isolation[53].

In response to the 2026 BDBV outbreak in the DRC, the ECDC published an interim case definition for use in the European Union/European Economic Area. Similar to the WHO framework, the ECDC definition incorporates clinical, epidemiological, and laboratory criteria but additionally introduces a dedicated high-risk exposure category and a "person under investigation" classification. These additional categories are intended to strengthen surveillance, facilitate early case detection, and support risk assessment activities[73].

In the guidelines of the Republic of Türkiye Ministry of Health, clinical findings and epidemiological risk history are assessed together, consistent with the approaches used by WHO and the CDC. However, unlike the WHO framework, the Turkish guidelines do not define a separate "suspected case" category. Cases meeting at least one epidemiological criterion and at least one clinical criterion are classified as "probable cases," whereas those confirmed by laboratory diagnostic testing are classified as "confirmed cases"[56]. Because these frameworks were developed for different surveillance, jurisdictional, and operational contexts, their criteria should not be considered fully interchangeable. Instead, the case definition specified by the relevant outbreak response framework and public health authority should be applied. A comparison of the case definitions used by WHO, CDC, ECDC, the Republic of Türkiye Ministry of Health, and UKHSA is presented in Table 4. A practical algorithm for the initial assessment and management of patients who meet the applicable clinical and epidemiological criteria for suspected EBOD is presented in Figure 1.

Treatment

Supportive Care and EBOV-Specific Therapies

Optimized supportive care was the cornerstone of EBOD management before the availability of specific therapeutics and remains essential even when species-specific therapies are available. During the West African EVD outbreak, early and comprehensive supportive care provided in advanced treatment centers was associated with survival rates of up to approximately 82%, underscoring the prognostic importance of timely, optimized care[75,76].

Optimized supportive care includes individualized fluid and electrolyte replacement, management of hypovolemia and shock, maintenance of adequate oxygenation, monitoring of glucose levels and renal function, and treatment of concomitant or secondary infections. Substantial crystalloid replacement may be required, particularly in patients with severe fluid loss resulting from vomiting and diarrhea; however, fluid therapy should be guided by repeated clinical and laboratory assessments. Early correction of hyponatremia, hypokalemia, and other electrolyte disturbances remains

Table 4. Comparison of EBOD case definitions.

Organization/ guideline	Scope	Case categories	Suspected/probable case approach	Confirmed case	Distinctive features
WHO[55,69]	Outbreak case definition for Ebola and Marburg diseases	Suspected, probable, confirmed, and discarded cases	A suspected case is defined as a person with sudden-onset high fever and a history of contact with an EBOD case or a sick or dead animal; a person with sudden-onset fever and at least three symptoms, including headache, weakness, loss of appetite, muscle or joint pain, abdominal pain, dysphagia, vomiting, dyspnea, diarrhea, or hiccups; or a person with unexplained bleeding or sudden unexplained death. A probable case is a suspected case evaluated by a clinician or a deceased suspected case with an epidemiological link to a confirmed case for whom a laboratory sample could not be obtained	Confirmed by detection of viral RNA by RT-PCR or EBOV-specific IgM antibodies.	Used for early case detection, isolation, and contact tracing in outbreak settings. Combines clinical findings, epidemiological exposure, and laboratory confirmation. Suspected or probable cases with negative laboratory results are classified as discarded cases
CDC[53]	General surveillance definition for VHFs, including Ebola	Suspected and confirmed cases	A suspected case is a case with compatible acute-onset clinical findings and an epidemiological link, or a case in which VHF or a VHF-causing agent is listed on the death record	A case with confirmatory laboratory evidence such as VHF-specific NAAT/genome sequencing, IgM positivity, a fourfold rise in IgG titer, or viral isolation	Not specific to Ebola; used for standardized classification of VHF cases in U.S. public health surveillance. Provides a broader VHF framework than the WHO definition
ECDC[73]	Interim surveillance case definition for the 2026 BVD outbreak in the DRC for use within the EU/EEA	PUI, probable, and confirmed cases	A PUI is a person who meets the clinical and epidemiological criteria or a person with a high-risk exposure who develops any compatible symptom, including fever of any grade. A probable case is a person fulfilling both the clinical and high-risk exposure criteria. Epidemiological criteria include travel to an affected area or contact with a probable or confirmed case within the previous 21 days	Laboratory confirmation by detection of BDBV nucleic acid with confirmatory testing or isolation of BDBV from a clinical specimen	Introduces a dedicated high-risk exposure category and a PUI classification to support surveillance and risk assessment activities
MoH-Türkiye[56]	National guidelines for EVD	No separate suspected case category; probable and confirmed cases are defined	A probable case is a case meeting at least one epidemiological criterion and at least one clinical criterion. Epidemiological criteria include contact with the blood/body fluids of an EVD case within the previous 21 days, residence in or travel to an area with active transmission, contact with bats/rodents/primates in endemic regions, or risky contact with sexual secretions of recovered patients. Clinical criteria include unexplained death, fever ≥38°C, or at least three compatible clinical findings	A case meeting the probable case definition and confirmed by laboratory diagnostic tests for EVD is classified as a confirmed case	Similar to the WHO and CDC definitions, it evaluates clinical findings together with epidemiological risk history. Unlike the WHO framework, it does not define a separate "suspected case" category. Because a single probable case is considered an outbreak, rapid notification is emphasized
UKHSA[74]	Case classification for health protection teams managing Ebola and Marburg cases	Possible, probable, and confirmed cases	A probable case is a person without laboratory results who has a clinically compatible illness and a history of high-risk exposure within the previous 21 days. Clinical findings may include fever > 37.5°C, severe weakness, headache, myalgia, abdominal pain, sore throat, vomiting, diarrhea, or unexplained bleeding. A possible case is a deceased person with epidemiological risk, or a person with compatible clinical illness and travel to an affected area or contact while using appropriate PPE	A positive real-time PCR result from blood or another body fluid is considered confirmation of a case	There is no "suspected case" category. Unlike the WHO framework, possible and probable cases are separated according to exposure risk level. The fever threshold is >37.5°C

CDC, Centers for Disease Control and Prevention; DRC, Democratic Republic of the Congo; EBOD, Ebola disease; EVD, Ebola virus disease; ECDC, European Centre for Disease Prevention and Control; EU/EEA, European Union/ European Economic Area; IgG, immunoglobulin G; IgM, immunoglobulin M; MoH-Türkiye, Republic of Türkiye Ministry of Health; NAAT, nucleic acid amplification test; PPE, personal protective equipment; PUI, person under investigation; RT-PCR, real-time reverse transcription polymerase chain reaction; UKHSA, UK Health Security Agency; VHF, viral hemorrhagic fever; WHO, World Health Organization; BDBV, Bundibugyo virus; EBOV, Ebola virus.

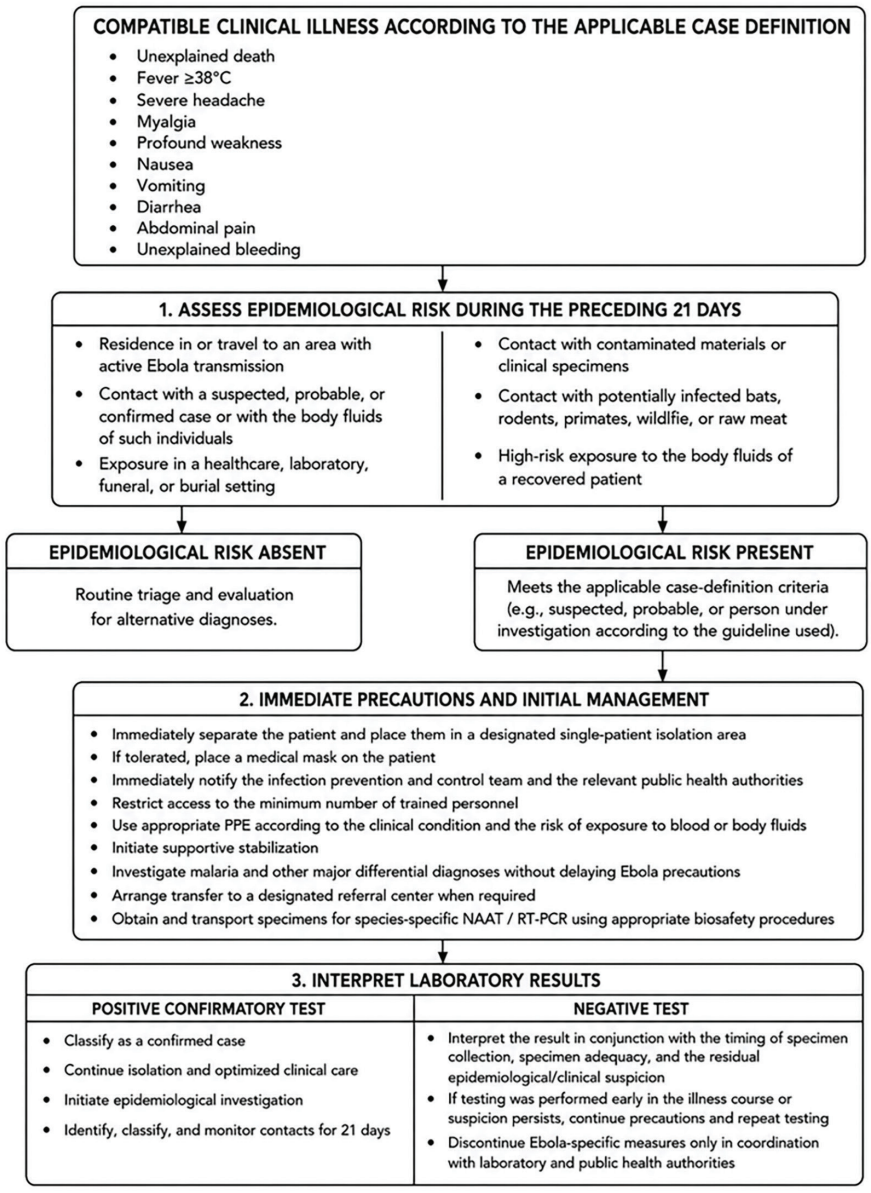

Figure 1. Practical algorithm for the initial assessment and management of a patient with suspected EBOD. The algorithm was developed by the authors based on current WHO guidance on diagnosis and infection prevention and control, relevant CDC clinical guidance, and the Republic of Türkiye Ministry of Health guideline for EVD. Case-category terminology varies across guidelines (WHO: suspected/probable/confirmed/discarded; CDC: suspected/confirmed; ECDC: PUI/probable/confirmed; Republic of Türkiye Ministry of Health: probable/confirmed; UKHSA: possible/probable/confirmed). Clinical findings are non-specific, and the identification of malaria or another infection does not necessarily exclude concomitant EBOD in the presence of compatible epidemiological exposure. NAAT, nucleic acid amplification test; RT-PCR, real-time reverse transcription polymerase chain reaction; PPE, personal protective equipment; PUI, person under investigation; EBOD, Ebola disease; WHO, World Health Organization; EVD, Ebola virus disease; ECDC, European Centers for Disease Control and Prevention; UKHSA, UK Health Security Agency.

critically important. WHO guidelines also identify early enteral nutrition, symptom control, empirical antimicrobial therapy when clinically indicated, and vasopressor support for refractory hypotension unresponsive to fluid resuscitation as components of optimized supportive care[77].

A major therapeutic advance emerged from the PALM trial conducted in the DRC. In this randomized controlled trial, mAb114 and REGN-EB3 reduced 28-day mortality compared with ZMapp, with mortality rates of 35.1% in the mAb114 group and 33.5% in the REGN-EB3 group[9,10]. Based on these findings, both monoclonal antibody therapies received approval from the U.S. Food and Drug Administration (FDA) for the treatment of EVD caused by EBOV. The 2022 WHO guideline strongly recommends either mAb114 (ansuvimab; Ebanga™) or REGN-

EB3 (atoltivimab, maftivimab, and odesivimab; Inmazeb™) for patients with RT-PCR-confirmed EBOV infection. These agents should be administered as early as possible, and both therapies should be delivered as a single intravenous infusion [11].

Remdesivir did not demonstrate clinical benefit as monotherapy in the PALM trial. Although preclinical and limited clinical evidence suggests potential roles for remdesivir in combination regimens and in the management of EBOV persistence, these uses remain investigational[14,78]. The evidence supporting mAb114 and REGN-EB3 is comparatively robust because it is based on randomized clinical trial data. However, this evidence is specific to EBOV and should not be extrapolated to BDBV or SUDV.

Treatment outcomes are also strongly influenced by disease stage and baseline viral load; in the PALM trial, higher viral load was strongly associated with mortality[9,79].

Investigational Therapies for SUDV and BDBV

Unlike EVD caused by EBOV, no approved therapeutic with demonstrated clinical efficacy is currently available for SVD or BVD[11,12].

For SVD, available evidence for candidate therapies remains predominantly preclinical. MBP134, a two-antibody pan-ebolavirus cocktail, reversed advanced SUDV disease in non-human primates when administered as a single intravenous or intramuscular dose[13]. In a separate non-human primate study, remdesivir combined with a pan-ebolavirus monoclonal antibody provided protection against advanced SUDV disease[14]. Although these findings provide a rationale for clinical evaluation, they do not establish therapeutic efficacy in humans[13,14].

For BVD, a WHO-convened Technical Advisory Group prioritized MBP134, maftivimab, and remdesivir for evaluation in clinical trials and recommended investigating remdesivir in combination with monoclonal antibody therapy[12]. In July 2026, the PARTNERS platform trial began enrolling patients with confirmed BVD in the DRC to evaluate MBP134 and remdesivir as monotherapies and in combination. Because the trial has only recently begun, BDBV-specific human efficacy data are not yet available, and these agents remain investigational[16].

For post-exposure prophylaxis, the WHO prioritized obeldesivir, an orally administered broad-spectrum antiviral candidate, for clinical evaluation in contacts of patients with confirmed BVD[12]. In non-human primate models, oral obeldesivir provided post-exposure protection against lethal EBOV infection when treatment was initiated 24 hours after exposure. However, these findings do not establish efficacy against BDBV in humans[80]. In July 2026, a separate clinical trial was initiated to evaluate obeldesivir as post-exposure prophylaxis in individuals who had been exposed to confirmed BVD cases but had not yet developed disease[15].

Beyond the candidates currently undergoing clinical evaluation, broadly neutralizing and bispecific antibodies targeting conserved glycoprotein epitopes across multiple *ebolavirus* species and other filoviruses represent another promising therapeutic strategy. However, most of these approaches remain in the preclinical or early clinical development stages, and their safety and efficacy against SVD or BVD in humans have not yet been established[81].

Infection Prevention and Control

IPC is a central component of preparedness for and response to EBOD outbreaks. Drawing on experience from previous filovirus outbreaks, WHO issued an updated IPC guideline for Ebola and Marburg diseases in 2026, with recommendations developed using the GRADE methodology[39]. The guideline emphasizes standard precautions, hand hygiene, protection of the eyes, nose, and mouth, fluid-resistant protective clothing, and appropriate respiratory protection during aerosol-generating procedures[39].

The WHO guideline does not limit IPC to the use of PPE. Instead, it adopts a hierarchy-of-controls approach that integrates engineering controls, environmental measures, administrative interventions, and appropriately selected PPE. The guideline provides a framework for screening and triage, patient placement, risk-based PPE selection, healthcare worker safety, environmental decontamination, occupational exposure management, and contact monitoring[39].

WHO recommends screening individuals presenting to healthcare facilities in outbreak-affected areas at the first point of contact, preferably using a “no-touch” approach, followed by prompt triage and isolation of suspected cases. Suspected or confirmed cases should preferably be placed in single rooms. In screening areas where a distance of at least 1 m can be maintained and direct contact with the patient or the patient’s environment is not anticipated, additional PPE beyond standard precautions may not be required. This risk-based approach aims to provide adequate protection while avoiding unnecessary PPE use, which may contribute to heat stress and fatigue and create communication difficulties and challenges during safe doffing[39].

When physical distancing cannot be maintained during screening, PPE should be selected according to the anticipated degree of contact and exposure. Direct care of patients with suspected or confirmed Ebola requires protection of the mucous membranes, along with fluid-resistant protective clothing and gloves. Additional protective barriers, including double gloves, should be used when warranted by the

procedure or the anticipated degree of exposure to blood and body fluids[39].

For healthcare facilities in the United States, CDC guidance also stratifies PPE requirements according to clinical stability and the likelihood of exposure to blood or body fluids. For clinically stable patients with suspected VHF who do not have bleeding, vomiting, or diarrhea, the minimum recommended ensemble includes a single-use, fluid-resistant gown or coverall, a facemask, a full-face shield, and two pairs of gloves. For patients with confirmed disease or suspected disease accompanied by clinical instability, bleeding, vomiting, or diarrhea, PPE should fully cover the skin and clothing and prevent exposure of the eyes, nose, and mouth. Care should be provided by trained personnel, with donning and doffing performed under the supervision of a trained observer[82].

CDC recommends placing patients with suspected or confirmed VHF in a single-patient room with a private bathroom, restricting access to essential personnel, and using dedicated patient-care equipment whenever possible. Aerosol-generating procedures should be avoided unless clinically necessary. When such a procedure is required, the number of personnel present should be minimized, and the procedure should be performed in a private room, ideally in an airborne infection isolation room when feasible. Healthcare workers should use a fit-tested N95 or higher-level respirator or a powered air-purifying respirator, together with the recommended barrier PPE[83].

One important update in the WHO guideline is the concept of the IPC ring approach. The IPC ring approach involves rapid assessment and targeted strengthening of IPC practices in healthcare facilities, households, and other sites epidemiologically linked to confirmed cases. Activities may include screening and isolation, environmental cleaning and disinfection, healthcare worker training, identification of IPC gaps, and rapid implementation of corrective measures. This targeted approach has been incorporated into recent filovirus outbreak responses, including the response to the 2022 Uganda SVD outbreak[39,84].

The continuing burden of infection among healthcare workers during the 2026 BVD outbreak illustrates the challenges of consistently implementing IPC measures in conflict-affected and resource-constrained settings. As of July 15, 2026, WHO had reported 119 confirmed infections and 36 deaths among healthcare workers in the DRC, highlighting persistent occupational and community exposure, as well as ongoing gaps in IPC implementation[26].

Accordingly, WHO does not recommend routine whole-body spraying of chlorine or other disinfectants onto PPE-clad healthcare workers before or during doffing. Evidence supporting the effectiveness of this practice in reducing contamination is insufficient, and spraying may cause skin or respiratory irritation while creating a false sense of security. Instead, visible contamination should be managed through validated, targeted decontamination procedures and safe doffing practices. For environmental cleaning, wiping methods that mechanically remove organic material are preferred to indiscriminate spraying. Alcohol-based hand rub or soap and water should be used for hand hygiene rather than chlorine solutions[39].

Environmental studies have detected viral RNA on gloves and frequently touched surfaces even in the absence of visible contamination. Although detection of viral RNA does not necessarily demonstrate the presence of viable virus, these findings support systematic cleaning and disinfection of potentially contaminated surfaces[39].

The evidence supporting several IPC interventions remains limited because comparative studies are ethically and operationally difficult to conduct during high-risk filovirus outbreaks. Consequently, some recommendations are necessarily based on a combination of indirect evidence, accumulated outbreak experience, feasibility, occupational safety considerations, and precautionary principles rather than on comparative effectiveness trials alone[39].

Contact Tracing

According to WHO, a contact is a person who has had direct contact with the blood, body fluids, secretions, or contaminated surfaces of a patient with EBOD during the symptomatic period. Living in the same household, unprotected contact during healthcare provision, laboratory exposure, unsafe burial practices, and contact with infected animals are also considered contact events. Contacts should be actively monitored for symptoms for 21 days after the last exposure, and individuals who develop compatible symptoms should be rapidly isolated and evaluated through laboratory testing[39]. Because the likelihood of detecting viral RNA in contacts who have not developed symptoms is low, routine diagnostic testing is not recommended for asymptomatic individuals[57]. Consistent with the WHO approach, the guideline of the Republic of Türkiye Ministry of Health also recommends classifying contacts as high-, intermediate-, or low-risk according to the level of exposure; implementing direct active monitoring of asymptomatic high- and intermediate-risk contacts; applying restrictions such as avoiding travel, public transportation, and crowded settings; and discontinuing follow-up on day 21 in contacts who remain asymptomatic[56].

Updated ECDC guidance for air travel–associated Ebola exposures recommends contact tracing only when the index case was symptomatic during the flight and was identified

within 21 days of travel. For confirmed cases, contact tracing is generally limited to passengers seated within one seat of the index case, cabin crew members working in the same cabin section, cleaning personnel, and individuals with direct exposure to body fluids[85]. Although contact tracing is central to outbreak containment, its effectiveness depends on timely case detection, complete identification of contacts, community trust, population mobility, data-sharing capacity, and the availability of trained personnel.

Vaccines

ERVEBO® (rVSVΔG-ZEBOV-GP) is a replication-competent, recombinant vesicular stomatitis virus (rVSV)-based live-vector vaccine that expresses the glycoprotein of EBOV belonging to the species *Orthoebolavirus zairense*. Following a positive assessment by the European Medicines Agency, ERVEBO® received EU-wide conditional marketing authorization in 2019 and was subsequently prequalified by WHO. ERVEBO® has been approved by the U.S. FDA for the prevention of disease caused by EBOV in individuals aged 12 months and older. It is currently the only licensed and WHO-prequalified vaccine available for EVD and does not provide protection against disease caused by other *Orthoebolavirus* species[7,86,87].

The two-dose Ad26.ZEBOV/MVA-BN-Filo regimen, comprising Zabdeno and Mvabea, received EU marketing authorization under exceptional circumstances in 2020. However, the marketing authorizations for both vaccines were withdrawn in May 2026 at the request of the marketing authorization holder for commercial reasons[8].

The PREVAIL I and PREVAC trials demonstrated that antibody responses were largely maintained for up to 12 months after vaccination. However, these immunogenicity findings do not establish the duration of clinical protection or determine the need for or optimal timing of booster vaccination[88,89].

In the United States, the Advisory Committee on Immunization Practices recommends pre-exposure vaccination with ERVEBO® for high-risk adults aged 18 years and older. These groups include responders to active EBOV outbreaks, personnel working with specimens that may contain replication-competent EBOV in BSL-4 or Laboratory Response Network laboratories, and healthcare workers involved in the care or transport of patients with suspected or confirmed EVD in Ebola treatment centers or special pathogen treatment centers. The vaccine should not be administered to individuals with a history of anaphylaxis to vaccine components or rice protein, and vaccination should be deferred in the presence of acute systemic infection or febrile illness. Decisions regarding vaccination during pregnancy, breastfeeding, and immunosuppression should be individualized according to the anticipated exposure risk and the potential benefits and harms[86,90].

During EBOV outbreaks, the ring vaccination strategy using ERVEBO® is based on the rapid identification and vaccination of close contacts of confirmed or probable EVD cases, as well as contacts of those contacts. In the ring vaccination trial conducted in Guinea, no cases of EVD with symptom onset occurring 10 days or more after randomization were observed among participants who received immediate vaccination. Vaccination outcomes during the outbreak that began in the DRC in 2018 further supported the effectiveness of this strategy for outbreak control[91,92]. However, the available evidence on clinical vaccine effectiveness is specific to EBOV and should not be extrapolated to SUDV or BDBV. No vaccine is currently licensed for SVD or BVD. In 2026, WHO concluded that the available evidence was insufficient to determine whether ERVEBO® provides clinically meaningful protection against BDBV and recommended against its programmatic use during BVD outbreaks outside controlled research settings [93].

Post-Ebola Syndrome and Long-Term Sequelae

Following recovery from acute EVD, EBOV RNA and, in some anatomical compartments, replication-competent virus may persist for prolonged periods in immunologically privileged or anatomically sequestered sites, including the testes, intraocular compartments, and central nervous system[39,78]. This is important not only because of potential late clinical complications but also because of the risk of sexual transmission, particularly as a result of viral persistence in semen. Because some cases reported during the later stages of the 2014-2016 outbreak were epidemiologically and molecularly linked to sexual transmission, WHO and CDC recommend semen testing, counseling on safer sexual practices, consistent condom use when abstinence is not chosen, and hand hygiene after contact with semen[94,95].

Post-Ebola syndrome may include multisystem sequelae such as fatigue, myalgia, arthralgia, headache, visual and auditory disturbances, neuropsychiatric symptoms, and uveitis. Uveitis is particularly important because it may lead to vision loss, and a high viral load during acute illness has been associated with an increased risk of ocular complications. In addition to physical sequelae, psychosocial outcomes, such as post-traumatic stress disorder, depression, anxiety, and stigmatization, are frequently reported among EVD survivors[3,96].

Although long-term sequelae following EBOV infection have been well characterized, data on long-term outcomes after BVD caused by BDBV remain limited. In a recent study evaluating survivors 16 years after the 2007–2008 outbreak in Uganda, persistent multisystem symptoms were reported, with neurological and musculoskeletal complaints representing the most common sequelae. Headache (35%) and visual

impairment (22.5%) were prominent findings, while 57.5% of participants reported persistent stigmatization. Hearing loss and gastrointestinal dysfunction, which are more commonly reported among EBOV survivors, were not prominent in this cohort. Although these findings suggest that some long-term manifestations may be shared across diseases caused by different *Orthoebolavirus* species, the evidence is derived from a limited observational survivor cohort and does not establish definitive species-specific differences in the spectrum or frequency of sequelae[97].

Future Challenges and Perspectives

Future research on EBOD should not be limited to agents with direct antiviral activity but should also encompass therapeutic strategies aimed at modulating the host response and reducing organ damage. In this context, immunomodulatory and other host-directed therapies represent important areas of investigation; however, their clinical role and optimal timing remain uncertain, particularly in BVD[3,98,99].

One of the most important limitations of current treatment options is that approved monoclonal antibodies have demonstrated efficacy only against EBOV infection. The PARTNERS trial has begun enrolling patients with confirmed BVD to evaluate MBP134 and remdesivir, administered as monotherapies and in combination[16]. A separate trial is evaluating obeldesivir as post-exposure prophylaxis among contacts of confirmed cases who have not yet developed the disease[15]. These studies may provide the first controlled human evidence on these interventions in BVD; however, their results are not yet available.

Therefore, the development of pan-filovirus therapeutics, clinical evaluation of broadly neutralizing *Orthoebolavirus* antibodies, investigation of antiviral-monoclonal antibody combination therapies, and development of interventions with wider therapeutic windows remain key priorities for future research[3,81,98].

Nevertheless, the development of effective treatments alone is not sufficient. Timely and equitable access to licensed therapies in outbreak-affected regions remains a major challenge. Therefore, post-approval implementation processes, including procurement, distribution, cold-chain infrastructure, cost, and health system capacity, must be strengthened[100,101].

For post-exposure chemoprophylaxis, EBOV-specific monoclonal antibodies, including REGN-EB3 and mAb114, have been used in limited case-based experiences. The absence of EBOD among 23 unvaccinated individuals with intermediate- or high-risk exposure supports the potential utility of this approach; however, the small sample size and uncontrolled study design mean that the available evidence remains insufficient to support routine use[102].

Conclusion

EBOD remains a major global public health threat because of its potential for recurrent outbreaks, high mortality, and the capacity of outbreaks to expand rapidly in settings characterized by population mobility, insecurity, limited laboratory access, and inadequate IPC capacity. The evolving 2026 BVD outbreak underscores that advances achieved for EBOV, including licensed vaccines and effective EBOV-specific monoclonal antibody therapies, cannot automatically be extrapolated to other *Orthoebolavirus* species.

Future preparedness should include earlier case detection, scalable and decentralized laboratory networks, strengthened IPC, protected healthcare workforces, pre-established cross-border data-sharing mechanisms, and pre-approved clinical research platforms that enable the rapid evaluation of candidate vaccines and therapeutics during emerging outbreaks. In parallel, the development of vaccines and therapeutics that are effective against multiple *Orthoebolavirus* species remains a major priority.

Footnotes

Authorship Contributions

Concept: A.B., Design: F.M.G.K., A.B., Data Collection or Processing: F.M.G.K., Analysis or Interpretation: F.M.G.K., A.B., Literature Search: F.M.G.K., A.B., Writing: F.M.G.K., A.B.

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Invisible Patient Safety Risk: A Point Prevalence Analysis of Antimicrobial-Related Potential Drug-Drug Interactions in Hospitalized Patients

Görünmeyen Bir Hasta Güvenliği Riski: Hastanede Yatan Hastalarda Antimikrobiyal ile İlişkili Potansiyel İlaç-İlaç Etkileşimlerinin Nokta Prevalans Analizi

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Abstract

Introduction: Antimicrobial-related potential drug-drug interactions (pDDIs) represent an important yet often underrecognized patient safety concern among hospitalized patients, particularly those receiving multiple medications. This study assessed the point prevalence, risk factors, and distribution of antimicrobial-related pDDIs among hospitalized adult patients.

Materials and Methods: This cross-sectional point prevalence study was conducted on March 4, 2026, at Şişli Hamidiye Etfal Training and Research Hospital. Adult inpatients receiving at least 1 systemic antimicrobial agent and at least 1 additional systemic medication were included. Potential drug–drug interactions were assessed using the UpToDate® Lexicomp® Drug Interactions Online Database. Interactions classified as category C, D, or X were considered pDDIs. Univariable and multivariable logistic regression analyses were performed to identify independent predictors of pDDIs.

Results: Among the 375 included patients, at least 1 antimicrobial-related pDDI was identified in 112 patients (29.9%). A total of 205 pDDI pairs were detected, of which 68% were category C (monitor therapy), 27% were category D (consider therapy modification), and 5% were category X (avoid combination) interactions. Overall, ceftriaxone (n = 44), clarithromycin (n = 33), and levofloxacin (n = 18) were the most frequently implicated agents. Clinically higher-risk category D and X interactions were predominantly associated with clarithromycin (n = 18), cefuroxime axetil (n = 17), linezolid (n = 9), and quinolones overall (n = 7). In the multivariable logistic regression analysis, the total number of medications was the strongest independent predictor of pDDIs (odds ratio, 1.40; 95% confidence interval, 1.28-1.54; $p < 0.001$).

Conclusion: Antimicrobial-related pDDIs were identified in 29.9% of hospitalized adult patients, with 32% of the identified pDDI pairs classified as category D or X interactions. Higher-risk interactions were predominantly associated with clarithromycin, cefuroxime axetil, linezolid, and quinolones. Routine screening for antimicrobial-related DDIs should be integrated into antimicrobial stewardship programs to enhance medication safety, particularly for patients receiving multiple medications.

Keywords: Drug interactions, anti-bacterial agents, polypharmacy, patient safety

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Öz

Giriş: Antimikrobiyal ile ilişkili potansiyel ilaç-ilaç etkileşimleri (pİİE), özellikle polifarmasi varlığında, hastanede yatan hastalarda önemli ancak sıklıkla gözden kaçan bir hasta güvenliği sorunudur. Bu çalışmada, hastanede yatan erişkin hastalarda antimikrobiyal ile ilişkili pİİE'lerin nokta prevalansı, risk faktörleri ve dağılımının değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Bu kesitsel nokta prevalans çalışması, 4 Mart 2026 tarihinde üçüncü basamak bir eğitim ve araştırma hastanesinde gerçekleştirildi. En az bir sistemik antimikrobiyal ajan ile birlikte en az bir ek sistemik ilaç kullanan erişkin yatan hastalar çalışmaya dahil edildi. Potansiyel ilaç–ilaç etkileşimleri, UpToDate® Lexicomp® Drug Interactions Online Database kullanılarak değerlendirildi. C, D ve X kategorisindeki etkileşimler pİİE olarak kabul edildi. pİİE gelişimiyle ilişkili bağımsız belirleyicileri saptamak amacıyla tek değişkenli ve çok değişkenli lojistik regresyon analizleri yapıldı.

Bulgular: Çalışmaya dahil edilen 375 hastanın 112'sinde (%29,9) en az bir antimikrobiyal ile ilişkili pİİE saptandı. Toplam 205 pİİE çifti tespit edildi; bunların %68'i C kategorisi (tedavinin izlenmesi önerilen), %27'si D kategorisi (tedavide değişiklik yapılması önerilen) ve %5'i X kategorisi (kombinasyondan kaçınılması gereken) etkileşimlerden oluşuyordu. Genel olarak en sık ilişkili ajanlar seftriakson (n = 44), klaritromisin (n = 33) ve levofloksasin (n = 18) iken; klinik açıdan daha yüksek riskli D ve X kategorisi etkileşimlerde klaritromisin (n = 18), sefuroksim aksetil (n = 17), linezolid (n = 9) ve kinolonlarla (toplam n = 7) ilişkiliydi. Çok değişkenli lojistik regresyon analizinde toplam ilaç sayısı, pİİE gelişiminin en güçlü bağımsız belirleyicisi olarak saptandı (olasılık oranı: 1,40, %95 güven aralığı: 1,28-1,54; $p < 0,001$).

Sonuç: Antimikrobiyal ile ilişkili pİİE'ler, hastanede yatan erişkin hastaların %29,9'unda saptanmış olup, belirlenen etkileşimlerin %32'si D veya X kategorisinde sınıflandırıldı. Klinik açıdan daha önemli D ve X kategorisi etkileşimler özellikle klaritromisin, sefuroksim aksetil, linezolid ve kinolonlarla ilişkiliydi. Bu bulgular, özellikle çoklu ilaç kullanan hastalarda antimikrobiyal ilişkili ilaç-ilaç etkileşimlerinin sistematik olarak taranmasının, antimikrobiyal yönetim ve hasta güvenliği uygulamalarının önemli bir bileşeni olması gerektiğini göstermektedir.

Anahtar Kelimeler: İlaç etkileşimleri, antibakteriyel ajanlar, polifarmasi, hasta güvenliği

Introduction

Drug-drug interactions (DDIs) are conditions in which concomitantly administered medications alter each other's pharmacokinetic or pharmacodynamic properties, resulting in changes in drug efficacy or toxicity[1,2]. A potential DDI (pDDI) refers to the concomitant use of 2 or more drugs with the potential to interact with one another[3]. Therefore, pDDIs do not necessarily result in clinically higher-risk interactions, and the proportion that progresses to actual DDIs may vary. DDIs are among the major causes of adverse drug reactions and are associated with increased morbidity and mortality, prolonged hospital stays, and higher healthcare costs[4,5]. Previous studies have reported that at least 1 pDDI has been identified in approximately one-third of hospitalized patients and in more than half of patients in intensive care units[3].

This risk is particularly pronounced during antimicrobial therapy. Antimicrobials, which are widely used in hospital settings, represent an important risk group for pDDIs because of their frequent use in combination with multiple medications. Agents such as quinolones, triazole antifungals, macrolides, and linezolid may cause clinically significant interactions, including QT prolongation, serotonin syndrome, and nephrotoxicity[6-9].

The frequency and clinical significance of antimicrobial-related pDDIs vary according to the study population, methodology, and interaction database used. Previous studies have reported that antimicrobials account for a substantial proportion of DDIs, with this contribution being particularly pronounced among patients in intensive care units[10]. Data from Türkiye indicate that antimicrobials account for approximately one-quarter of all drug interactions and that a limited number of agents, including quinolones, triazole antifungals, metronidazole, linezolid, and clarithromycin, are responsible for the majority of these interactions[11]. In addition, advanced age and a greater total number of medications have been identified as independent risk factors for the development of pDDIs[12].

In light of these findings, determining the prevalence and predictors of antimicrobial-related pDDIs in hospitalized patients is important for developing strategies to improve patient safety. In this study, the point prevalence and clinical determinants of antimicrobial-related pDDIs were evaluated among hospitalized adult patients. In addition, the distribution of risk categories and the most frequently implicated agents were systematically analyzed.

Materials and Methods

Study Design and Patient Selection

This study used a cross-sectional point prevalence design and was conducted on March 4, 2026, at Şişli Hamidiye Etfal Training and Research Hospital to evaluate antimicrobial-related pDDIs. Adult patients aged 18 years or older who were hospitalized on the study date and were receiving at least 1 systemic antimicrobial agent and at least 1 additional systemic medication were included. Topical, ophthalmic, and intranasal medications were excluded from the evaluation.

Data Collection

Patient data were collected by the investigators from the hospital information management system and medical records. Demographic characteristics (age and sex), hospitalization ward, and all systemic medications and antimicrobials administered on the study date were recorded. Polypharmacy was defined as the concomitant use of 5 or more systemic medications.

Identification of pDDIs

All concomitantly administered medications were analyzed using the UpToDate® Lexicomp® Drug Interactions Online Database. Lexicomp was selected because it is a widely used and regularly updated clinical decision support database that provides comprehensive information on DDIs and is commonly used in clinical practice and research. The database classifies pDDIs into 5 risk categories: A (no known interaction), B (no action needed), C (monitor therapy), D (consider therapy modification), and X (avoid combination). In this study, interactions classified as categories C, D, and X were considered pDDIs. Interactions classified as categories D and X were considered clinically higher-risk interactions. Drug pairs associated with pDDIs and their potential clinical consequences were systematically recorded.

Ethics Approval

The study was approved by the University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital Ethics Committee (decision number: 3409; date: March 3, 2026) and was conducted in accordance with the principles of the Declaration of Helsinki. Because anonymized data obtained from hospital records were used, the requirement for informed consent was waived by the ethics committee.

Statistical Analysis

Continuous variables were presented as mean ± standard deviation or median (interquartile range [IQR]), according to data distribution, whereas categorical variables were expressed as numbers and percentages. Between-group differences were evaluated using the Mann-Whitney U test for continuous variables and the Pearson chi-square test or Fisher exact test for categorical variables.

Univariable and multivariable logistic regression analyses were performed to identify independent risk factors associated with antimicrobial-related pDDIs. Variables with $p < 0.20$ in the univariable analysis, as well as variables considered clinically relevant, were included as candidate variables in the multivariable model. A bidirectional stepwise selection method (forward and backward) based on the Akaike information criterion (AIC) was used to construct the final model. Because of the structural relationship between the total number of medications and polypharmacy (defined as a binary variable based on the use of ≥5 medications), these 2 variables were not included simultaneously in the same model. The total number of medications was included in the final model because it retained the characteristics of a continuous variable and provided a better model fit. Multicollinearity was assessed using the variance inflation factor (VIF), and VIF values <2 were confirmed for all variables. Model fit was evaluated using the AIC, likelihood ratio (LR) chi-square test, and Hosmer-Lemeshow goodness-of-fit test. A p value <0.05 was considered statistically significant. All analyses were performed using R statistical software (version 4.5.0; R Foundation for Statistical Computing, Vienna, Austria) and the gtsummary package.

Results

Patient Characteristics

At the time of the study, a total of 719 patients were hospitalized, excluding those in pediatric wards. After excluding patients who were not receiving antimicrobials and those receiving antimicrobial therapy without concomitant medications, 375 patients (52.2%) met the inclusion criteria and were included in the study. The median age was 65 years (IQR, 49-77 years), and 202 patients (53.9%) were male. Of the included patients, 70 (18.7%) were hospitalized in the intensive care unit, 127 (33.9%) in medical wards, and 178 (47.5%) in surgical wards. The median length of hospital stay was 4 days (IQR, 1-9 days).

A total of 350 patients (93.3%) were receiving 3 or more medications, 253 (67.5%) were receiving 5 or more medications, and 82 (21.9%) were receiving 10 or more medications. The median total number of medications per patient was 6 (IQR, 4-7). Regarding antimicrobial use, 259 patients (69.1%) received 1 antimicrobial agent, 100 (26.7%) received 2, and 16 (4.3%) received 3 or more antimicrobial agents. The median number of antimicrobials per patient was 1 (IQR, 1-1).

The median total number of medications was 9 (IQR, 7-12) among patients hospitalized in the intensive care unit, compared with 5 (IQR, 4-8) among those hospitalized in other wards ($p < 0.001$). Similarly, the median number of antimicrobials was 2 (IQR, 1–2) among intensive care unit patients and 1 (IQR, 1-2) among patients hospitalized in other wards ($p < 0.001$).

Prevalence and Distribution of pDDIs

A total of 205 antimicrobial-related pDDI pairs were identified. At least 1 pDDI was present in 112 patients (29.9%). When interactions were evaluated according to risk category, 5% (n = 10) were classified as category X, 27% (n = 55) as category D, and 68% (n = 140) as category C.

When all risk categories were considered, the most frequently implicated agents in pDDIs were ceftriaxone (n = 44), clarithromycin (n = 33), and levofloxacin (n = 18), followed by cefuroxime axetil (n = 17) and moxifloxacin (n = 15).

When clinically higher-risk category D and X interactions were considered together, the most frequently implicated agents were clarithromycin (n = 18), cefuroxime axetil (n = 17), and linezolid (n = 9) (Table 1; Figure 1).

Patients with pDDIs were older and had longer hospital stays and a greater number of medications and antimicrobials used than patients without pDDIs ($p < 0.001$ for all comparisons). In addition, the proportions of patients hospitalized in the intensive care unit and those with polypharmacy were significantly higher among patients with pDDIs ($p = 0.041$ and $p < 0.001$, respectively) (Table 2).

Identification of Independent Risk Factors for pDDIs: Multivariable Logistic Regression Analysis

In the univariable analysis, age [odds ratio (OR), 1.02; 95% confidence interval (CI), 1.01-1.03; $p < 0.001$], female sex (OR, 0.60; 95% CI, 0.38-0.95; $p = 0.029$), intensive care unit hospitalization (OR, 1.75; 95% CI, 1.02-3.00; $p = 0.041$), total number of medications (OR, 1.39; 95% CI, 1.29-1.51; $p < 0.001$), number of antimicrobials (OR, 2.45; 95% CI, 1.69-3.61; $p < 0.001$), and presence of polypharmacy (OR, 8.76; 95% CI, 4.47-19.3; $p < 0.001$) were significantly associated with pDDIs. In the multivariable model, the total number of medications (OR, 1.40; 95% CI, 1.28-1.54; $p < 0.001$) and female sex (OR, 0.57; 95% CI, 0.33-0.95; $p = 0.034$) were identified as independent predictors. Intensive care unit hospitalization showed an inverse association in the multivariable model (OR, 0.50; 95% CI, 0.25-0.98; $p = 0.049$). Age and number of antimicrobials were no longer statistically significant in the multivariable model. The results of the univariable and multivariable logistic regression analyses are presented in Table 3.

In the goodness-of-fit analyses, the Hosmer–Lemeshow test showed no significant discrepancy between the model and the observed data ($p > 0.05$). The LR chi-square test demonstrated that the model provided a significantly better fit than the null model ($p < 0.001$). The Nagelkerke R^2 value supported the explanatory power of the model.

Discussion

In this study, at least 1 antimicrobial-related pDDI was identified in 29.9% of hospitalized adult patients receiving systemic antimicrobial therapy. Nearly one-third of these interactions were classified as category D or X, suggesting that they may have clinically important implications and warrant careful attention from a patient safety perspective. Furthermore, the finding that the total number of medications was the strongest independent predictor of pDDIs in the multivariable analysis highlights the important role of overall medication burden in the occurrence of these interactions.

The 29.9% prevalence of pDDIs identified in our study appears to be lower than the rates reported in studies evaluating drug interactions more broadly, likely because our analysis was limited to antimicrobial-related interactions. Indeed, studies investigating drug interactions at the hospital level have reported substantially higher prevalence rates. A multicenter prospective study conducted in Norway identified at least 1 drug-related problem in 81% of patients[13], whereas studies evaluating all drug interactions in medical intensive care units reported prevalence rates as high as 46.3%[14]. In contrast, lower rates have been reported in studies focusing specifically on antimicrobial-related interactions. In one study evaluating interactions between antimicrobials and other medications, the prevalence of pDDIs was 21.4%[15], whereas a multicenter point prevalence study conducted in Türkiye reported a prevalence of 22.7%[11]. However, prevalence rates appear to increase in specific patient populations with a high medication burden. The proportion of patients with at least 1 pDDI has been reported to reach 88% among intensive care unit patients receiving antimicrobials[16], while a prevalence of 42% was reported in a geriatric population[12]. Taken together, these findings suggest that the prevalence of pDDIs varies not only according to the scope of the analysis but also according to patient characteristics and, particularly, the burden of polypharmacy. Although the prevalence observed in our study was lower than that reported in broader analyses, its similarity to findings from antimicrobial-focused studies supports the potential influence of patient characteristics and medication burden on the occurrence of pDDIs.

The identification of the total number of medications as an independent predictor of pDDIs in the multivariable analysis suggests that the effects of factors such as age, number of antimicrobials, and intensive care unit hospitalization, which were significant in the univariable analysis, may be partly explained by their relationship with overall medication burden. The literature similarly identifies the number of medications as one of the strongest and most consistent predictors of DDIs[17,18]. Indeed, some variables, such as advanced age, have been shown to lose much of their independent predictive value after adjustment for the number of medications. Nevertheless, some studies have reported that advanced age and intensive care unit hospitalization remain independent risk factors regardless of medication count, and these discrepancies may be attributable to differences in patient populations, clinical settings, and analytical methods[15,19].

Table 1. Clinically higher-risk antimicrobial-related pDDIs (Categories D and X): Interaction mechanisms and potential clinical consequences.

Antimicrobial	Interacting drug	n	Risk rating	Interaction mechanism/clinical consequences
Clarithromycin	Papaverine hydrochloride	1	**X**	Pharmacodynamic additive effect; QT prolongation and torsades de pointes risk
	Nimodipine	1	**X**	CYP3A4 inhibition; increased drug exposure and hypotension risk
	Quetiapine	1	**X**	CYP3A4 inhibition; QT prolongation risk
	Amiodarone	1	**X**	Pharmacodynamic additive QT effect; QT prolongation and torsades de pointes risk
	Silodosin	1	**X**	CYP3A4 inhibition; increased drug exposure and hypotension risk
	Lercanidipine	2	**X**	CYP3A4 inhibition; increased drug exposure and hypotension risk
	Tamsulosin	1	**X**	CYP3A4 inhibition; increased tamsulosin exposure and hypotension risk
	Budesonide	5	**D**	CYP3A4 inhibition; increased systemic corticosteroid exposure and adrenal suppression risk
	Atorvastatin	4	**D**	CYP3A4/SLCO1B1 inhibition; increased atorvastatin exposure and rhabdomyolysis/myopathy risk
	Tolterodine	1	**D**	CYP3A4 inhibition (CYP2D6-poor metabolizers); increased drug exposure and anticholinergic toxicity risk
Cefuroxime axetil	Proton pump inhibitors	17	**D**	Increased gastric pH resulting in reduced absorption; decreased antibiotic efficacy
Ceftriaxone	Calcium salts	3	**D**	Precipitation with calcium; risk of vascular precipitate formation
Meropenem	Valproic acid	1	**D**	Reduced valproate enterohepatic recirculation; subtherapeutic valproic acid levels and seizure risk
Ciprofloxacin	Clozapine	1	**D**	CYP1A2 inhibition and additive QT effect; increased clozapine levels and QT prolongation risk
Levofloxacin	Quetiapine	3	**D**	Additive QT-prolonging effect; QT interval prolongation and torsades de pointes risk
Daptomycin	Statins	5	**D**	Additive skeletal muscle toxicity; myopathy and rhabdomyolysis risk
Metronidazole	Diazepam	1	**X**	Ethanol-acetaldehyde accumulation; disulfiram-like reaction
Linezolid	Tramadol	1	**X**	Linezolid MAOI-like activity with a serotonergic opioid; serotonin syndrome risk
	Remifentanil	5	**D**	Linezolid MAOI-like activity; serotonin syndrome and opioid toxicity (respiratory depression) risk
	Sympathomimetics	3	**D**	Linezolid MAOI-like activity; hypertensive crisis risk
Moxifloxacin	Iron preparations	1	**D**	Chelation forming an insoluble complex; reduced absorption and decreased antibiotic efficacy
	Magnesium salts	2	**D**	Chelation with insoluble complex formation; reduced antibiotic absorption
Fluconazole	Clopidogrel	1	**D**	CYP2C19 inhibition; reduced clopidogrel active metabolite and diminished antiplatelet effect
	Quetiapine	1	**D**	CYP3A4 inhibition and additive QT effect; increased quetiapine exposure and QT prolongation risk
Posaconazole	Proton pump inhibitors	1	**D**	Increased gastric pH, reducing absorption; decreased posaconazole exposure and risk of prophylaxis failure
Voriconazole	Trazodone	1	**D**	CYP3A4 inhibition; increased trazodone exposure, sedation, and QT prolongation risk

pDDI, potential drug-drug interaction; CYP, cytochrome P450; CYP1A2, cytochrome P450 1A2; CYP2C19, cytochrome P450 2C19; CYP2D6, cytochrome P450 2D6; CYP3A4, cytochrome P450 3A4; D, therapy modification recommended; MAOI, monoamine oxidase inhibitor; n, number of identified interactions; QT, QT interval; SLCO1B1, solute carrier organic anion transporter family member 1B1; X, avoid combination.

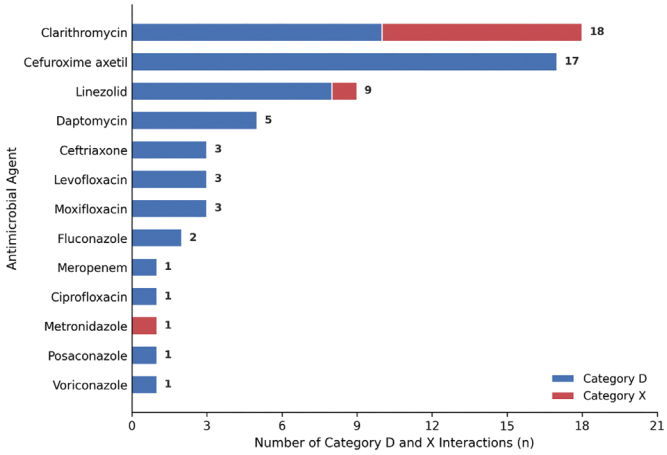

Figure 1. Distribution of clinically higher-risk (categories D and X) antimicrobial-related pDDIs according to antimicrobial agent. Blue bars represent category D interactions (therapy modification recommended), whereas red bars represent category X interactions (avoid combination). pDDIs, potential drug-drug interactions.

In our study, each 1-unit increase in the total number of medications was associated with approximately 1.40-fold higher odds of pDDIs, indicating a cumulative increase in interaction risk. This finding is consistent with the literature[20] and supports prioritizing systematic interaction screening, particularly among patients receiving multiple medications.

The finding that female sex was associated with lower odds of pDDIs in the multivariable analysis is noteworthy. The relationship between sex and the risk of DDIs remains unclear in the literature. While some studies have reported a higher risk of DDIs among women, others have found no significant association[21]. In our study, this finding may be related to sex-specific differences in comorbidity distribution, prescribing patterns, or clinical presentation. However, given the observational design of the study, this association should not be interpreted as causal.

The finding that intensive care unit hospitalization was positively associated with pDDIs in the univariable analysis but inversely associated with pDDIs in the multivariable model may initially appear counterintuitive. Patients admitted to the intensive care unit had a higher medication burden, and the total number of medications was the strongest independent predictor of pDDIs. Therefore, adjustment for medication burden may have attenuated the independent contribution of intensive care unit hospitalization and contributed to the reversal in the direction of the association. Marked multicollinearity is unlikely to explain this finding, as the VIF values were below 2 for all variables included in the model. Accordingly, the adjusted association should not be interpreted as evidence of a true protective effect, and residual confounding may also have contributed to this finding.

In our study, the majority of antimicrobial-related pDDIs were classified as category C (68%), followed by categories D (27%) and X (5%). This distribution is consistent with previous reports showing that most interactions fall within category C[22,23]. Nevertheless, the finding that category D and X interactions together accounted for 32% of all identified pDDI pairs is clinically important. Previous studies have reported rates ranging from 8% to 32% for category D interactions and from 1% to 13% for category X interactions[24-27]. The relatively high proportion of category D interactions observed in our study may be related to the heterogeneous nature of the study population, high medication burden, and widespread antimicrobial use. Although the proportion of category X interactions in our study (5%) appears consistent with previously reported rates, their

Table 2. Comparison of patients with and without pDDIs.

Variable	Patients without pDDIs (n = 263)	Patients with pDDIs (n = 112)	p value
Age, years	62 (46-76)	71 (55.8-80)	**<0.001**
Sex			**0.029**
Male	132 (50.2)	70 (62.5)	
Female	131 (49.8)	42 (37.5)	
Ward type			**0.041**
General ward	221 (84.0)	84 (75.0)	
Intensive care unit	42 (16.0)	28 (25.0)	
Length of hospital stay, days	4 (1-7)	6 (3-12.3)	**<0.001**
Total number of medications	5 (4-7)	9.5 (7-12)	**<0.001**
Number of antimicrobials	1 (1-1)	1 (1-2)	**<0.001**
Polypharmacy			**<0.001**
No	114 (43.3)	9 (8.0)	
Yes	149 (56.6)	103 (92.0)	

Data are presented as median (IQR) or n (%).IQR, interquartile range; pDDI, potential drug-drug interaction.

Table 3. Univariable and multivariable logistic regression analyses of factors associated with the development of pDDIs.

	Univariable		Multivariable	
Characteristics	**OR (95% CI)**	**p value**	**OR (95% CI)**	**p value**
Age (per 1-year increase)	1.02 (1.01-1.03)	<0.001	1.00 (0.99-1.02)	0.601
Sex				
Male	Reference	—	Reference	—
Female	0.60 (0.38–0.95)	0.029	0.57 (0.33-0.95)	0.034
Ward type				
General ward	Reference	—	Reference	—
Intensive care unit	1.75 (1.02-3.00)	0.041	0.50 (0.25-0.98)	0.049
Length of hospital stay (per 1-day increase)	1.02 (1.00-1.04)	0.078	—	—
Total number of medications	1.39 (1.29-1.51)	<0.001	1.40 (1.28-1.54)	<0.001
Number of antimicrobials	2.45 (1.69-3.61)	<0.001	1.36 (0.89-2.09)	0.156
Polypharmacy				
No	Reference	—	—	—
Yes	8.76 (4.47-19.3)	<0.001	—	—

CI, confidence interval; OR, odds ratio; pDDI, potential drug-drug interaction.

potential clinical importance should not be underestimated because these interactions represent contraindicated combinations. These findings support the need for systematic evaluation of DDIs, particularly when planning antimicrobial therapy for high-risk patients.

When all risk categories were considered, the highest numbers of interactions were associated with ceftriaxone (n = 44), clarithromycin (n = 33), and levofloxacin (n = 18). This finding may be explained by the widespread clinical use of these agents. Although ceftriaxone was the most frequently implicated agent, most of the interactions involving ceftriaxone were classified as category C, suggesting that their clinical impact may be limited. This finding is consistent with studies demonstrating that electronic interaction databases may identify a large number of low-risk interactions and that only a proportion of reported interactions are associated with actual clinical consequences[28].

Among the category D and X interactions, clarithromycin (n = 18), cefuroxime axetil (n = 17), and linezolid (n = 9) were the most frequently implicated agents. The prominent contribution of clarithromycin is likely related to its potent CYP3A4 inhibitory effect, and its interaction potential, particularly with statins and other CYP3A4 substrates, has been well described in the literature[29]. All interactions observed with cefuroxime axetil involved proton pump inhibitors, which is consistent with the pH-dependent absorption profile of this agent[30]. Given the widespread use of proton pump inhibitors in hospitalized patients, this interaction may be encountered frequently in routine clinical practice. Therefore, when prescribing cefuroxime axetil to patients receiving proton pump inhibitor therapy, clinicians should be aware of the potential for reduced drug absorption.

In addition, the finding that agents such as quinolones and linezolid were associated with higher-risk interaction categories despite their relatively lower frequency of use suggests that interaction risk does not necessarily parallel prescribing frequency. The interaction potential of quinolones through QT prolongation and cytochrome enzyme pathways, as well as the serotonergic interactions associated with linezolid through monoamine oxidase inhibition, may explain this finding[31,32]. These findings support the need for systematic evaluation of not only causative microorganisms and resistance patterns but also concomitant medication use when planning antimicrobial therapy.

However, these findings should be interpreted in light of an important limitation of the present study. Because only pDDIs were evaluated, treatment modifications, drug discontinuation, and adverse drug events associated with these interactions could not be assessed. Nevertheless, identifying high-risk antimicrobial-related pDDIs may facilitate timely medication review, support appropriate treatment modifications when necessary, and help prevent potentially avoidable adverse drug events. Therefore, integrating routine pDDI screening into antimicrobial stewardship programs and daily clinical practice may contribute to safer prescribing and improved patient safety, particularly among patients receiving multiple concomitant medications.

Study Limitations

This study has several limitations. Its cross-sectional and single-center design precludes assessment of causal relationships. Furthermore, because data were collected at a single time point, the findings represent a snapshot of prescribing practices and antimicrobial-related pDDIs on the study day and may not fully reflect temporal changes in prescribing patterns. In addition, the study was limited to the identification of pDDIs and did not capture whether these interactions resulted in clinically relevant consequences, including treatment modification, discontinuation of therapy, or adverse drug events. Therefore, the actual clinical significance of the identified pDDIs could not be determined. Furthermore, although Lexicomp is a widely used and regularly updated clinical decision support database, pDDI assessment was performed using a single electronic database. Differences in the identification and risk classification of DDIs among available databases may therefore have influenced the results. Nevertheless, our study provides contemporary real-world data on the burden of antimicrobial-related pDDIs and associated risk factors in a large hospital population.

Conclusion

In conclusion, antimicrobial-related pDDIs were identified in 29.9% of hospitalized adult patients, with 32% of the identified interactions classified as category D or X. Clinically important category D and X interactions were predominantly associated with clarithromycin, cefuroxime axetil, linezolid, and quinolones, highlighting the need for careful evaluation of concomitant medications when these agents are prescribed. The total number of medications was the strongest independent predictor of pDDIs, underscoring the central role of medication burden in interaction risk. These findings support the importance of systematic DDI screening during antimicrobial therapy. Accordingly, integrating routine pDDI screening into hospital antimicrobial stewardship programs, supported by multidisciplinary medication review involving clinical pharmacists, may facilitate the early identification and management of clinically higher-risk interactions and contribute to safer antimicrobial prescribing and improved patient safety.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital Ethics Committee (decision number: 3409; date: March 3, 2026) and was conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Because anonymized data obtained from hospital records were used, the requirement for informed consent was waived by the ethics committee.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.D.D., H.M.T., Concept: N.D.D., H.M.T., Design: N.D.D., H.M.T., Data Collection or Processing: N.D.D., H.M.T., Analysis or Interpretation: O.D., Writing: N.D.D.

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Antifungal Susceptibility Patterns and Minimum Inhibitory Concentrations of Clinically Important Yeasts Using Sensititre YeastOne in a Tertiary Care Cancer Hospital

Üçüncü Basamak Kanser Hastanesinde Klinik Açıdan Önemli Mayaların Sensititre YeastOne Kullanılarak Antifungal Duyarlılık Paternlerinin ve Minimum İnhibitör Konsantrasyonlarının Ölçülmesi

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Abstract

Introduction: To evaluate antifungal susceptibility patterns and minimum inhibitory concentrations (MICs) of clinically important yeasts.

Materials and Methods: This laboratory-based observational study, involving prospective collection of yeast isolates and retrospective analysis of anonymized laboratory data, was conducted from March to December 2024 at a tertiary care cancer hospital. A total of 100 non-duplicate yeast isolates were included. Yeast isolates were initially identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the clinical microbiology laboratory. All isolates were reidentified using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) targeting the internal transcribed spacer region of fungal ribosomal DNA . The accuracy of PCR-RFLP was validated by molecular sequencing of selected isolates, including rare and uncommon species. Antifungal susceptibility testing was performed using the Sensititre YeastOne Antifungal Susceptibility Test system. MIC values were interpreted according to applicable Clinical and Laboratory Standards Institute species-specific clinical breakpoints. MIC_{50} and MIC_{90} values were calculated.

Results: *Candida albicans* (37%) and *Candida tropicalis* (32%) were the most frequently isolated species. Blood was the predominant specimen source (43%), followed by urine (37%). Echinocandins exhibited the lowest MIC values across most species, indicating consistent *in vitro* activity. Fluconazole showed the widest MIC range (0.12-256 µg/mL) and the highest MIC_{90} values, particularly among *Candida glabrata* and *Candida krusei*. Azole agents demonstrated strong positive correlations in MIC values, whereas amphotericin B showed weak correlations with other antifungal classes.

Conclusion: Non-*albicans Candida* species constituted a substantial proportion of yeast isolates and exhibited variable azole susceptibility. These findings highlight the importance of species-level identification and susceptibility-guided antifungal therapy. Ongoing local surveillance and routine susceptibility testing remain essential for optimizing antifungal therapy.

Keywords: *Candida*, echinocandin, azole resistance, antifungal susceptibility, Sensititre YeastOne

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Öz

Giriş: Klinik açıdan önemli mayaların antifungal duyarlılık paternlerini ve minimum inhibitör konsantrasyonlarını (MIC) değerlendirmek.

Gereç ve Yöntem: Bu laboratuvar tabanlı gözlemsel çalışma, prospektif olarak toplanan maya izolatlarını ve anonimleştirilmiş laboratuvar verilerinin retrospektif analizini içermekte olup, Mart-Aralık 2024 tarihleri arasında üçüncü basamak bir kanser hastanesinde gerçekleştirilmiştir. Toplam 100 adet tekrarlanmayan maya izolatı dahil edilmiştir. Maya izolatları başlangıçta klinik mikrobiyoloji laboratuvarında MALDI-TOF MS kullanılarak tanımlanmıştır. Tüm izolatlar, mantar rDNA'sının ITS bölgesini hedefleyen PCR-RFLP kullanılarak yeniden tanımlanmıştır. PCR-RFLP'nin doğruluğu, nadir ve yaygın olmayan türler de dahil olmak üzere seçilen izolatların moleküler dizilemesi ile doğrulanmıştır. Antifungal duyarlılık testi, Sensititre YeastOne Antifungal Duyarlılık Testi sistemi kullanılarak gerçekleştirildi. MIC değerleri, ilgili CLSI türe özgü klinik kırılma noktalarına göre yorumlandı. MIC_{50} ve MIC_{90} hesaplandı.

Bulgular: *Candida albicans* (%37) ve *Candida tropicalis* (%32) en sık izole edilen türlerdi. Kan, en yaygın örnek kaynağıydı (%43), bunu idrar (%37) izledi. Ekinokandinler, tüm türlerde en düşük MIC değerlerini göstererek tutarlı *in vitro* aktivite sergiledi. Flukonazol, en geniş MIC aralığını (0,12-256 µg/mL) ve özellikle *Candida glabrata* ve *Candida krusei* üzerinde en yüksek MIC_{90} değerlerini gösterdi. Azol ajanları güçlü pozitif MIC korelasyonları gösterirken, amfoterisin B diğer antifungal sınıflarla zayıf korelasyon gösterdi.

Sonuç: *Candida albicans* dışı türler, maya izolatlarının önemli bir bölümünü oluşturmuş ve değişken azol duyarlılığı göstermiştir. Bu bulgular, mevcut tedavi kılavuzlarıyla uyumludur; ancak klinik yönetim, klinik bağlama göre bireyselleştirilmelidir. Antifungal tedaviyi en verimli hale getirmek için, sürekli yerel izleme ve rutin duyarlılık testleri hâlâ büyük önem taşımaktadır.

Anahtar Kelimeler: *Candida*, ekinokandin, azol direnci, antifungal duyarlılık, Sensititre YeastOne

Introduction

Invasive fungal infections are a major cause of morbidity and mortality among hospitalized patients, particularly those with malignancies, immunosuppression, or prolonged hospital stays[1,2]. Patients undergoing chemotherapy or intensive care are at increased risk of developing opportunistic fungal infections. Among these pathogens, *Candida* species are the leading cause of invasive fungal infections worldwide.

Over the past two decades, the epidemiology of candidiasis has changed substantially, with an increasing proportion of infections being caused by non-*albicans Candida* species. These species frequently exhibit reduced susceptibility to commonly used antifungal agents, particularly azoles, which may complicate the selection of appropriate antifungal therapy[3,4].

Several surveillance studies have demonstrated geographic variation in the distribution of *Candida* species. However, local data from cancer centers in Pakistan remain limited, particularly with respect to minimum inhibitory concentration (MIC) distributions and cross-class MIC correlations. In several Asian countries, *Candida tropicalis* has emerged as one of the predominant causes of candidemia[5,6].

Accurate identification of *Candida* species is essential because antifungal susceptibility profiles can vary substantially among species. Conventional phenotypic identification methods may not reliably distinguish closely related species, potentially affecting the selection of appropriate antifungal therapy. Molecular techniques, such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), provide rapid and reliable identification of medically important yeast species[7,8].

Antifungal susceptibility testing is critical for guiding appropriate therapy. The reference broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) is widely used for determining antifungal susceptibility; however, commercial systems such as the Sensititre YeastOne Antifungal Susceptibility Test offer a more convenient alternative and have demonstrated good correlation with reference methods[9,10]. Therefore, we aimed to characterize the species distribution, antifungal MIC patterns, and cross-drug MIC correlations among clinical yeast isolates obtained from a tertiary care cancer hospital.

Materials and Methods

Study Design and Sample Size

This laboratory-based observational study involved the prospective collection of yeast isolates and retrospective analysis of anonymized laboratory data. It was conducted from March to December 2024 at a tertiary care cancer hospital. A total of 100 non-duplicate clinical yeast isolates were included during the study period. The sample size was determined based on the feasibility of collection within the defined study period and is acknowledged as a limitation of the study. Ethical approval was obtained from Shaukat Khanum Memorial Trust Review Board (protocol number: IRB-23-46, date: 11.12.2023). Patient consent was waived by the Institutional Review Board because the patient data were analyzed retrospectively and all identifiers were removed before analysis.

Identification of Yeasts

Yeast isolates obtained from clinical specimens, including blood, urine, pus, tissue, and body fluids, were included in the study. All isolates were cultured on Sabouraud dextrose agar

and incubated at 35-37 °C for 24-48 hours before identification and antifungal susceptibility testing.

Initial species identification was performed using matrix-assisted laser desorption ionization–time-of-flight mass spectrometry in the clinical microbiology laboratory. All isolates were subsequently reidentified using PCR-RFLP targeting the internal transcribed spacer (ITS) region of fungal ribosomal DNA, a widely accepted universal DNA barcode marker for fungi[7,8]. PCR amplification was performed using the universal primers ITS1 and ITS4. The amplified products were digested with the restriction enzyme MspI to generate species-specific fragment patterns, which were analyzed by agarose gel electrophoresis and compared with reference profiles for species identification.

Rare and uncommon yeast species, such as *Kodamaea ohmeri* and *Dirkmeia churashimaensis*, were confirmed by Sanger sequencing.

Reference strains of *Candida albicans* (ATCC 10231), *Candida parapsilosis* (ATCC 90018), *Candida glabrata* (ATCC 15126), *Candida tropicalis* (ATCC 1369), and *Candida auris* (CDC B11903) were used as quality-control strains.

Antifungal Susceptibility Testing

Antifungal susceptibility testing of clinically significant yeasts was performed using the Sensititre™ YeastOne™ system (Thermo Fisher Scientific), which provides standardized, reproducible, and quantitative MIC results for multiple antifungal agents[9]. The antifungal panel included fluconazole, voriconazole, itraconazole, posaconazole, 5-flucytosine, amphotericin B, and the echinocandins caspofungin, micafungin, and anidulafungin.

A broad range of *Candida* species, including *Candida albicans, Candida famata, Candida glabrata, Candida guilliermondii, Candida krusei, Candida orthopsilosis, Candida parapsilosis, Candida pelliculosa,* and *Candida tropicalis*, as well as non-*Candida* yeasts such as *Papiliotrema laurentii, Cryptococcus neoformans,* and *Dirkmeia churashimaensis*, were tested.

A yeast suspension equivalent to 0.5 McFarland was prepared from a pure 24-hour culture. Subsequently, 20 µL of the suspension was transferred into 11 mL of YeastOne broth. Using the Sensititre™ AIM system, 100 µL of the inoculated broth was dispensed into each well of the Sensititre plate. The plates were sealed and incubated at 35 °C in ambient air and were read after 24 hours using the Sensititre™ Vizion system.

Individual MIC values were recorded for each antifungal agent. MIC results obtained using the Sensititre™ YeastOne™ system were interpreted using species-specific CLSI clinical breakpoints outlined in CLSI M60, Performance Standards for *Antifungal Susceptibility Testing of Yeasts*, with categorical interpretations performed only when established breakpoints were available [10,11].

CLSI clinical breakpoints have been established primarily for selected *Candida* species and specific antifungal agents. For non-*Candida* yeasts and organism–drug combinations for which validated breakpoints were unavailable, MIC values were reported descriptively without categorical interpretation. Where applicable, epidemiological cutoff values were considered to distinguish wild-type from non-wild-type isolates.

Statistical Analysis

MIC values were $\log_2$-transformed before analysis. MIC_{50}, MIC_{90}, and geometric mean MIC values were calculated for each antifungal agent.

Isolates with missing MIC values for a given antifungal agent were excluded from the calculations for that specific drug. All analyses were performed at the overall isolate level and were not stratified by clinical setting (e.g., intensive care unit, inpatient, or outpatient).

Pearson correlation coefficients were calculated to assess relationships among $\log_2$-transformed MIC values for azole and echinocandin agents. Amphotericin B was excluded from the correlation analysis because cross-class MIC correlations with azoles have limited clinical interpretability and no validated CLSI clinical breakpoint is available for amphotericin B in *Candida* species.

Results

Species Distribution and Specimen Sources

A total of 100 yeast isolates were included in the study. Blood (43%) and urine (37%) were the predominant specimen sources, followed by pus, tissue, tips, and other specimens.

The species distribution was as follows: *Candida albicans* (n = 37, 37%), *Candida tropicalis* (n = 32, 32%), *Candida krusei* (n = 6, 6%), *Candida famata* (n = 5, 5%), *Candida parapsilosis* (n=5, 5%), *Candida glabrata* (n = 4, 4%), and *Candida pelliculosa* (n = 3, 3%). Less frequently isolated species included *Cryptococcus neoformans* (n = 2), *Candida orthopsilosis* (n = 1), *Candida guilliermondii* (n=1), *Papiliotrema laurentii* (formerly *Cryptococcus laurentii*) (n = 1), *Kodamaea ohmeri* (n = 1), and *Dirkmeia churashimaensis* (n = 1). One mixed yeast infection was also identified. No clinical *Candida auris* isolates were identified during the study period.

Overall Antifungal Susceptibility Profile

The overall *in vitro* antifungal activity against all isolates is summarized in Table 1 and Figure 1.

The distribution of MIC values (Figure 1) demonstrated substantial variability for fluconazole, whereas the echinocandins exhibited tightly clustered low MIC values, indicating consistent *in vitro* activity.

Amphotericin B showed relatively narrow variability, suggesting consistent antifungal activity.

Fluconazole MICs ranged from 0.12 to 256 µg/mL, with an MIC_{50} of 1 µg/mL, an MIC_{90} of 4 µg/mL, and a geometric mean MIC of 1.56 µg/mL. Voriconazole, itraconazole, and posaconazole demonstrated low MIC_{50} values (≤0.06 µg/mL) with narrow MIC distributions. All three echinocandins—caspofungin, micafungin, and anidulafungin—showed excellent *in vitro* activity, with MIC_{50} values of 0.03 µg/mL and low geometric mean MICs. Amphotericin B retained consistent activity, with an MIC_{50} of 0.25 µg/mL.

Table 1. Overall geometric mean minimum inhibitory concentrations (MICs) of the antifungal agents tested against yeast isolates.

Drug	n	MIC_{50}	MIC_{90}	Geometric mean (rounded)
Fluconazole	89	1	4	1.5
Voriconazole	90	0.06	0.5	0.07
Caspofungin	90	0.03	0.12	0.04
Micafungin	90	0.03	0.06	0.04
Anidulafungin	90	0.03	0.12	0.03
Amphotericin B	90	0.25	0.5	0.3
Itraconazole	90	0.06	0.25	0.07
Posaconazole	90	0.06	0.25	0.05

n varies by antifungal agent because of missing values. MIC values were generated using the Sensititre™ YeastONE™ system; MIC_{50} and MIC_{90} values are reported using the nearest twofold dilution step available on the YeastONE tray.

MIC analysis included 89–90 isolates per antifungal agent, depending on the availability of valid MIC results. Isolates with missing MIC values were excluded only from the analysis of the respective drug.

CLSI categorical interpretations were applied only to *Candida* species for which established clinical breakpoints were available. For non-*Candida* yeasts, including *Cryptococcus* and other rare species, MIC values are presented descriptively because validated clinical breakpoints are unavailable.

Based on applicable CLSI species-specific clinical breakpoints, most *Candida albicans* isolates were categorized as susceptible to echinocandins and azoles for which applicable CLSI clinical breakpoints were available. Reduced fluconazole susceptibility was observed primarily among non-*albicans Candida* species. For *Candida glabrata*, fluconazole results were categorized as susceptible-dose dependent or resistant according to CLSI criteria.

Candida albicans: Isolates generally demonstrated low MICs for azoles and echinocandins. Fluconazole MICs ranged from 0.12 to 8.0 µg/mL, with an MIC_{50} of 1 µg/mL and an MIC_{90} of 4 µg/mL. All echinocandins demonstrated potent activity, with

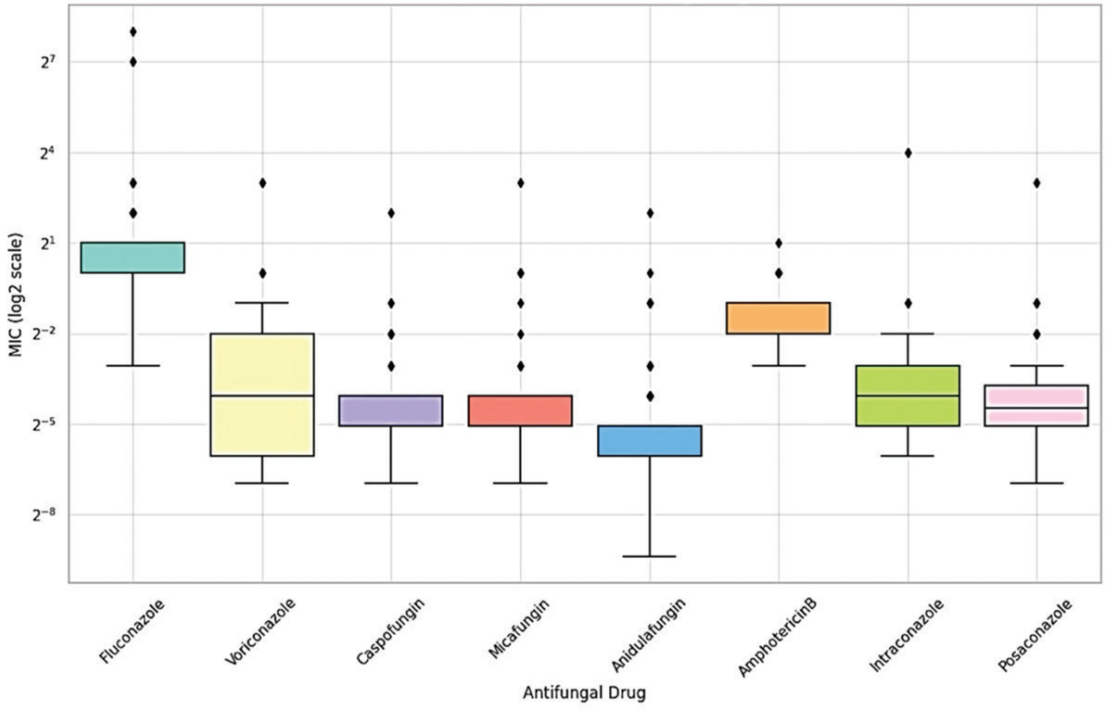

Figure 1. Boxplot showing the overall distribution of minimum inhibitory concentration (MIC) values (log_2 scale) for antifungal agents.

MIC_{50} values of ≤0.03 µg/mL (Table 2). Amphotericin B also demonstrated consistent activity.

Candida tropicalis: Isolates demonstrated preserved susceptibility to most antifungal agents. Fluconazole MICs ranged from 0.5 to 8.0 µg/mL, with MIC_{50} and MIC_{90} values of 1 µg/mL and 4 µg/mL, respectively.

Candida krusei: Isolates demonstrated intrinsically elevated fluconazole MICs (1–128 µg/mL), with an MIC_{50} of 64 µg/mL and an MIC_{90} of 128 µg/mL. Voriconazole and the echinocandins demonstrated lower MIC values.

Candida glabrata: Isolates exhibited intrinsically reduced susceptibility to fluconazole, with MICs ranging from 4 to 256 µg/mL and a high geometric mean MIC of 11.31 µg/mL.

Cryptococcus neoformans: Isolates showed a narrow fluconazole MIC range (0.5–1.0 µg/mL).

Other non-*Candida* species and rare yeasts demonstrated variable MIC distributions across the antifungal agents tested. For these yeasts, MIC values are presented descriptively only and should not be interpreted as indicating clinical susceptibility.

Most *Candida albicans* and *Candida tropicalis* isolates remained susceptible to fluconazole and the echinocandins, whereas *Candida glabrata* predominantly exhibited susceptible-dose dependent or resistant fluconazole phenotypes. *Candida krusei* was intrinsically resistant to fluconazole.

Percentages were calculated only for isolates with valid MIC results for which categorical interpretation was possible. Therefore, the totals may not correspond to the total number of isolates within a species because isolates with missing MIC values or without applicable CLSI breakpoints were excluded from the categorical analysis.

Comparative Analysis

Figure 2 presents a bubble plot showing the geometric mean MICs of the antifungal agents across yeast species.

Species-specific comparisons showed higher fluconazole MIC values for *Candida glabrata* and *Candida krusei*, whereas the echinocandins maintained low MIC values across most species. This pattern highlights the variability in azole susceptibility and the consistently low MICs observed with echinocandins.

Antifungal MIC Correlation Analysis

Figure 3 illustrates the pairwise Pearson correlation coefficients among antifungal MIC values. Strong correlations were observed only between certain azole pairs (fluconazole–itraconazole r = 0.78, fluconazole–posaconazole r = 0.78, itraconazole–posaconazole r = 0.86). Other azole pairs showed weaker correlations (e.g., voriconazole–itraconazole r = 0.20, voriconazole–posaconazole r = 0.20). Echinocandin correlations ranged from weak to moderate (r = −0.02 to 0.65). Amphotericin B was excluded from the correlation

Table 2. Species-specific geometric mean MICs (µg/mL) of antifungal agents against clinically relevant yeast species.

Species (n)	Amphotericin B	Anidulafungin	Caspofungin	Fluconazole	Itraconazole	Micafungin	Posaconazole	Voriconazole
Candida albicans (n = 37)	0.291	0.025	0.033	1.084	0.057	0.036	0.038	0.045
Candida famata (n = 5)	0.214	0.03	0.03	2.297	0.07	0.04	0.08	0.14
Candida glabrata (n = 4)	0.595	0.072	0.061	11.31	0.49	0.102	0.291	0.589
Candida krusei (n = 6)	0.376	0.034	0.046	13.45	0.16	0.046	0.092	0.162
Candida orthopsilosis (n = 1)	0.5	0.12	0.03	4	0.12	0.03	0.25	1
Candida parapsilosis (n = 5)	0.25	0.015	0.015	2	0.12	0.03	0.06	0.25
Candida pelliculosa (n = 3)	0.5	0.015	0.021	0.707	0.042	0.03	0.042	0.021
Candida tropicalis (n = 32)	0.284	0.029	0.04	1.368	0.072	0.042	0.051	0.058
Candida guilliermondii (n = 1)	NA	NA	NA	NA	NA	NA	NA	NA
Cryptococcus neoformans (n = 2)	0.25	0.042	0.015	0.707	0.042	0.03	0.03	0.03
Papiliotrema laurentii (n = 1)	NA	NA	NA	NA	NA	NA	NA	NA
Dirkmeia churashimaensis (n = 1)	0.25	0.015	0.03	4	0.25	0.03	0.5	0.12
Kodamaea ohmeri (n = 1)	0.25	0.03	0.03	1	0.03	0.06	0.008	0.03
Mixed yeast infection (n = 1)	NA	NA	NA	NA	NA	NA	NA	NA

NA, Not applicable geometric mean MIC could not be calculated because susceptibility results were unavailable for the isolate(s).

analysis because cross-class MIC correlations with azoles were considered to have limited clinical interpretability in the absence of validated clinical breakpoints and treatment-outcome data.

Discussion

The antifungal susceptibility patterns observed in this study support the continued role of echinocandins in the management of invasive candidiasis. Consistently low MIC values across most *Candida* species indicate preserved *in vitro* activity of this antifungal class, consistent with existing evidence demonstrating their potent activity and the globally low prevalence of echinocandin resistance, which is largely attributable to the low frequency of *FKS* gene mutations[12,13].

The relatively higher MICs observed in the *Candida parapsilosis* complex are consistent with its intrinsically reduced susceptibility, which is associated with naturally occurring polymorphisms in the β-(1,3)-D-glucan synthase enzyme rather than acquired resistance mechanisms[12,13].

The categorical susceptibility findings (Table 3) further support the preserved activity of echinocandins against the major *Candida* species. However, the greater variability in azole susceptibility, particularly among *Candida glabrata* and *Candida krusei*, underscores the importance of species-level identification and susceptibility-guided therapy.

Echinocandin resistance and antifungal susceptibility trends among *Candida* species have been widely studied in recent years, highlighting the emergence of resistance mechanisms

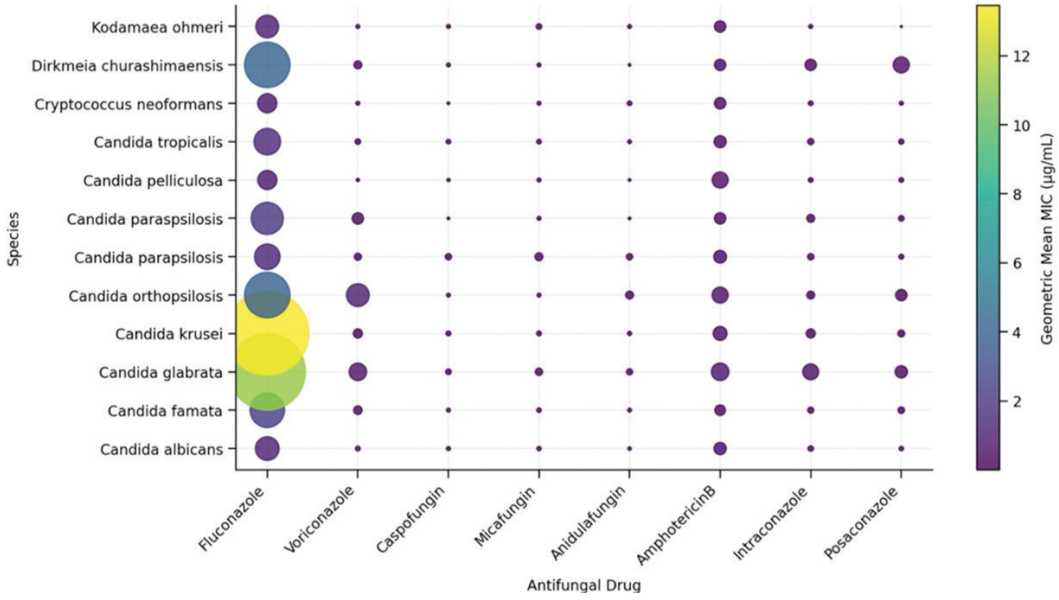

Figure 2. Species-wise antifungal susceptibility patterns based on geometric mean minimum inhibitory concentration values.

Table 3. Species-specific antifungal susceptibility categories among Candida isolates.

Species (n)	Fluconazole, S/SDD/R, n (%)	Voriconazole, S/I/R, n (%)	Anidulafungin, S/I/R, n (%)	Caspofungin, S/I/R, n (%)	Micafungin, S/I/R, n (%)
Candida albicans **(n = 37)**	29 (78.4) / 7 (18.9) / 1 (2.7)	26 (70.3) / 11 (29.7) / 0	32 (86.5) / 5 (13.5) / 0	33 (89.2) / 4 (10.8) / 0	33 (89.2) / 4 (10.8) / 0
Candida tropicalis **(n = 32)**	27 (84.4) / 3 (9.4) / 2 (6.2)	22 (68.8) / 10 (31.2) / 0	29 (90.6) / 3 (9.4) / 0	30 (93.8) / 2 (6.2) / 0	29 (90.6) / 3 (9.4) / 0
Candida glabrata **(n = 4)**	0 / 3 (75.0) / 1 (25.0)	NA	3 (75.0) / 1 (25.0) / 0	3 (75.0) / 1 (25.0) / 0	3 (75.0) / 1 (25.0) / 0
Candida krusei **(n = 6)**	IR: 6 (100)	4 (66.7) / 2 (33.3) / 0	6 (100) / 0 / 0	6 (100) / 0 / 0	6 (100) / 0 / 0
Candida parapsilosis **(n = 5)**	5 (100) / 0 / 0	3 (60.0) / 2 (40.0) / 0	5 (100) / 0 / 0	5 (100) / 0 / 0	5 (100) / 0 / 0
Other/rare yeasts	NA	NA	NA	NA	NA

S, susceptible; SDD, susceptible-dose dependent; I, intermediate; R, resistant; IR, intrinsic resistance; NA: indicates that no categorical interpretation was assigned because no applicable CLSI breakpoint. *Candida krusei* is intrinsically resistant to fluconazole (IR).

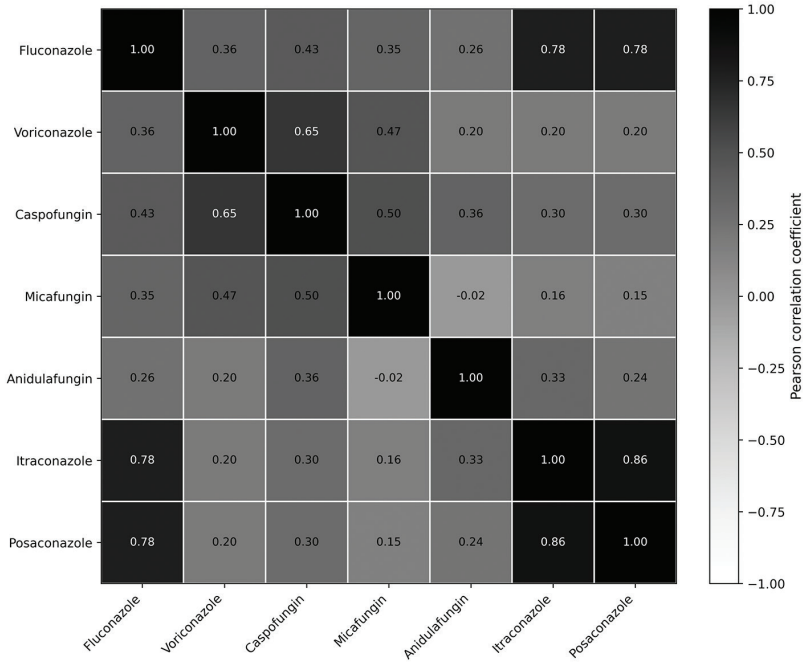

Figure 3. Correlation heat map showing pairwise relationships between minimum inhibitory concentration values of antifungal agents.

and the need for continued surveillance[14]. Amphotericin B maintained stable *in vitro* activity across most isolates in this study, consistent with previous reports indicating preserved susceptibility[15].

Overall, these findings are consistent with the role of echinocandins in empirical therapy for high-risk cancer patients, with subsequent therapy guided by species-level identification and local susceptibility patterns[16].

The antifungal MIC correlation analysis further supported these findings, with strong associations only between certain azole pairs (fluconazole–itraconazole, fluconazole–posaconazole, itraconazole–posaconazole), while other azole pairs were weakly correlated. *Echinocandin correlations* ranged from weak to moderate (r = −0.02 to 0.65). Amphotericin B was excluded from the correlation analysis because cross-class correlations with azoles have limited clinical interpretability in the absence of validated clinical breakpoints and treatment-outcome data. These findings highlight the value of considering species-specific susceptibility patterns when interpreting antifungal MIC data[17,18].

Clinical manifestations and treatment outcomes of candidemia vary according to the infecting *Candida* species, emphasizing the importance of species-level identification in guiding appropriate clinical management[19].

Consistently low MIC values across most *Candida* species indicate preserved *in vitro* activity of echinocandins, with no substantial evidence of reduced susceptibility in the isolates tested[20].

Azole antifungals, particularly fluconazole, demonstrated considerable variability in MIC values. Recent multicenter studies have reported increasing azole resistance among *Candida* species, particularly *Candida parapsilosis*[21]. Species-specific differences in efflux pump activity and alterations in ergosterol biosynthesis may contribute to reduced azole susceptibility, further emphasizing the importance of early species identification and susceptibility-guided antifungal therapy[22]. The high frequency of *Candida tropicalis* in our cohort is consistent with reports from Pakistan and India, where *Candida tropicalis* has been identified as a leading *Candida* species, including among patients with hematologic malignancies[23,24].

It is also important to note that *Candida glabrata* exhibits intrinsically reduced susceptibility to fluconazole, which should be considered when interpreting its elevated MICs. Its occurrence in oncology populations may be associated with factors such as neutropenia, prolonged hospitalization, broad-spectrum antibiotic exposure, and intensive healthcare exposure, although these factors were not specifically evaluated in our study.

From a clinical perspective, the susceptibility findings support the use of an echinocandin as appropriate empirical therapy for high-risk oncology patients with suspected invasive candidiasis, particularly given the consistently low

echinocandin MICs observed among the major *Candida* species. Subsequent therapy should be individualized based on species identification, susceptibility results, and clinical response, with de-escalation considered when clinically appropriate[16].

Rare yeast species identified in this study, including *Dirkmeia churashimaensis*, *Kodamaea ohmeri*, and *Papiliotrema laurentii*, are uncommon but recognized opportunistic human pathogens, particularly in immunocompromised and oncology patients. Reported infections include fungemia and catheter-related bloodstream infections, while *Dirkmeia churashimaensis* has also been associated with an outbreak of bloodstream infection[25-27]. The *Dirkmeia churashimaensis* and *Kodamaea ohmeri* isolates identified in our study were confirmed by sequencing. This molecular confirmation reduces the likelihood of species misidentification and supports the reliability of these uncommon findings. However, because these species were represented by single isolates and clinical outcome data were unavailable, their clinical significance in our cohort cannot be determined.

Study Limitations

This study has several limitations. First, it was conducted at a single center and included a relatively small sample size, which may limit the generalizability of the findings. Second, clinical outcome data were unavailable, precluding assessment of the relationship between antifungal susceptibility results and treatment response or patient outcomes. Third, the absence of CLSI clinical breakpoints for several species-drug combinations restricted categorical interpretation for some isolates. The lack of stratification by clinical setting may also limit the interpretation of setting-specific antifungal resistance patterns. Despite these limitations, the study provides valuable local epidemiological and antifungal susceptibility data that may inform clinical practice and future surveillance efforts. The recovery of single isolates of rare yeast species, including *Dirkmeia churashimaensis*, also limited species-specific assessment of their clinical significance and susceptibility patterns.

The taxonomy of *Candida famata* has undergone substantial revision, with isolates historically identified as *Candida famata* now largely assigned to *Debaryomyces hansenii* and related taxa. Therefore, species-level interpretation of the *Candida famata* isolates should be approached with caution[28].

Conclusion

This study highlights the predominance of non-*albicans Candida* species and the variability in antifungal susceptibility patterns in a tertiary care oncology setting. Echinocandins demonstrated consistent *in vitro* activity, whereas azole resistance remains a concern. These findings underscore the importance of species-level identification and routine antifungal susceptibility testing to guide appropriate antifungal therapy.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Shaukat Khanum Memorial Trust Review Board (protocol number: IRB-23-46, date: 11.12.2023).

Informed Consent: Patient consent was waived by the Institutional Review Board because the patient data were analyzed retrospectively and all identifiers were removed before analysis.

Footnotes

Authorship Contributions

Concept: H.F., A.R., F.S., Design: H.F., S.N., R.Q., Data Collection or Processing: H.F., S.N., N.M., M.M., A.F., M.I., Analysis or Interpretation: H.F., R.Q., S.N., N.M., A.R., M.M., A.F., M.I., F.S., Literature Search: R.Q., F.S., Writing: H.F., R.Q., S.N., N.M., A.R., M.M., A.F., M.I., F.S.

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CASE REPORT / OLGU SUNUMU

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Molecular identification of fatal *Clostridioides mangenotii* bloodstream infection in an immunocompromised patient: A case report

Bağışıklık sistemi baskılanmış bir hastada ölümcül *Clostridioides mangenotii* kan dolaşımı enfeksiyonunun genomik olarak tanımlanması: Bir vaka raporu

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Abstract

Immunocompromised patients are at increased risk of rare opportunistic and emerging infections that can be difficult to diagnose using conventional methods. Molecular approaches can facilitate the identification of unusual pathogens that warrant further investigation. A 36-year-old woman with metastatic invasive ductal carcinoma who had received broad-spectrum antimicrobials for recurrent febrile neutropenia was acutely admitted to the hospital with fever, tachycardia, and hypotension. Septic workup revealed anaerobic Gram-positive bacilli that could not be identified by Matrix-assisted laser desorption/ionization time-of-flight, whereas the VITEK 2 automated system suggested *Clostridioides difficile*. Despite broad-spectrum antibiotics targeting presumed clostridial infection, including piperacillin/tazobactam, meropenem, vancomycin, and metronidazole, her condition rapidly deteriorated to septic shock, and she died within 72 hours of its onset. Because of the unusual presentation and discordant microbiological results, 16S ribosomal RNA (rRNA) gene amplification and sequencing were performed. The isolate was provisionally identified as *Clostridioides mangenotii*. This environmental pathogen is closely related to *Clostridioides difficile* but has rarely been implicated in human disease. This case highlights the importance of molecular diagnostics in identifying rare invasive pathogens in immunocompromised hosts.

Keywords: *Clostridioides difficile, Clostridioides mangenotii*, bloodstream infection, immunocompromised

Öz

Bağışıklık sistemi baskılanmış hastalar, geleneksel yöntemlerle değerlendirilmesi zor olan nadir fırsatçı enfeksiyonlar ve yeni ortaya çıkan enfeksiyonlar açısından artmış risk altındadır. Moleküler yaklaşımlar, daha fazla araştırma gerektiren alışılmadık patojenlerin tanımlanmasını kolaylaştırabilir. Tekrarlayan ateşli nötropeni nedeniyle geniş spektrumlu antimikrobiyaller alan metastatik invaziv duktal karsinomlu 36 yaşında bir kadın, ateş, taşikardi ve hipotansiyon ile akut olarak hastaneye yatırıldı. Sepsis araştırması, MALDI-TOF ile tanımlanamayan anaerobik Gram-pozitif basiller ortaya koyarken, VITEK 2 otomatik sistemi *Clostridioides difficile*'yi düşündürdü. Geniş spektrumlu antibiyotikler (piperasilin/tazobaktam, meropenem, vankomisin ve metronidazol dahil) ile Clostridium enfeksiyonuna yönelik olası tedavilere rağmen, hasta hızla septik şoka girdi ve başlangıcından itibaren 72 saat içinde hayatını kaybetti. Olağandışı tablo ve uyumsuz mikrobiyolojik sonuçlar nedeniyle, 16S rRNA gen amplifikasyonu ve sekanslama yapıldı. İzolat geçici olarak *Clostridioides mangenotii* olarak tanımlandı. Bu çevresel patojen, *Clostridioides difficile* ile yakından ilişkilidir ancak insan hastalığında nadiren rol oynamıştır. Bu vaka, bağışıklık sistemi baskılanmış hastalarda nadir görülen invaziv patojenlerin tanımlanmasında moleküler tanılamanın önemini vurgulamaktadır.

Anahtar Kelimeler: *Clostridioides difficile, Clostridioides mangenotii*, kan dolaşımı enfeksiyonu, bağışıklık sistemi baskılanmış

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Introduction

Clostridioides mangenotii is a Gram-positive, spore-forming anaerobic bacillus that grows under mesophilic, strictly anaerobic conditions. Although closely related to *Clostridioides difficile*, genomic reclassification has identified it as a distinct species[1]. While *Clostridioides difficile* is a recognized human pathogen, *Clostridioides mangenotii* is primarily an environmental organism and has been isolated from soil, water, and animal stool[2]. Its endospore-forming ability enables it to survive under extreme environmental conditions, including temperature variation, pH shifts, chemical exposure, and radiation[3-5]. Although rare, members of the genus *Clostridioides* have been reported to cause invasive infections, such as bacteremia and septicemia[6]. The broader use of molecular diagnostics and the increasing number of immunocompromised patients may explain the increased recognition of these uncommon pathogens.

To our knowledge, *Clostridioides mangenotii* has not previously been reported as a cause of human bloodstream infection, and this may represent the first reported fatal case in an immunocompromised patient. After repeated failure of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) to identify the organism, it was provisionally identified by 16S ribosomal RNA (rRNA) sequencing and bioinformatic comparison with reference 16S databases.

Case Report

A 36-year-old woman with stage IV invasive ductal carcinoma metastatic to the lungs, lymph nodes, bones, and brain was receiving palliative gemcitabine/carboplatin and whole-brain radiotherapy. Four days after her second chemotherapy cycle, she was admitted with fever, myalgia, and malaise. Diagnosed with febrile neutropenia, she received piperacillin/tazobactam (4.5 g every 6 hours) and was discharged after neutrophil recovery and negative cultures.

Before her third cycle, she was readmitted with diffuse pain and general deterioration. She appeared unwell and distressed but was afebrile, with stable hemodynamics and oxygenation. Multiple enlarged lymph nodes were palpable in the cervical and inguinal regions. She had no diarrhea, abdominal pain, vomiting, or other gastrointestinal symptoms, and there was no clinical evidence of mucositis or colitis. Because she had no gastrointestinal symptoms, *Clostridioides difficile* toxin testing was not performed. She did not have a central venous catheter. On day 3 of admission, she developed fever (38.9°C), tachycardia (133 bpm), tachypnea (24/min), oxygen saturation of 92%-93% on room air, and hypotension (96/66 mm Hg). Blood cultures were obtained, and piperacillin/tazobactam (4.5 g every 6 hours) was started with intravenous fluids. Laboratory findings are summarized in Table 1.

Microbiological Investigations

Two sets of blood cultures were collected from two separate peripheral venipuncture sites (right and left upper limbs), with both anaerobic bottles flagging positive after 15 hours of incubation. Gram staining of the positive bottles demonstrated Gram-positive bacilli (Figure 1a). Subculture under anaerobic conditions yielded metronidazole-sensitive gray colonies after 48 hours on anaerobic blood agar (Figure 1b). MALDI-TOF mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Billerica, MA, USA) repeatedly failed to identify the organism, both directly from the positive blood culture bottles and from pure colony growth. The isolate was then tested using the VITEK 2 automated system (bioMérieux, Marcy-l'Étoile,

Table 1. Laboratory findings on admission and day 3.

Laboratory parameter	On admission	Day 3 of admission	Reference range
Hemoglobin	7.1 g/dL	7.4 g/dL	13–17 g/dL
WBC	5.3 × 10^3/µL	4.0 × 10^3/µL	4.0–10 × 10^3/µL
ANC	4.1 × 10^3/µL	3.1 × 10^3/µL	2.0–4.7 × 10^3/µL
Platelets	24 × 10^3/µL	16 × 10^3/µL	150–410 × 10^3/µL
Urea	4.8 mmol/L	1.8 mmol/L	2.5–7.8 mmol/L
Creatinine	37 µmol/L	26 µmol/L	62–106 µmol/L
ALT	57 U/L	50 U/L	0–41 U/L
AST	38 U/L	62 U/L	0–40 U/L
CRP	70 mg/L	242 mg/L	0–5 mg/L
Procalcitonin	0.09 ng/mL	0.57 ng/mL	—
Lactate	—	5.10 mmol/L	0.36–1.60 mmol/L
Fibrinogen	—	2.70 g/L	2–4 g/L

WBC, white blood cell; ANC, absolute neutrophil count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein.

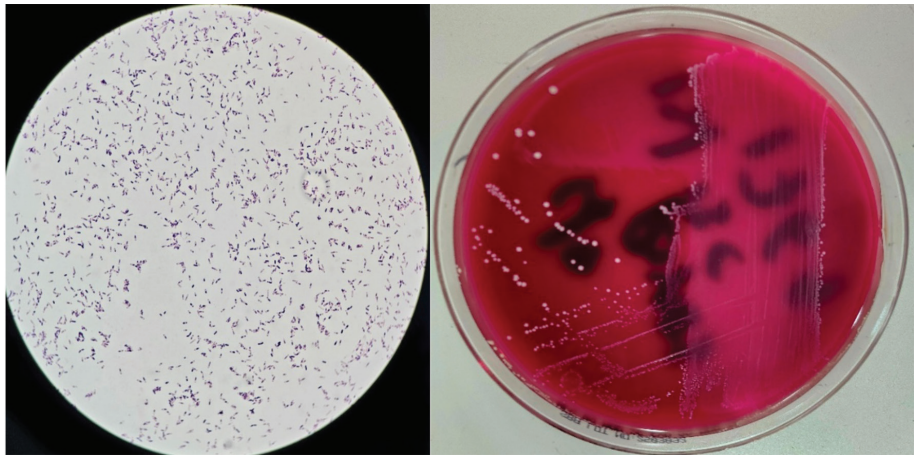

Figure 1. (a) Gram stain showing Gram-positive bacilli. (b) Colony morphology of *Clostridioides mangenotii* on anaerobic blood agar after 48 hours of incubation.

France), which identified it as *Clostridioides difficile* and reported susceptibility to amoxicillin-clavulanate, piperacillin/tazobactam, meropenem, and metronidazole, with resistance to clindamycin. Antimicrobial susceptibility results were interpreted according to Clinical and Laboratory Standards Institute criteria.

The patient continued to deteriorate, with hypotension, respiratory distress, altered mentation, and escalating oxygen requirements, progressing from supplemental oxygen via nasal cannula and a non-rebreather mask to non-invasive ventilatory support with continuous positive airway pressure. Her quick Sequential Organ Failure Assessment score was 3. Antibiotic therapy was escalated to meropenem (1 g every 8 hours) and intravenous metronidazole (500 mg every 8 hours), with the addition of oral vancomycin for presumed severe *Clostridioides difficile* infection. Vasopressor support was not initiated. Disseminated intravascular coagulation was not clinically suspected. Given her advanced metastatic malignancy and continued clinical deterioration, the family subsequently opted for comfort-focused management, and no further radiological investigations, laboratory testing, or invasive procedures were pursued. A do-not-attempt-resuscitation order was instituted. Despite broad-spectrum antimicrobial therapy and supportive care, she continued to deteriorate and died 72 hours after the onset of septic shock. An autopsy was not performed.

Molecular Identification of the Isolate

Because of the atypical clinical presentation, repeated failure of MALDI-TOF identification, and concern regarding possible misidentification by VITEK 2, molecular characterization of the isolate was undertaken. Genomic DNA was extracted from a colony of the bacterial isolate using the Qiagen Virus Mini Purification Kit 2.0 (Qiagen, Hilden, Germany) and quantified using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). A DNA concentration of 10 ng was achieved by buffer modification.

DNA was barcoded using the SQK-16S024 kit (Oxford Nanopore Technologies, Oxford, UK), which contains 16S-specific primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') for amplification of the complete 16S rRNA gene. Sequencing was performed on a MinION platform (Mk1C MC110319; Oxford Nanopore Technologies, Oxford, UK) using a FLO-MIN106D Spot-On Flow Cell (R9 version; Oxford Nanopore Technologies, Oxford, UK). A 50-µL reaction volume was used, and the purified library was loaded onto the flow cell according to the manufacturer's protocol.

Live base calling was performed using Guppy. Taxonomic assignment was generated using the wf-metagenomics workflow on the EPI2ME platform (Oxford Nanopore Technologies, Oxford, UK), with comparison against the National Center for Biotechnology Information 16S rRNA bacterial database. The isolate showed 95% 16S rRNA sequence homology with published *Clostridioides mangenotii* reference sequences and clustered with *Clostridioides mangenotii* in phylogenetic analysis (Figure 2). The 95% sequence homology is below the commonly accepted threshold for definitive species-level identification and was therefore interpreted in conjunction with the phylogenetic clustering. Additionally, a recent study reported 96.36% diagnostic accuracy for Nanopore 16S rRNA sequencing compared with Sanger sequencing, supporting its utility in identifying previously unrecognized bacterial isolates[7]. The sequencing data generated in this study were deposited in the Sequence Read Archive under accession number SRR34968737.

Review of Literature

Members of the genera *Clostridioides* and *Clostridium* are ubiquitous, Gram-positive, anaerobic, spore-forming rods

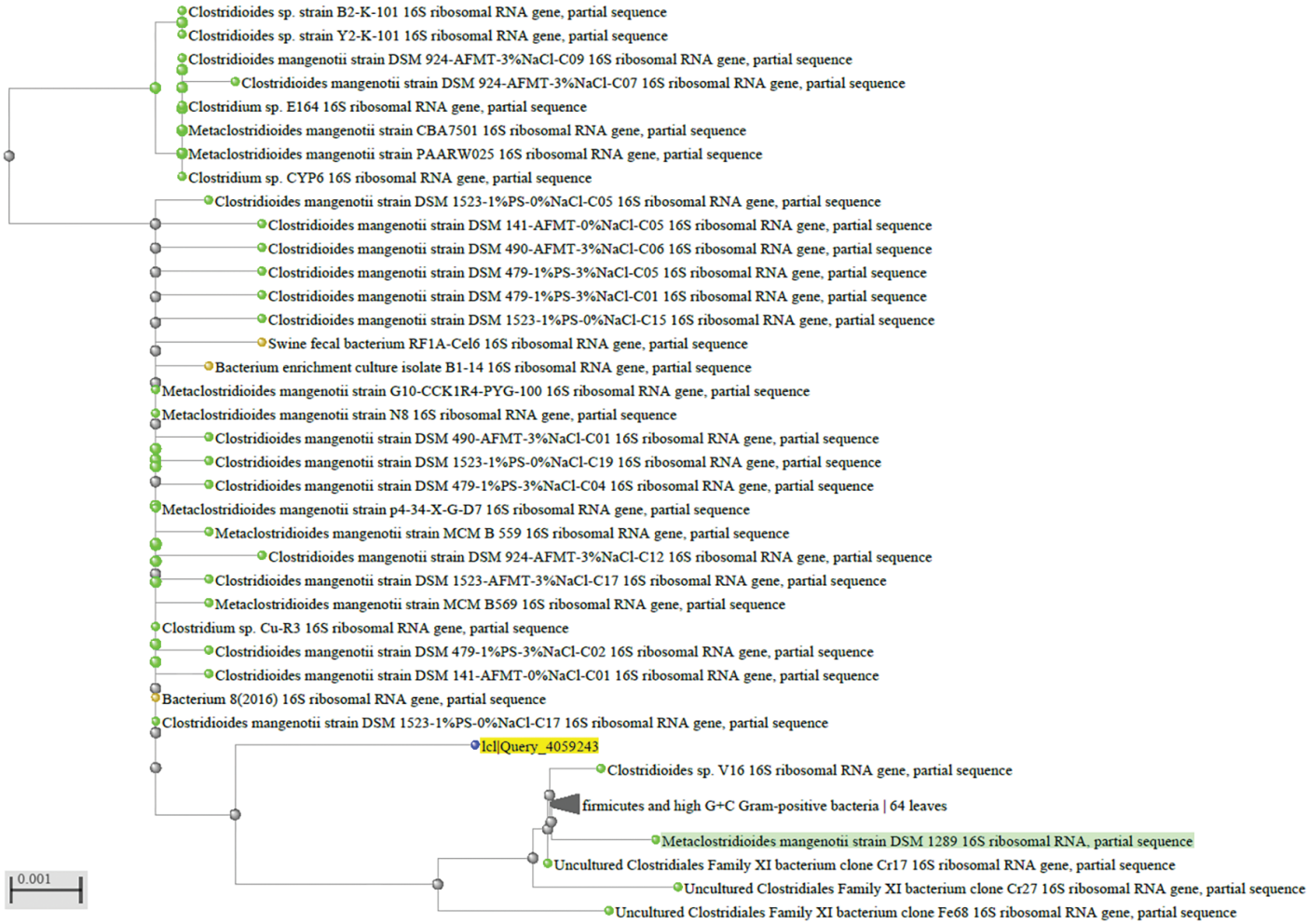

Figure 2. Phylogenetic identification of *Clostridioides mangenotii* using 16S rRNA sequencing.

found in soil, water, and the gastrointestinal tracts of animals and humans. These genera collectively contain more than 200 species, among which *Clostridioides difficile*, *Clostridium perfringens*, *Clostridium botulinum*, and *Clostridium tetani* are most commonly associated with human disease. Their key virulence traits include spore formation, which enhances environmental survival, and the production of potent exotoxins that contribute to the rapid and sometimes fatal course of infection[8].

Clostridioides mangenotii was initially grouped with *Clostridioides difficile* because of their close genotypic and phenotypic similarities. However, advances in molecular analysis, including whole-genome sequencing (WGS), have supported its classification as a distinct species. In 2016, Lawson et al.[1] analyzed 16S rRNA sequences and reported 95% identity between *Clostridioides difficile* and *Clostridioides mangenotii*, indicating a close phylogenetic relationship. In contrast, Knight et al.[9] demonstrated through whole-genome analysis that the two species share only 77% average nucleotide identity, well below the 95% threshold for species delineation, supporting their taxonomic separation. Subsequent phylogenomic studies further proposed reclassifying *Clostridioides mangenotii* into a new genus, *Metaclostridioides*, based on low amino acid identity and the presence of unique conserved signature indels[10].

The pathogen has primarily been isolated from environmental sources such as soil and animal feces but has not previously been associated with human infections[2]. Genomic analysis of the timber rattlesnake isolate revealed 68 genes related to virulence, defense mechanisms, and antimicrobial resistance, including resistance to vancomycin, fluoroquinolones, zinc, and arsenic[2]. Despite these findings, its virulence mechanisms remain poorly understood because of the rarity of reported cases. Among other uncommon clostridial pathogens, *Clostridium tertium* has been particularly associated with immunocompromised and neutropenic patients; in a recent

cohort, it accounted for 62 of 601 (10%) *Clostridium* species bacteremias, with 50% of patients having neutropenia or hematologic malignancy[11].

Discussion

Our patient initially presented with diffuse myalgia followed by fever and signs of sepsis. Despite early microbiological evidence of Gram-positive anaerobic bacteremia, conventional identification methods failed to provide a reliable diagnosis. MALDI-TOF repeatedly failed to identify the isolate, whereas VITEK 2 suggested *Clostridioides difficile*, leading to treatment directed at presumed severe clostridial infection. As her condition rapidly deteriorated with profound hypotension, respiratory distress, and altered consciousness, treatment was escalated to meropenem with additional agents directed against presumed severe *Clostridioides difficile* infection[12].

A major strength of this case is the use of molecular testing to resolve discordant conventional microbiological results. MALDI-TOF is a widely accepted rapid diagnostic tool for common bacterial pathogens, including Gram-positive anaerobes such as *Clostridioides difficile*, with accuracy approaching 100%[13]. The repeated failure to identify the isolate, despite its presence in the instrument library, suggests the possibility of a distinct or atypical species. Although the VITEK 2 system identified the organism as *Clostridioides difficile*, misidentification remains possible because of the close genetic homology between the two pathogens, and comparative studies have consistently demonstrated higher accuracy with MALDI-TOF[14]. Because of this discrepancy, the isolate underwent full-length 16S rRNA gene sequencing and phylogenetic analysis, which supported its provisional identification as *Clostridioides mangenotii*. However, WGS was not performed and would have enabled more robust species-level confirmation, representing a limitation of this case. Furthermore, the 95% 16S rRNA sequence homology is below the commonly accepted threshold for definitive species-level identification and should therefore be interpreted with caution.

The antimicrobial susceptibility results should also be interpreted carefully because the isolate was initially identified by VITEK 2 as *Clostridioides difficile*, and validated species-specific susceptibility criteria for *Clostridioides mangenotii* have not been established. The isolate was reported as susceptible to piperacillin/tazobactam, meropenem, and metronidazole, all of which were administered during the clinical course. Therefore, the poor clinical outcome was likely multifactorial, reflecting the patient's advanced metastatic malignancy, profound immunocompromised status, and severity of sepsis.

The absence of documented human infections and limited understanding of the pathogenesis of *Clostridioides mangenotii* likely contributed to the diagnostic delay. This underscores the critical role of molecular diagnostics in detecting rare or unusual pathogens, particularly when conventional methods have limited capacity to identify uncommon or previously unrecognized pathogens[15]. This diagnostic challenge extends beyond rare anaerobic bacteria in hemato-oncological patients, with recent literature also highlighting unusual and emerging invasive fungal pathogens, including the *Fusarium proliferatum* species complex, which may pose significant diagnostic and therapeutic challenges in immunocompromised hosts[16].

Clinically, the patient presented with systemic signs of sepsis, including fever, altered consciousness, profound hypotension, and respiratory distress, progressing to multiorgan failure and death. This contrasts with primary *Clostridioides difficile* infection, which typically presents with gastrointestinal symptoms, mainly abdominal pain and diarrhea resulting from colitis. The absence of gastrointestinal symptoms in this case complicated the diagnosis. The patient's rapid deterioration despite aggressive therapy suggests that *Clostridioides mangenotii* may possess significant virulence and may be capable of causing severe sepsis and septic shock, particularly in immunocompromised individuals.

To our knowledge, this may represent the first reported human case of fatal *Clostridioides mangenotii* bloodstream infection in an immunocompromised patient. Its misidentification by conventional methods highlights the potential for diagnostic confusion with closely related organisms, underscoring the limitations of standard diagnostic methods and the need for broader adoption of molecular and genomic testing in atypical infections.

Given the pathogen's rarity, further research is warranted. Future studies should explore the pathogenic mechanisms of *Clostridioides mangenotii*, particularly the role of its 68 identified virulence-related genes. Investigating its antimicrobial resistance profile is crucial, particularly because validated species-specific susceptibility criteria are lacking. Furthermore, given the limited clinical data, additional case reports facilitated by advances in identification techniques and genomic studies will help improve understanding, evaluation, and management of this emerging pathogen.

Conclusion

Immunocompromised patients are particularly vulnerable to rare, opportunistic, and emerging infections that may evade detection by conventional methods. Incorporating molecular approaches can facilitate the identification of unusual pathogens that warrant further investigation. To our knowledge, this may represent the first reported human case of fatal *Clostridioides mangenotii* bloodstream infection in an immunocompromised patient.

Ethics

Informed Consent: Written informed consent was obtained from the patient's family for publication of this report in accordance with the journal's patient consent policy.

Footnotes

Authorship Contributions

Concept: S.Z., A.A.B., S.A.J., M.A.M., Design: S.Z., A.A.B., S.A.J., M.A.M., Data Collection or Processing: S.Z., P.C., Y.P.H., Analysis or Interpretation: S.Z., A.A.B., P.C., F.E., Y.P.H., Literature Search: S.Z., H.A., Writing: S.Z., H.A., P.C., F.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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Predictors of Mortality in *Staphylococcus aureus* Bacteremia: A Retrospective Cohort Study from a Tertiary-Care Center

Staphylococcus aureus Bakteriyemisinde Ölüm Oranını Belirleyen Faktörler: Üçüncü Basamak Hastaneden Retrospektif Kohort Çalışması

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Abstract

Introduction: *Staphylococcus aureus* bacteremia (SAB) remains a serious infectious condition associated with substantial early mortality despite advances in antimicrobial therapy. This study evaluated clinical, laboratory, and management-related factors associated with 28-day mortality among adults with SAB.

Materials and Methods: A total of 151 adult patients with clinically significant SAB identified by blood culture between January 1, 2023, and October 24, 2024, were retrospectively analyzed. The primary outcome was 28-day all-cause mortality. Univariable and multivariable logistic regression analyses were performed to identify independent predictors of mortality. Model discrimination was assessed using the area under the receiver operating characteristic curve (AUC).

Results: The median age of the cohort was 67 years (interquartile range, 55–78), 60% of patients were male, and 33% were managed in the intensive care unit (ICU). Overall mortality was 54.3%, and 28-day mortality was 40.7%. Compared with 28-day survivors, non-survivors were older and more frequently had ICU admission, neurological comorbidities, healthcare-associated infection, an elevated neutrophil-to-lymphocyte ratio, and higher serum lactate levels, whereas fever and chills were more frequent among survivors. Source control and infectious disease (ID) consultation within the first 24 hours were also more frequently provided to survivors. In multivariable analysis, advanced age [odds ratios (OR), 1.04; 95% confidence intervals (CI), 1.00–1.09], ICU admission (OR, 6.37; 95% CI, 1.85–24.2), and higher serum lactate levels (OR, 1.55 per mmol/L; 95% CI, 1.08–2.56) were independently associated with increased 28-day mortality. Source control and early ID consultation demonstrated protective trends after adjustment. The final model showed good discriminatory performance (AUC, 0.85; 95% CI, 0.75–0.95).

Conclusion: This study suggests that early mortality in SAB is driven primarily by host characteristics and disease severity rather than by microbiological resistance patterns alone. Advanced age, ICU admission, and elevated serum lactate were the strongest independent predictors of early mortality, whereas source control and early ID consultation demonstrated protective trends that did not reach statistical significance after adjustment. These findings support a standardized, multidisciplinary approach to SAB management that extends beyond antimicrobial therapy and emphasizes timely optimization of modifiable clinical care processes.

Keywords: *Staphylococcus aureus* bacteremia, mortality, intensive care unit, lactate, source-control, infectious disease consultation

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Öz

Giriş: *Staphylococcus aureus* bakteriyemisi (SAB), antimikrobiyal tedavideki gelişmelere rağmen erken dönemde yüksek mortalite ile seyreden ciddi bir enfeksiyondur. Bu çalışmada, erişkin SAB hastalarında 28 günlük mortalite ile ilişkili klinik, laboratuvar ve yönetimsel faktörler değerlendirildi.

Gereç ve Yöntem: 1 Ocak 2023 ile 24 Ekim 2024 tarihleri arasında kan kültürlerinde klinik olarak anlamlı SAB saptanan toplam 151 erişkin hasta retrospektif olarak incelendi. Birincil sonlanım noktası 28 günlük tüm nedenlere bağlı mortaliteydi. Bağımsız öngördürücüleri belirlemek amacıyla tek değişkenli ve çok değişkenli lojistik regresyon analizleri yapıldı. Modelin ayırt edici gücü, alıcı işletim karakteristiği eğrisi altında kalan alan (AUC) ile değerlendirildi.

Bulgular: Kohortun ortanca yaşı 67 yıl (çeyrekler arası açıklık: 55–78) olup hastaların %60'ı erkekti; %33'ü yoğun bakım ünitesinde izlendi. Genel mortalite %54,3, Yirmi sekiz günlük mortalite ise %40,7 idi. 28 günlük mortalite durumuna göre yapılan karşılaştırmalarda ileri yaş, yoğun bakım ünitesine yatış, nörolojik komorbidite, sağlık hizmeti ilişkili enfeksiyon, yüksek nötrofil-lenfosit oranı ve serum laktat düzeyi gruplar arasında farklılık gösterirken, ateş ve titreme 28 günlük mortalitesi olmayan hastalarda daha sık görüldü. Odak kontrolü ve ilk 24 saat içinde enfeksiyon hastalıkları konsültasyonu da 28 günlük mortalitesi olmayan hastalarda daha sıktı. Çok değişkenli analizde ileri yaş (olasılık oranı [OR] 1,04; %95 güven aralığı [GA] 1,00–1,09), yoğun bakım ünitesine yatış (OR 6,37; %95 GA 1,85–24,2) ve yüksek serum laktat düzeyi (mmol/L başına OR 1,55; %95 GA 1,08–2,56), 28 günlük mortalitenin bağımsız öngördürücüleri olarak saptandı. Odak kontrolü ve erken enfeksiyon hastalıkları konsültasyonu, düzeltme sonrasında koruyucu yönde eğilim gösterdi. Nihai model iyi ayırt edicilik gösterdi (AUC 0,85; %95 GA 0,75–0,95).

Sonuç: SAB'de erken mortalite, mikrobiyolojik direnç paternlerinden çok konakla ilişkili faktörler ve hastalık şiddeti tarafından belirlenmektedir. İleri yaş, yoğun bakım gereksinimi ve yüksek serum laktat düzeyi erken ölümün en güçlü bağımsız öngördürücüleri olarak öne çıkarken, odak kontrolü ve erken enfeksiyon hastalıkları konsültasyonu düzeltme sonrasında istatistiksel anlamlılığa ulaşmayan koruyucu eğilimler göstermiştir. Bulgular, SAB yönetiminde antibiyotik tedavisinin ötesine geçen, standartlaştırılmış ve multidisipliner bir yaklaşımı desteklemektedir.

Anahtar Kelimeler: *Staphylococcus aureus* bakteriyemisi, mortalite, yoğun bakım ünitesi, laktat, odak kontrolü, enfeksiyon hastalıkları konsültasyonu

Introduction

Staphylococcus aureus is one of the most important causative agents of both community-acquired and healthcare-associated infections, causing a broad spectrum of clinical conditions ranging from skin and soft-tissue infections to endocarditis, bacteremia, and sepsis. Despite advances in antimicrobial therapy and infection control practices, *Staphylococcus aureus* bacteremia (SAB) remains a serious clinical problem associated with substantial morbidity and mortality. Mortality rates reported in the literature generally range from 20% to 40%, depending on patients' comorbid conditions, the source of infection, and the timing and effectiveness of treatment[1,2].

Complications such as infective endocarditis, pneumonia, and deep tissue abscesses are relatively common and can substantially increase mortality. Comorbid conditions, including advanced age, malignancy, chronic kidney disease, diabetes mellitus, and immunosuppressive therapy, are also closely associated with poor prognosis. In addition, healthcare-associated infection, inappropriate empirical antibiotic therapy, and delays in source control have been identified as modifiable risk factors associated with increased mortality in patients with SAB[3,4].

In recent years, notable changes have been observed in the epidemiology of SAB. Although the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) has declined in many countries, methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteremia has been reported with increasing frequency, particularly among older individuals and those with multiple comorbidities. The narrowing difference in mortality between MRSA and MSSA bacteremia suggests that clinical outcomes cannot be explained solely by antimicrobial susceptibility and that early diagnosis, appropriate empirical therapy, and timely source control continue to play critical roles in patient prognosis[5,6].

Accurate identification of clinical and laboratory indicators is essential for predicting mortality risk. Persistent bacteremia, elevated C-reactive protein (CRP) or procalcitonin levels, lymphopenia, and delays in source control have been independently associated with mortality in patients with SAB[2]. However, data on the epidemiological characteristics, clinical course, and determinants of mortality in patients with SAB in Türkiye remain limited. Given the steadily increasing proportion of elderly patients and individuals with multiple comorbidities, generating local data is essential to inform and improve empirical treatment approaches, infection control strategies, and patient management algorithms.

In this context, the present study aimed to comprehensively evaluate the demographic, clinical, and laboratory characteristics of patients diagnosed with SAB at University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital and to identify factors associated with mortality.

Materials and Methods

Study Design and Setting

This single-center, retrospective cohort study included adult inpatients diagnosed with SAB at University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital between January 1, 2023, and October 24, 2024.

Study Population

All patients aged ≥18 years with at least one blood culture bottle positive for *Staphylococcus aureus* who received treatment for clinically significant bacteremia were eligible for inclusion. For patients with more than one episode of SAB, only the first episode was included in the analysis. Patients younger than 18 years, those with blood culture results considered to represent contamination, those with unavailable primary outcome data, and those with polymicrobial bacteremia in whom pathogens other than *Staphylococcus aureus* were considered to have significantly influenced the clinical course were excluded.

Definitions and Clinical Variables

The index time was defined as the time of collection of the first blood culture positive for *Staphylococcus aureus*. Antimicrobial susceptibility testing was performed using an automated system (e.g., VITEK 2, bioMérieux, France), and methicillin resistance was confirmed using cefoxitin (30 µg) disk diffusion testing. Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing criteria, and isolates were classified as MRSA or MSSA.

Persistent bacteremia was defined as continued growth of *Staphylococcus aureus* in blood cultures obtained 48–72 hours after initiation of appropriate antimicrobial therapy. Primary bloodstream infection was defined as SAB without an identifiable infectious focus after clinical and diagnostic evaluation. Early appropriate empirical therapy was defined as initiation of at least one antimicrobial agent with in vitro activity against the isolated strain within the first 24 hours after blood culture collection. Adequate source control was defined as removal of an infected intravascular catheter and/or performance of surgical intervention or drainage procedures, when indicated, preferably within the first 72 hours.

The timing of targeted antimicrobial therapy was categorized as initiation before SAB diagnosis, on the same day as SAB diagnosis, one day after diagnosis, or two or more days after diagnosis, with the day of blood culture collection used as the reference. Whether an infectious disease (ID) and clinical microbiology consultation was obtained within the first 24 hours was also recorded.

Data Collection

Data were retrospectively collected from electronic medical records, microbiology laboratory databases, infection control surveillance records, and pharmacy and antimicrobial utilization systems. The collected variables included demographic characteristics (age and sex); comorbidities (diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease, malignancy, and neurological diseases); clinical findings [intensive care unit (ICU) admission, fever, chills, and signs of sepsis or septic shock]; laboratory parameters [white blood cell count, neutrophil-to-lymphocyte ratio (NLR), CRP, procalcitonin, and serum lactate levels]; microbiological characteristics (MRSA or MSSA status); and management-related variables (empirical and targeted antimicrobial therapy, early ID consultation, echocardiography, and source-control interventions).

Outcomes

The primary outcome was all-cause mortality within 28 days after the index blood culture. Secondary outcomes included in-hospital mortality, ICU admission, length of hospital stays, and the development of complications, including persistent bacteremia and endocarditis. Patients who remained alive at the end of the 28-day follow-up period, including those who died subsequently after day 28, were classified as 28-day survivors. Death occurring on day 28 was considered a 28-day mortality event; thus, the 28-day follow-up period was defined as inclusive of day 28, with day 0 corresponding to the date of the index blood culture.

Ethical Approval

Ethical approval was obtained from the University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital Clinical Research Ethics Committee of the Health Practice and Research Center (approval number: 3305; date: January 6, 2026). Because of the retrospective nature of the study, individual informed consent was not obtained. The study protocol was reviewed and approved by the ethics committee with a waiver of the informed consent requirement.

Statistical Analysis

Data distributions were assessed to determine the appropriate statistical tests. Categorical variables were summarized as numbers and percentages, whereas continuous variables were reported as mean ± standard deviation or median [interquartile range (IQR)], as appropriate. Comparisons between 28-day survivors and non-survivors were performed using the chi-square test or Fisher's exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables.

Univariable logistic regression analyses were initially performed for all candidate variables to identify potential predictors of 28-day mortality. Variables associated with mortality at $p < 0.10$ in the univariable analyses, along with clinically relevant covariates selected a priori based on previous literature, were entered into the initial multivariable model. The final model was developed through manual model reduction without using automated forward, backward, or stepwise variable-selection procedures. Model refinement was based on adjusted effect estimates, likelihood ratio tests, assessment of multicollinearity, and clinical interpretability. Multicollinearity among predictors was evaluated using variance inflation factors. Model discrimination was assessed using the area under the receiver operating characteristic curve (AUC), with 95% confidence intervals (CIs) calculated using the DeLong method. Model calibration was evaluated using the Hosmer–Lemeshow goodness-of-fit test, calibration intercept and slope, and bootstrap-corrected calibration plots based on 1,000 bootstrap resamples. Overall model performance was further assessed using Akaike's Information Criterion (AIC), pseudo-R^2 statistics, the Brier score, and mean absolute calibration error. Potential clinical utility was evaluated using decision curve analysis (DCA) across a range of threshold probabilities. Results are presented as odds ratios (ORs) with 95% CIs, and a two-sided p value < 0.05 was considered statistically significant.

Statistical Software and Reporting

All statistical analyses were performed using R version 4.5.2 (R Foundation for Statistical Computing, Vienna, Austria) through RStudio/Posit Cloud. The gtsummary package was used to generate and report summary statistics and regression tables[7].

Results

Demographic and Clinical Characteristics of the Study Population

A total of 151 patients diagnosed with SAB were included in the study. One patient was excluded from the 28-day mortality analysis because of missing follow-up data. Among the 150 patients with available 28-day outcome data, 59.3% (n = 89) were male, and the median age was 67 years (IQR, 55–78). Of these patients, 66.7% (n = 100) were admitted to general medical wards, whereas 33.3% (n = 50) were managed in the ICU.

The most common comorbidities among the 150 patients with available 28-day outcome data were hypertension (52.0%, n = 78) and diabetes mellitus (44.0%, n = 66), followed by chronic kidney disease (28.0%), coronary artery disease (24.0%), malignancy (21.3%), and neurological comorbidities (18.7%). Microbiological evaluation identified MRSA in 33.3% of patients (n = 50) and MSSA in 66.7% (n = 100).

During the study period, the overall mortality rate was 54.3% (n = 82), whereas 28-day mortality was 40.7% (n = 61 among the 150 patients with complete 28-day follow-up data). One additional patient was confirmed to have died but had an unknown time of death and was therefore excluded from the 28-day mortality analysis. For the prespecified 28-day endpoint, the 20 patients who died after day 28 were classified as 28-day survivors. Accordingly, the binary 28-day outcome comprised 89 28-day survivors and 61 28-day non-survivors.

At presentation, the most frequently reported clinical symptoms were chills (30.0%) and fever (28.0%). Less frequent symptoms included nausea and vomiting (14.7%), decreased oral intake (11.3%), and rash (3.3%). With respect to risk factors, 28.7% of patients had an intravascular catheter in place, 6.0% had a history of trauma, and intravenous drug use was identified in 2.0% of cases.

Laboratory evaluation showed a median white blood cell count of 11,675 cells/μL (IQR, 8,600–17,350) and a median NLR of 15.0 (IQR, 7.5–22.0). Among the inflammatory markers, the median CRP level was 160.5 mg/L (IQR, 71.8–235.8), the median PCT level was 1.7 ng/mL (IQR, 0.4–8.0), and the median serum lactate level was 2.0 mmol/L (IQR, 1.3–2.8).

Evaluation of infection sources showed that catheter-related infections were the most common source (21.3%), followed by skin and soft-tissue infections (13.3%) and pneumonia (9.3%). Bone and joint infections were identified in 2.7% of patients, whereas central nervous system (CNS) infections were among the least frequent sources, occurring in 2.0% (n = 3) of patients. Endocarditis was detected in 10.0% (n = 4) of the 40 patients for whom echocardiographic data were available. No identifiable infectious focus was found in 75 patients (50%), who were therefore classified as having primary bloodstream infection (Table 1).

Comparisons Between 28-Day Survivors and Non-survivors

Comparisons of 28-day survivors and non-survivors revealed several significant differences between the two groups. ICU admission was substantially more frequent among 28-day non-survivors than among 28-day survivors (60.7% vs. 14.6%, $p < 0.001$).

A significant difference was also observed in age between the groups. The median age was higher among 28-day non-survivors than among 28-day survivors [72 (IQR, 64–81) vs. 61 (IQR, 50–73) years, $p < 0.001$].

Regarding underlying comorbidities, malignancy was more frequent among 28-day non-survivors than among 28-day survivors (26.2% vs. 18.0%), although the difference did not reach statistical significance ($p = 0.2$). In contrast, neurological comorbidities were significantly more common among 28-

Table 1. Baseline demographic, clinical, microbiological, and treatment characteristics of 28-day survivors and non-survivors.

Characteristic	n	Overall n = 150[1]	28-day survivors n = 89	28-day non-survivors n = 61	p value[2]
Admission location	150				**<0.001**
General ward		100 (66.7%)	76 (85.4%)	24 (39.3%)	
ICU		50 (33.3%)	13 (14.6%)	37 (60.7%)	
Sex	150				>0.9
Female		61 (40.7%)	36 (40.4%)	25 (41.0%)	
Male		89 (59.3%)	53 (59.6%)	36 (59.0%)	
Age, years	150	67 (55, 78)	61 (50, 73)	72 (64, 81)	**<0.001**
Comorbidities					
Diabetes mellitus	150	66 (44.0%)	40 (44.9%)	26 (42.6%)	0.8
Hypertension	150	78 (52.0%)	45 (50.6%)	33 (54.1%)	0.7
Chronic kidney disease	150	42 (28.0%)	26 (29.2%)	16 (26.2%)	0.7
Coronary artery disease	150	36 (24.0%)	17 (19.1%)	19 (31.1%)	0.090
Heart failure	150	16 (10.7%)	8 (9.0%)	8 (13.1%)	0.4
Malignancy	150	32 (21.3%)	16 (18.0%)	16 (26.2%)	0.2
Chronic obstructive pulmonary disease	150	13 (8.7%)	9 (10.1%)	4 (6.6%)	0.4
Heart valve dysfunction	150	5 (3.3%)	3 (3.4%)	2 (3.3%)	>0.9
Neurological comorbidity	150	28 (18.7%)	11 (12.4%)	17 (27.9%)	**0.017**
History of trauma	150	9 (6.0%)	4 (4.5%)	5 (8.2%)	0.5
Intravascular catheter	150	43 (28.7%)	29 (32.6%)	14 (23.0%)	0.2
Intravenous drug use	150	3 (2.0%)	3 (3.4%)	0 (0.0%)	0.3
Clinical symptoms					
Fever	150	42 (28.0%)	33 (37.1%)	9 (14.8%)	**0.003**
Chills	150	45 (30.0%)	35 (39.3%)	10 (16.4%)	**0.003**
Vertebral/hip pain	150	10 (6.7%)	8 (9.0%)	2 (3.3%)	0.2
Decreased oral intake	150	17 (11.3%)	7 (7.9%)	10 (16.4%)	0.11
Nausea/vomiting	150	22 (14.7%)	16 (18.0%)	6 (9.8%)	0.2
Rash	150	5 (3.3%)	3 (3.4%)	2 (3.3%)	>0.9
Laboratory parameters					
WBC, cells/µL	150	11,675 (8,600, 17,350)	11,220 (8,300, 16,150)	13,010 (8,710, 19,500)	0.2
Neutrophils, %	150	86.7 (80.4, 91.3)	85.5 (78.1, 91.0)	88.0 (85.0, 91.8)	0.017
Lymphocytes, %	150	6.5 (4.0, 11.1)	7.6 (4.3, 12.3)	5.4 (3.4, 8.9)	0.040
NLR	149	15.0 (7.5, 22.0)	12.0 (6.0, 21.0)	17.2 (10.0, 30.0)	**0.008**
Lactate, mmol/L	145	2.0 (1.3, 2.8)	1.8 (1.2, 2.5)	2.5 (1.5, 3.4)	**0.005**
CRP, mg/L	150	160.5 (71.8, 235.8)	146.0 (63.1, 227.3)	186.2 (86.5, 258.0)	0.10
PCT, ng/mL	132	1.7 (0.4, 8.0)	1.4 (0.3, 10.9)	2.2 (0.7, 8.0)	0.5
Microbiological characteristics					
Staphylococcus aureus resistance	150				0.8
MRSA		50 (33.3%)	29 (32.6%)	21 (34.4%)	
MSSA		100 (66.7%)	60 (67.4%)	40 (65.6%)	
Source of bacteremia					
Catheter-related infection	150	32 (21.3%)	22 (24.7%)	10 (16.4%)	0.2
Skin and soft-tissue infection	150	20 (13.3%)	17 (19.1%)	3 (4.9%)	**0.012**

Table 1. Continued.

Characteristic	n	Overall n = 150[1]	28-day survivors n = 89	28-day non-survivors n = 61	p value[2]
Pneumonia	150	14 (9.3%)	5 (5.6%)	9 (14.8%)	0.059
CNS infection	150	3 (2.0%)	1 (1.1%)	2 (3.3%)	0.6
Bone and joint infection	150	4 (2.7%)	2 (2.2%)	2 (3.3%)	>0.9
Endocarditis	40	4 (10.0%)	3 (12.5%)	1 (6.3%)	0.6
Primary bloodstream infection	150	75 (50%)	41 (46.1%)	34 (55.7%)	0.3
Infection acquisition	150				**0.007**
Community-associated infection		37 (24.7%)	29 (32.6%)	8 (13.1%)	
Healthcare-associated infection		113 (75.3%)	60 (67.4%)	53 (86.9%)	
Treatment and management					
Appropriate empiric therapy within 24 h	150	101 (67.3%)	63 (70.8%)	38 (62.3%)	0.3
Persistent bacteremia	84	48 (57.1%)	29 (49.2%)	19 (76.0%)	**0.023**
Infectious disease consultation within the first 24 h	150	112 (74.7%)	73 (82.0%)	39 (63.9%)	**0.012**
Targeted antibiotic therapy	146				0.3
None		4 (2.7%)	2 (2.3%)	2 (3.3%)	
Before SAB diagnosis		19 (13.0%)	15 (17.4%)	4 (6.7%)	
Same day as SAB diagnosis		77 (52.7%)	46 (53.5%)	31 (51.7%)	
1 day after SAB diagnosis		25 (17.1%)	13 (15.1%)	12 (20.0%)	
≥2 days after SAB diagnosis		21 (14.4%)	10 (11.6%)	11 (18.3%)	
Source control within 72 h	85				**0.007**
No		48 (56.5%)	27 (46.6%)	21 (77.8%)	
Yes		37 (43.5%)	31 (53.4%)	6 (22.2%)	
Surgical source-control procedure	90				**0.009**
No procedure required		43 (47.8%)	21 (35.6%)	22 (71.0%)	
Drainage/operation		14 (15.6%)	11 (18.6%)	3 (9.7%)	
Catheter removal or replacement		14 (15.6%)	13 (22.0%)	1 (3.2%)	
Procedure indicated but not performed		19 (21.1%)	14 (23.7%)	5 (16.1%)	

[1]n (%); median (Q1, Q3).

[2]Wilcoxon rank-sum test; Pearson's chi-squared test; Fisher's exact test, as appropriate.

Percentages for variables with missing data were calculated using available-case denominators. Patients who were alive at the end of the 28-day follow-up window, including those who subsequently died after day 28, were classified as 28-day survivors.

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus;* ICU, intensive care unit; NLR, neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; PCT, procalcitonin; IQR, interquartile range; COPD, chronic obstructive pulmonary disease; CNS, central nervous system; ID, infectious diseases.

day non-survivors than among survivors (27.9% vs. 12.4%, p = 0.017).

Analysis of clinical presentation showed that fever and chills were significantly more frequent among 28-day survivors. Fever was reported in 14.8% of 28-day non-survivors compared with 37.1% of 28-day survivors (p = 0.003). Similarly, chills were reported in 16.4% and 39.3% of the two groups, respectively (p = 0.003).

Comparison of laboratory parameters demonstrated a significantly higher NLR among 28-day non-survivors than among 28-day survivors [median, 17.2 (IQR, 10.0–30.0) vs. 12.0 (IQR, 6.0–21.0), p = 0.008]. Serum lactate levels were also significantly higher among 28-day non-survivors [median, 2.5 (IQR, 1.5–3.4) vs. 1.8 (IQR, 1.2–2.5) mmol/L, p = 0.005].

When the mode of infection acquisition was examined, healthcare-associated SAB was significantly more frequent among 28-day non-survivors than among 28-day survivors (86.9% vs. 67.4%, p = 0.007). However, the mode of infection acquisition was not independently associated with mortality after multivariable adjustment.

Regarding treatment and management, receipt of appropriate empirical antibiotic therapy within the first 24 hours was not significantly associated with 28-day mortality (70.8% among 28-day survivors vs. 62.3% among 28-day non-survivors, p

= 0.3). In contrast, ID consultation within the first 24 hours was significantly more frequent among 28-day survivors than among non-survivors (82.0% vs. 63.9%, p = 0.012).

Source control was achieved more frequently among 28-day survivors than among 28-day non-survivors (53.4% vs. 22.2% among patients with available source-control data, p = 0.007). Persistent bacteremia, in contrast, was more common among 28-day non-survivors than among survivors (76.0% vs. 49.2% among patients with available follow-up blood culture data, p = 0.023).

Variables with $p < 0.10$ in the univariable analyses, together with clinically relevant variables identified a priori, were entered into the initial candidate multivariable model. The final model was subsequently developed through clinically guided manual model reduction, without using automated forward, backward, or stepwise variable-selection procedures.

Multivariable Logistic Regression Analysis for 28-Day Mortality

Among the 150 patients with available 28-day follow-up data, 61 (40.7%) died within 28 days. In the multivariable logistic regression analysis, increasing age (OR, 1.04; 95% CI, 1.00–1.09; p = 0.050), ICU admission (OR, 6.37; 95% CI, 1.85–24.2; p = 0.004), and higher serum lactate levels (OR, 1.55 per mmol/L increase; 95% CI, 1.08–2.56; p = 0.044) were independently associated with increased odds of 28-day mortality. Source control (OR, 0.38; 95% CI, 0.10–1.31; p = 0.13) and ID consultation within the first 24 hours (OR, 0.26; 95% CI, 0.05–1.10; p = 0.071) yielded protective effect estimates but did not reach conventional statistical significance (Table 2).

No evidence of problematic multicollinearity was identified, with all VIF values <1.1. The final multivariable model demonstrated good discrimination, with an AUC of 0.85 (95% CI, 0.75–0.95). Model fit was satisfactory (AIC, 82.1; McFadden pseudo-R^2, 0.30; Nagelkerke R^2, 0.44), while calibration analyses indicated excellent agreement between predicted and observed risks (Hosmer–Lemeshow $\chi^2 = 4.21$, p = 0.84; calibration intercept ≈ 0; calibration slope = 1.00). Bootstrap-corrected calibration curves demonstrated minimal optimism, with a mean absolute calibration error of 0.044. Furthermore, DCA demonstrated a positive net clinical benefit across a broad range of clinically relevant threshold probabilities, supporting the potential clinical utility of the model for risk stratification in patients with SAB (Figure 1).

Discussion

In the present study, the mortality burden among adults with SAB was higher than that reported in much of the contemporary literature. The overall mortality rate was 82/151 (54%), whereas 28-day mortality was 61/150 (41%). In contrast, a large meta-analysis by Bai et al., which evaluated approximately 500,000 patients across 341 studies and examined 30-year mortality trends in SAB, demonstrated a substantial decline in 28-day mortality during the post-2011 period (18%) compared with the pre-2001 era (31%). However, despite this improvement in early mortality, the same analysis found that reductions in longer-term outcomes, including 3-month and 1-year mortality, had not yet reached clinically meaningful levels. Compared with the post-2011 data, mortality in our cohort remained notably higher[8]. Conversely, although the literature indicates that 30-day mortality in SAB generally ranges from approximately 15% to 31% across most series and has remained largely stable in recent years, mortality consistently increases with a greater proportion of critically ill patients and a higher comorbidity burden[9]. In our cohort, the relatively high

Table 2. Univariable and multivariable analyses of factors associated with 28-day mortality.

	Univariable				Multivariable		
Characteristic	n	OR	95% CI	p value	OR	95% CI	p value
Age	150	1.04	1.02, 1.07	<0.001	1.04	1.00, 1.09	0.050
Lactate	145	1.42	1.14, 1.88	0.006	1.55	1.08, 2.56	0.044
Source control	85						
No		1.00	—		—	—	
Yes		0.25	0.08, 0.67	0.009	0.38	0.10, 1.31	0.13
ID consultation within the first 24 h	150						
No		1.00	—		—	—	
Yes		0.39	0.18, 0.82	0.014	0.26	0.05, 1.10	0.071
ICU admission	150	9.01	4.23, 20.3	<0.001	6.37	1.85, 24.2	0.004

Reference category: No. Odds ratios represent the odds of 28-day mortality. Candidate variables initially evaluated for multivariable modeling included age, malignancy, neurological comorbidity, ICU admission, infection acquisition, source control, NLR, lactate, and early infectious disease consultation. CI, confidence interval; OR, odds ratio; ICU, intensive care unit; NLR, neutrophil-to-lymphocyte ratio; ID, infectious disease.

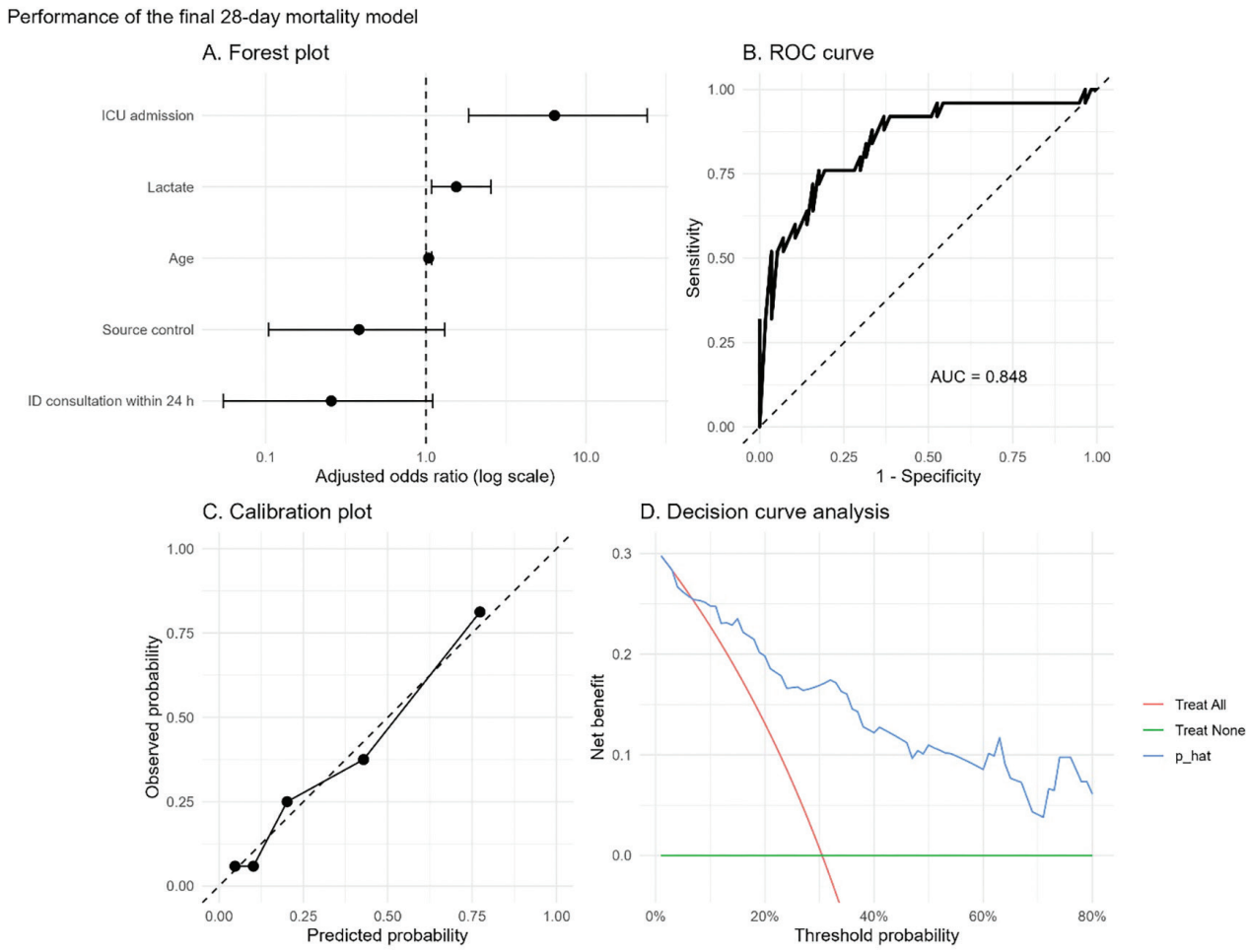

Figure 1. Performance of the final 28-day mortality model. (A) Forest plot of adjusted odds ratios from the multivariable model; (B) receiver operating characteristic (ROC) curve demonstrating an area under the curve (AUC) of 0.85; (C) calibration plot showing agreement between predicted and observed risks; and (D) decision curve analysis (DCA) demonstrating net clinical benefit across a range of threshold probabilities.

proportion of patients requiring ICU admission, together with the notable prevalence of conditions associated with increased vulnerability, such as malignancy, may help explain why the observed mortality rate approached the upper range reported in the literature. Regional differences may also contribute to variation in reported mortality rates. Despite recent improvements, when North America is used as a reference, the higher risk estimates reported from regions such as Europe and Asia suggest that mortality rates in these settings may remain above the global average[8]. These findings underscore the continued need for research and structured programs aimed at improving the quality of care for SAB, which remains associated with substantial mortality.

In our study, age emerged as an important determinant of mortality, consistent with previous literature. Increasing age was associated with mortality in the univariable analyses, and this association remained independent in the 28-day mortality model, with each additional year of age associated with an approximately 4% increase in the odds of mortality. Similarly, previous studies and meta-analyses have consistently identified increasing age as an important predictor of mortality in SAB[10]. Advanced age increases the risk of mortality through several mechanisms, including immune system decline, reduced physiological reserve, and the accumulation of comorbid conditions[10,11]. However, in a case–control study conducted by Tacconelli et al.[12], mortality was higher among older patients despite otherwise comparable characteristics, including comorbid conditions, suggesting that advanced age may independently predict mortality irrespective of comorbidity burden.

The requirement for ICU admission, one of the strongest indicators of clinical severity, emerged as one of the strongest independent predictors of mortality in the final multivariable model among patients with SAB. ICU admission occurred in

60.7% of patients who died within 28 days compared with 14.6% of those who survived beyond 28 days, underscoring the important role of disease severity in determining early outcomes. This finding was also supported by the univariable analysis, in which ICU admission was associated with markedly increased odds of 28-day mortality, and by the multivariable analysis, in which it remained the strongest independent predictor. ICU admission has consistently been identified as one of the most robust predictors of mortality in SAB[9]. In particular, among patients with SAB presenting with a septic clinical picture, the early development of hemodynamic instability and organ dysfunction contributes substantially to early mortality[1].

In patients admitted to the ICU, infection is often at an advanced stage by the time of diagnosis, with delayed source control and an increased risk of persistent bacteremia, all of which are major contributors to adverse outcomes. Consistent with these findings, previous studies have demonstrated that sepsis and septic shock associated with SAB independently increase mortality and that survival is substantially reduced among patients requiring ICU admission[4,13].

In light of these findings, the need for ICU admission in patients with SAB may serve as an early marker of increased mortality risk[11].

In contrast to NLR, serum lactate remained independently associated with 28-day mortality in the final multivariable model, consistent with its established role as a marker of tissue hypoperfusion and sepsis severity. Elevated lactate reflects impaired tissue perfusion and mitochondrial dysfunction associated with sepsis-related organ dysfunction, and persistent lactate elevation is a well-established predictor of poor outcomes across diverse infectious etiologies. These findings support incorporating lactate, together with ICU admission and age, into early risk-stratification strategies for patients with SAB[14].

In our cohort, source control was achieved more frequently among 28-day survivors than among 28-day non-survivors, supporting the concept that prognosis may depend less on the presence of an infectious source itself than on its early recognition and effective control[15-17]. In the final multivariable model for 28-day mortality, source control showed a similarly protective point estimate but did not reach conventional statistical significance after adjustment for the other variables included in the model. Although statistical significance was not retained after adjustment, the consistent direction of the association suggests that source control may remain clinically relevant. This attenuation may reflect the reduced statistical power of the analytic sample (n = 82) because of missing source-control data, as well as residual confounding by disease severity; patients who are too unstable to undergo source-control procedures may inherently have a higher risk of death regardless of the intervention.

ID consultation within the first 24 hours was more frequent among 28-day survivors than among 28-day non-survivors. In the final multivariable model, early ID consultation retained a protective effect estimate that approached but did not reach conventional statistical significance. Given the wide CI, this finding should be interpreted cautiously, particularly in the context of the reduced complete-case analytic sample. Nevertheless, the direction of the association is consistent with previous evidence indicating that ID consultation improves several components of SAB management[18,19].

In our study, fever and chills were significantly more frequent among 28-day survivors than among 28-day non-survivors. These findings should not be interpreted as independent protective effects because fever and chills were not included as candidate variables in the multivariable model. Instead, their association with survival may reflect earlier recognition of infection, more prompt presentation for medical care, and timely initiation of diagnostic and therapeutic interventions.

Conversely, in vulnerable populations—particularly older patients, those with malignancy, and those requiring intensive care—SAB may present with attenuated or atypical symptoms, potentially resulting in diagnostic delays and poorer outcomes. Consistent with previous reports, the absence or blunted expression of fever has been associated with poorer prognosis in SAB, underscoring that the absence of fever or chills should not be considered a reassuring clinical feature in this setting[1,4,9].

Although both the NLR and serum lactate levels were significantly higher among 28-day non-survivors than among 28-day survivors, these biomarkers diverged in the multivariable analysis. This finding may reflect their shared roles as early indicators of systemic inflammation and tissue hypoperfusion in SAB. Both NLR and lactate were evaluated as candidate variables; however, lactate was retained in the final model, whereas NLR was not. CRP and procalcitonin were not significantly different between 28-day survivors and non-survivors in the unadjusted analyses. Collectively, these biomarkers may function as intermediate indicators associated with the dominant clinical determinants of outcome, particularly disease severity and the need for intensive care.

Several variables, including neurological comorbidity, healthcare-associated acquisition, fever, chills, and NLR, differed significantly between 28-day survivors and non-survivors. Neurological comorbidity, healthcare-associated acquisition, and NLR were evaluated as candidate variables but were not retained in the final model, whereas fever

and chills were not entered as candidate variables. These findings suggest that their unadjusted associations may have been partly explained by stronger determinants, including advanced age, ICU admission, serum lactate levels, and overall disease severity. Accordingly, these variables may remain clinically relevant for initial risk assessment but should not be interpreted as independent predictors of early mortality after adjustment.

Malignancy was numerically more frequent among 28-day non-survivors than among 28-day survivors (26.2% vs. 18.0%), although this difference was not statistically significant. Although malignancy is a recognized marker of host vulnerability in SAB, our findings suggest that its association with early mortality was weaker than those of age, ICU requirement, and serum lactate in this cohort[8,10].

Although male sex predominated in our cohort, sex was not associated with mortality. This finding is consistent with evidence suggesting that outcomes in SAB are driven primarily by disease severity, comorbidity burden, and modifiable processes of care rather than by biological sex alone. However, in an observational study of 1,293 patients with SAB conducted between 1988 and 2007, mortality was higher among women than among men; notably, this excess mortality could not be explained by differences in demographic characteristics, underlying conditions, infection severity, management strategies, or septic complications[10]. Therefore, a definitive association between biological sex and mortality could not be established in our study.

Although MRSA accounted for 33% of cases in our cohort, MRSA status was not associated with mortality. Although several meta-analyses have reported a higher risk of mortality in MRSA bacteremia than in MSSA bacteremia, these findings are characterized by substantial heterogeneity[20,21]. Our findings support the concept that antimicrobial resistance phenotype alone does not determine mortality; rather, outcomes appear to be driven primarily by host vulnerability, particularly advanced age, and by markers of severe disease and tissue hypoperfusion, including ICU admission and elevated serum lactate[1].

In our study, healthcare-associated SAB was more frequent among 28-day non-survivors than among 28-day survivors. This finding is consistent with previous reports indicating that healthcare-associated SAB occurs more frequently among older patients and those with a greater burden of comorbidities and more severe clinical illness[22]. Accordingly, infection acquisition was not retained as an independent predictor in the multivariable analysis after adjustment for age, ICU admission, lactate, and other clinically relevant variables. The close association of healthcare-associated infections with invasive procedures, central venous catheters, and ICU admission may contribute to a more complex clinical context and poorer early outcomes. However, the lack of independent significance of infection origin in the multivariable analysis suggests that the adverse prognosis associated with healthcare-associated SAB may be driven less by the acquisition setting itself than by underlying host vulnerability and disease severity. These findings underscore the importance of interpreting infection origin in conjunction with clinical severity and management processes.

Risk factors such as the presence of intravascular catheters, a history of trauma, and intravenous drug use were observed in our cohort; however, catheter presence alone was not associated with mortality. The critical distinction lies not in the mere presence of a catheter but in whether the catheter represents the actual source of infection and whether timely removal and adequate source control are achieved[17]. In our study, although catheter-related infections constituted a substantial proportion of cases, mortality appeared to be driven not by any single risk factor but by the overall severity of illness and the quality and timeliness of clinical management.

The distribution of infectious foci differed between the outcome groups. Skin and soft-tissue infections were more frequent among 28-day survivors, whereas pneumonia showed a non-significant trend toward greater frequency among 28-day non-survivors. Catheter-related infection and endocarditis were not significantly associated with 28-day mortality in the present cohort. Nevertheless, previous studies have demonstrated that endovascular and deep-seated infectious foci are clinically important because of their association with persistent bacteremia, metastatic complications, and adverse outcomes[17,23].

Appropriate empirical antibiotic therapy within the first 24 hours was not significantly associated with 28-day mortality in our cohort. This finding should not be interpreted as evidence against the importance of timely, appropriate antimicrobial therapy[24]. Rather, it suggests that early outcomes in SAB are influenced by multiple interacting factors, including disease severity, source control, and other components of clinical management.

Persistent bacteremia was significantly more frequent among 28-day non-survivors than among 28-day survivors (76.0% vs. 49.2%, $p = 0.023$). However, because follow-up blood culture data were unavailable for a substantial proportion of patients and persistent bacteremia is closely interrelated with source control and disease severity, it was not included in the final multivariable model. Previous studies have consistently demonstrated that persistent SAB is associated with complications, metastatic foci, and increased mortality[3,25].

In this study, pathogen-directed antibiotic therapy did not emerge as an independent predictor of mortality. Its potential effect may have been attenuated in patients with inadequate source control, persistent bacteremia, or ICU requirement[15,16].

From a clinical utility perspective, the final model demonstrated good discrimination (AUC, 0.85; 95% CI, 0.75–0.95) and excellent calibration, indicating close agreement between predicted and observed risks. DCA further demonstrated potential net clinical benefit across a broad range of threshold probabilities, supporting the potential applicability of the model for risk stratification in patients with SAB. The independently associated predictors—age, ICU admission, and serum lactate—are routinely available in clinical practice, while the model also incorporates source control and early ID consultation as clinically meaningful management-related variables. This combination supports pragmatic bedside interpretation while acknowledging the reduced complete-case sample used for the multivariable analysis.

Strengths of the Study

A major strength of this study is the comprehensive evaluation of clinical, laboratory, and management-related variables among patients with SAB. The use of 28-day mortality as the primary outcome enabled a focused assessment of early deaths, which represent a particularly critical period in the clinical course of SAB. The final multivariable model demonstrated good discrimination and calibration, while DCA indicated potential clinical utility across a broad range of threshold probabilities using variables that are routinely available in clinical practice.

In addition, the inclusion of directly modifiable process-of-care factors, such as ID consultation, source control, and the appropriateness of empirical therapy, enhances the practical relevance of the findings. Thus, the study extends beyond prognostic description by providing potentially actionable insights for improving clinical management. Although conducted at a single center, the study reflects real-world data from a University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital with a high proportion of critically ill patients, making the findings particularly relevant to similar high-acuity settings. Collectively, these strengths position the study as a meaningful contribution to the existing literature on SAB, particularly regarding the early identification of high-risk patients and the implementation of targeted intervention strategies.

Study Limitations

Several limitations of this study should be acknowledged. First, the retrospective design inherently limits causal inference and is susceptible to missing data arising from routine clinical documentation. In particular, the relatively high proportion of missing data for source control, persistent bacteremia, and endocarditis limited the precision of analyses involving these variables. Source control was included in the final multivariable model, whereas persistent bacteremia and endocarditis were not included. Furthermore, the limited number of patients with documented endocarditis restricted our ability to fully assess the impact of this important complication on mortality. Because source control and other variables included in the model had missing observations, the final multivariable model for 28-day mortality was based on 82 patients with complete data for all variables included in the final model. This reduction in sample size limited statistical power and widened the CI around several estimates, particularly those for source control and ID consultation, and should be considered when interpreting the magnitude and precision of these associations.

Additionally, empirical and targeted antibiotic variables had to be simplified because of small sample sizes within certain subgroups, limiting a more detailed assessment of individual antimicrobial regimens. Finally, the single-center nature of the study may limit the generalizability of the findings. However, the advanced age and high comorbidity burden of the cohort may preserve their clinical relevance to tertiary-care and intensive care–oriented institutions.

Conclusion

Overall, these findings underscore that mortality in SAB reflects a complex interplay of host vulnerability, disease severity, and management-related factors rather than microbiological determinants alone. Advanced age, ICU admission, and elevated serum lactate emerged as the strongest independent predictors of 28-day mortality, whereas modifiable process-of-care factors, including timely source control and early ID consultation, demonstrated consistent protective trends. The fact that most deaths occurred within the first 28 days further underscores that SAB is a clinical condition requiring early, aggressive, and comprehensive management. In this context, implementing a standardized SAB management bundle that extends beyond antimicrobial therapy to incorporate early risk stratification, systematic source evaluation, and multidisciplinary follow-up may represent a rational strategy for reducing mortality.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital Clinical Research Ethics Committee of the Health Practice and Research Center (approval number: 3305; date: January 6, 2026).

Informed Consent: Because of the retrospective nature of the study, individual informed consent was not obtained. The study protocol was reviewed and approved by the ethics committee with a waiver of the informed consent requirement.

Footnotes

Authorship Contributions

Surgical and Medical Practices: C.A.T., S.A., O.D., Ö.G., N.D.D., H.M.T., A.Ö., D.Y.S., İ.D., Concept: C.A.T., S.A., O.D., Ö.G., A.Ö., D.Y.S., B.B., İ.D., Design: C.A.T., Ö.G., N.D.D., H.M.T., A.Ö., D.Y.S., B.B., İ.D., Data Collection or Processing: C.A.T., S.A., H.M.T., B.B., Analysis or Interpretation: O.D., Literature Search: C.A.T., Ö.G., H.M.T., Writing: C.A.T., N.D.D.

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Factors Influencing Influenza Vaccine Acceptance Among Healthcare Workers

Sağlık Çalışanlarında İnfluenza Aşısı Kabulünü Etkileyen Faktörler

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Abstract

Introduction: This study aimed to assess attitudes and vaccination behaviors among primary healthcare workers toward seasonal influenza vaccination and to examine the psychological antecedents underlying their vaccination decisions using the Psychological Antecedents of Vaccination (5C) framework.

Materials and Methods: This cross-sectional study included 181 healthcare workers employed at primary healthcare institutions affiliated with a provincial health directorate. Data were collected using a structured questionnaire assessing sociodemographic characteristics, knowledge, attitudes, and behaviors related to influenza vaccination, together with the seven-point Likert-type 5C scale. Participants who reported having received an influenza vaccine at least once in previous seasons were compared with those who had never been vaccinated. Participants were also compared according to their vaccination status during the 2025-2026 season. Variables showing significant associations in univariable analyses were subsequently entered into a multivariable binary logistic regression model.

Results: The participants had a mean age of 38.1 ± 7.2 years, and 75.1% were women. Overall, 33.1% had received an influenza vaccine at least once, whereas only 16.6% had been vaccinated during the 2025-2026 season. Participants who had received an influenza vaccine at least once had higher collective responsibility scores ($p = 0.045$). Those vaccinated during the current season had lower constraints scores ($p = 0.006$) and higher calculation scores ($p = 0.030$). Significant differences across occupational groups were observed in confidence, complacency, constraints, and collective responsibility ($p = 0.002$, $p < 0.001$, $p = 0.002$, and $p = 0.022$, respectively). In the multivariable analysis, recommending influenza vaccination [adjusted odds ratio (OR): 7.677; 95% confidence interval (CI), 3.024-19.490; $p < 0.001$], female sex (adjusted OR: 2.852; 95% CI, 1.115-7.296; $p = 0.029$), and being a physician (adjusted OR: 2.904; 95% CI, 1.160-7.272; $p = 0.023$) were independently associated with having received an influenza vaccine at least once.

Conclusion: Influenza vaccination uptake among primary healthcare workers was low, both historically and during the 2025-2026 season. Having recommended influenza vaccination to others, being female, and being a physician were independently associated with previous influenza vaccination, while higher collective responsibility was associated with vaccination history. These findings support the need for multicomponent interventions that address psychological, behavioral, and practical barriers to vaccination while promoting collective responsibility among healthcare workers.

Keywords: Influenza vaccines, health personnel, vaccination hesitancy, primary health care, attitude of health personnel

Öz

Amaç: Bu çalışmanın amacı, birinci basamak sağlık hizmetlerinde görev yapan sağlık çalışanlarının mevsimsel influenza aşısına yönelik tutum ve davranışlarını belirlemek ve aşılanma kararlarının psikolojik öncüllerini "Aşılanmanın Psikolojik Öncülleri (5C)" modeli çerçevesinde incelemektir.

Gereç ve Yöntemler: Kesitsel tipteki bu çalışma, bir il sağlık müdürlüğüne bağlı birinci basamak sağlık kurumlarında görev yapan 181 sağlık çalışanı ile yürütülmüştür. Veriler; sosyodemografik özellikler, influenza aşısına ilişkin bilgi, tutum ve davranışlar ile yedili Likert tipi 5C ölçeği'ni içeren

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yapılandırılmış bir anket ile toplanmıştır. Geçmiş sezonlarda en az bir kez aşılananlar hiç aşılanmayanlarla karşılaştırılmış, aynı özellikler 2025-2026 sezonundaki aşılanma durumuna göre de değerlendirilmiştir. Tek değişkenli analizlerde anlamlı bulunan değişkenler çok değişkenli ikili lojistik regresyon modeline alınmıştır.

Bulgular: Katılımcıların yaş ortalaması 38,1 ± 7,2 yıl olup %75,1'i kadındır. Katılımcıların %33,1'i en az bir kez influenza aşısı yaptırmış, 2025-2026 sezonunda aşılanma oranı ise yalnızca %16,6 olarak saptanmıştır. En az bir kez aşılananlarda kolektif sorumluluk puanı hiç aşılanmayanlara göre daha yüksek ($p = 0,045$); içinde bulunulan sezonda aşılananlarda ise kısıtlılık puanı daha düşük ($p = 0,006$) ve hesaplama puanı daha yüksek ($p = 0,030$) bulunmuştur. Meslek grupları arasında güven, kayıtsızlık, kısıtlılık ve kolektif sorumluluk alt boyutlarında anlamlı farklılık saptanmıştır (sırasıyla $p = 0,002$, $p < 0,001$, $p = 0,002$ ve $p = 0,022$). Çok değişkenli analizde influenza aşısı önermek [düzeltilmiş olasılık oranı (OR): 7,677; %95 güven aralığı (GA), 3,024-19,490; $p < 0,001$], kadın cinsiyet (düzeltilmiş OR: 2,852; %95 GA, 1,115-7,296; $p = 0,029$) ve hekim olmak (düzeltilmiş OR: 2,904; %95 GA, 1,160-7,272; $p = 0,023$) en az bir kez influenza aşısı yaptırmış olma ile bağımsız olarak ilişkili bulunmuştur.

Sonuç: Birinci basamak sağlık çalışanlarında influenza aşılanma oranları düşüktür. Aşılanma; aşıyı başkalarına önerme, kadın cinsiyet, hekim olmak ve daha yüksek kolektif sorumluluk ile ilişkili bulunmuştur. Psikolojik ve davranışsal belirleyicilerin yanı sıra uygulamadaki engelleri de ele alan ve kolektif sorumluluğu güçlendiren çok bileşenli müdahalelere ihtiyaç vardır.

Anahtar Kelimeler: İnfluenza aşıları, sağlık personeli, aşı kararsızlığı, birinci basamak sağlık hizmetleri, sağlık personelinin tutumu

Introduction

Influenza is a viral disease characterized by acute respiratory tract infection. The virus, which infects millions of people worldwide each year, causes approximately 290,000-650,000 deaths[1]. One in five unvaccinated children and one in ten unvaccinated adults contract seasonal influenza each year[2]. Although the symptoms of the disease can be mild, influenza can cause severe illness and death in high-risk individuals and is associated with high morbidity and mortality in children, older adults, individuals with chronic diseases, and pregnant women[1,3]. Due to its high transmission rate and pandemic potential, influenza poses a significant public health risk. Seasonal influenza vaccination reduces the occurrence of severe symptoms and hospitalization by providing immunity against the disease[4,5].

Healthcare workers are at a higher risk of seasonal influenza than other adults[6]. This occupational group, which plays a critical role in the nosocomial transmission cycle because of its close contact with infected patients, may transmit the virus to high-risk patients. Furthermore, significant workforce losses and associated economic burdens occur among healthcare personnel infected with seasonal influenza[7,8]. Considering these risks, seasonal influenza vaccination for healthcare workers is recommended by authorities in many countries, and in some countries, it is mandatory for employment[9]. A recent survey of 34 European countries found that 33 have an influenza vaccination policy for healthcare personnel, and 16 mandate at least one vaccination for this group, with nine requiring vaccination as a prerequisite for employment[10]. In Türkiye, healthcare workers are among the risk groups for whom the seasonal influenza vaccine is provided free of charge, and vaccination is voluntary[11].

Despite recommendations from health authorities and the free provision of the vaccine, seasonal influenza vaccine uptake among healthcare workers remains low[12,13]. In the most recent survey of European Union and European Economic Area countries, healthcare worker vaccination coverage was reported by only nine countries that recommended vaccination and ranged from 16.6% to 62.8%, with a median of 32%[14]. A systematic review and meta-analysis estimated the pooled influenza vaccine uptake among nurses at 44%[15], whereas coverage among healthcare personnel in the United States reached 76.3% in the 2024-2025 season[16]. Vaccine hesitancy is defined as a delay in acceptance or refusal of vaccination despite the availability of vaccination services[17]. In studies examining seasonal influenza vaccine hesitancy among healthcare workers, factors such as doubts about vaccine effectiveness, concerns regarding vaccine safety, and a low perceived personal risk of influenza have emerged as prominent factors[18,19]. Interventions designed to improve vaccine uptake have produced heterogeneous results, and their effectiveness appears to depend strongly on the behavior-change methods employed[20,21]. In this context, identifying the underlying causes of vaccine hesitancy among healthcare workers and developing effective strategies to address them are critically important for reducing influenza cases among both healthcare personnel and the general public.

The vaccine acceptance process is multidimensional, and the Psychological Antecedents of Vaccination (5C) model was developed by Betsch et al.[22]. The model conceptualizes vaccination decisions across five psychological dimensions: confidence, meaning trust in vaccines and the vaccination system; complacency, meaning a low perceived risk of vaccine-preventable diseases; constraints, meaning practical or psychological barriers to vaccination; calculation, meaning extensive searching for information about the risks and benefits of vaccination; and collective responsibility, meaning the willingness to protect others through one's own vaccination[22].

This framework provides a comprehensive approach to understanding the psychological factors underlying vaccination decisions and has been shown to explain vaccination behavior better than sociodemographic variables alone[23].

The aim of this study is to determine the factors affecting the attitudes and vaccination behaviors of healthcare workers regarding the seasonal influenza vaccine. Furthermore, this study aimed to investigate the psychological processes underlying participants' decisions to accept or reject the seasonal influenza vaccine based on the 5C model.

Materials and Methods

The population of this cross-sectional study consisted of physicians and allied healthcare personnel (midwives, nurses, health officers, medical secretaries, workers, and others) working at primary healthcare institutions affiliated with the İstanbul Provincial Health Directorate. The study aimed to reach the entire population (n = 222). To evaluate the applicability of the questionnaire developed by the researchers, a pilot study was conducted with 15 healthcare workers. The questionnaire, which was finalized after the pilot implementation, was administered using the supervised questionnaire method. This study was approved by İstanbul Medipol University Non-Interventional Clinical Research Ethics Committee (decision number: 1391, date: 13.11.2025). Written informed consent was obtained from all participants before administration. Data were collected over two days, March 2 and 3, 2026, toward the end of the 2025-2026 influenza season, so that vaccination status for that season could be considered essentially complete at the time of data collection. A total of 181 healthcare workers actively working in primary care settings who fully completed the questionnaire were included in the study, corresponding to a response rate of 81.5%.

Data Collection Tool

The questionnaire, prepared by the researchers based on a literature review, consists of 18 questions divided into three sections and is provided in full, along with an English translation, in Supplementary File 1.

Demographic information: This section consists of 10 questions regarding sociodemographic characteristics, such as age, sex, occupation, and educational status.

Knowledge, attitudes, and behaviors regarding vaccination: This section included questions aimed at evaluating participants' knowledge, attitudes, and behaviors related to vaccination. Vaccination training, influenza vaccine recommendation behavior, frequency of seasonal influenza vaccination, Coronavirus Disease 2019 (COVID-19) vaccination status, and whether the vaccinations of participants' children and/or first-degree relatives were complete were evaluated. Vaccination in previous seasons was assessed using a single item with three response options (vaccinated regularly every year, vaccinated occasionally but not regularly, or never vaccinated); participants who selected either of the first two options were classified as having been vaccinated at least once.

Psychological antecedents of vaccination (5C) scale: The psychological antecedents of vaccination (5C) scale was developed by Betsch et al.[22] based on the World Health Organization's 3C model[17]. The scale assesses five psychological antecedents that influence vaccination behavior. The confidence subdimension reflects trust in the effectiveness and safety of vaccines, the system that delivers them, and the motivations of policymakers; complacency reflects the perception that vaccine-preventable diseases pose a low risk and that vaccination is therefore unnecessary; constraints refer to perceived structural and psychological barriers that make vaccination difficult; calculation reflects the extent to which individuals engage in extensive information searching about the risks and benefits of vaccination; and collective responsibility reflects the willingness to protect others through one's own vaccination[22].

Each subdimension consists of three items, and the scale comprises a total of 15 items. The items are rated on a seven-point Likert-type scale (1 = strongly disagree, 7 = strongly agree). Therefore, each sub-dimension score ranges from 3 to 21, with higher scores indicating a stronger presence of the respective antecedent. In the original validation study, Cronbach's alpha coefficients for the subdimensions ranged from 0.71 to 0.85, whereas values ranging from 0.71 to 0.82 were reported in the Turkish validity and reliability study[24].

In the original validation study, higher confidence scores were positively associated with vaccination behavior, whereas higher complacency and constraints scores were negatively associated with vaccination behavior; calculation and collective responsibility were not independent predictors in that sample. The calculation subdimension reflects individuals' tendency to seek and evaluate information regarding the risks and benefits of vaccines[22].

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences for Windows (version 27.0; Armonk, NY: IBM Corp., USA). Descriptive statistics are presented as frequencies, percentages, means, and standard deviations. The normality of numerical variables was assessed using skewness and kurtosis values. Categorical variables were compared using the Pearson chi-square test, and Fisher's exact test was used when the minimum expected cell count was less than five. Student's t-test and one-way analysis of variance were used

to compare normally distributed numerical variables, and the Bonferroni test was used for post-hoc pairwise comparisons. For comparisons of the 5C subdimensions, professions were categorized into three groups: physicians, midwives/nurses, and other healthcare personnel (health officers, medical secretaries, workers, and others). For the binary logistic regression analysis, professions were dichotomized as physicians and non-physician healthcare workers. Sex, profession, and the behavior of recommending influenza vaccination, all of which were significant in univariable analyses, were entered into the primary multivariable binary logistic regression model using the enter method. Model fit was assessed using the omnibus test of model coefficients, the Hosmer–Lemeshow goodness-of-fit test, and the Nagelkerke R^2 value. Odds ratios (ORs) were presented with 95% confidence intervals (CIs). Statistical significance was set at $p < 0.05$.

Results

A total of 181 of the 222 eligible healthcare workers completed the questionnaire, corresponding to a response rate of 81.5%. The participants had a mean age of 38.1 ± 7.2 years (range, 23-63 years), and 75.1% (n = 136) were women. A university degree or higher was reported by 77.9% (n = 141) of the participants. Of the participants, 17.1% (n = 31) were physicians or specialist physicians, 23.8% (n = 43) were midwives or nurses, and 59.1% (n = 107) belonged to other occupational groups. In addition, 60.8% (n = 110) were married, and 50.3% (n = 91) had children.

Overall, 33.1% (n = 60) of the healthcare workers had received an influenza vaccine at least once, whereas only 16.6% (n = 30) had received the seasonal influenza vaccine during the 2025-2026 season. A COVID-19 vaccine had been received by 96.1% (n = 174) of the participants.

Participants who had received an influenza vaccine at least once (n = 60) were compared with those who had never been vaccinated (n = 121) (Table 1). The proportion of women was significantly higher among participants who had been vaccinated at least once than among those who had never been vaccinated (85.0% vs. 70.2%, p = 0.031). Similarly, the proportions of physicians (28.3% vs. 11.6%, p = 0.005) and participants who recommended influenza vaccination to their close contacts (90.0% vs. 49.6%, p < 0.001) were significantly higher in the previously vaccinated group. No significant differences were observed between the two groups in age, educational level, marital status, having children, presence of chronic disease, presence of chronic disease in a first-degree relative, receipt of vaccination training, COVID-19 vaccination status, or completeness of vaccinations among their children and/or first-degree relatives.

When the same characteristics were compared according to influenza vaccination status during the 2025-2026 season, no statistically significant association was observed with sex, educational level, marital status, having children, occupational group, presence of chronic disease, presence of chronic disease in a first-degree relative, or receipt of vaccination training (Supplementary Table 1). In contrast, participants who recommended influenza vaccination to their close contacts were significantly more likely to have received the influenza vaccine during the 2025-2026 season (p < 0.001). Furthermore, participants who had previously received an influenza vaccine at least once were significantly more likely to have received the vaccine during the 2025-2026 season than those who had never been vaccinated (p < 0.001). The 5C sub-dimension

Table 1. Comparison of participants' sociodemographic and vaccination-related characteristics according to history of influenza vaccination.

Characteristic	Never vaccinated (n = 121)	Vaccinated at least once (n = 60)	p
Age (years)*	38.7 ± 7.3	36.8 ± 6.9	0.081
Female	85 (70.2)	51 (85.0)	0.031
University degree or higher	92 (76.0)	49 (81.7)	0.390
Married	70 (57.9)	40 (66.7)	0.253
Having children	62 (51.2)	29 (48.3)	0.713
Physician/specialist physician	14 (11.6)	17 (28.3)	0.005
Presence of chronic disease	28 (23.1)	12 (20.0)	0.632
Chronic disease in a first-degree relative	81 (66.9)	39 (65.0)	0.795
Received vaccination training	18 (14.9)	12 (20.0)	0.383
Recommends influenza vaccination to close contacts	60 (49.6)	54 (90.0)	<0.001
Received COVID-19 vaccine†	115 (95.0)	59 (98.3)	0.428
Vaccinations complete for children and/or first-degree relatives†	115 (95.0)	56 (93.3)	0.732

Data are presented as n (%) unless otherwise indicated. *, Mean ± standard deviation, compared using Student's t-test; †, Fisher's exact test; all other categorical comparisons were performed using the Pearson chi-square test.

scores according to participant characteristics are presented in Table 2. No statistically significant differences were observed between women and men or between participants with different educational levels for any of the five subdimensions.

Statistically significant differences were observed among occupational groups in the confidence, complacency, constraints, and collective responsibility sub-dimension scores ($p = 0.002$, $p < 0.001$, $p = 0.002$, and $p = 0.022$, respectively). Post-hoc comparisons showed that complacency and constraints scores were significantly higher among other healthcare personnel (health officers, medical secretaries, workers, and others) than among both physicians and midwives/nurses. In contrast, confidence scores were significantly lower among other healthcare personnel than among midwives/nurses. Although the overall analysis of variance for collective responsibility was statistically significant, none of the pairwise comparisons reached statistical significance after Bonferroni correction.

Participants who had received the influenza vaccine during the 2025-2026 season had lower constraints scores (4.9 ± 2.3 vs. 6.8 ± 3.5, $p = 0.006$) and higher calculation scores (18.4 ± 2.9 vs. 16.8 ± 3.8, $p = 0.030$) than those who had not received the vaccine (Table 2).

When collective responsibility sub-dimension scores were examined according to seasonal influenza vaccination status in previous seasons, the mean score was significantly higher among participants who had been vaccinated at least once than among those who had never been vaccinated (17.8 ± 3.8 vs. 16.7 ± 3.6, $p = 0.045$). No statistically significant differences were observed between the two groups in the remaining four subdimensions.

Participants who recommended the influenza vaccine to their close contacts had higher confidence scores (17.1 ± 3.8 vs. 14.4 ± 4.2, $p < 0.001$) and collective responsibility scores (18.2 ± 2.7 vs. 15.1 ± 4.2, $p < 0.001$) and lower complacency scores (7.1 ± 3.2 vs. 8.5 ± 3.6, $p = 0.009$) than those who did not recommend the vaccine (Table 2).

The mean collective responsibility sub-dimension score was 17.2 ± 3.6 among participants who had received a COVID-19 vaccine and 13.4 ± 4.5 among those who had not ($p = 0.008$). Similarly, the mean complacency sub-dimension scores were 7.5 ± 3.4 and 10.6 ± 3.1, respectively ($p = 0.020$); only seven participants had not received a COVID-19 vaccine. In addition, participants who reported that their children's and/or first-degree relatives' vaccinations were complete had higher confidence and collective responsibility sub-dimension scores and lower complacency scores (all $p < 0.001$). Ten participants reported that these vaccinations were not complete (Table 2).

In univariable analyses, female sex, being a physician, and recommending influenza vaccination were significantly associated with having received an influenza vaccine at least once. Sex, profession, and vaccine recommendation were entered into the primary multivariable model (Table 3). Participants who recommended influenza vaccination had 7.7-fold higher odds of having received an influenza vaccine at least once than those who did not (adjusted OR: 7.677; 95% CI, 3.024-19.490; $p < 0.001$). Female sex (adjusted OR: 2.852; 95% CI, 1.115-7.296; $p = 0.029$) and being a physician (adjusted OR: 2.904; 95% CI, 1.160-7.272; $p = 0.023$) were also independently associated with vaccination. The model was statistically significant (omnibus test, $p < 0.001$), demonstrated adequate goodness of fit (Hosmer–Lemeshow test, $p = 0.849$), and explained 27.8% of the variance (Nagelkerke $R^2 = 0.278$; -2 log likelihood = 189.541).

Discussion

In this study, which included 181 healthcare workers employed in primary healthcare services, participants' attitudes toward the seasonal influenza vaccine and the factors affecting their vaccination behaviors were evaluated within the framework of the Psychological Antecedents of Vaccination (5C) model. The findings indicate that influenza vaccine acceptance among healthcare workers cannot be explained solely by sociodemographic characteristics; rather, psychological antecedents and behavioral factors are also associated with vaccination behavior.

Healthcare workers are at high risk for seasonal influenza infection because of their occupational exposure. However, despite recommendations from health authorities and the free provision of the vaccine in Türkiye, influenza vaccination rates among healthcare workers remain below desired levels[11]. In our study, the influenza vaccination rate during the 2025-2026 season was 16.6%, whereas 33.1% of participants had received an influenza vaccine at least once.

Our findings are consistent with the limited but growing body of national evidence. In a study of 370 healthcare workers at two hospitals in Türkiye, 36.1% reported having received an influenza vaccine at least once, a proportion similar to the 33.1% observed in our study[11]. In a more recent cross-sectional study of 428 healthcare workers at a university hospital in Türkiye, only 22% had received the influenza vaccine for the 2024 season, whereas 91% had received a COVID-19 vaccine[25]. A similar contrast was observed in our sample, in which 96.1% of participants had received a COVID-19 vaccine, compared with only 16.6% who had received the influenza vaccine during the current season. A recent national survey of adults in

Türkiye likewise reported an influenza vaccination rate of 28%, with being a healthcare worker identified as an independent predictor of vaccination[26]. Regional evidence points in the same direction: in a multinational study of 721 healthcare providers led from Türkiye, 28% were classified as hesitant regarding their own vaccination[27]. International figures vary considerably, ranging from a median of 32% across European countries reporting healthcare worker vaccination coverage[14] to 76.3% among healthcare personnel in the United States[16].

The recent experience of England provides an example of a multicomponent, institution-level strategy implemented in a setting where healthcare worker influenza vaccination uptake subsequently increased. During the 2025-2026 season, seasonal influenza vaccine uptake among frontline healthcare workers in National Health Service trusts in England reached 45.0% (523,410 of 1,162,139 staff), representing a 7.5-percentage-point increase from the 37.5% recorded in the previous season and the first national increase after four consecutive seasons of decline[28]. The national campaign preceding this

Table 2. Comparison of 5C psychological antecedents of vaccination sub-dimension scores according to participant characteristics.

Characteristic		Confidence	Complacency	Constraints	Calculation	Collective responsibility
Sex	Male (n = 45)	15.6 ± 4.2	8.3 ± 3.5	6.5 ± 3.2	17.5 ± 4.1	16.4 ± 3.7
	Female (n = 136)	16.3 ± 4.2	7.4 ± 3.3	6.5 ± 3.5	16.9 ± 3.6	17.3 ± 3.7
	p	0.324	0.138	0.945	0.353	0.156
Educational level	Vocational school or below (n = 40)	15.1 ± 4.6	8.3 ± 3.8	6.7 ± 3.2	17.2 ± 3.5	16.2 ± 3.7
	University degree or above (n = 141)	16.4 ± 4.0	7.5 ± 3.3	6.4 ± 3.5	17.0 ± 3.8	17.3 ± 3.7
	p	0.091	0.179	0.672	0.750	0.118
Profession	Physician (n = 31)	17.1 ± 5.0[a,b]	6.1 ± 2.0[a]	5.5 ± 2.7[a]	16.3 ± 5.1	17.9 ± 4.3
	Midwife/nurse (n = 43)	17.6 ± 3.0[b]	6.6 ± 3.4[a]	5.4 ± 2.8[a]	17.5 ± 2.7	18.0 ± 2.8
	Other healthcare personnel (n = 107)	15.2 ± 4.1[a]	8.5 ± 3.5[b]	7.2 ± 3.7[b]	17.1 ± 3.6	16.4 ± 3.7
	p	0.002	<0.001	0.002	0.341	0.022
Received vaccination training	Yes (n = 30)	16.8 ± 3.9	6.7 ± 3.0	5.6 ± 2.6	17.8 ± 3.5	18.2 ± 3.6
	No (n = 151)	16.0 ± 4.2	7.8 ± 3.5	6.7 ± 3.5	16.9 ± 3.8	16.8 ± 3.7
	p	0.300	0.100	0.116	0.274	0.055
Vaccinated during the 2025-2026 season	Yes (n = 30)	16.2 ± 4.6	7.9 ± 3.5	4.9 ± 2.3	18.4 ± 2.9	18.1 ± 3.0
	No (n = 151)	16.1 ± 4.1	7.6 ± 3.4	6.8 ± 3.5	16.8 ± 3.8	16.8 ± 3.8
	p	0.860	0.601	0.006	0.030	0.080
Influenza vaccination in previous seasons	Vaccinated at least once (n = 60)	16.2 ± 5.0	7.4 ± 3.7	6.8 ± 4.0	16.9 ± 4.3	17.8 ± 3.8
	Never vaccinated (n = 121)	16.1 ± 3.7	7.7 ± 3.3	6.4 ± 3.1	17.2 ± 3.5	16.7 ± 3.6
	p	0.810	0.576	0.520	0.557	0.045
Recommends influenza vaccination	Yes (n = 114)	17.1 ± 3.8	7.1 ± 3.2	6.2 ± 3.2	17.3 ± 3.5	18.2 ± 2.7
	No (n = 67)	14.4 ± 4.2	8.5 ± 3.6	7.0 ± 3.7	16.8 ± 4.2	15.1 ± 4.2
	p	<0.001	0.009	0.130	0.376	<0.001
Received the COVID-19 vaccine	Yes (n = 174)	16.2 ± 4.2	7.5 ± 3.4	6.6 ± 3.4	17.1 ± 3.6	17.2 ± 3.6
	No (n = 7)	13.4 ± 4.0	10.6 ± 3.1	4.7 ± 2.4	17.7 ± 6.8	13.4 ± 4.5
	p	0.083	0.020	0.160	0.650	0.008
Vaccinations complete for children and/or first-degree relatives	Yes (n = 171)	16.5 ± 3.8	7.4 ± 3.2	6.3 ± 3.1	17.1 ± 3.6	17.4 ± 3.4
	No (n = 10)	9.3 ± 5.2	11.3 ± 4.6	10.3 ± 6.0	16.0 ± 5.8	11.8 ± 5.1
	p	<0.001	<0.001	0.065	0.347	<0.001

Data are presented as mean ± standard deviation. Each sub-dimension score ranges from 3 to 21. Two-group comparisons were performed using Student's t-test and three-group comparisons were performed using one-way analysis of variance. Within the profession block, values that do not share a common superscript letter differ significantly in post-hoc comparisons (Bonferroni test, $p < 0.05$). For collective responsibility, the overall analysis of variance was significant ($p = 0.022$) whereas no pairwise comparison reached significance after Bonferroni correction; therefore, superscript letters are not shown. COVID-19, Coronavirus Disease 2019.

season required each trust to designate a named executive lead responsible for staff vaccination, establish an explicit improvement target of at least five percentage points over the previous year, ensure that an accessible and convenient vaccination offer was available to all staff from October 1 to March 31, and monitor trust-level uptake through a central data platform[29]. This combination of accountability, a quantified target, convenient access, and routine monitoring is consistent with the broader intervention literature. A systematic review of 86 studies evaluating interventions targeting healthcare workers for influenza vaccination found that incentive-based, nudging, and persuasive communication methods were the most effective, whereas mandates, although effective, were not the most effective approach[20]. A meta-analysis of 180 studies involving more than two million participants reported an overall relative risk of vaccination of 1.26 (95% CI, 1.21-1.32) for intervention vs. control, with provider-directed interventions showing the largest effect among healthcare workers[21].

It should be noted that the evidence that vaccinating healthcare workers protects the patients in their care remains uncertain. An updated Cochrane review of four cluster-randomized trials including 8,468 long-term care residents found that offering influenza vaccination to healthcare workers probably reduces all-cause mortality among residents from 9% to 6% but has little or no effect on laboratory-confirmed influenza in residents. The review authors noted that this reduction in all-cause mortality could not be explained by changes in influenza-specific outcomes and considered the effects on influenza-specific outcomes uncertain[30]. The rationale for vaccinating healthcare workers therefore rests substantially on protecting the workers themselves and reducing workforce losses, alongside their professional role as vaccination advocates[31,32].

Low influenza vaccination uptake among healthcare workers has been associated with multiple factors, including doubts about vaccine effectiveness, concerns about adverse effects, and practical barriers to vaccination[33-39]. Although influenza vaccination is provided free of charge to healthcare workers in Türkiye, our study showed that constraints scores were significantly higher among participants who had not been vaccinated during the current season (6.8 ± 3.5 vs. 4.9 ± 2.3). This finding suggests that barriers to vaccination may extend beyond financial or physical access and may include lack of time, forgetting, failure to prioritize vaccination, and other practical obstacles. A recent study applying the 5C model to 550 nurses, albeit for a different vaccine, likewise found that constraints were the only psychological antecedent independently associated with vaccination status, in addition to the influence of colleagues[40]. Isolated measures appear insufficient on their own: a randomized trial of mailed educational letters and a single large incentive found no effect on vaccination rates among hospital employees[41]. Considered together with the experience of England, our findings support the implementation of multicomponent strategies in Türkiye that combine convenient workplace vaccination[42,43] and reminder systems[21] with interventions targeting psychological and behavioral determinants.

Our findings indicate that professional roles within healthcare institutions are associated with attitudes and behaviors toward influenza vaccination. In our study, confidence and collective responsibility scores were higher among physicians and midwives/nurses, whereas complacency and constraints scores were higher among other healthcare personnel. Consistent with these findings, physicians had approximately threefold higher odds than non-physician healthcare workers of having received an influenza vaccine at least once in both univariable and multivariable analyses. Recent evidence from Türkiye supports these observations. In a study of 1,712 adults attending family health centers in which the same 5C scale was used, higher confidence and collective responsibility were associated with willingness to be vaccinated, whereas a higher calculation score was associated with hesitancy[44]. In another recent Turkish study of 374 healthcare workers who were also parents, physicians had significantly lower vaccine hesitancy scores than midwives, nurses, and public health officers[45]. These findings are also consistent with previous studies reporting that a stronger sense of responsibility for protecting public health among healthcare workers is associated with higher vaccine acceptance and that physicians have more positive attitudes toward influenza vaccination than other

Table 3. Univariable and multivariable binary logistic regression analysis of factors associated with having received at least one influenza vaccination.

Variable	Category	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p
Sex	Female (reference: male)	2.400 (1.069-5.388)	0.034	2.852 (1.115-7.296)	0.029
Profession	Physician (reference: non-physician)	3.022 (1.370-6.664)	0.006	2.904 (1.160-7.272)	0.023
Influenza vaccine recommendation	Recommends (reference: does not recommend)	9.150 (3.662-22.860)	<0.001	7.677 (3.024-19.490)	<0.001

Sex, profession, and vaccine recommendation were entered simultaneously using the enter method. The omnibus test of model coefficients was significant (χ^2 = 40.413, df = 3, $p < 0.001$); -2 log likelihood = 189.541, the Hosmer–Lemeshow goodness-of-fit test was p = 0.849, and Nagelkerke R^2 was 0.278. The model included 60 events and three covariates (20 events per variable). OR, odds ratio; CI, confidence interval.

healthcare workers[46,47]. Taken together, influenza vaccination programs in healthcare settings should not be limited to on-site vaccine provision but should also be supported by targeted educational activities and reminder systems aimed at reducing perceived barriers and strengthening motivation and collective responsibility.

In addition, participants who recommended the influenza vaccine to others had approximately 7.7-fold higher odds of having been vaccinated themselves. Personal vaccination behavior may serve not only the purpose of individual protection but also reflect a professional attitude supported by the responsibility to protect public health. Indeed, in our study, collective responsibility sub-dimension scores, which reflect perceptions of herd immunity and societal benefit, were higher among participants who recommended the vaccine. Similarly, participants who had received a COVID-19 vaccine and those who reported that their children's and/or first-degree relatives' vaccinations were complete had higher collective responsibility and lower complacency scores. These findings suggest that vaccine acceptance is not merely a behavior specific to a particular vaccine or disease; rather, it is closely related to individuals' general approach to vaccination, health behaviors, and perceptions of societal protection. Interventions aimed at strengthening awareness of collective responsibility among healthcare workers may therefore increase not only individual vaccination rates but also, through the role-modeling and counseling effects of healthcare workers, vaccine acceptance in the wider community.

In our study, no significant associations were found between sociodemographic variables, such as educational level and marital status, and influenza vaccination status. Female healthcare workers had higher vaccination rates than male healthcare workers. In contrast, the literature reports that older age, male sex, being married, and a higher educational level are associated with higher influenza vaccination rates[19]. Furthermore, although that study emphasized that physicians working in hospitals had higher vaccination rates than other healthcare professionals, our study similarly found higher vaccination rates among physicians[19].

Study Limitations

This study has several limitations. First, because of its cross-sectional design, a cause-and-effect relationship cannot be established. This limitation is particularly relevant to the strong association observed between recommending the influenza vaccine and having been vaccinated, as the direction of this relationship cannot be determined from our data. Healthcare workers who are vaccinated may be more inclined to recommend vaccination, while those who recommend vaccination may, in turn, be more inclined to be vaccinated themselves. Second, the study included primary healthcare workers from a single province, which may limit the generalizability of the findings to other regions and healthcare settings. Third, although the response rate was acceptable, approximately 19% of eligible participants did not complete the questionnaire, raising the possibility of non-response bias. Fourth, vaccination status was self-reported and was not verified against vaccination records, which may have introduced recall or social desirability bias. Fifth, two of the subgroups compared in Table 2—participants who had not received a COVID-19 vaccine (n = 7) and those who reported that the vaccinations of their children and/or first-degree relatives were incomplete (n = 10)—were small; therefore, the corresponding comparisons should be interpreted with caution. Despite these limitations, evaluating influenza vaccine acceptance among healthcare workers using the 5C scale and examining psychological antecedents alongside behavioral factors represent important strengths of this study.

Conclusion

The influenza vaccination rate among primary healthcare workers in this study remained well below targeted levels. The findings suggest that vaccination behavior is associated with perceived practical barriers and a sense of collective responsibility rather than with sociodemographic characteristics alone. Participants who were vaccinated during the current season reported fewer constraints and greater information-seeking behavior, whereas those who had received an influenza vaccine at least once demonstrated a stronger sense of collective responsibility. Although confidence was not directly associated with vaccination status, it differed significantly across occupational groups and was markedly higher among healthcare workers who recommended the vaccine to others, suggesting that confidence may influence vaccine uptake indirectly through professional attitudes. To increase vaccination rates, strategies extending beyond informational campaigns alone are warranted. Multicomponent approaches that combine convenient and accessible workplace vaccination, institutional targets and monitoring, reminder systems, and interventions aimed at strengthening collective responsibility may be more effective in promoting influenza vaccination among healthcare workers.

Ethics

Ethics Committee Approval: This study was approved by İstanbul Medipol University Non-Interventional Clinical Research Ethics Committee (decision number: 1391, date: 13.11.2025).

Informed Consent: Written informed consent was obtained from all participants before administration of the questionnaire.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.A., E.A., Concept: B.A., E.A., Design: B.A., E.A., P.K., Data Collection or Processing: B.A., E.A., P.K., Analysis or Interpretation: B.A., E.A., P.K., Literature Search: B.A., E.A., P.K., Writing: B.A., E.A., P.K.

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Supplementary Table: https://d2v96fxpocvxx.cloudfront.net/cf9d60d6-523c-458a-a2e6-78728d3ffbb0/content-images/5a038ad2-8a25-46f9-88cf-bdccfd35e15c.pdf

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Valganciclovir Dose Appropriateness Based on Estimated Glomerular Filtration Rate in Kidney Transplant Recipients: A Real-World Study

Böbrek Transplant Alıcılarında Tahmini Glomerüler Filtrasyon Hızına Göre Valgansiklovir Doz Uygunluğu: Gerçek Yaşam Çalışması

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Abstract

Introduction: Rapid fluctuations in estimated glomerular filtration rate (eGFR) during the early post-transplant period can complicate valganciclovir dosing, which requires adjustment according to renal function. This study aimed to evaluate the appropriateness of valganciclovir prophylaxis dosing based on eGFR in kidney transplant recipients and to examine its associations with cytomegalovirus (CMV) DNAemia, hematological parameters, and changes in kidney function.

Materials and Methods: This single-center retrospective study included 71 adult kidney transplant recipients who underwent transplantation between October 2023 and May 2025 and received valganciclovir prophylaxis for at least 3 months. Dose appropriateness was evaluated using Chronic Kidney Disease Epidemiology Collaboration-based eGFR values (mL/min/1.73 m^2) automatically reported by the hospital laboratory, consistent with routine clinical practice at our center. Hematological parameters and eGFR values measured at prophylaxis initiation and completion were compared. CMV DNA polymerase chain reaction surveillance was conducted every 2 weeks during prophylaxis and monthly thereafter through 1 year after transplantation.

Results: The median patient age was 40 years [interquartile range (IQR), 32–52.5], and 63.4% were male. At prophylaxis initiation, 35 (49.3%) patients received an eGFR-appropriate valganciclovir dose, whereas 34/71 (47.9%) received a higher-than-recommended dose and 2/71 (2.8%) received a lower-than-recommended dose. By prophylaxis completion, the proportion receiving an appropriate dose had increased to 66/71 (93.0%); 5/71 (7.0%) continued to receive a higher-than-recommended dose, and none received a lower-than-recommended dose. Late-onset CMV DNAemia developed in 7 (9.9%) patients at a median of 6 months (IQR, 5–9) after transplantation. Among patients who initially received eGFR-inappropriate dosing and had complete paired data, white blood cell count and neutrophil counts decreased significantly, whereas lymphocyte counts and eGFR increased significantly during prophylaxis (all $p < 0.001$). At prophylaxis completion, however, hematological parameters did not differ significantly between patients receiving appropriate and inappropriate doses.

Conclusion: eGFR-inappropriate valganciclovir dosing was frequent at prophylaxis initiation but decreased substantially over the course of follow-up. Because eGFR can change dynamically during the early post-transplant period, valganciclovir dosing should be reassessed regularly to ensure continued dose appropriateness. Multidisciplinary monitoring and antiviral stewardship strategies may help maintain appropriate valganciclovir dosing throughout prophylaxis.

Keywords: Kidney transplantation, valganciclovir, cytomegalovirus, estimated glomerular filtration rate, drug dosage calculations

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Öz

Giriş: Böbrek transplantasyonu sonrası erken dönemde tahmini glomerüler filtrasyon hızı (eGFR)'nda meydana gelen hızlı değişiklikler, renal fonksiyona göre doz ayarlaması gereken valgansiklovirin uygun dozlanmasını güçleştirebilmektedir. Bu çalışmada, böbrek transplant alıcılarında valgansiklovir profilaksisinin eGFR'ye göre doz uygunluğunun değerlendirilmesi ve doz uygunluğunun sitomegalovirüs (CMV) DNAemisi, hematolojik parametreler ve böbrek fonksiyonlarındaki değişikliklerle ilişkisinin incelenmesi amaçlandı.

Gereç ve Yöntem: Bu tek merkezli retrospektif çalışmaya Ekim 2023–Mayıs 2025 tarihleri arasında böbrek transplantasyonu yapılan ve en az üç ay valgansiklovir profilaksisi alan 71 erişkin hasta dahil edildi. Valgansiklovir doz uygunluğu, hastane laboratuvarı tarafından Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) denklemi kullanılarak otomatik olarak raporlanan eGFR değerlerine göre değerlendirildi. Profilaksi başlangıcı ve sonundaki hematolojik parametreler ve eGFR değerleri karşılaştırıldı. CMV DNA polimeraz zincir reaksiyonu izlemi profilaksi süresince iki haftada bir, profilaksi tamamlandıktan sonra ise transplantasyon sonrası birinci yıla kadar aylık olarak yapıldı.

Bulgular: Hastaların medyan yaşı 40 [çeyrekler arası aralık (IQR): 32–52,5] yıl olup %63,4'ü erkekti. Profilaksi başlangıcında 35 (%49,3) hasta eGFR'ye göre uygun valgansiklovir dozu alırken, 34/71 (%47,9) hasta önerilenden yüksek, 2/71 (%2,8) hasta önerilenden düşük doz aldı. Profilaksi sonunda 66/71 (%93,0) hasta uygun, 5/71 (%7,0) hasta önerilenden yüksek doz alırken, önerilenden düşük doz alan hasta yoktu. Takipte 7 (%9,9) hastada geç başlangıçlı CMV DNAemisi gelişti ve medyan CMV DNAemi zamanı transplantasyon sonrası 6 (IQR: 5–9) aydı. Başlangıçta eGFR'ye göre uygunsuz dozlanan ve eşleştirilmiş verileri bulunan hastalarda profilaksi süresince lökosit ve nötrofil sayıları anlamlı olarak azalırken, lenfosit sayısı ve eGFR anlamlı olarak arttı (tümü $p < 0,001$). Profilaksi sonunda uygun ve uygunsuz doz grupları arasında hematolojik parametreler açısından anlamlı fark saptanmadı.

Sonuç: Böbrek transplant alıcılarında eGFR'ye göre valgansiklovir doz uygunsuzluğu profilaksi başlangıcında sık görülmekle birlikte, takip sürecinde doz uygunluğu belirgin şekilde artmaktadır. Transplantasyon sonrası erken dönemde eGFR değerinde dinamik değişimi nedeniyle valgansiklovir dozunun düzenli olarak yeniden değerlendirilmesi önemlidir. Multidisipliner takip ve antiviral stewardship yaklaşımları uygun dozlamanın sürdürülmesine katkı sağlayabilir.

Anahtar Kelimeler: Böbrek transplantasyonu, valgansiklovir, sitomegalovirüs, tahmini glomerüler filtrasyon hızı, ilaç doz hesaplamaları

Introduction

Cytomegalovirus (CMV) infection remains one of the most common opportunistic infections affecting solid organ transplant (SOT) recipients. It can cause serious disease in transplant recipients and, through its immunomodulatory effects, adversely affect both short- and long-term graft function[1,2]. In particular, kidney transplant recipients who are CMV-seropositive and receive lymphocyte-depleting agents such as anti-thymocyte globulin (ATG) for induction therapy are at substantial risk of CMV DNAemia because latent CMV can be reactivated in the setting of profound ATG-induced immunosuppression[1]. Therefore, CMV prevention is a major focus of care in this high-risk patient population.

Current guidelines recommend the early initiation of valganciclovir prophylaxis in high-risk kidney transplant recipients. This prophylactic strategy effectively reduces CMV DNAemia and CMV disease during periods of high risk and limits the indirect effects of CMV, including graft dysfunction, acute rejection, and other opportunistic infections[2-4]. However, prolonged antiviral exposure requires careful monitoring because of potential drug toxicity, particularly hematological adverse effects, as well as the risk of antiviral resistance[5]. Thus, optimizing valganciclovir use is essential. Higher-than-recommended doses increase the risk of drug-related bone marrow suppression, whereas inadequate dosing may result in clinically significant CMV infection and the development of valganciclovir resistance[1,2,6,7].

Myelotoxicity is one of the major limitations of valganciclovir prophylaxis and may manifest as neutropenia, leukopenia, or anemia[5]. Although myelotoxicity can also occur with other medications commonly used in SOT recipients, it has been reported significantly more frequently among patients receiving valganciclovir prophylaxis[5]. These adverse effects may necessitate premature discontinuation of prophylaxis, potentially increasing the risk of late CMV infection, particularly among patients receiving higher drug doses and those with impaired kidney function[8,9]. Because valganciclovir is primarily eliminated by the kidneys, its dose must be adjusted according to renal function. However, maintaining appropriate dosing in kidney transplant recipients can be challenging because kidney function may change rapidly during the early post-transplant period.

Previous real-world studies have demonstrated that discordance between prescribed valganciclovir doses and renal function-based dosing recommendations is common after kidney transplantation. Rissling et al.[10] reported frequent valganciclovir underdosing, particularly in the context of improving renal function. Hammer et al.[11] found that fewer than half of weekly dose assessments were appropriate. More recent studies by Rigon et al.[12] and Forsyth et al.[13] further suggested that suboptimal valganciclovir dosing may be associated with CMV DNAemia and hematological toxicity. Collectively, these findings underscore the difficulty of maintaining appropriate valganciclovir dosing during the

early post-transplant period, when renal function can change rapidly[10-13].

However, real-world data on longitudinal changes in valganciclovir dose appropriateness and their relationship with CMV DNAemia, hematological parameters, and renal function during prophylaxis remain limited. We hypothesized that valganciclovir prophylaxis dosing may frequently be discordant with estimated glomerular filtration rate (eGFR)-based dosing categories during the early period after kidney transplantation because of rapidly changing renal function. Therefore, this study aimed to evaluate the appropriateness of valganciclovir prophylaxis dosing based on routinely reported eGFR values in kidney transplant recipients and to assess its relationship with CMV DNAemia, hematological parameters, and changes in kidney function.

Materials and Methods

In this single-center retrospective study, adult patients who underwent kidney transplantation at the Ankara Etlik City Hospital Organ Transplant Center between October 2023 and May 2025 were evaluated. Patients included in the study had received valganciclovir for at least 3 months as post-transplant CMV prophylaxis and had a follow-up period of 1 year after transplantation. Patients with complete clinical and laboratory data from the initiation to the completion of prophylaxis were assessed. Patients younger than 18 years, those who underwent multiple organ transplantation, those with active CMV infection before prophylaxis initiation, and those with missing required data were excluded. The study was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (decision number: AEŞH-BADEK2-2025-291; approval date: 22.07.2025). The study was conducted in accordance with the principles of the Declaration of Helsinki. Given the retrospective nature of the study and the use of anonymized patient data, the requirement for individual informed consent was waived by the ethics committee.

The reporting of this study followed the Strengthening the Reporting of Observational Studies in Epidemiology statement.

Demographic and clinical data were retrospectively obtained from the electronic patient record system. Data on age, gender, etiology of end-stage renal failure, type of transplant, CMV donor and recipient serologies, induction and maintenance immunosuppressive therapy, use of ATG and pulse steroids, concomitant bacterial or fungal infections, rejection episodes, and CMV DNAemia status were recorded. At our center, CMV prophylaxis is routinely administered to CMV-seropositive recipients and/or patients receiving lymphocyte-depleting induction therapy. In this study, CMV prophylaxis was initiated within the first 10 days after transplantation.

Valganciclovir prophylaxis and dose adjustments were planned through joint assessment by the nephrology and infectious diseases teams. Dose modifications were reviewed during outpatient visits based on the patients' current kidney function. Kidney function tests were routinely performed during hospitalization and after discharge during outpatient visits, and the valganciclovir dose was adjusted accordingly when necessary. In routine clinical practice at our center, renal function for valganciclovir dose assessment is evaluated using the eGFR automatically reported by the hospital laboratory based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Accordingly, in this retrospective real-world study, valganciclovir dose appropriateness was assessed using the recorded eGFR values (mL/min/1.73 m^2). Although conventional valganciclovir renal dose-adjustment recommendations are based on creatinine clearance, the corresponding renal function thresholds were applied to the routinely reported eGFR values to classify the prescribed dose as appropriate, lower than recommended, or higher than recommended[2] (Box).

Dose appropriateness was assessed at two predefined time points: at prophylaxis initiation and at prophylaxis completion, using the contemporaneous eGFR at each time point. Dose appropriateness between these two time points was not systematically assessed over time.

At our center, CMV DNA polymerase chain reaction (PCR) surveillance was routinely performed every 2 weeks during valganciclovir prophylaxis and monthly after prophylaxis completion until 1 year post-transplantation. Additional CMV DNA PCR testing was performed outside the scheduled surveillance when clinical signs or symptoms suggestive of CMV infection were present. CMV infection was defined as the detection of CMV replication regardless of symptoms, whereas CMV disease was defined as the presence of clinical findings related to CMV infection or end-organ involvement. CMV DNAemia was defined as the detection of viral DNA at any level by quantitative PCR[2].

CMV DNA was quantified in plasma using a quantitative nucleic acid test on the cobas 8800 System with real-time PCR. The linear range of the assay was 34.5–10,000,000 IU/mL, with 1 IU/mL corresponding to 1.1 copies/mL.

CMV risk groups were classified based on the donor (D) and recipient (R) anti-CMV immunoglobulin G results:

- Low risk: D−/R−
- Moderate risk: D+/R+ or D−/R+
- High risk: D+/R−

All patients with detected CMV DNAemia received valganciclovir/ganciclovir treatment.

White blood cell (WBC) count, absolute neutrophil count, lymphocyte count, hemoglobin level, platelet count, and eGFR were evaluated at the start and end of prophylaxis.

The primary endpoint of the study was to assess whether the valganciclovir dose at prophylaxis initiation was appropriate according to the routinely reported CKD-EPI-based eGFR. Secondary endpoints included changes in dose appropriateness at the end of prophylaxis, development of late-onset CMV DNAemia, changes in hematological parameters, and changes in kidney function.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows (Version 26, IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Shapiro–Wilk test and by examining histograms. Non-normally distributed continuous variables were presented as medians and interquartile ranges (IQRs), whereas categorical variables were expressed as numbers and percentages. The Mann–Whitney U test was used to compare independent groups, and the chi-square test or Fisher's exact test was used for comparisons of categorical variables. The Wilcoxon signed-rank test was used to compare paired measurements before and after prophylaxis. A two-sided $p < 0.05$ was considered statistically significant.

Results

Between October 2023 and May 2025, a total of 75 adult patients who underwent kidney transplantation were evaluated. One patient was excluded because they received valganciclovir prophylaxis for less than 3 months, another because they had less than 1 year of post-transplant follow-up, and two because of missing clinical or laboratory data. Thus, a total of 71 patients who met the inclusion criteria were included in the analysis.

The median age of the patients was 40 years (IQR, 32–52.5), and 45 (63.4%) were male. The most common cause of kidney failure was an unknown etiology, identified in 34 (47.9%) patients, followed by glomerulonephritis in 15 (21.1%) and diabetes mellitus in 8 (11.3%). Of the transplants, 58 (81.7%) were from living donors. Sixty-seven recipients (94.4%) were CMV IgG seropositive (R+), whereas 4 (5.6%) were seronegative (R–). The median duration of CMV prophylaxis was 3 months (IQR, 3–6). During follow-up, CMV DNAemia occurred in 7 (9.9%) patients, and rejection episodes developed in 13 (18.3%). Concomitant bacterial infections were observed in 38 (53.5%) patients and fungal infections in 5 (7.0%) (Table 1).

After prophylaxis, 7 (9.9%) patients developed late-onset CMV DNAemia. The median time to CMV DNAemia was 6 months after transplantation (IQR, 5–9 months). The median peak CMV DNA level was 1200 IU/mL (IQR, 652–9380 IU/mL). Among patients with CMV DNAemia, 2 (28.6%) had concurrent rejection episodes, 2 (28.6%) received ATG therapy, and 2 (28.6%) were treated with pulse steroids. Four (57.1%) patients with CMV DNAemia were receiving an eGFR-appropriate dose, whereas 3 (42.9%) were receiving a higher-than-recommended dose of valganciclovir. No CMV DNAemia was observed among patients receiving a lower-than-recommended dose based on eGFR (Table 1).

At prophylaxis initiation, 35 (49.3%) patients received an appropriate valganciclovir dose according to eGFR, whereas 36 (50.7%) had inappropriate dosing; 34/71 (47.9%) received a higher-than-recommended dose, and 2/71 (2.8%) received a lower-than-recommended dose. By prophylaxis completion, 66/71 (93.0%) patients received an appropriate dose, 5/71 (7.0%) received a higher-than-recommended dose, and none received a lower-than-recommended dose. This increase in dose appropriateness reflects assessment against the contemporaneous eGFR and does not necessarily indicate that valganciclovir dose modifications were made in all patients whose classification changed. Individual dose modifications and their specific reasons were not systematically evaluated in this study (Table 1). Patients were grouped according to valganciclovir dose appropriateness at prophylaxis initiation, and laboratory parameters measured at prophylaxis completion were compared between these baseline-defined groups. At baseline, patients receiving inappropriate eGFR-based dosing had significantly lower lymphocyte counts (95 [55–160] vs. 240 [110–945]/mm^3, $p = 0.002$), lower platelet counts (154,500 [133,500–176,000] vs. 184,000 [144,500–240,500]/mm^3, $p = 0.030$), and substantially lower eGFR (14 [10–18] vs. 48 [30–57] mL/min/1.73 m^2, $p < 0.001$) than those receiving appropriate dosing. By the end of prophylaxis, none of the between-group differences were statistically significant, including eGFR (49 [40–63] vs. 58 [49–70] mL/min/1.73 m^2, $p = 0.056$). WBC, neutrophil, and hemoglobin values did not differ significantly between the two groups at either time point (all $p > 0.05$) (Table 2). Among the 35 patients with eGFR-inappropriate valganciclovir dosing at prophylaxis initiation and complete paired data, all four parameters showed statistically significant changes from prophylaxis initiation to completion. WBC and neutrophil counts decreased significantly (median changes, −4830/mm^3 [−47.2%] and −6310/mm^3 [−63.9%], respectively; both $p < 0.001$), whereas lymphocyte counts and eGFR increased significantly (+1070/mm^3 [+1188.9%] and +35 mL/min/1.73 m^2 [+250%], respectively; both $p < 0.001$) (Table 3; Figure 1).

Table 1. Demographic, clinical, and CMV-related characteristics of kidney transplant recipients.

Variable	Value
Age (years), median (IQR)	40.0 (32.0–52.5)
Sex, n (%)	
Female	26 (36.6)
Male	45 (63.4)
White blood cell count (/μL) at prophylaxis initiation, median (IQR)	10,150.0 (7,250.0–12,330.0)
Neutrophil count (/μL) at prophylaxis initiation, median (IQR)	9,280.0 (6,095.0–11,750.0)
Lymphocyte count (/μL) at prophylaxis initiation, median (IQR)	130.0 (75.0–370.0)
Hemoglobin (g/dL) level at prophylaxis initiation, median (IQR)	9.7 (8.3–10.5)
Platelet count (/μL) at prophylaxis initiation, median (IQR)	161,000 (138,500–196,000)
White blood cell count (/μL) at prophylaxis completion, median (IQR)	5,915.0 (4,770.0–8,540.0)
Neutrophil count (/μL) at prophylaxis completion, median (IQR)	3,770.0 (2,830.0–6,490.0)
Lymphocyte count (/μL) at prophylaxis completion, median (IQR)	1,260.0 (910.0–1,770.0)
Hemoglobin (g/dL) level at prophylaxis completion, median (IQR)	11.5 (9.8–12.6)
Platelet count (/μL) at prophylaxis completion, median (IQR)	260,000 (203,000–294,000)
eGFR at prophylaxis initiation, median (IQR)	20.0 (14.0–46.5)
Patients receiving an appropriate valganciclovir dose according to eGFR, n (%)	35 (49.3)
eGFR at prophylaxis completion, median (IQR)	54.5 (45.0–65.0)
Patients receiving an appropriate valganciclovir dose according to eGFR at prophylaxis completion, n (%)	66 (93.0)
Indication for kidney transplantation, n (%)	
Unknown etiology	34 (47.9)
Glomerulonephritis	15 (21.1)
Diabetes mellitus	8 (11.3)
Hypertension	5 (7.0)
Familial Mediterranean fever	4 (5.6)
Other	5 (7.0)
Living-donor kidney transplantation, n (%)	58 (81.7)
Kidney replacement therapy before transplantation, n (%)	
Hemodialysis	61 (85.9)
Peritoneal dialysis	10 (14.1)
Duration of chronic kidney disease (years), median (IQR)	3.0 (2.0–7.5)
Recipient CMV IgG serostatus, n (%)	
Negative	4 (5.6)
Positive	67 (94.4)
Donor CMV IgG serostatus, n (%)	
Negative	0 (0.0)
Positive	57 (80.3)
Not available	14 (19.7)
Recipient CMV risk	
Moderate risk D+/R+	53 (74.6)
High risk D+/R−	4 (5.6)
Donor serostatus unavailable	14 (19.7)
Duration of CMV prophylaxis after transplantation (months), median (IQR)	3.0 (3.0–6.0)
Patients with CMV DNAemia, n (%)	7 (9.9)
Acute rejection during CMV DNAemia, n (%)	2 (28.6)
ATG administration during CMV DNAemia, n (%)	2 (28.6)
Pulse steroid therapy during CMV DNAemia, n (%)	2 (28.6)
Acute rejection after transplantation, n (%)	13 (18.3)
ATG administration after transplantation, n (%)	50 (70.4)
Pulse steroid therapy after transplantation, n (%)	26 (36.6)

CMV risk classification was based on available donor and recipient serostatus; donor CMV serostatus was unavailable in 14 recipients. IQR, interquartile range; eGFR, estimated glomerular filtration rate; CMV, cytomegalovirus; IgG, immunoglobulin G; ATG, antithymocyte globulin; D, donor; R, recipient (D+/R+: donor positive/recipient positive; D+/R−: donor positive/recipient negative).

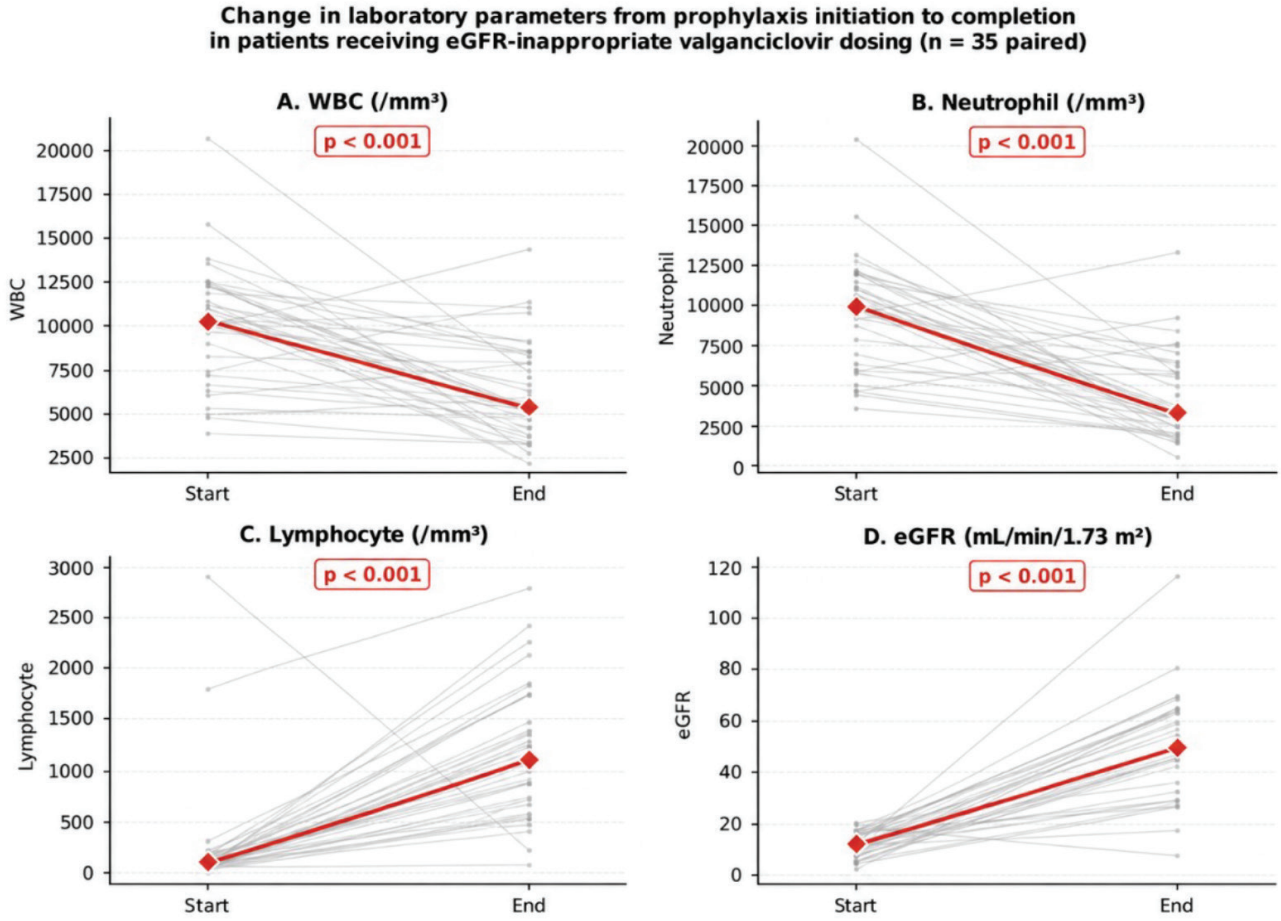

Figure 1. Individual patient trajectories (gray lines) and group medians (red diamonds) for WBC, neutrophil, and lymphocyte counts and eGFR from prophylaxis initiation (Start) to completion (End) in the 35 patients with eGFR-inappropriate valganciclovir dosing at prophylaxis initiation and complete paired data. The interval between prophylaxis initiation and completion varied according to the duration of valganciclovir prophylaxis. WBC and neutrophil counts decreased significantly, whereas lymphocyte counts and eGFR increased significantly over the prophylaxis period (all $p < 0.001$, Wilcoxon signed-rank test).

WBC, white blood cell count; eGFR, estimated glomerular filtration rate.

Discussion

Valganciclovir prophylaxis is the standard approach recommended by current guidelines for preventing CMV infection, particularly in kidney transplant recipients who receive ATG and are CMV-seropositive[2]. However, the need for dose adjustment according to renal function, combined with rapid changes in kidney function during the early post-transplant period, makes maintaining appropriate dosing challenging in clinical practice. In our study, real-world valganciclovir prophylaxis data from kidney transplant recipients were evaluated, and although approximately half of the patients received an inappropriate dose based on eGFR at prophylaxis initiation, the incidence of CMV DNAemia was low, and most patients achieved appropriate dosing during follow-up. Additionally, among patients who initially received an eGFR-inappropriate dose, significant changes in hematological parameters and an increase in eGFR were observed during follow-up.

In our study, the median time to CMV DNAemia among patients who developed CMV DNAemia was 6 months after transplantation. In a study evaluating the frequency of CMV infection following valganciclovir prophylaxis in 481 high-risk CMV D+/R− kidney transplant recipients, CMV infection developed in 38% of patients. The infection occurred at a median of 264 days after transplantation and 84 days after the end of prophylaxis[14]. The occurrence of CMV DNAemia within approximately 3 months after prophylaxis in our study highlights the importance of close monitoring after prophylaxis completion.

In a study evaluating weekly laboratory data from kidney transplant recipients receiving valganciclovir prophylaxis

Table 2. Laboratory parameters at the initiation and completion of valganciclovir prophylaxis according to baseline eGFR-based dose appropriateness.

Variables	Appropriate dose at baseline eGFR (n = 35)	Inappropriate dose at baseline eGFR (n = 36)	p value
At prophylaxis initiation			
WBC (/mm^3)	8,990 (6,925–12,115)	10,525 (7,800–12,330)	0.280
Neutrophil (/mm^3)	7,360 (5,575–11,210)	9,890 (6,830–12,025)	0.171
Lymphocyte (/mm^3)	240 (110–945)	95 (55–160)	0.002
Hemoglobin (g/dL)	9.5 (8.0–10.4)	9.8 (8.9–10.6)	0.337
Platelet (/mm^3)	184,000 (144,500–240,500)	154,500 (133,500–176,000)	0.030
eGFR (mL/min/1.73 m^2)	48 (30–57)	14 (10–18)	< 0.001
At prophylaxis completion			
WBC (/mm^3)	6,200 (4,800–9,025)	5,410 (4,780–8,400)	0.363
Neutrophil (/mm^3)	4,140 (3,190–6,630)	3,570 (2,595–6,125)	0.280
Lymphocyte (/mm^3)	1,330 (940–1,735)	1,160 (785–1,650)	0.353
Hemoglobin (g/dL)	11.6 (10.3–12.6)	11.2 (9.4–12.6)	0.477
Platelet (/mm^3)	265,000 (211,500–312,500)	240,000 (190,500–286,000)	0.335
eGFR (mL/min/1.73 m^2)	58 (49–70)	49 (40–63)	0.056

Data are presented as median (interquartile range, IQR). Group comparisons were performed using the Mann–Whitney U test. A p-value < 0.05 was considered statistically significant. eGFR, estimated glomerular filtration rate; IQR, interquartile range; WBC, white blood cell count.

Table 3. Within-group changes in laboratory parameters from valganciclovir prophylaxis initiation to completion among patients receiving eGFR-inappropriate dosing (n = 35 with complete paired data).

Variable	At initiation	At completion	Δ Median	Δ %	p value
WBC (/mm^3)	10,525 (7,995–12,320)	5,410 (4,780–8,400)	−4,830	−47.2%	<0.001
Neutrophil (/mm^3)	9,890 (6,875–12,018)	3,570 (2,595–6,125)	−6,310	−63.9%	<0.001
Lymphocyte (/mm^3)	95 (58–160)	1,160 (785–1,650)	+1,070	+1,188.9%	<0.001
eGFR (mL/min/1.73 m^2)	14 (10–17)	49 (40–63)	+35	+250.0%	<0.001

Data are presented as median (interquartile range, IQR). Paired comparisons were performed using the Wilcoxon signed-rank test. One patient who died before completing prophylaxis was excluded from the paired analysis. A p value < 0.05 was considered statistically significant. eGFR, estimated glomerular filtration rate; IQR, interquartile range; WBC, white blood cell count; Δ, change between time points.

Box. Valganciclovir prophylaxis dosing according to eGFR categories used for dose assessment in the study.

eGFR used for dose assessment (mL/min/1.73 m^2)	Valganciclovir prophylaxis dose
≥60	900 mg once daily
40–59	450 mg once daily
25–39	450 mg every 2 days
10–24	450 mg twice weekly

eGFR, estimated glomerular filtration rate.

at two centers, more than half of the dose assessments were not concordant with renal function-based dosing recommendations. Of 1,032 weekly dose assessments based on weekly kidney function tests, 460 (44.6%) involved an appropriate valganciclovir dose, whereas 234 (22.7%) involved underdosing (below the recommended dose) and 338 (32.8%) involved overdosing (above the recommended dose)[11]. A recent retrospective study showed that kidney transplant recipients received valganciclovir doses outside the recommended range on approximately half (49%) of treatment days during prophylaxis[15]. Similarly, in our study, approximately half of the patients (50.7%) were initially found to have inappropriate dosing based on eGFR. Although conventional valganciclovir renal dose-adjustment recommendations are based on creatinine clearance, dose appropriateness in our study was evaluated using the eGFR routinely used for dose assessment at our center, thereby reflecting real-world clinical practice.

These findings suggest that the method used to assess kidney function during the early post-transplant period, together with rapid changes in renal function, may influence valganciclovir dose classification and subsequent dose adjustments.

The optimal dosing strategy for valganciclovir prophylaxis has not yet been fully established. Although some studies suggest that low-dose prophylaxis may provide efficacy comparable to that of standard doses in selected patient populations while reducing myelosuppressive adverse effects[16-18], higher-than-recommended doses are particularly associated with hematological toxicity resulting from bone marrow suppression[2,11,19]. In another recently published retrospective study, kidney transplant recipients received the optimal dose during only 51% of the follow-up period while receiving valganciclovir prophylaxis; doses below the recommended level were associated with breakthrough CMV DNAemia, whereas higher-than-recommended doses were associated with the development of neutropenia[13]. Taken together, these findings suggest that variability in renal function makes it challenging to maintain an appropriate valganciclovir dose and that dose inadequacy may affect clinical outcomes.

Based on available evidence, current guidelines recommend adjusting the valganciclovir dose according to kidney function and reassessing the dose as renal function changes[2]. Therefore, the recommended approach is to adjust the dose according to kidney function and regularly reassess treatment in response to changes in renal function[1,2,7]. Nevertheless, real-world data continue to show that dose mismatches based on eGFR are common among kidney transplant recipients[11,19].

In our study, among patients who initially received an eGFR-inappropriate, predominantly higher-than-recommended dose of valganciclovir, WBC and neutrophil counts decreased significantly during prophylaxis, whereas lymphocyte counts and eGFR increased significantly. These findings represent longitudinal changes observed during the prophylaxis period and should not be interpreted as direct effects of valganciclovir dosing. In a study evaluating more than 1,500 patients who underwent transplantation between 2010 and 2016 at two major transplant centers, patients who received valganciclovir prophylaxis were compared with those who did not. The valganciclovir prophylaxis group had significantly higher rates of myelosuppression-related adverse effects, including leukopenia, severe leukopenia, and thrombocytopenia[5]. In a study evaluating dosing differences in pediatric patients, it was emphasized that variations in dosing strategies may not always affect CMV outcomes but may have significant effects on hematological toxicity[19]. However, the hematological changes observed in our cohort cannot be attributed solely to valganciclovir, as concomitant myelosuppressive and immunosuppressive therapies may also have contributed. The decreases in leukocyte and neutrophil counts observed during prophylaxis are consistent with the known myelosuppressive effects of valganciclovir. However, the absence of significant differences in hematological parameters between the appropriate- and inappropriate-dose groups at prophylaxis completion suggests that these changes may not be attributable solely to valganciclovir dose, but may also have been influenced by concomitant immunosuppressive treatments and clinical changes during the early post-transplant period. Similarly, in a real-world, two-center study based on weekly dose assessments by Hammer et al.[11], no strong correlation was found between valganciclovir overdose and leukopenia or neutropenia. The authors proposed that the observed hematological changes might reflect the cumulative and time-dependent myelosuppressive effects of agents such as valganciclovir, mycophenolate mofetil, and thymoglobulin[11]. Given that 70.4% of our patients received ATG induction therapy and that other potentially myelosuppressive treatments, including mycophenolate mofetil and trimethoprim-sulfamethoxazole, are commonly used during the early post-transplant period, the hematological changes observed in our study are likely multifactorial. In addition, the increases in lymphocyte count and eGFR may partly reflect expected hematological and renal recovery during the early post-transplant period rather than the effects of valganciclovir dose appropriateness.

Study Limitations

Our study has some limitations. First, because of its retrospective and single-center design, it does not allow causal relationships to be established, and the generalizability of the results is limited. Second, the relatively small number of patients, particularly the small number who developed CMV DNAemia, may have reduced the statistical power of some analyses. Additionally, when evaluating hematological toxicity, the potential contributions of ATG, mycophenolate mofetil, trimethoprim-sulfamethoxazole, and other concomitant immunosuppressive treatments could not be analyzed separately. Similarly, the observed increase in eGFR may partly reflect the expected recovery of renal function during the early post-transplant period and cannot be attributed to valganciclovir dose adjustment. Another limitation is that valganciclovir dose appropriateness was assessed using the CKD-EPI-based eGFR routinely reported by our hospital laboratory, whereas conventional valganciclovir renal dose-adjustment recommendations are based on creatinine clearance. Because eGFR and creatinine clearance are not interchangeable, the use of eGFR may have resulted in different dose-appropriateness classifications in some patients. Dose appropriateness was assessed at two predefined time points, prophylaxis initiation and completion, and dose appropriateness during the intervening prophylaxis period

was not analyzed in a time-dependent manner. Predefined clinical thresholds for cytopenias were not used; therefore, hematological findings were evaluated as longitudinal changes in laboratory parameters rather than as predefined toxicity events. Finally, because plasma levels of valganciclovir or ganciclovir were not measured, the relationship between dosing and actual drug exposure could not be evaluated.

Conclusion

The substantial increase in valganciclovir dose concordance from prophylaxis initiation to completion highlights the dynamic nature of dosing during the early post-transplant period. This improvement may reflect several factors, including stabilization of kidney function over time and ongoing clinical reassessment, and cannot be attributed solely to multidisciplinary follow-up. These findings indicate that valganciclovir dosing during the early period after kidney transplantation is not a one-time decision but rather a dynamic process that requires regular reassessment as kidney function changes. Although the effects of multidisciplinary follow-up or antiviral stewardship were not directly evaluated in our study, such strategies may support appropriate valganciclovir dosing in clinical practice. Our study adds real-world data by evaluating valganciclovir dose appropriateness based on eGFR together with longitudinal hematological changes. Larger, multicenter, prospective studies are needed to further clarify the clinical impact of optimal dosing strategies and structured monitoring approaches.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (decision number: AEŞH-BADEK2-2025-291; approval date: 22.07.2025).

Informed Consent: Given the retrospective nature of the study and the use of anonymized patient data, the requirement for individual informed consent was waived by the ethics committee.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.D., Concept: G.K., E.I.S., G.D., E.O., Design: G.K., E.O., F.A.Y., P.B.Ü., Data Collection or Processing: P.B.Ü., Analysis or Interpretation: F.A.Y., Literature Search: G.K., E.I.S., E.O., Writing: G.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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Molecular Detection of *UreC* and *mrpA* Genes in *Proteus mirabilis* Isolated from Kidney Stone Patients

Böbrek Taşı Hastalarından İzole Edilen *Proteus mirabilis*'teki *ureC* ve *mrpA* Genlerinin Moleküler Yöntemlerle Tespiti

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Abstract

Introduction: Nephrolithiasis is one of the most common chronic kidney disorders in patients with hypertension. This study aimed to identify and isolate *Proteus mirabilis* from the urine samples of kidney stone patients, investigate the presence of the *ureC* and *mrpA* genes using polymerase chain reaction (PCR), and assess the levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and CCL2 in these patients.

Materials and Methods: Fifty urine samples were obtained from the Urology Department of Baghdad Teaching Hospital in Baghdad Governorate between March 2025 and the end of November 2025. Twenty healthy individuals participated in this study as controls. Blood samples were acquired from the patients and controls to assess their TNF-α, IL-6, and CCL2 levels.

Results: The results of the present study indicated that *Proteus mirabilis* was the most abundant bacterium, as identified in 10 (20%) cases, followed by *Escherichia coli* and *Staphylococcus aureus*, each present in seven instances (14%). *Pseudomonas aeruginosa* was identified in six (12%) cases, while *Klebsiella pneumoniae* was detected in five (10%) cases. Molecular approaches using PCR to target the *ureC* and *mrpA* genes yielded results indicating that the *ureC* gene was present in 70% of the samples, while *mrpA* was detected in 60% of the samples. Furthermore, these findings indicated elevated levels of TNF-α, IL-6, and CCL2 in kidney stone patients.

Conclusion: It was revealed that *Proteus mirabilis* was the predominant bacterium in patients with renal stones and that it possesses the *ureC* and *mrpA* genes, which contribute to the pathogenicity of bacteria. This study also showed that TNF-α, IL-6, and CCL2 tend to increase in kidney stone patients.

Keywords: IL-6, kidney stone, *mrpA*, *Proteus mirabilis*, TNF-α, *ureC*, virulence genes

Öz

Giriş: Nefrolitiyazis, hipertansiyonu olan hastalarda en sık görülen kronik böbrek hastalıklarından biridir. Bu çalışma, böbrek taşı hastalarının idrar örneklerinden *Proteus mirabilis*'i izole edip tanımlamayı, polimeraz zincir reaksiyonu (PCR) kullanarak *ureC* ve *mrpA* genlerinin varlığını araştırmayı ve bu hastalardaki TNF-α, IL-6 ve CCL2 düzeylerini değerlendirmeyi amaçlamıştır.

Gereç ve Yöntem: Mart 2025 ile Kasım 2025 sonu arasında Bağdat Valiliği'ndeki Bağdat Eğitim Hastanesi Üroloji bölümünden 50 idrar örneği alınmıştır. Ayrıca, çalışmaya kontrol grubu olarak 20 sağlıklı birey dahil edilmiştir. TNF-α, IL-6 ve CCL2 düzeylerinin incelenmesi amacıyla hastalardan ve kontrol grubundan kan örnekleri alınmıştır.

Bulgular: Çalışma sonuçları, en sık görülen bakterinin *Proteus mirabilis* olduğunu (10 olguda, %20) ve bunu her biri 7 olguda (%14) tespit edilen *Escherichia coli* ile *Staphylococcus aureus*'un izlediğini göstermiştir. *Pseudomonas aeruginosa* 6 olguda (%12), *Klebsiella pneumoniae* ise 5 olguda (%10) tespit edilmiştir. *ureC* ve *mrpA* genlerini hedef alan PCR tabanlı moleküler analizler, örneklerin %70'inde *ureC*, %60'ında ise *mrpA* geninin bulunduğunu ortaya koymuştur. Mevcut araştırma, böbrek taşı hastalarında TNF-α, IL-6 ve CCL2 düzeylerinin arttığını göstermiştir.

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Sonuç: Bu araştırma, böbrek taşı olan hastalarda baskın bakterinin *Proteus mirabilis* olduğunu ortaya koymuştur. Sonuçlar, *Proteus mirabilis*'in bakterinin patojenitesine katkıda bulunan *ureC* ve *mrpA* genlerini taşıdığını göstermektedir. Ayrıca çalışma, böbrek taşı hastalarında TNF-α, IL-6 ve CCL2 düzeylerinde artış olduğunu ortaya koymuştur.

Anahtar Kelimeler: IL-6, böbrek taşı, *mrpA*, *Proteus mirabilis*, TNF-α, *ureC*, virülans genleri

Introduction

Renal stones, also known as urolithiasis and kidney stones, are solid aggregates that form in the kidneys from dissolved urinary minerals[1]. These stones can cause severe pain, hematuria, and complications, such as urinary tract infections (UTIs) and kidney disease. The lifetime risk of developing kidney stones is approximately 12% for men and 7% for women, with recurrence rates up to 50% within 5–10 years after the first episode. Many risk factors have been proven to precipitate renal stones, including genetic, dietary, and environmental factors[2,3].

Although kidney stones smaller than 5 mm flow readily through the urinary tract, those larger than 5 mm can precipitate, resulting in calculus development[4]. The most prevalent types of stones are calcium oxalate and calcium phosphate, constituting at least 80% of all kidney stones. The remaining urinary stones consist of uric acid, cysteine, and mixed forms. The acute edges of oxalate calculi tend to injure the urinary tract epithelium and promote the proliferation of organisms by creating a nidus for infectious agents[5,6].

The prevalent and misguided application of antibiotics has contributed to the issues associated with UTIs[7]. The rise of antibiotic resistance constitutes a significant challenge for the medical community and poses a substantial threat to public health[8]. Urease-producing bacteria, including *Proteus mirabilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, are consistently associated with the formation and recurrence of struvite stones[9]. The pathogenicity of *Proteus mirabilis* is influenced by a variety of virulence genes that promote adhesion, colonization, immune evasion, and tissue damage, advancing the infectious process in diverse host conditions[10,11]. *ureC* and *mrpA* are unique virulence genes associated with the pathogenicity of *Proteus mirabilis*[12,13]. The *ureC* gene encodes the alpha subunit of urease, which promotes the formation of bladder and kidney stones by catalyzing the hydrolysis of urea[14]. The ureolytic activity of *Proteus mirabilis* generates ammonia and carbonate ions, increasing the urine pH[15] and precipitating calcium and magnesium phosphates. Microcrystals become ensnared in biofilms, resulting in the development of crystalline biofilms that can encrust and obstruct urinary catheters. Obstructed catheters can cause urine retention in the bladder and retrograde flow to the kidneys, resulting in recurrent UTIs. Failure to replace obstructed catheters may result in pyelonephritis, septicemia, and endotoxic shock[16].

Chemokines are low-molecular-weight proteins whose principal function is to direct the migration of immune cells to specific target areas. CCL2, or monocyte chemoattractant protein-1 (MCP-1), is considered a crucial regulatory element in inflammatory responses. Consistent with previous observations, renal tubular epithelial cells can synthesize CCL2 in response to calcium oxalate stimulation[17-19]. Interleukin-6 (IL-6) is a multifunctional cytokine that modulates immunological responses and is regarded as an endogenous pyrogen that induces fever in infected individuals. Following infection and inflammation, IL-6 is initially produced, with its levels swiftly increasing to a peak within 2 hours[20].

Tumor necrosis factor-alpha (TNF-α) is a macrophage-derived endogenous modulator of several host responses to infection and inflammation[21]. Several studies have reported that renal stones may induce renal cells to release inflammatory mediators in vitro, including MCP-1 (often referred to as CCL2), TNF-α, and IL-6. Consequently, the inflammatory responses of renal tissue significantly contribute to the pathogenesis of urolithiasis[22].

Although the association between *Proteus mirabilis* and UTIs and the formation of infectious kidney stones has been well established, published data on the molecular distribution of certain virulence-related genes among the isolates of this bacterium from kidney stone patients, particularly in Iraq, have been limited. Thus, further research on these two genes may contribute to a deeper understanding of the virulence characteristics of these bacteria and their potential contribution to infection and stone formation.

Materials and Methods

Fifty urine samples were obtained from the Urology Department of Baghdad Teaching Hospital in Baghdad Governorate between March 2025 and the end of November 2025. Their ages ranged from 35 to 64 years, encompassing both sexes.

Sample Collection

During sample collection, the initial urine drops were disregarded, and the midstream urine was collected and stored in sterile containers. Subsequently, it was sent to the Postgraduate Microbiology Laboratory at the College of

Science, Department of Biology, for culture and diagnostic purposes. Moreover, 5 mL of venous blood was drawn from each specimen using a sterile disposable syringe and permitted to coagulate at ambient temperature. The tubes underwent centrifugation at 3000 rpm for a duration of 15 minutes. Subsequently, the tubes were stored at a temperature of -20 °C until the assay, which encompassed the following parameters: TNF-α, IL-6, and CCL2.

Inclusion and Exclusion Criteria

This study included only those patients for whom the attending urologist diagnosed kidney stones on the basis of clinical examination and imaging (ultrasonography or computed tomography) findings. Only adult patients (35–64 years old) who had not been exposed to any antibiotic treatment in the preceding 2 weeks before sampling were included. The exclusion criteria were patients with chronic inflammatory disease, autoimmune disorders, malignancies, severe renal failure, or any other systemic infection. The control group comprised apparently healthy subjects with no history of kidney stones or UTIs.

Isolation and Identification

The diagnosis was made based on[16] the morphological characteristics, such as growth on blood agar, MacConkey agar, and mannitol salt agar. Moreover, microscopic examination and biochemical tests were performed. The identification results were subsequently confirmed using the VITEK 2 automated identification system.

DNA Extraction and Molecular Detection of the *ureC* and *mrpA* Genes

Proteus mirabilis was cultivated in brain heart infusion broth and incubated for 24 hours at a temperature of 37 °C. In accordance with the manufacturer's instructions, genomic DNA was extracted using the FavorPrep Blood/Cultured Cell Genomic DNA Extraction Mini Kit. To ensure the sample quality for downstream molecular investigations, the extracted DNA concentration and purity were assessed using the Quantus™ Fluorometer. Table 1 shows the primers used to detect *Proteus mirabilis* and its virulence gene using polymerase chain reaction (PCR): The initial denaturation was 1 cycle at a temperature of 94°C for 3 min, followed by 35 cycles of 40 seconds each of denaturation at 94 °C, annealing at 60 °C, and elongation at 72 °C. The reaction was finished with 1 cycle of 72°C elongation for 7 minutes. The samples were electrophoresed on a 1.5% agarose gel to validate the reaction and create predicted bands.

Table 1. The primers used in this investigation.

Gene	Sequence	Product size	Reference
ureC	5′-CCGGAACAGAAGTTGTCGCTG GA-3′	533 bp	[16]
	5′-GGGCTCTCCTACCGACTTGATC-3′		
mrpA	5′-TTCTTACTGATAAGACATTG-3′	410 bp	[16]
	5′-ATTTCAGGAAACAAAAGATG-3′		

Evaluation of TNF-α, IL-6, and CCL2

All study samples were measured using enzyme-linked immunosorbent assay (Elabscience®, Wuhan, China) in accordance with the manufacturer's instructions.

Ethical Approval

This study was conducted on urine samples collected as part of routine laboratory investigations, with no direct patient intervention. Thus, formal ethical approval was not required according to the institutional guidelines. All procedures were performed in accordance with relevant regulations and ethical standards.

Statistical Analysis

Statistical analyses were performed using the IBM SPSS Statistics software (version 26.0; IBM Corp., Armonk, NY, USA). Data were expressed as mean ± standard deviation. The normality of the data was established using the Shapiro-Wilk test. For the normally distributed continuous variables, the independent samples t-test was used, whereas for the categorical variables, the chi-square test or Fisher's exact test may be used, depending on the circumstances. Furthermore, statistical significance was set at a p value of <0.05.

Results

A total of 50 urine samples were analyzed. Bacterial growth was detected in 35 (70%) samples, whereas 15 (30%) samples showed no bacterial growth. Among the positive cultures, *Proteus mirabilis* was found to be the most common bacterium in 10 isolates (20%), followed by *Escherichia coli* and *Staphylococcus aureus* (7 isolates each; 14%), *Pseudomonas aeruginosa* in 6 isolates (12%), and *Klebsiella pneumoniae* in 5 isolates (10%) (Table 2).

Table 2. Frequency of bacterial isolates in the kidney stone patients.

Type of bacteria	Number of isolates	Percentage (%)
Proteus mirabilis	10	20%
Escherichia coli	7	14%
Staphylococcus aureus	7	14%
Pseudomonas aeruginosa	6	12%
Klebsiella pneumoniae	5	10%
No bacterial growth	15	30%
Total	50	100%

Differential media were used to distinguish *Proteus mirabilis* from other Enterobacteriaceae: the bacterial isolates on blood agar showed swarming and hemolysis, whereas on MacConkey agar, they were non-lactose fermenters and had pale, round, convex, smooth colonies with a fish-like odor (Figures 1 and 2). The biochemical test was used to describe the *Proteus mirabilis* isolates. All 10 isolates tested positive for catalase, citrate, and urease but negative for indole and oxidase (Table 3).

The findings of the present investigation suggest that seven (70%) of the *Proteus mirabilis* isolates had the *ureC* gene detected by amplifying bacterial DNA using the PCR method at 533 bp, whereas three isolates (30%) were negative (Figure 3). Moreover, six (60%) of the *Proteus mirabilis* isolates had the *mrpA* gene detected by amplifying the bacterial DNA for this gene using the PCR method at 410 bp, while four isolates (40%) did not carry this gene, as shown in Figure 4.

In the present study, the mean serum level of IL-6 in the kidney stone patients was 7.62 ± 11.5 ng/mL, while the mean serum level of the control group was 5.05 ± 4.9 ng/mL, as shown in Figure 5. This result was highly significant ($p = 0.002$). The mean serum levels of TNF-α and CCL2 in the present study among the kidney stone patients were 18.61 ± 0.21 ng/mL and 211 ± 19.21 ng/mL, respectively, compared with 3.46 ± 0.13 ng/mL and 76.4 ± 5.3 ng/mL in the controls. These differences were also deemed statistically significant ($p = 0.001$), as shown in Figures 6 and 7, respectively.



Figure 1. *Proteus mirabilis* on MacConkey agar.



Figure 2. *Proteus mirabilis on blood agar.*

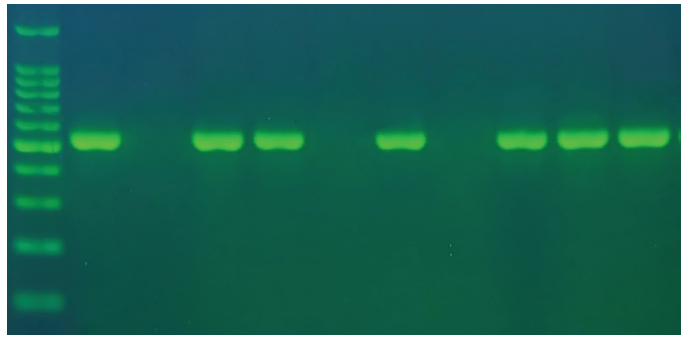

Figure 3. The polymerase chain reaction product exhibits a band size of 533 base pairs. The sample was subjected to electrophoresis on 1.5% agarose using 1 TBE buffer for 1 hour and 30 minutes. N: DNA ladder (100 base pairs).

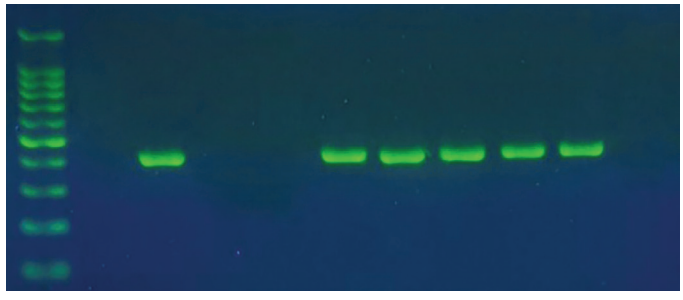

Figure 4. The polymerase chain reaction result shows a band size of 410 base pairs. The sample was subjected to electrophoresis on 1.5% agarose using 1 TBE buffer for 1 hour and 30 minutes. N: DNA ladder (100 base pairs).

Table 3. Biochemical test for *Proteus mirabilis*

Catalase	Oxidase	Citrate	Indole	Urease	MR/VP	TSI
+	-	+	-	+	-/+	H_2S+

R, methyl red; VP, Voges–Proskauer; TSI, triple sugar iron; H_2S, hydrogen sulfide.

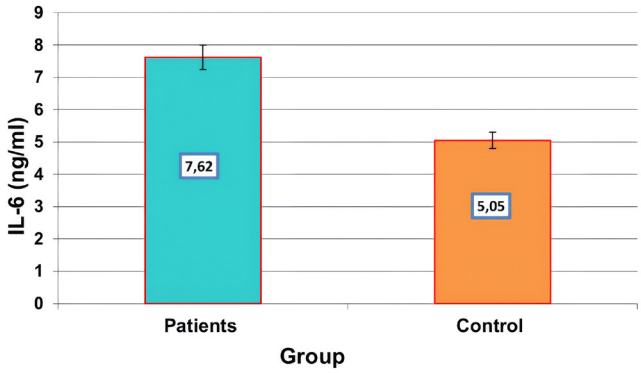

Figure 5. Mean level of IL-6 (ng/mL) in the kidney stone patients and controls. IL-6, interleukin-6.

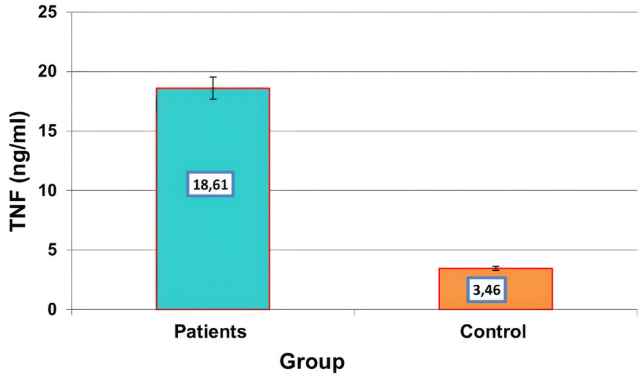

Figure 6. Mean level of TNF-α (ng/mL) in the kidney stone patients and controls. TNF-α, tumor necrosis factor-alpha.

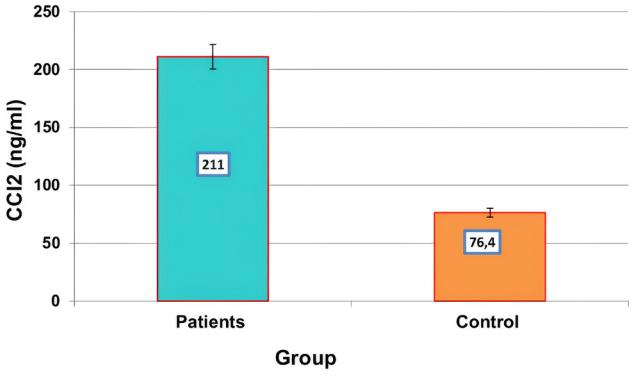

Figure 7. Mean level of CCL2 (ng/mL) in the kidney stone patients and controls.

Discussion

Until recently, it was suggested that only urease-producing bacteria were involved in the pathophysiology of urolithiasis and the formation of struvite stones. Researchers believe that bacteria might help make all kinds of stones, even non-struvite stones. This is because struvite stones are not as common as the other types of stones and people with urolithiasis are more likely to get UTIs and positive urine cultures[23]. The findings of the present study showed that *Proteus mirabilis* was the predominant bacterium, followed by *Escherichia coli* and *Staphylococcus aureus*.

The present study is consistent with previous studies conducted by[24], which reported that the percentage of isolated *Proteus mirabilis* was 19.04%, and with the study conducted by[25], which indicated that *Proteus mirabilis* is the most abundant organism in patients with kidney stones. Another study[26] showed a correlation between renal stones and *Escherichia coli*, *Enterococci*, *Micrococcus luteus*, and *Staphylococci* spp. This result contradicts that of the study conducted by[27], which indicated that *Staphylococcus* spp. was the predominant genus in the stones and urine of patients with kidney stones. The formation of these outcomes can be attributed to multiple factors, including environmental conditions, host characteristics, and microbial interactions[28]. Antibiotic usage, hospitalization, and host immunological condition may affect the predominance of specific bacterial species. Furthermore, the transmission dynamics of these bacteria in healthcare environments or communities may influence their differing prevalence rates[29]. *Proteus mirabilis* possesses several virulence genes associated with infection. The results reveal that the prevalence rate of *ureC* genes was seven (70%) and that of *mrpA* genes was six (60%). Urease (*ureC*) is a virulence gene crucial to the pathogenesis of *Proteus mirabilis*. This enzyme facilitates the formation of kidney stones. It is also crucial for urolithiasis, as it hydrolyzes urea to release ammonia, hence increasing urine pH, which results in the precipitation of calcium and magnesium compounds and the production of kidney stones[12]. The presence of these genes may contribute to the pathogenicity and virulence of the bacterial strains[17]. The *mrpA* gene, for instance, has been related with certain features, such as adhesion, biofilm formation, and resistance to host defenses[18]. The findings of the present study are consistent with those of a previous study[30] that revealed detection rates of the *mrpA* gene of 75% but contradict with the findings of[16],

which reported a 100% detection rate. The *mrpA* gene encodes a mannose-resistant *Proteus*-like fimbriae component needed for urinary tract bacterial adhesion, colonization, and biofilm formation. These fimbriae help bacteria settle in the urinary system and weaken the immune system, prolonging infection. MRPA may increase virulence, which may affect the treatment outcomes by increasing bacterial resistance to human defenses and antimicrobials[31].

The present study indicated an increase in the TNF-α, IL-6, and CCL2 levels in the kidney stone patients compared with the controls. The results are consistent with those of[32-34] indicating increased levels of TNF-α, IL-6, and CCL2. This may result from inflammatory responses induced by the kidney stones, perhaps leading to other urinary system infections. TNF-α is associated with various renal pathologies, and its association with clinical factors has been documented in chronic kidney disease[35]. Ahuja et al.[36] reported that circulating IL-6 drives inflammatory responses through CXCL1 synthesis following acute renal damage in mice. The results indicated that the plasma levels of TNF-α were downregulated, while the plasma concentrations of IL-6 were increased in patients following percutaneous nephrolithotripsy relative to the preoperative values[37]. Boonla et al.[38] showed an increased mRNA expression of IL-6 and MCP-1 in renal biopsy tissue from the kidneys with calculi. The role of IL-6 in stone formation, whether it is released due to the mechanical stimulation of epithelial cells or contributes to renal stone development, remains ambiguous[39,40].

Study Limitations

This study has several limitations that should be addressed. The sample size was relatively limited, and the samples were obtained from a single center, which may affect the generalizability of the findings. The cross-sectional nature of the study does not allow the establishment of causal relationships. However, the use of both microbiological and molecular techniques, along with the evaluation of key inflammatory biomarkers (TNF-α, IL-6, and CCL2), provides valuable insights into the studied condition. Thus, further studies with larger sample sizes and multicenter designs are recommended to further validate these findings.

Conclusion

The present study showed that *Proteus mirabilis* was the most prevalent bacterium in kidney stone patients. The results indicated that *Proteus mirabilis* carries the *ureC* and *mrpA* genes, which contribute to the pathogenicity of bacteria. Furthermore, this study also suggested that TNF-α, IL-6, and CCL2 tend to increase in kidney stone patients.

Ethics

Ethics Committee Approval: This study was conducted on urine samples collected as part of routine laboratory investigations, with no direct patient intervention. Thus, formal ethical approval was not required according to the institutional guidelines. All procedures were performed in accordance with relevant regulations and ethical standards.

Informed Consent: As the study was conducted using urine samples collected during routine laboratory investigations, without direct patient intervention, the requirement for informed consent was waived in accordance with institutional guidelines.

Footnotes

Authorship Contributions

Concept: F.A.A.A., A.D.A., Design: A.A.H., Data Collection or Processing: F.A.A.A., A.A.H., A.D.A., Analysis or Interpretation: F.A.A.A., A.A.H., Literature Search: A.A.H., A.D.A., Writing: F.A.A.A., A.D.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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REVIEW / DERLEME

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Scoping Review of Lactic Acid Bacteria Use in Preventing Antibiotic-Associated Diarrhea: Mechanisms and Recent Clinical Trials

Antibiyotik İlişkili İshalin Önlenmesinde Laktik Asit Bakterilerinin Kullanımına İlişkin Kapsamlı Derleme: Mekanizmalar ve Güncel Klinik Çalışmalar

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Abstract

Dysbiosis of the gut microbiota is a potential adverse effect of antimicrobial therapy and can manifest as antibiotic-associated diarrhea (AAD). The use of lactic acid bacteria (LAB) as probiotics is one of the most extensively investigated interventions for the prevention and management of AAD. This review summarizes the existing literature on LAB use in AAD prevention and management, drawing on *in vivo* studies and clinical trials. A literature search was conducted in the PubMed and Scopus databases using the following keywords: ("lactic acid bacteria" OR "lactobacillus" OR "bifidobacterium" OR "probiotic") AND ("antibiotic-associated diarrhea"). Full-text articles reporting *in vivo* studies and randomized clinical trials published in English between 2000 and 2024 were considered. A total of 43 articles were included in the review. LAB may help prevent and manage AAD through several mechanisms, including modulation of tryptophan metabolism, increased short-chain fatty acid production, intestinal colonization, competition with pathogenic species, and bacteriocin production. However, clinical trials investigating LAB as probiotics for AAD prevention and management have yielded inconsistent results. Some trials have reported reductions in AAD incidence, severity, and duration, as well as improvements in other symptoms. Several factors complicate the assessment of the clinical benefits of LAB probiotics, including heterogeneity in the LAB strains and species used, whether administered individually or in combination; differences in probiotic dosages and formulations; variation in gut microbiota composition among populations; and a lack of outcome assessments examining changes in microbiota composition following probiotic administration, such as LAB colonization. The effectiveness of LAB in preventing AAD and reducing its incidence remains inconclusive. Further studies are needed to determine the optimal use of LAB probiotics for the prevention and management of AAD.

Keywords: Lactic acid bacteria, Lactobacillales, *Bifidobacterium*, antibiotic-associated diarrhea, probiotics

Öz

Bağırsak mikrobiyotasındaki disbiyoz, antimikrobiyal tedavinin olası bir yan etkisidir ve antibiyotik ilişkili ishal (Aİİ) olarak kendini gösterir. Probiyotik olarak laktik asit bakterilerinin (LAB) uygulanması, Aİİ'nin hem önlenmesinde hem de tedavisinde en çok araştırılan müdahalelerden biridir. Bu makale, Aİİ'nin önlenmesi ve yönetiminde LAB kullanımına dair mevcut literatürü, in vivo çalışmalar ve klinik araştırmalar ışığında gözden geçirmektedir. PubMed ve Scopus veri tabanlarında ("lactic acid bacteria" OR "lactobacillus" OR "bifidobacterium" OR "probiotic") VE ("antibiotic-associated diarrhea") anahtar kelimeleri kullanılarak bir literatür taraması yapılmıştır. 2000-2024 yılları arasında İngilizce dilinde yayımlanmış, in vivo çalışmaları ve randomize klinik araştırmaları içeren tam metin makaleler incelenmiştir. Derlemeye toplam 43 makale dahil edilmiştir. LAB; triptofan metabolizmasını düzenleyerek, kısa zincirli yağ asidi üretimini artırarak, bağırsak lümenine yerleşerek, patojen türlere karşı rekabetçi bir

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büyüme sergileyerek ve bakteriyosin üreterek Aİİ'nin önlenmesi ve tedavisinde etkili olmaktadır. Bununla birlikte, probiyotik olarak LAB kullanımına dair klinik çalışmalar farklı sonuçlar ortaya koymuştur. Bazı klinik çalışmalar, LAB kullanımının Aİİ insidansını, şiddetini ve süresini azalttığını, ayrıca diğer semptomları hafiflettiğini bildirmiştir. Probiyotik olarak LAB'lerin klinik faydalarının değerlendirilmesindeki zorluklar arasında; kullanılan LAB suş ve türlerinin (tek başlarına veya kombinasyon halinde) heterojenliği, probiyotik dozajı ve preparatlarındaki farklılıklar, popülasyonlar arasındaki bağırsak mikrobiyotası çeşitliliği ve probiyotik kullanımından sonra mikrobiyota kompozisyonundaki değişimlere (örneğin LAB kolonizasyonu) dair sonuçların yeterince değerlendirilmemiş olması yer almaktadır. LAB'lerin Aİİ'yi önlemedeki ve insidansını azaltmadaki faydaları henüz kesinlik kazanmamıştır. Aİİ'nin önlenmesi ve tedavisinde probiyotik olarak LAB kullanımından optimal klinik fayda sağlanabilmesi için daha fazla çalışmaya ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Laktik asit bakterileri, Lactobacillales, *Bifidobacterium*, antibiyotik ilişkili ishal, probiyotikler

Introduction

The human gut harbors a highly diverse microbial ecosystem (approximately 10^{11} bacteria per gram of intestinal content). This ecosystem, known as the gut microbiota, plays an important role in maintaining host health[1]. When antibiotics are used to eradicate pathogenic bacteria during bacterial infections, they may also affect part of the gut microbiota, disrupting its diversity and balance. This condition, known as dysbiosis, increases the risk of antibiotic-associated diarrhea (AAD). AAD is defined as diarrhea occurring within hours after the initiation of antibiotic therapy and up to 8 weeks after its discontinuation. The incidence of AAD ranges from 5% to 25%, depending on the antibiotics used. A previous meta-analysis found that approximately 27% of AAD cases among hospitalized patients were caused by *Klebsiella oxytoca*, followed by *Clostridioides difficile* (19.6%), *Clostridium perfringens* (14.9%), and *Staphylococcus aureus* (5.2%)[2]. However, the meta-analysis included only 56 English-language articles, most of which were from Asia (26 studies) and Europe (15 studies). AAD-attributable mortality among inpatients ranges from 12.03% to 18.7%, and 65.47% of patients with AAD require longer hospital stays[3,4].

The burden of AAD resulting from gut microbiota dysbiosis has prompted research into strategies to reduce antibiotic-induced dysbiosis. Probiotics have potential as preventive and therapeutic interventions for AAD. Lactic acid bacteria (LAB) are among the microorganisms most commonly used in probiotic preparations. They are also commonly used to ferment dairy, meat, and vegetable products[5]. Common LAB genera include *Lactobacillus* and *Bifidobacterium*. However, their effectiveness in treating gastrointestinal diseases remains unclear because findings from existing studies are inconsistent[6]. This review aims to summarize the mechanisms of action of LAB and their clinical use in the prevention and management of AAD.

Materials and Methods

This article used a scoping review design and was written according to the PRISMA Extension for Scoping Reviews (PRISMA-ScR) guidelines. Articles were screened through searches of the PubMed and Scopus databases using the keywords ("lactic acid bacteria" OR "lactobacillus" OR "bifidobacterium" OR "probiotic") AND ("antibiotic-associated diarrhea"). We included *Lactobacillus* and *Bifidobacterium* as keywords because 90% of probiotic formulations contain bacteria from these genera[7]. Although some studies do not classify *Bifidobacterium* as LAB[8,9], others do[10,11]. We included *Bifidobacterium* in this review because it is widely used as a probiotic agent alongside *Lactobacillus*.

The mechanisms of action of LAB against AAD were reviewed based on articles reporting *in vivo* studies, whereas updates on the use of LAB for AAD prevention and treatment were based on clinical trials. Research articles reporting *in vivo* studies and clinical trials published in English between 2000 and 2024 that investigated the use of LAB against AAD were screened for inclusion. The year 2000 was chosen as the starting year because research on probiotics was expanding rapidly. The definition of probiotics was also updated and introduced in 2001[12]. The final literature search was conducted on December 31, 2024. Articles involving interventions with non-LAB species or studies involving pregnant women, immunodeficient patients, patients with cancer, or patients with a history of gastrointestinal surgery were excluded. The initial search retrieved 575 articles. During screening, the following articles were excluded: duplicates (225), review articles (186), articles irrelevant to the review topic (75), articles without LAB interventions (31), articles without full-text access (5), articles that did not report AAD incidence (6), articles that did not report *in vivo* studies or randomized clinical trials (3), and articles that did not clarify probiotic content (1). A total of 43 articles were included in this review. All included clinical trial articles were assessed for risk of bias using the Cochrane Risk of Bias 2 Tool. The article screening process is presented in Figure 1.

Results

LAB Overview

LAB are a group of Gram-positive cocci or bacilli that are aerotolerant, non-spore-forming, nonmotile, acid-resistant, and catalase-negative[13]. LAB are widely distributed in nature and can be found in decomposing plant matter, human and

animal orifices (oral, genital, intestinal, and respiratory tracts), and other environments, such as water. LAB can also be found in vegetables, juice, dairy products, fermented meat and fish, mash, and sourdough[14]. LAB can survive across a wide temperature range (5°C–45°C), at an optimum pH of 4.0–4.5, in high-salinity environments, and in the presence of additive compounds[15].

Currently, LAB comprise six main families: *Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Leuconostocaceae, Lactobacillaceae,* and *Streptococcaceae*[15]. A common application of LAB is in starter cultures. Starter cultures are preparations containing numerous microbial cells from one or more microbial species that are added to a product to utilize the microbes' enzymatic or metabolic activities[16]. The defining characteristic of LAB is the production of lactic acid as the main end product of carbohydrate fermentation. LAB lack functional cellular respiration systems and instead generate energy through substrate-level phosphorylation via two metabolic pathways for hexose fermentation: homofermentative and heterofermentative pathways[13]. The homofermentative pathway produces lactic acid as the main product without additional metabolites, such as gas and flavor compounds. In contrast, heterofermentative LAB produce lactic acid and other products, such as ethanol, acetic acid, and carbon dioxide[15].

The ability of LAB to synthesize various beneficial metabolites, including lactic acid, supports their use in biotechnological, food, and therapeutic products[13]. Common products made using LAB include dairy products such as yogurt, fermented

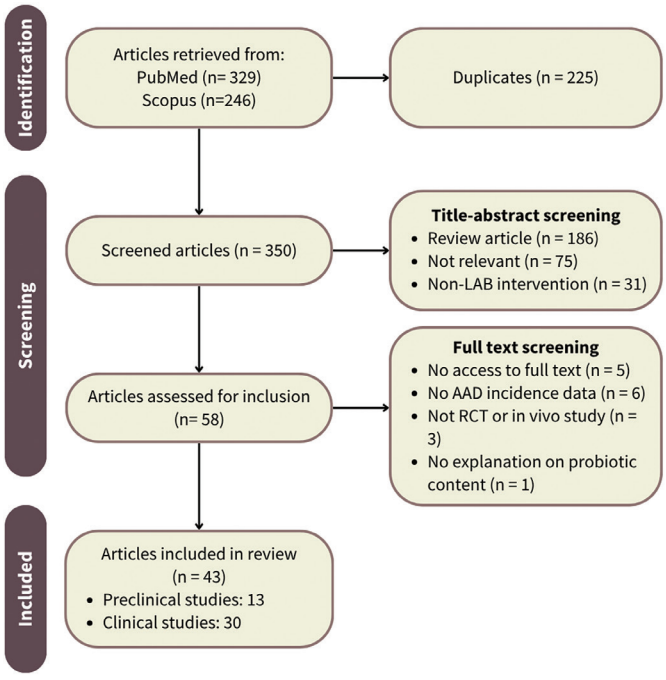

Figure 1. Article screening process.

milk, Yakult, tarhana, kefir, *acidophilus* milk, cheese, butter, and buttermilk (Figure 2)[17]. LAB play an important role in dairy fermentation by contributing to product preservation, flavor, and consistency. Some LAB species possess active proteolytic enzymes in milk and other food matrices, which increase the concentrations of bioactive peptides and polyamines. These peptides may exert antihypertensive, antithrombotic, immunomodulatory, and antioxidant effects. In addition, LAB may synthesize vitamins and help prevent intestinal infections[18].

LAB can also synthesize compounds with antimicrobial effects, particularly lactic acid and acetic acid. These compounds exhibit bioactive properties in their protonated forms at low pH. Other antimicrobial compounds are produced by LAB at concentrations below their minimum inhibitory concentrations, and they may act synergistically with lactic acid and acetic acid. Organic acids inhibit many pathogenic bacteria and other spoilage microorganisms through mechanisms associated with the creation of an acidic environment, which lowers the pH below the threshold required for microbial metabolism and growth. Additionally, lactic acid may inhibit cellular metabolism and proliferation by increasing the osmotic pressure of the medium. Organic acids may also alter the electrochemical balance and plasma membrane permeability, ultimately leading to microbial cell death[19].

LAB Mechanisms of Action Against AAD

Antibiotic administration directly affects the gut microbiota by decreasing the intestinal bacterial count, allowing pathogenic bacteria to grow and proliferate. Certain antibiotics also reduce gut bacterial diversity and abundance, leading to dysbiosis[20]. Short-term antibiotic use, such as a 7-day course of clindamycin, can cause a significant decrease in *Bacteroides* and enterococcal populations that persists for more than 2 years after treatment. This is also accompanied by an increase in antibiotic-resistant strains. In a small study (n = 10), a 10-day course of clindamycin decreased *Bifidobacterium* and *Lactobacillus* populations. The abundance of *Bifidobacterium* did not return to normal even 1 year after antibiotic therapy[21].

Some animal studies have shown that antibiotic therapy causes several pathological changes in the intestines, including crypt structural damage, submucosal edema, decreased villus number and density, mucosal thinning, and inflammatory cell infiltration. The severity of these changes depends on the antibiotic class and dosage. Antibiotic administration in animal models also caused changes in inflammatory markers. Pro-inflammatory mediators, such as interleukin (IL)-6, IL-1β, IL-17, and tumor necrosis factor-alpha (TNF-α), increased, whereas anti-inflammatory markers, such as IL-10, IL-4, transforming growth factor-beta (TGF-β), and interferon-gamma, decreased.

A summary of the pathological findings and changes in inflammatory markers after antibiotic administration in animal models is presented in Table 1.

Imbalances in pro-inflammatory and anti-inflammatory mediators may cause intestinal tissue damage. Pro-inflammatory mediators, such as IL-1β, IL-6, and TNF-α, play

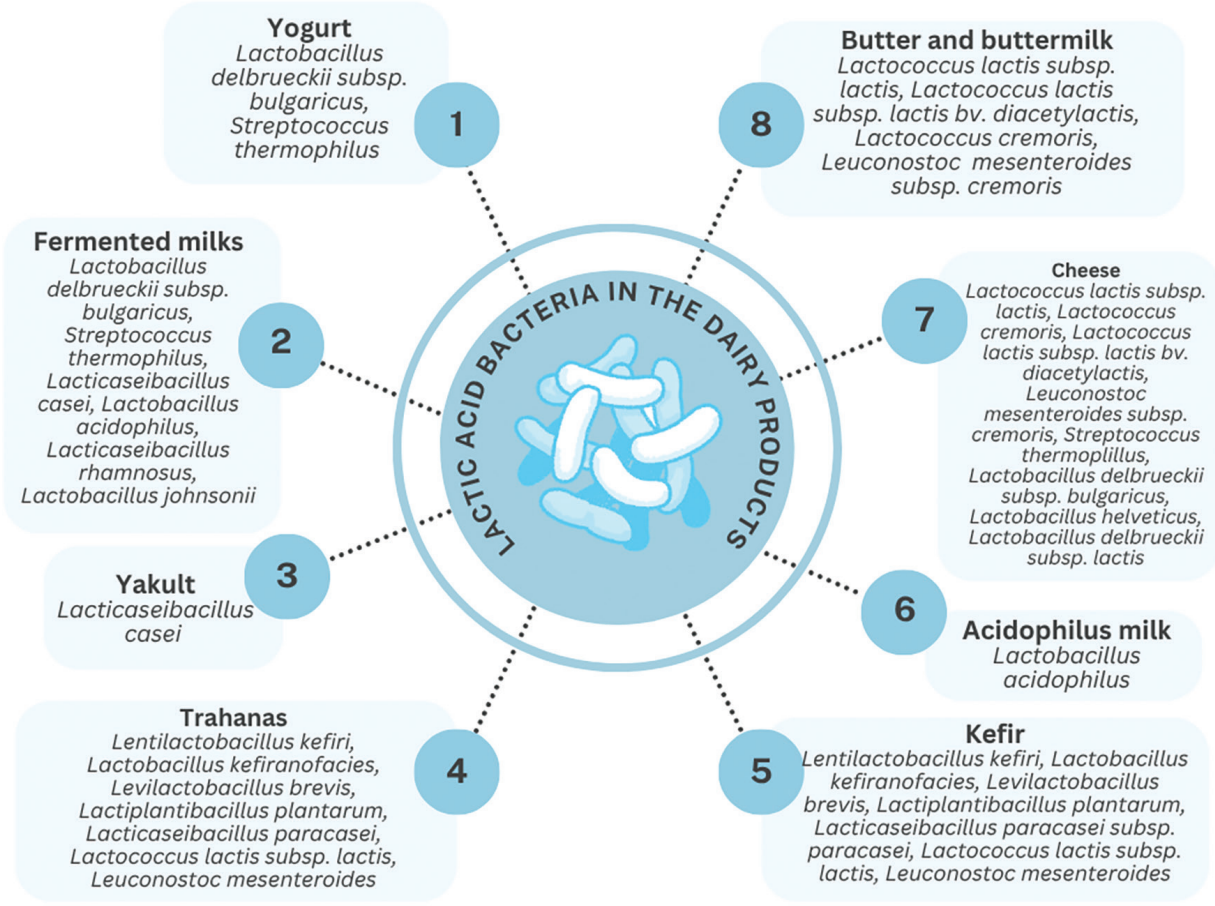

Figure 2. Use of LAB in various dairy products (data obtained from reference [17]).

Table 1. Summary of antibiotic-induced impacts on animal models.

Pathological findings	Inflammatory markers	Ref
• Crypt structure damage and inflammatory cell infiltration in colon tissue • ↓ Gut microbiota diversity • ↓ *Firmicutes* and *Desulfobacterota* populations • ↑ *Bacteroidetes* and *Verrucomicrobiota* populations	• ↑ IL-6 and IL-1β • ↓ IL-10 and TGF-β	[22]
• Severe damage of small intestinal mucosa and sparsely damaged villi • ↑ LPS and D-La titers • ↓ Total protein, serum albumin, and sIgA • ↓ ZO-1, occludin, claudin-1, and MUC2 expression • ↓ *Bacteroidetes* and *Proteobacteria* populations	• ↑ IL-6, TNF-α and IL-1β • ↓ IL-10	[23]
• Mild submucosal edema, accompanied by inflammatory cell infiltration and decreased gland cells • ↑ LPS titers • ↓ NHE3, occludin, and Mucin-2 expression • ↓ Acetic, propionic, isobutyric, and isovaleric acid titers • ↓ Gut microbiota diversity	• ↑ IL-6, IL-17, IL-1β, and TNF-α	[24]

Table 1. Continued

Pathological findings	Inflammatory markers	Ref
• Homogenous, integrated villi damage; most colon epithelial cells had severe swelling and some were ruptured • ↓ *Bacteroidetes/Prevotella, Clostridium* cluster XIVab and XI, *Faecalibacterium prausnitzii, Bifidobacterium* spp. populations • ↑ *Enterococcus* spp. and *Enterobacteriaceae* populations	• ↑ TNF-α • ↓ IL-10 and IFN-γ	[25]
• Shortening and loosening of intestinal villi, serous and muscular tissue thinning, and inflammatory cell infiltration • ↑ D-La, ET, and DAO titers • ↓ *Firmicutes* population • ↑ *Proteobacteria* population	• ↑ TNF-α • ↓ IL-10 and IFN-γ	[26]
• Mucosal epithelial shedding, loosely arranged intestinal glands, and slight edema accompanied by inflammatory cell infiltration • ↓ Acetic acid, propionic acid, butyric acid, and total SCFA titers • ↑ Regulation of TLR4/MyD88/NF-κB/IκBα signaling • ↑ Regulation of p-PI3K/p-AKT/NF-κB signaling • ↓ *Bacillota* population • ↑ *Bacteroidota* spp. and *Pseudomonadota* populations	• ↑ TNF-α • ↓ IL-10	[27]
• Cecum enlargement • ↓ Butyrate receptor GPR109A and butyrate transporter SLC5A8 expression • ↓ SLC16A3, AQP4, and NHE3 ion exchangers expression • ↓ ZO-1 and occluding expression	Not measured	[28]
• Thinning of colon mucosa and decreased goblet cell count • ↓ sIgA and IgG • ↑ LPS, D-La, and DAO • ↓ ZO-1, occludin, claudin-1, and MUC2 expression • ↑ p-Smad3 and ↓ Smad7 titers • ↑ *Bacteroidetes, Blautia, and Corpococcus* populations • ↓ *Firmicutes, Proteobacteria, Lactobacillus, Allobaculum,* and *Bifidobacterium* populations	• ↑ IL-4, IL-17A, TGF-β1	[29]
• ↑ *Firmicutes* population	• ↓ IL-4 and IL-10 • ↑ TNF-α and IFN-γ	[30]
• ↑ Cecum size and length • ↓ *Firmicutes* population • ↑ *Bacteroidetes* population	Not measured	[31]
• ↓ Acetic, propionic, and butyric acid titers • ↓ *Lactobacillaceae, Muribaculaceae, and Lachnospiraceae* populations	Not measured	[32]
• ↓ Cecum thickness • ↑ Surface gland degeneration and atrophy • ↑ *Bacteroides* population	• ↑ TNF-α • IL-6	[33]
• ↓ Ratio villous height to crypt depth • ↑ *Firmicutes* population • ↓ AQP1, AQP8, ZO-1, occluding, and Claudin-1 expression	Not measured	[34]

DAO, diamine oxidase; D-La, D-lactate; ET, endotoxin; IFN-γ, interferon-gamma; IL-1β, interleukin-1 beta; LPS, lipopolysaccharide; SCFA, short-chain fatty acid; sIgA, secretory immunoglobulin A; TGF-β, transforming growth factor-beta; TGF-β1, transforming growth factor-beta 1; TNF-α, tumor necrosis factor-alpha; ZO-1, zonula occludens-1; MUC2, mucin 2; NHE3, sodium-hydrogen exchanger 3; TLR4, toll-like receptor 4; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa B; IκBα, inhibitor of nuclear factor kappa B alpha; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; GPR109A, G protein-coupled receptor 109A; SLC5A8, solute carrier family 5 member 8; SLC16A3, solute carrier family 16 member 3; AQP1, aquaporin 1; AQP4, aquaporin 4; AQP8, aquaporin 8; Smad3, SMAD family member 3; Smad7, SMAD family member 7.

crucial roles in the inflammatory process. IL-6 is considered a major mediator involved in chronic inflammatory diseases, autoimmune disorders, cancer, and cytokine storms[35]. IL-1β can induce intestinal inflammation by disrupting tight junctions in the intestinal epithelium and increasing intestinal permeability[22].

In addition, IL-1β and TNF-α can activate transcription factors that induce IL-6 secretion. A combination of IL-6 and TGF-β is required to differentiate naïve CD4+ T cells into Th17 cells, whereas IL-6 inhibits Treg cell differentiation. An increased Th17/Treg ratio is considered important in disorders of immunological tolerance and the progression of inflammatory and autoimmune diseases[36]. TNF-α is secreted mainly by macrophages and monocytes, although other cells, including T-cell subsets, NK cells, dendritic cells, B cells, cardiomyocytes, fibroblasts, and astrocytes, may also secrete TNF-α. When TNF-α binds to its receptors (TNFR1 and TNFR2), several intracellular pathways are activated, mediating cell death and/or cell survival responses[37].

Probiotics can prevent AAD through several mechanisms. Some probiotic preparations containing *Bifidobacterium* and *Limosilactobacillus reuteri* can prevent colonic inflammation and alleviate inflammatory responses by increasing tryptophan concentrations and the production of indole-3-carboxaldehyde through the tryptophan metabolism pathway[22]. Tryptophan metabolites, including kynurenine and indole, are important for the binding and activation of the aryl hydrocarbon receptor (AhR) and for increasing the expression of the effector molecules CYP1A1 and CYP1B1, which are involved in intestinal immune regulation. A porcine study involving intraperitoneal lipopolysaccharide (LPS) injection showed that tryptophan administration increased the expression of AhR, CYP1A1, and CYP1B1 genes and decreased IL-1β, IL-6, IL-8, and TNF-α levels compared with those in controls that did not receive tryptophan[38]. Another study found that AhR activation inactivated actin-regulatory proteins, such as ezrin, resulting in decreased myosin II-induced actin contractility. This mechanism may also help maintain tight and adherens junctions, thereby reducing epithelial permeability[39]. Furthermore, indole derivatives, particularly indole propionic acid, bind to the pregnane X receptor, which may inhibit local inflammation and increase tight junction expression[40].

Probiotics may also exert beneficial effects by producing short-chain fatty acids (SCFAs) that help maintain intestinal barrier integrity. SCFAs are carboxylic acids with aliphatic tails containing fewer than six carbon atoms. Acetate (C2), propionate (C3), and butyrate (C4) are the main end products of fermentation by intestinal microbes. These molecules can be readily absorbed by the colon, where they provide energy for colonic cells and help repair epithelial injury. SCFAs are also involved in regulating glucose and lipid metabolism, thereby contributing to host energy homeostasis[41]. Each SCFA has distinct functions. Propionate may inhibit LPS-induced TNF-α and other cytokine production, as well as NF-κB activation. Butyrate may decrease IL-8 gene expression, improve intestinal permeability by activating peroxisome proliferator-activated receptors, inhibit NF-κB, upregulate MUC1–MUC4 expression, and promote the formation of regulatory T cells. Acetate may stimulate crypt cell proliferation, inhibit NF-κB activity, suppress IL-6 secretion, and serve as a precursor for butyrate formation[42].

Probiotics may also compete with pathogenic bacteria for nutrients. In addition, probiotics can secrete antimicrobial substances, such as bacteriocins and H_2O_2[43,44]. Bacteriocins are antimicrobial peptides produced by ribosomes and have various mechanisms of action. Some bacteriocins, such as nisin (secreted by *Lactococcus lactis*), plantaricin (*Lacticaseibacillus plantarum*), and lacticin B (*Lactobacillus acidophilus*), have demonstrated biological activity against foodborne enteropathogens, such as *Listeria*, *Clostridium*, *Staphylococcus aureus*, and vancomycin-resistant enterococci. Bacteriocins exert their biological effects by attaching to microbial cells and penetrating the phospholipid membrane, owing to their small size and varying hydrophobicity. They may then alter bacterial cytoplasmic membrane permeability, leading to the leakage of cellular contents. Bacteriocins may also inhibit RNA and DNA synthesis and/or cell wall synthesis[45]. Bacteriocins such as nisin have also shown inhibitory activity against *Clostridioides difficile* spores and vegetative growth[46,47].

Probiotics may also play a role in regulating electrolyte concentrations. SCFAs, particularly butyrate, have been shown to reduce cholera toxin-induced electrolyte and water secretion. Butyrate may bind to and activate GPR109A, thereby reducing intracellular cAMP levels. cAMP is a major signaling molecule in the gastrointestinal tract and is involved in regulating water and electrolyte absorption. Increased intracellular cAMP levels in the gastrointestinal tract can cause secretory diarrhea. In addition, butyrate may activate ion exchangers, such as SLC26A3, which triggers Na^+ absorption in the colon[28]. An *in vivo* study reported that mice infected with *Citrobacter rodentium* showed decreased expression of ion transporters, accompanied by reductions in serum chloride and sodium levels. The study also showed that *L. acidophilus* administration increased the expression and function of NHE3 (Na^+/H^+ exchanger 3) and DRA (Cl^-/HCO_3^- exchanger)[48]. An overview of the mechanisms through which probiotics containing LAB exert their effects against AAD is presented in Figure 3. A summary of *in vivo* studies, interventions, and the beneficial effects of probiotic administration in animal models is presented in Table 2.

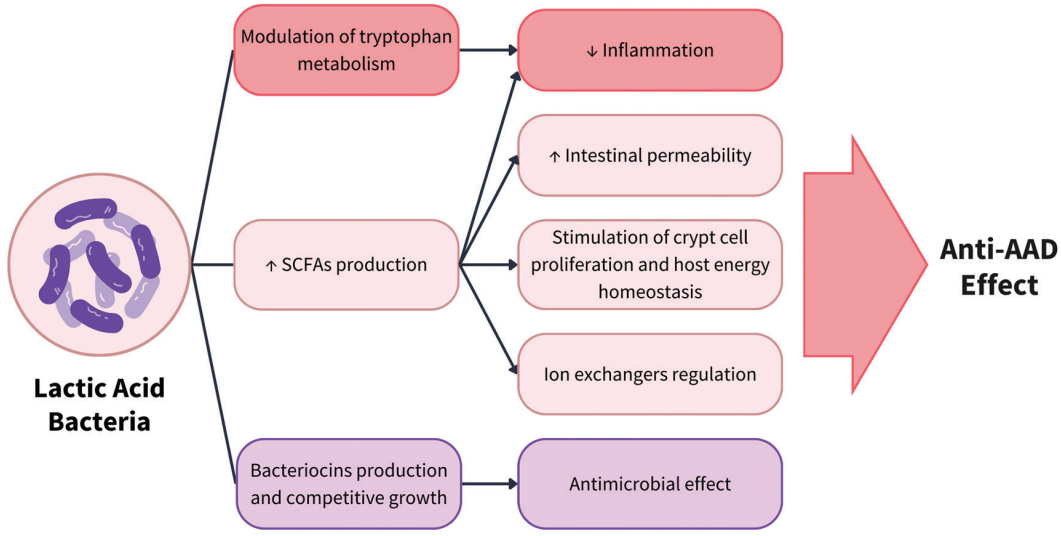

Figure 3. Overview of the main mechanisms of lactic acid bacteria action against AAD. SCFAs, short-chain fatty acids; AAD, antibiotic-associated diarrhea.

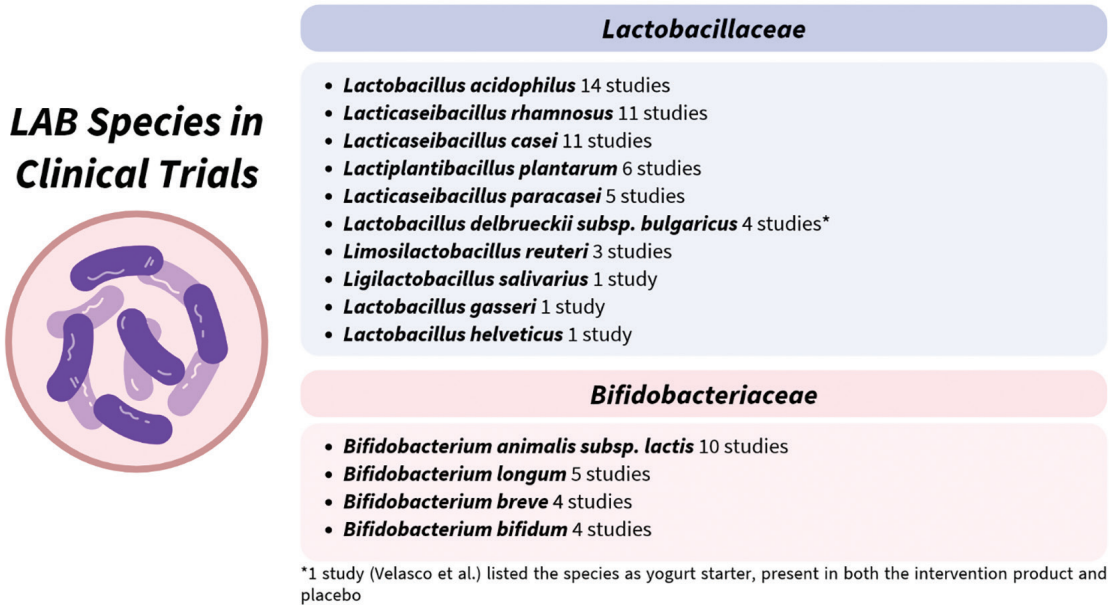

Figure 4. Lactic acid bacteria (LAB) species used in the clinical trials reviewed in this article.

Clinical Trials of LAB Use in AAD

Ten species of Lactobacillaceae and six species of Bifidobacteriaceae were used across the 30 clinical trials reviewed in this article (Figure 4). In addition, several studies included non-LAB species, such as *Streptococcus thermophilus* (six studies), *Bacillus coagulans* (one study), and *Saccharomyces boulardii* (one study), in their intervention products. Twenty-two studies used blends of multiple species and/or strains[49-62,66,68-73,78], whereas the remaining eight used single-strain interventions[63-65,67,74-77]. Details of the populations, interventions, and results of all reviewed studies are provided in Table 3.

Table 2. Preclinical testing of probiotic effects

No	Intervention	Probiotic benefits observed	Ref.
1.	• **Subject:** Mice • **Interventions:** AAD was induced by the administration of lincomycin hydrochloride G1: normal control (normal saline); G2: AAD + normal saline; G3: AAD + *Lacticaseibacillus plantarum* H-6; G4: AAD + *Weissella viridescens* J-1 • **Intervention duration:** 7 days	↓ diarrhea status score and significantly ↑ weight in G3 compared to G2 and G4 Repair of colon damage and ↓ inflammatory cell infiltration in G3 ↑ anti-inflammatory (IL-10 and TGF-β) and ↓ pro-inflammatory (IL-1β and IL-6) cytokines in G3 ↑ reduced riboflavin, threoninyl-methionine, L-tryptophan, and ↓ diethyl succinate levels in G3 ↑ abundance and diversity of colon microbiota in G3, approaching normal (G1)	[22]
2.	• **Subject:** BALB/c mice • **Interventions:** AAD was induced by administration AAD was induced by administration of ampicillin (2x/day, dose: 11,2 g/kg BW) G1: normal control (normal saline); G2: AAD mice + normal saline; G3: AAD mice + Bifico (contains *Bifidobacterium longum, Lactobacillus acidophilus,* and *Enterococcus faecalis);* 3 groups receiving complex probiotics with 3 doses: low (2.5×10^6 CFU, G4), medium (5×10^6 CFU, G5), and high (1×10^7 CFU, G6). • **Intervention duration:** 14 days	↓ fecal water content (most notably in G6) ↑ body weight (most notably in G6) ↓ lipopolysaccharide (LPS) and D-lactate (D-LA) levels (most notably in G3) ↑ total protein and albumin levels ↑ sIgA and IL-10 and ↓ IL-6 levels, (most notably in G6) ↑ ZO-1, occludin, claudin-1, and MUC2 mRNA expressions, (most notably in G6) ↑ abundance and diversity of gut microbiota in G6	[23]
3.	• **Subject:** Mice • **Interventions:** AAD was induced by the administration of lincomycin hydrochloride G1: normal control (phosphate buffer saline, PBS); G2: AAD mice + PBS; G3-G9: AAD mice + intervention regimens comprising various *Bifidobacterium* species, including *Bacteroides intestinalis* (Bi), *Bacteroides uniformis* (Bu), *Bacteroides adolescentis* (Ba), *Bacteroides bifidum* (Bb), and their combinations. • **Intervention duration:** 3 days	↓ diarrhea status score in groups receiving probiotic therapy, most notably in the group receiving Bu + Ba ↓ fecal water content, most notably in groups receiving Bi + Ba and Bu + Ba No significant pathological lesions from colon tissues retrieved from groups receiving Bu or Bu + Ba ↓ IL-6 levels in the Bu + Ba group ↓ LPS levels, most notably in the Bi + Ba group ↑ occludin and Mucin-2 expression, most notably in the Bu+Ba group ↑ NHE3 expression, most notably in groups receiving Bi, Bi +Ba, and Bu +Ba ↑ production of short-chain fatty acid/SCFA (acetic acid, propionic acid, isobutyric acid, and isovaleric acid) in groups receiving Bu + Ba and Bi + Bb ↑ abundance and diversity of gut microbiota in groups receiving probiotics	[24]
4.	• **Subject:** C57BL/6 mice • **Interventions:** AAD was induced by the administration of ceftriaxone 8 g/kg bw • G1: normal control (normal saline); G2: mice AAD; G3: AAD mice + sterile saline; G4: AAD mice + *Clostridium butyricum;* G5: AAD mice + *Bifidobacterium infantis;* G6: AAD mice + combination of probiotics *(Clostridium butyricum* + *Bifidobacterium infantis)*	Repair of edematous and ruptured colon tissue structures in G6 ↑ IL-10 and IFN-γ levels ↓ IL-1β and TNF-α levels Recovery of fecal microbiota diversity in the group receiving combination of probiotics	[25]
5.	• **Subject:** SPF Balb/c mice • **Interventions:** AAD was induced by the administration of lincomycin hydrochloride G1: normal control (normal saline); G2: AAD mice + normal saline; G3: AAD mice + suspension of *Streptococcus thermophilus* DMST-H2; G4: AAD mice + yogurt containing *Streptococcus thermophilus* DMST-H2 • **Intervention duration:** 6 days	Improves fecal consistency ↑ IL-10 and IFN-γ levels ↓ TNF-α levels ↓ infiltration of inflammatory cells ↓ cecum index ↑ *Firmicutes* and ↓ *Proteobacteria* populations	[26]

Table 2. Continued

No	Intervention	Probiotic benefits observed	Ref.
6.	• **Subject:** C57BL/6 mice • **Interventions:** AAD was induced by administration of amoxicillin 500 mg/kg + clindamycin 400 mg/kg + streptomycin 350 mg/kg G1: normal control (normal saline); G2: AAD mice; G3: AAD mice + *Lacticaseibacillus plantarum* 0.1 mL/10 g • **Intervention duration:** 14 days	↓ infiltration of inflammatory cells in colonic tissue, ↓ edema, and repair of intestinal gland tissue ↓ TNF-α and IL-1β levels ↑ SCFA production (acetic acid, butyric acid, total SCFA) Inhibition of TLR4/NF-κB signaling Inhibition of PI3K/AKT/NF-κB phosphorylation and activation ↑ abundance and diversity of gut microbiota	[27]
7.	• **Subject:** C57BL/6 mice • **Interventions:** AAD was induced by the administration of metronidazole + vancomycin + neomycin sulfate (in G2) Probiotics used: *Lacticaseibacillus rhamnosus* strain GG G1: mice without antibiotic therapy, divided into 4 interventional subgroups: G1a: control; G1b: probiotics; G1c: tributyrin; G1d: probiotics + tributyrin. G2: mice receiving antibiotic therapy divided into interventional subgroups similar to G1. • **Intervention duration:** 7 days	Prevents the decline of SLC5A8 butyrate transporter and GPR109A butyrate receptor expressions in the gut Prevents the decline of ion exchanger SLC26A3, NHE3, and water channel AQP4 expressions.	[28]
8.	• **Subject:** C57BL/6 mice • **Interventions:** AAD was induced by the administration of amoxicillin + clindamycin + streptomycin G1: normal control (normal saline); G2: AAD mice + normal saline; G3: AAD mice + *Lacticaseibacillus plantarum* ELF051; G4: AAD mice + *A.* polysaccharide; G5: AAD mice + *Lacticaseibacillus plantarum* + *A.* polysaccharide • **Intervention duration:** 14 days	↓ cecum index ↑ mucous membrane area and goblet cells in the colon ↑ sIgA and IgG levels ↓ IL-17A and IL-4 levels ↓ diamine oxidase, d-lactate, lipopolysaccharides, and TGF-β1 levels ↓ p-Smad3 and ↑ Smad7 proteins ↑ *Firmicutes, Lactobacillus, Bifidobacterium,* and Protebacteria populations, also ↓ *Bacteroides,* Coprococcus, and Blautia populations These effects are more pronounced in combination with Astragalus polysaccharide.	[29]
9.	• **Subject:** SPF KM mice • **Interventions:** AAD was induced by the administration of cefradine + gentamicin sulfate G1: normal control (normal saline); G2: AAD mice; G3: AAD mice + normal saline; G4: AAD mice + *Lacticasebacillus plantarum* ($1x10^7$ CFU/mL); G5: AAD mice + *Lacticasebacillus plantarum* ($1x10^8$ CFU/mL); G6: AAD mice + *Lacticaseibacillus plantarum* ($1x10^9$ CFU/mL)	Speeds up diarrhea resolution ↑ body weight and appetite ↑ IL-4 and IL-10 ↓ TNF-α and IFN-γ ↑ abundance and diversity of gut microbiota	[30]
10.	• **Subject:** Kunming mice • **Interventions:** AAD was induced by the administration of lincomycin + gentamicin sulfate G1: normal control (normal saline); G2: AAD mice; G3: AAD mice + tablet Lacidophilin *(Lactobacillus);* G4: AAD mice + ambrosial yogurt *(Streptococcus thermophilus);* G5: AAD mice + bifid triple capsule *(Bifidobacterium longum, Enterococcus faecalis, Lactobacillus acidophilus)* • **Intervention duration:** 10 days	↓ duration of diarrhea ↑ body weight and ↓ cecum enlargement (most notably in G4) ↑ abundance of microbial communities ↑ populations with *Firmicutes* and *Bacteroidetes*	[31]
11.	• **Subject:** C57BL/6 mice • **Interventions:** AAD was induced by the administration of amoxicillin G1: normal control (normal saline); G2: AAD mice + normal saline; G3: AAD mice + probiotic solution containing *Bifidobacterium lactis* BB-12; G4: AAD mice + yogurt without *Bifidobacterium lactis;* G5: AAD mice + yogurt with *Bifidobacterium lactis* BB-12 • **Intervention duration:** 10 days	↑ body weight in male mice ↑ *Streptococcaceae* and ↓ Erwiniaceae populations ↓ formate, glycine, and glutamate metabolite levels (approaching controls)	[32]

Table 2. Continued

No	Intervention	Probiotic benefits observed	Ref.
12.	• **Subject:** Wistar rats • **Interventions:** AAD induced by Lincomycin G1: Control (distilled water) G2: AAD rats + distilled water G3: AAD rats + distilled water G4: AAD rats + *Lactobacillus acidophilus* + *Lacticaseibacillus casei* + *Bifidobacterium bifidum* preparation with 3×10^9 CFU/g per strain density • **Intervention duration:** 5 days	↓ duration of diarrhea ↑ cecum thickness ↑ body weight ↓ TNF-α and IL-6 ↑ serum and tissue IgA ↑ population with *Firmicutes* and genera *Lactobacillus, Bacillus, and Romboutsia* ↓ *Bacteroides* population	[33]
13.	• **Subject:** ICR mice • **Interventions:** AAD induced by ceftriaxone sodium G1: Control (saline) G2: AAD mice + saline G3: AAD mice + unfermented apple juice G4: AAD mice + *Lacticaseibacillus plantarum* G5: AAD mice + fermented apple juice • **Intervention duration:** 7 days	↓ duration and severity of diarrhea Repair of intestinal damage (↑ villi count and height) *Effects were most significant in G5.	[34]

AAD, antibiotic-associated diarrhea; AQP4, aquaporin 4; BALB/c, Bagg albino/c mouse strain; CFU, colony-forming unit; G1–G9, groups 1–9; GPR109A, G protein-coupled receptor 109A; ICR, Institute of Cancer Research mouse strain; IFN-γ, interferon-gamma; IgA, immunoglobulin A; IgG, immunoglobulin G; IL-1β, interleukin-1 beta; KM, Kunming mice; LPS, lipopolysaccharide; MUC2, mucin 2; NF-κB, nuclear factor kappa B; NHE3, sodium-hydrogen exchanger 3; PBS, phosphate-buffered saline; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; SCFA, short-chain fatty acid; sIgA, secretory immunoglobulin A; SLC5A8, solute carrier family 5 member 8; SLC26A3, solute carrier family 26 member 3; SPF, specific pathogen-free; TGF-β, transforming growth factor-beta; TGF-β1, transforming growth factor-beta 1; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-alpha; ZO-1, zonula occludens-1.

Table 3. Summary of clinical trials of the benefits of probiotics in the treatment of AAD.

No.	Intervention	AAD definition in study	Probiotic effects	Risk of bias	Ref.
1.	• Participants: 350 pediatric patients aged 3 months–18 years receiving oral or intravenous broad-spectrum antibiotics • Probiotics used: Ecologic AAD 612, containing combination of 8 species: *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Lactobacillus acidophilus* W37, *Lactobacillus acidophilus* W55, *Lacticaseibacillus paracasei* W20, *Lactiplantibacillus plantarum* W62, *Lacticaseibacillus rhamnosus* W71, and *Ligilactobacillus salivarius* W24 (formerly known as *Lactobacillus salivarius* W24) • Preparation form: Sachet • Dose: 5×10^9 CFU/sachet, 2 /days (10^{10} CFU/day) • Follow-up duration: 1 week after cessation of antibiotic therapy	"AAD defined as 3 or more loose or watery stools (a score of A on the AISS or 5–7 on the BSFS) per day in a 24-hour period, caused either by *Clostridioides difficile* or of otherwise unexplained etiology, after testing for common, predefined diarrheal pathogens." Testing for *Clostridioides difficile:* - Detection of glutamate dehydrogenase - Detection of toxins A and B via immunoassay	↓ all diarrhea cases regardless of etiology (20.9% vs. 32.3% [pro vs. pla], 95% CI: 0.44–0.94, *p:* 0.02) and AAD incidence (14.6% vs. 18.1% [pro vs. pla], 95% CI: 0.49–1.33) No significant difference in CDAD (0.6% vs. 1.9%, 95% CI: 0.05–2.26) ↓ number of patients requiring rehydration therapy	Low	[49]
2.	• Participants: 36 adult patients (≥18 years) receiving amoxicillin-clavulanic acid therapy for 7 days, divided into 2 groups: placebo and intervention groups • Probiotics used: *Lactobacillus acidophilus* NCFM, *Lacticaseibacillus rhamnosus* Lr-32, *Bifidobacterium breve* M-16V, *Bifidobacterium longum* BB536, *Bifidobacterium lactis* BI-04, and *Bifidobacterium bifidum* Bb-02 • Preparation form: Capsule • Dose: 10^9 CFU/capsule, 1×/day • Intervention duration: 30 days	"AAD was defined as at least 3 watery/fluid stools in a 24-h period."	Delay in diarrhea onset by 5.39 days ($p = 0.002$) compared to placebo No significant difference in defecation frequency per day	High	[50]

Table 3. Continued

No.	Intervention	AAD definition in study	Probiotic effects	Risk of bias	Ref.
3.	• Participants: 255 adult patients (50–70 years) receiving penicillin, cephalosporin, or clindamycin therapy, divided into 3 groups receiving 2 capsules of probiotics (G1); 1 probiotic + 1 placebo capsules (G2); or 2 placebo capsules (G3) per day • Probiotics used: *Lactobacillus acidophilus* CL1285 + *Lacticaseibacillus casei* LBC80R • Preparation form: Capsule • Dose: 5×10^9 CFU/capsule • Intervention duration: starting within 36 hours since antibiotic therapy initiation, up to 5 days after antibiotic cessation • Follow-up duration: Up to 21 days after the intervention stopped.	"Diagnosis of AAD was made when a patient produced three or more liquid stools in a 24-h period after antibiotic treatment with no other obvious reason for diarrhea." Testing for *Clostridioides difficile:* cytotoxin assay	↓ AAD (28.6% vs. 23% vs. 9.1%, [G3 vs. G2 vs. G1]) and CDAD incidence (19.1% vs. 9.5% vs. 0) Faster relief of symptoms, most notably in G1	Low	[51]
4.	• Participants: 89 adult inpatients receiving at least 3 days of systemic antibiotic therapy, divided into 2 groups receiving probiotics and placebo • Probiotics used: *Lacticaseibacillus casei* and *Lactobacillus acidophilus* • Preparation form: Fermented milk beverage • Dose: 50×10^9 CFU/container, 98 g/day (49 g/day for first 2 days) • Intervention duration: starting within 48 hours since antibiotic therapy initiation, up to antibiotic cessation • Follow-up duration: Up to 21 days after the intervention stopped.	"AAD was defined as three or more liquid stools in a 24-h period." Testing for *Clostridioides difficile:* cytotoxin assay	↓ AAD (15.9% vs. 35.6%, [pro vs. pla], 95% CI: 0.125–0.944, p = 0.05) and CDAD incidence (2.3% vs. 15.6%, 95% CI: 0.020–1.109, p = 0.06) No significant effect of probiotic use on the length of stay	Low	[52]
5.	• Participants: 450 adult inpatients receiving antibiotic therapy, divided into 3 groups receiving high-dose probiotics (G1), low-dose probiotics (G2), or placebo (G3) • Probiotics used: *Lactobacillus acidophilus, Lacticaseibacillus paracasei* Lpc-37, *Bifidobacterium lactis* Bi-07, *Bifidobacterium lactis* BI-04 • Preparation form: Capsule • Dose: 1.70×10^{10} CFU (high dose) or 4.17×10^9 CFU (low dose) • Intervention duration: starting at antibiotic therapy initiation, up to 7 days after antibiotic cessation • Follow-up duration: Up to 3 weeks after the intervention stopped	"AAD was defined as three or more liquid stools in a 24-h period. Testing for *Clostridioides difficile:* Cytotoxic and antigen detection assays	↓ AAD incidence (24.6% vs 19.6% vs. 12.5% [in G3 vs. G2 vs. G1], p = 0.005 in G1 vs. G3) ↓ CDAD incidence (4.8% vs 1.8% vs. 1.8%, [G3 vs. G2 vs G1], p = 0.04 in G1 vs. G3) ↓ defecation frequency High-dose probiotics shorten the AAD duration	Low	[53]
6.	• Participants: 437 adult inpatients receiving antibiotic therapy, divided into 2 groups receiving probiotics or placebo • Probiotics used: *Lacticaseibacillus casei* and *Lactobacillus acidophilus* • Preparation form: Fermented milk beverage • Dose: 50×10^9 CFU/container, 98 g/day (49 g/day for first 2 days) • Intervention duration: starting at antibiotic therapy initiation and administered for 4 weeks (28 days). • Follow-up duration: Up to 2 weeks after the intervention stopped.	"Diarrhea was defined as one or more episodes of unformed or liquid stool in a 24-h period." Testing for *Clostridioides difficile:* detection of toxin A or B	↓ AAD incidence (21.8% vs. 31.7% [pro vs. pla]; 95% CI: 0.405–0.971; p = 0.037) Shortens the AAD duration (in days, after adjusted analysis) No significant difference in CDAD incidence (6.2% vs. 13.3%, p = 0.645).	Some concerns	[54]

Table 3. Continued

No.	Intervention	AAD definition in study	Probiotic effects	Risk of bias	Ref.
7.*	• Participants: 555 adult outpatients receiving 1 or 2 antibiotics, divided into 2 groups receiving probiotics or placebo • Probiotics used: 7 species of *Lactobacillus* spp., 4 species of *Bifidobacterium* spp.; *Bacillus coagulans, Saccharomyces boulardii* • Preparation form: Enterosolvent capsule • Dose: Each capsule contains 7 species of *Lactobacillus* spp. (56×10^9 CFU), 4 species of *Bifidobacterium* spp. (38×10^9 CFU); *Bacillus coagulans* (1×10^9 CFU); *Saccharomyces boulardii* (5×10^9 CFU), given 1×/day (2 capsules per day for the duration of antibiotic therapy followed by 1 capsule/day for 14 days after antibiotics are stopped) • Intervention duration: starting at antibiotic therapy initiation and administered up to 14 days after antibiotic cessation • Follow-up duration: Up to 21 ± 2 days after cessation of antibiotics	"AAD was defined as ≥3 loose or liquid stools (types 5–7) according to the Bristol Stool Form Scale [BSFS] over a period of 24 hours."	↓ incidence (9.2% vs. 25.3%, [pro vs. pla], $p < 0.001$) and severity of AAD (1.1% vs. 8.4%, $p < 0.001$). Shortens the AAD duration Alleviation of gastrointestinal symptoms	Low	[55]
8.	• Participants: 113 adult inpatients receiving antibiotic therapy, divided into 2 groups receiving probiotics or no probiotics • Probiotics used: *Limosilactobacillus reuteri* LMG P-27481 + *Lacticaseibacillus* GG ATCC 53103 • Preparation form: Oral stick • Dose: 2×10^{10} CFU, 1/day • Intervention duration: starting within 24 hours from antibiotic therapy initiation, administered up to antibiotic cessation • Follow-up duration: Up to 4 weeks after discharge from the hospital (by phone)	"AAD was considered in cases of unexplained diarrhea, stool test negativity, or the presence of CDI toxin, occurring from the start of antibiotic therapy until 4 weeks after the end of antibiotic therapy." Testing for *Clostridioides difficile:* detection of toxins A and B	↓ AAD (11% vs. 28% [pro vs. pla], $p < 0.01$) and CDAD incidence (0 vs. 11% [pro vs. pla], $p < 0.01$) Alleviates gastrointestinal symptoms	High	[56]
9.	• Participants: 340 adult patients receiving antibiotic therapy, divided into 2 groups receiving probiotics or placebo • Probiotics used: *Lacticaseibacillus rhamnosus* GG + *Bifidobacterium longum* BB536 • Preparation form: Capsule • Dose: 1×10^{10} CFU/capsule, 2/day • Intervention duration: starting within 24 hours from antibiotic therapy initiation, administered up to antibiotic cessation.	Not available	↓ AAD incidence (18.23% vs. 31.17% [pro vs. pla]) Shortens the diarrhea duration No significant difference in diarrhea severity and length of stay	Low	[57]
10.	• Participants: 146 otherwise-healthy adults receiving amoxicillin-clavulanate therapy, divided into 2 groups receiving probiotics or placebo • Probiotic used: *Lactobacillus helveticus* and *Lacticaseibacillus rhamnosus* • Preparation form: capsule • Doses: *Lactobacillus helveticus* (2.0×10^8 CFU/mL) and *Lacticaseibacillus rhamnosus* (3.8×10^9 CFU/mL), twice daily • Intervention duration: 2 weeks • Follow-up duration: 10 weeks	"A diarrhea-like defecation, as defined, corresponded to a stool frequency ≥3/d and/or stool consistency ≥5 (on the BSS) for at least 2 consecutive days"	↓ diarrhea-like defecation duration (2.70 vs. 3.71 [pro vs. pla], $p = 0.037$)	Low	[58]

Table 3. Continued

No.	Intervention	AAD definition in study	Probiotic effects	Risk of bias	Ref.
11.	• Participants: 72 children receiving antibiotic therapy, divided into 2 groups receiving probiotics or placebo • Probiotic used: *Lacticaseibacillus rhamnosus, Bifidobacterium lactis,* and *Lactobacillus acidophilus* Placebo: pasteurized yogurt containing *S. thermophilus* (4.4×10^4 CFU/day) and *Lactobacillus bulgaricus* (1.2×10^8 CFU/day) • Preparation form: yogurt • Doses: *Lacticaseibacillus rhamnosus* (5.2×10^9 CFU/day), *Bifidobacterium lactis* (5.9×10^9 CFU/day), and *Lactobacillus acidophilus* (8.3×10^9 CFU/day), twice daily • Intervention duration: 1 week	Various definitions of diarrhea. These included: (A) stool consistency ≥5 and stool frequency ≥2/day for more than 2 days; (B) stool consistency ≥5 and stool frequency ≥3/day for more than 2 days; (C) stool consistency ≥6 and stool frequency ≥2/day for more than 2 days; and (D) stool consistency ≥6 and stool frequency ≥3/day for more than 2 days.	Less participants with defecation frequency ≥2×/day and fecal consistency score ≥5 in the probiotics group (significant in all definitions)	Low	[59]
12.	• Participants: 122 adult inpatients receiving systemic antibiotics, divided into 2 groups receiving probiotics or placebo • Probiotics used: *Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lacticaseibacillus plantarum, Lacticaseibacillus paracasei, L. delbrueckii* ssp. *Bulgaricus,* and *Streptococcus thermophilus* • Preparation form: powdered sachet • Doses: 4.5×10^{11} CFU/sachet, twice daily • Intervention duration: during the course of antibiotic therapy, resumed until 7 days after antibiotic cessation • Follow-up: 28 days after antibiotic cessation	"AAD was defined as more than two liquid stools (Bristol stool chart types 6 and 7) in excess of normal for each patient a day for two or more days."	↓ AAD incidence (0 vs. 11.4% [pro vs pla], p = 0.006)	Low	[60]
13.	• Participants: 63 adult patients receiving antibiotics for at least 7 days, divided into 2 groups receiving probiotics or placebo • Probiotic used: *Lacticaseibacillus rhamnosus, Lactobacillus acidophilus, and Bifidobacterium* • Preparation form: milk • Doses: *Lacticaseibacillus rhamnosus, Bifidobacterium* (10^8 CFU/mL), *Lactobacillus acidophilus* (10^7 CFU/mL), 250 mL/day • Intervention duration: 14 days	"Diarrhea defined as at least three fluid stools/day for at least 2 days during the test period" Testing for *Clostridioides difficile:* detection of toxin A via chromatographic assay, bacterial culture	↓ AAD incidence (5.9% vs. 27.6% [pro vs. pla], p = 0.035) No significant difference in CDAD incidence	High	[61]
14.	• Participants: 113 inpatients aged >50 years receiving antibiotic therapy, divided into 2 groups receiving probiotics or placebo • Probiotic used: *Lacticaseibacillus casei, Lactobacillus bulgaricus,* and *Streptococcus thermophilus* • Preparation form: Yogurt • Doses: *Lacticaseibacillus casei* and *Streptococcus thermophilus* (10^8 CFU/mL) and *Lactobacillus bulgaricus* (10^7 CFU/mL), twice daily • Intervention duration: 2 days after the start of antibiotic therapy up to 1 week after antibiotic cessation • Follow-up duration: 4 weeks following discharge	"Diarrhoea was defined as more than two liquid stools a day for three or more days in quantities in excess of normal for each patient." Testing for *Clostridioides difficile:* detection of toxins A and B via immunoassay	↓ AAD (12% vs. 34% [pro vs. pla], p = 0.007) and CDAD (0 vs. 17% [pro vs. pla], p = 0.001) incidence	Low	[62]

Table 3. Continued

No.	Intervention	AAD definition in study	Probiotic effects	Risk of bias	Ref.
15.	• Participants: 258 (study 1) and 60 (study 2) adult inpatients in the Internal Medicine wards • Probiotics used: Study 1: *Lacticaseibacillus casei* DN-114001 against placebo drink Study 2: *Lacticaseibacillus casei* DN-114001 (G1), *Lacticaseibacillus casei* Shirota (G2). • Preparation form: Probiotic drinks • Dose: 10^{10} CFU/container of drinks, 2×/day • Intervention duration: Study 1: 3 months Study 2: during the course of antibiotic therapy	"AAD was defined as at least 3 watery/fluid stools in a 24-h period."	Study 1: • ↓ AAD incidence (6.5% vs. 28.4% [pro vs. pla], $p < 0.001$) • ↓ AAD duration (1.7 vs. 3.1 days [pro vs. pla], $p = 0.015$) • No significant difference in length of hospital stay Study 2: • ↓ AAD incidence (6.7% vs. 33.3% [pro vs. pla], $p = 0.021$) • No significant difference in length of stay and AAD duration between the 2 groups	High	[63]
16.	• Participants: 23 adult inpatients receiving antibiotic therapy, divided into groups receiving probiotics or placebo • Probiotics used: *Limosilactobacillus reuteri* ATCC 55730 • Preparation form: Chewable tablet • Dose: 1×10^8 CFU/tablet, 2/day • Intervention duration: starting at antibiotic therapy initiation and administered for 4 weeks (28 days) • Follow-up duration: Up to 2 weeks after the intervention stopped.	"Diarrhea was defined as stool output meeting or exceeding 3 or more Type 6 (fluffy pieces with ragged edges and a mushy stool) or Type 7 (watery) bowel movements per day for 2 consecutive days."	↓ AAD incidence (8% vs. 50% [pro vs. pla], $p = 0.04$) ↓ defecation frequency No significant difference in gastrointestinal symptoms (nausea, abdominal pain/cramps, bloating, gas, vomiting, constipation, and reflux)	High	[64]
17.	• Participants: 158 adult patients with spinal cord injuries receiving antibiotics, divided into 2 groups receiving probiotics or placebo • Probiotic used: *Lacticaseibacillus casei* Shirota • Preparation form: beverages • Doses: 6.5×10^9 CFU/bottle, 1×/day • Intervention duration: 24 hours from the first antibiotic administration to 7 days after antibiotic cessation • Follow-up: 30 days after antibiotic cessation	"diarrhoea defined as more than two liquid stools (Bristol Stool Chart type 5, 6, or 7) a day for 3 or more days, in quantities in excess of normal." Testing for *Clostridioides difficile:* detection of glutamate dehydrogenase and toxin	↓ AAD incidence (17.1% vs. 54.9% [pro vs. pla], $p < 0.001$) No significant difference in CDAD incidence (0 vs. 1.2%)	Some concerns	[65]
18.	• Participants: 157 hospitalized infants (6–36 months) receiving antibiotic therapy, divided into groups receiving probiotic formula or placebo • Probiotics used: *Bifidobacterium lactis* and *Streptococcus thermophilus* • Preparation form: Formula milk • Dose: 10^7 viable cells of *Bifidobacterium lactis* and 10^6 viable cells of *Streptococcus thermophilus* • Intervention duration: starting at antibiotic therapy initiation and being administered for 15 days.	"Diarrhea was defined as a change in bowel habits with the passage of three or more liquid stools per day for at least 2 consecutive days."	↓ AAD incidence (16.3% vs 31.2% [pro vs. pla], $p = 0.044$) and dehydration (69.2% vs. 95.8%, $p = 0.039$) No significant difference in the duration or incubation period of AAD	High	[66]

Table 3. Continued

No.	Intervention	AAD definition in study	Probiotic effects	Risk of bias	Ref.
19.†	• Participants: 163 patients receiving antibiotic therapy, divided into groups receiving drinks with no probiotics or with probiotics • Probiotics used: *Lacticaseibacillus plantarum* • Preparation form: Beverage • Dose: 5×10^7 CFU/mL, 200 mL/day (total 10^{10} CFU/day) • Intervention duration: starting within 48 hours from antibiotic therapy initiation and administered up to 1 week after antibiotic cessation • Follow-up duration: up to 3 days after the intervention stopped	"Diarrhea was defined as at least 3 loose or watery stools per day for at least 2 consecutive days." Testing for *Clostridioides difficile:* detection of toxin via enzyme-linked immunosorbent assay	Probiotics did not affect diarrhea incidence (7.5% vs 6.0% [pro vs. pla], p = 0.86) ↓ risk of watery stool ↓ risk of nausea No significant difference in toxin-producing *Clostridioides difficile* between the groups	High	[67]
20.	• Participants: 88 adult patients with *Helicobacter pylori* infection receiving antibiotic therapy were divided into 3 groups receiving acidified milk without probiotics (G1), pasteurized fermented milk (G2), or fermented milk with probiotics (G3) • Probiotics used: *Lactobacillus acidophilus* LA-5, *B. animalis* subsp *Bifidobacterium lactis,* and *Streptococcus thermophilus* • Preparation form: Fermented milk beverage • Dose: 10^6 CFU/g, 2 × 125 g/day • Intervention duration: 5 weeks • Follow-up duration: up to 56 days after the initiation of the intervention.	"Diarrhoea was defined as three or more watery stools on two or more consecutive days."	No significant reduction in the incidence of diarrhea (4 vs. 2 vs. 3 [G3 vs. G2 vs. G1]) Shortens the diarrhea duration Alleviates abdominal pain (observed both in G2 and G3) Increased *Bifidobacterium* population in G1 and G3 Increased *Lactobacillus acidophilus* population in G3	Some concerns	[68]
21.	• Participants: 70 adult patients receiving antibiotic therapy, divided into 2 groups: placebo and probiotic groups • Probiotics used: *Lacticaseibacillus casei* and *Bifidobacterium breve* • Preparation form: Sachet • Dose: 2×10^7 to 10^9 cells of *Lactobacillus casei* and 5×10^7 to 10^9 cells of *Bifidobacterium breve,* 1×/day	"All of them contracted diarrhea during the use or in the next 7 days after the suspension of antimicrobial medication, which had been induced for at least 24h. This occurred with watery or semi-liquid stools on two or more occasions, in the last 24h."	No significant difference in diarrhea duration (4.87 vs. 4.52 days [pro vs. pla], p > 0.05) or recurrence (34.3% vs. 22.8% [pro vs. pla], p > 0.05) was found.	Some concerns	[69]
22.	• Participants: 314 adult patients receiving amoxicillin-clavulanate or levofloxacin antibiotic therapy, divided into 3 groups receiving no yogurt (G1), placebo yogurt with *Streptococcus thermophilus* + *L. delbrueckii* subsp. *Bulgaricus* as starters (G2), and probiotic yogurt (same as G2 with addition of probiotics) • Probiotics used: *Lactobacillus acidophilus* La-5, *B. animalis* subsp. *lactis* Bb-12, *Lacticaseibacillus casei* subsp. *casei* Lc-01 • Preparation form: Yogurt • Dose: *Lactobacillus acidophilus* La-5 (5×10^6 CFU/mL), *L. casei* Lc-01 (1×10^7 CFU/mL), and *Bifidobacterium lactis* Bb-12 (1×10^8 CFU/mL), given in 150–200 ml of yogurt per day • Intervention duration: starting within 48 hours of antibiotic therapy initiation up to 5 days after therapy cessation • Follow-up duration: up to 1 month after the cessation of antibiotic therapy (by phone)	"Definition of diarrhea was used: ≥3 loose or watery stools per day for ≥2 days."	Probiotics did not decrease the duration of AAD (3.2 vs. 3.7 days [pro vs. pla], p = 0.742), prevent AAD (28% vs. 22% [pro vs. pla], p = 0.30), or decrease the length of stay (2.4% vs. 0 [pro vs. pla], p = 0.243).	Low	[70]

Table 3. Continued

No.	Intervention	AAD definition in study	Probiotic effects	Risk of bias	Ref.
23.	• Participants: 172 adult patients with respiratory tract infections within 48 hours of receiving antibiotic therapy, divided into groups receiving probiotics and without probiotics • Probiotics used: *Lacticaseibacillus rhamnosus* R0011 + *Lactobacillus acidophilus* R0052 • Preparation form: Capsule • Dose: 2 × 10^9 CFU/capsule, 2/day • Intervention duration: 14 days • Follow-up duration: 14 days	Definition in the primary outcome "AAD-1 was defined as loose or watery stool more than 3 times per day for at least 2 days within 14 days of enrollment." Definition in the secondary outcome "AAD-2 was defined as loose or watery stool more than 2 times per day for at least 2 days within 14 days of enrollment."	No significant difference in AAD-1 (3.9% vs. 7.2% [pro vs. pla], p = 0.44) and AAD-2 (8.7% vs. 14.4% [pro vs. pla], p = 0.28) incidence	Low	[71]
24.	• Participants: 78 pediatric patients (5 months-16 years) receiving antibiotic therapy, divided into groups receiving probiotics and placebo • Probiotics used: *Lacticaseibacillus rhamnosus* KL53A + *Bifidobacterium longum* PL03 + *Lacticaseibacillus plantarum* PL02 • Preparation form: Food supplement • Dose: 10^8 CFU *[Bifidobacterium longum* PL03 (33%), *Lacticaseibacillus rhamnosus* KL53A (33%), and *Lacticaseibacillus plantarum* PL02 (34%)].	"Diarrhoea was defined as ≥ 3 loose or watery stools per day for a minimum of 48 h, occurring during and/or up to 2 weeks after the end of the antibiotic therapy."	Probiotic use did not decrease AAD incidence (2.5% vs. 5.2% [pro vs. pla], p > 0.05, 95%CI -0.8-15) Defecation frequency decreased with probiotic use.	Low	[72]
25.	• Participants: 2941 elderly patients (age ≥65 years) receiving antibiotics, divided into 2 groups receiving probiotics or placebo • Probiotic used: 2 strains of *Lactobacillus acidophilus* and 2 strains of *Bifidobacterium* • Preparation form: vegetarian capsule • Doses: 6 × 10^{10} live bacteria (proportion between strains/species not specified), once daily • Intervention duration: 21 days • Follow-up duration: 12 weeks	"Diarrhea as three or more loose stools (consistency 5–7 on the Bristol Stool Form Scale) in a 24 h period or as stools described as looser than normal." Testing for *Clostridioides difficile:* culture and detection of toxin via immunoassay	No significant difference was found in AAD (10.8% vs. 10.4% [pro vs. pla], p = 0.72, 95% CI: 0.83-1.32) and CDAD (0.8% vs. 1.2% [pro vs. pla], p = 0.35, 95% CI: 0.34-1.48) incidence Similar duration and severity of diarrhea between groups	Low	[73]
26.	• Participants: 32 adult ICU patients receiving one or more antibiotics during ICU care, divided into 2 groups with no probiotics and with probiotics • Probiotics used: *Lacticaseibacillus paracasei* • Preparation form: Commercial yogurt drink • Dose: 10^{10} CFU/container, 2 containers/day • Intervention duration: starting within 48 hours of antibiotic therapy initiation up to 7 days after therapy cessation • Follow-up duration: 1 month after the cessation of antibiotic therapy	"Diarrhoea was defined as three or more bowel movements per day or greater than 750 mL of liquid stool." Testing for *Clostridioides difficile:* detection of toxin A or B via immunoassay	There was no significant difference in AAD incidence (68.8% vs. 62.5% [pro vs. pla], p = 1.000), duration (2.13 vs. 3.69 days [pro vs. pla], p = 0.241), and CDAD in ICU (6.0% vs. 12.5%, p = 0.600).	High	[74]

Table 3. Continued

No.	Intervention	AAD definition in study	Probiotic effects	Risk of bias	Ref.
27.	• Participants: 438 pediatric outpatients receiving antibiotic therapy, divided into groups receiving probiotics and placebo • Probiotics used: *Lacticaseibacillus plantarum* DSM9843 • Preparation form: Capsule • Dose: 1×10^{10} CFU/capsule • Intervention duration: starting with antibiotic therapy initiation, up to 1 week after antibiotic cessation • Follow-up duration: Up to 1 week after the intervention was stopped (range: 15–28 days)	"Diarrhea was defined as ≥3 loose/watery stools/24 hours starting after initiation of antibiotic treatment."	No significant difference in AAD incidence (2.8% vs. 4.1% [pro vs. pla], p = 0.4), frequency of defecation, and other gastrointestinal symptoms.	Low	[75]
28.	• Participants: 267 adult inpatients receiving antibiotic therapy, divided into 2 groups receiving probiotics or placebo • Probiotics used: *Lactobacillus* GG • Preparation form: Capsule • Dose: 10×10^9 CFU/capsule, 2/day • Intervention duration: starting within the first 24 hours from antibiotic therapy initiation and being administered for 2 weeks • Follow-up duration: Up to 21 days after the intervention stopped	"Diarrhea was defined as watery or liquid stools (bowel movement consistency of 1, 2, or 3 on the Stool Consistency Continuum) for 2 or more consecutive days in a patient with normal stools (≤1 per day) at baseline or 3 or more bowel movements more than the patient's normal daily number of bowel movements." Testing for *Clostridioides difficile:* detection of toxin	No significant effect of probiotic use in preventing AAD incidence (29.3% vs. 29.9% [pro vs. pla], p = 0.93), reducing severity (3.1% vs. 7.1%, p = 0.47), and preventing CDAD (1.5% vs. 2.2%, p > 0.99).	Low	[76]
29.	• Participants: 359 adult patients with SC injuries receiving antibiotic therapy, divided into 2 groups receiving probiotics or placebo • Probiotic used: *Lacticaseibacillus casei* Shirota • Preparation form: beverage • Doses: 65×10^9 CFU/mL, once daily • Intervention duration: 7 days • Follow-up duration: up to 30 days after antibiotic cessation.	"Diarrhoea was defined as more than two liquid stools (Bristol stool scale type 5, 6, or 7) in any 24 h period." Testing for *Clostridioides difficile:* detection of glutamate dehydrogenase, toxin A and/or B via immunoassay, and polymerase chain reaction to detect toxin A and B	No significant difference in AAD (45% vs. 42.1% [pro vs. pla], p = 0.639) and CDAD (1.8% vs. 1.9%, p = 0.963) No difference in the duration and frequency of diarrheal episodes between groups Probiotic use only decreased AAD incidence in patients receiving routine PPI therapy (28% vs. 52.2%, p = 0.015).	Low	[77]
30.	• Participants: 854 patients aged >55 years receiving antibiotics 7 days before admission up to 72 hours since admission, divided into 2 groups receiving probiotics or placebo • Probiotic used: *Lacticaseibacillus casei, L. delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* • Preparation form: yoghurt • Doses: *Lacticaseibacillus casei* (10^8 CFU/mL), *L. delbrueckii, and Streptococcus thermophilus* (10^6 CFU/mL), 2×100 mL/day • Intervention duration: 7 days • Follow-up duration: 2 weeks	"Diarrhoea was initially defined in the protocol as more than two liquid stools a day for three or more days in quantities in excess of normal for each patient." Testing for *Clostridioides difficile:* detection of toxin via enzyme-linked immunosorbent assay	No significant difference in AAD (19.3% vs. 17.9% [pro vs. pla], 95% CI 0.82-1.49, p = 0.53) and CDAD incidence (0.2% vs. 0.45%)	Some concerns	[78]

* Studies using mix of LAB and non-LAB probiotics.

† Studies numbered 19-30 showed no significant effect of probiotics in AAD prevention.

AAD, antibiotic-associated diarrhea; AISS, Amsterdam Infant Stool Scale; BSFS, Bristol Stool Form Scale; BSS, Bristol Stool Scale; CDAD, *Clostridioides difficile-associated* diarrhea; CDI, *Clostridioides difficile* infection; CFU, colony-forming unit; CI, confidence interval; ICU, intensive care unit; LAB, lactic acid bacteria; PPI, proton pump inhibitor; SC, spinal cord; pro, probiotic; pla, placebo.

Most studies (18 of 30 trials) reported a decrease in AAD incidence following the administration of LAB as probiotics[49-66]. Wong et al.[77] reported a decrease in AAD incidence only among patients with spinal cord injury who received routine proton pump inhibitor (PPI) therapy. Among the 15 studies specifically assessing AAD incidence due to *Clostridioides difficile* infection (CDI)[49,51-54,56,61,62,65,67,73,74,76-78], only five reported a decrease in CDI incidence with LAB probiotic use[51-53,56,62]. Other outcomes assessed across clinical trials included diarrhea duration, length of hospital stay, occurrence of other gastrointestinal symptoms, and dehydration.

Several studies (6 of 30 trials) showed that LAB administration as probiotics reduced AAD duration[51,54,55,57,63,68]. Szymański et al.[72] reported that although probiotic use did not reduce AAD incidence, it notably decreased diarrhea frequency among pediatric patients receiving probiotics. Cimperman et al.[64] and Ouwehand et al.[53] also reported significant reductions in diarrhea frequency among adults receiving LAB-containing probiotics. Regarding the onset of diarrhea, García Trallero et al.[50] reported a 5.39-day delay in the onset of diarrhea in the group receiving probiotics. However, no difference in daily diarrhea frequency was observed between patients receiving probiotics and those receiving placebo[50]. Hodzhev et al.[55] found a mean reduction of 1.12 days in diarrhea duration in the group receiving multispecies probiotics, but no changes in diarrhea frequency were observed.

Some clinical trials involving inpatients also assessed length of hospital stay among patients receiving LAB as probiotics. Dietrich et al.[63] conducted two studies, one comparing probiotics with placebo and the other comparing two probiotic drinks containing different *Lacticaseibacillus casei* strains. Neither study found a significant difference in length of hospital stay between groups. A study involving adult ICU patients in Canada also found no significant difference in length of hospital stay following probiotic use[74].

Several studies compared the incidence of gastrointestinal symptoms accompanying AAD in patients receiving LAB probiotics with that in patients not receiving them. A study by de Vrese et al.[68] involving patients with *Helicobacter pylori* infection reported a decrease in abdominal pain among patients receiving fermented milk containing LAB probiotics. However, this decrease was also observed in the group receiving pasteurized fermented milk[68]. Gao et al.[51] reported reductions in several symptoms, including abdominal pain and distension, loose stools, and constipation, following LAB probiotic use. These reductions were more pronounced in the group receiving a higher probiotic dose[51]. Hodzhev et al.[55] also reported that multistrain probiotic use reduced nausea, abdominal pain and distension, and gas formation. In a study by Saviano et al.[56], LAB probiotic administration reduced nausea but did not significantly affect abdominal pain. Lönnermark et al. found a reduced risk of nausea and watery stool formation among patients receiving drinks containing *Lacticaseibacillus plantarum*[67]. However, Olek et al.[75] found no significant changes in gastrointestinal symptoms, including abdominal pain and distension, nausea, and flatulence, among pediatric patients receiving *Lacticaseibacillus plantarum*. In addition, two studies assessed the effects of LAB probiotic use on dehydration incidence in patients with AAD. A multicenter study in the Netherlands and Poland involving pediatric patients reported a reduction in the proportion of patients requiring rehydration therapy in the group receiving probiotics[49]. A Brazilian study involving infants also reported a decrease in dehydration incidence following the administration of formula milk containing LAB probiotics[66].

Discussion

Probiotics exert antibacterial effects by secreting antimicrobial peptides and competing with pathogens for nutrients. They also possess antibiofilm properties by binding pathogens into aggregates, thereby preventing pathogen colonization and biofilm formation. Probiotics prevent bacterial adhesion by competing for nutrients and binding sites (receptors) in the intestine. Antimicrobial substances, such as organic acids, oxidant catabolites (hydrogen peroxide), and antimicrobial peptides (bacteriocins), also prevent pathogen adhesion. A previous study reported that *Lacticaseibacillus plantarum* and *Lacticaseibacillus* rhamnosus inhibited the colonization of *Escherichia coli* and *Salmonella enterica serovar Typhimurium* by up to 30%–90%[79].

In vivo studies reviewed in this article showed that LAB exert beneficial effects by reducing diarrhea incidence, symptoms, and inflammation. However, clinical trials have yielded varying results, which may be attributable to several factors. First, phylogenetic and metagenomic analyses have revealed that, despite similarities in the genera present in the gut microbiota of mice and humans, their relative abundances differ substantially. The murine gut microbiota is characterized by the predominance of Lactobacillus and Turicibacter, whereas the human gut microbiota is dominated by Faecalibacterium. In addition, the mouse forestomach is lined with various Lactobacillus strains. This contrasts with the human stomach, which is colonized by Helicobacter *pylori*, *Prevotella* spp., and *Streptococcus* spp.[80]. These differences may affect the mechanisms by which probiotics act against AAD, including the modulation of SCFA production, secretion of antimicrobial peptides, and suppression of pro-inflammatory cytokines. Further studies are warranted to explore and confirm these effects.

Several challenges remain in generalizing findings across clinical studies. In the present review, probiotics showed beneficial effects in 15 of 25 studies involving adults and 3 of 5 studies involving pediatric patients. Thus, the benefits of probiotics in pediatric patients remain unclear. In one study, only three patients developed AAD (one in the probiotic group and two in the placebo group)[72]. Another study using a single-species probiotic found no significant preventive effect against AAD[75]. Numerous previous meta-analyses have examined the use of LAB as probiotics for AAD prevention. Most concluded that probiotics were effective in preventing AAD in adults[81-88]. However, their efficacy in pediatric and older adult populations remained controversial[83-85,89,90]. A meta-analysis by Guo et al.[91] reported that the benefits of probiotics in preventing AAD in pediatric patients were significant only when the probiotics were administered at doses of $\geq 5 \times 10^9$ colony-forming unit (CFU)/day. These studies also highlighted several limitations of existing clinical trials, including variations in AAD definitions, the use of single- and multistrain probiotics, differences in probiotic doses, and limited reporting of adverse events.

Variations in AAD definitions have been a challenge when applying clinical trial results in practice. Studies may use different definitions of AAD based on the number of bowel movements and stool consistency[84,86,92]. However, Jafarnejad et al.[84] concluded in a meta-analysis that AAD was most commonly defined in probiotic trials as the "abrupt onset of ≥3 loose or watery stools following antibiotic administration". This broader definition may help in interpreting trial results in clinical practice. In addition, the included clinical trials did not report the identification of causative pathogens of AAD (such as *Klebsiella oxytoca*, *Staphylococcus aureus*, or others) in all patients. Different causative agents of AAD may have affected the clinical outcomes of probiotic administration. Future studies should address this issue by identifying the causative agents of AAD to help establish a more specific relationship between AAD pathogens and the probiotic species used.

Current guidelines outline the use of probiotics for managing or preventing AAD or CDAD. The 2018 guidelines of the Infectious Diseases Society of America allow probiotic use in immunocompetent adults and pediatric patients (with weak evidence for adults and moderate evidence for pediatric patients),[93] but do not recommend probiotics to prevent AAD caused by *Clostridioides difficile*[94]. Meanwhile, the updated 2021 guidelines of the European Society of Clinical Microbiology and Infectious Diseases do not recommend probiotics for the prevention or management of AAD, particularly cases caused by *Clostridioides difficile*[95]. Nevertheless, a recent Cochrane meta-analysis by Esmaeilinezhad et al.[96] reported that CDAD incidence was 50% lower in groups receiving probiotics than in control groups (1.6% vs. 3.2%, respectively). In the present review, most studies on CDAD (10 of 15 studies) found that probiotics were not effective in preventing CDAD. However, methods for detecting *Clostridioides difficile* varied across studies, and most clinical trials did not distinguish between true infection and asymptomatic carriage. Globally, the prevalence of *Clostridioides difficile* colonization is estimated to be around 7.6%, while colonization occurs in 1.5% of healthy populations. This prevalence may be higher in vulnerable populations, such as patients with cancer or cystic fibrosis, older adults, inpatients, and ICU patients[97].

Currently available tests for CDI do not have sufficient sensitivity and specificity when used alone, so more than one testing method may be necessary. In addition, *Clostridioides difficile* may cause asymptomatic colonization, and available tests cannot clearly distinguish asymptomatic colonization from active infection[1]. Pattani et al.[87] also noted in a meta-analysis that cases of community-associated CDI may be excluded from trials because hospital-based trials often exclude outpatients who received antibiotics in the preceding weeks. Future trials should address these issues to provide better evidence on the use of probiotics for CDI prevention.

Most studies in this review involved hospitalized inpatients. This finding was similar among studies showing beneficial effects of probiotics for AAD (14 of 18 studies) and those showing no significant effects (9 of 12 studies). A meta-analysis by Blaabjerg et al.[92] found that probiotics were beneficial for AAD prevention in outpatients compared with placebo (8% vs. 17.7%; 95% CI, 0.36–0.66). Another meta-analysis found that probiotics reduced the pooled relative risk (RR) of AAD in both inpatient studies (RR, 0.67; 95% CI, 0.58–0.77) and outpatient studies (RR, 0.67; 95% CI, 0.49–0.92)[84]. Our findings suggest that probiotics may be used for AAD prevention in both inpatient and outpatient settings. However, the appropriate target population must be considered to ensure the safe and effective use of probiotics. Patients with certain conditions, such as immunocompromised status, a history of gastrointestinal surgery, or other relevant comorbidities, have been underrepresented in clinical trials because of medical risks. Therefore, careful consideration of the patient's clinical history and presentation is necessary before probiotic use.

Eight studies used only one LAB species as a probiotic, whereas 22 used combinations of two or more LAB species. One publication by Dietrich et al.[63] reported two studies: the first compared a single LAB species (*Lacticaseibacillus casei* DN-114001) with placebo, whereas the second compared two different strains of the same LAB species (*Lacticaseibacillus casei* DN-114001 and *Lacticaseibacillus casei* Shirota). In the present review, most studies showing beneficial effects of probiotics for AAD prevention or treatment (15 of 18 studies) used multispecies probiotics. The LAB species that showed the

most consistent beneficial effects was *Lactobacillus acidophilus* (10 of 18 studies), which was administered in combination with other Lactobacillaceae and *Bifidobacterium* spp. The use of multiple species and/or strains in probiotic preparations makes it difficult to independently associate each species and/or strain with a reduced risk of AAD. This limitation has also been noted in existing meta-analyses[81,83,84,87,92]. The selection of LAB species for probiotic combinations must consider possible inhibitory interactions between species. Chapman et al.[98] previously reported inhibitory interactions between LAB species in a multispecies probiotic. For instance, *Bifidobacterium breve* was shown to inhibit the growth of *Lacticaseibacillus casei*. Such interactions may reduce the effectiveness of a probiotic preparation. Therefore, although probiotics may provide overall benefits for AAD prevention, identifying the most effective agents through head-to-head comparisons remains an important area for future research.

All trials in the present review used Lactobacillaceae and *Bifidobacterium* spp. as probiotics, with only a few studies using multispecies probiotics that included other organisms, such as *Saccharomyces boulardii*, *Bacillus coagulans*, and *Streptococcus thermophilus*. No study in the present review used *Leuconostoc* spp. The beneficial effects of *Lactobacillus* spp. and *Bifidobacterium* spp. as probiotics vary by species and strain[99]. These differences may result from variations in bacteriocins, SCFAs, or other specific compounds secreted by LAB. For instance, plantaricin, a type of bacteriocin, contributes to the alleviation of colitis symptoms and a decrease in Mucispirillum abundance. Enterocin A, another bacteriocin secreted by *Enterococcus*, exerts its effects by eliminating some multidrug-resistant bacteria. In the context of AAD prevention, different bacteriocins may have distinct effects. In addition, bacteriocin production depends heavily on temperature and pH[100], so different probiotic formulations may result in different levels of bacteriocin secretion. This warrants further investigation into how different LAB species and genera interact and produce these metabolites, including Lactobacillaceae and *Bifidobacterium*. Future studies are needed to investigate the roles of specific compounds secreted by LAB as anti-AAD agents and to identify the optimal combinations of bacterial species for maximum benefit.

Currently, the assessment of strain specificity and benefits is hampered by variability in the strains or strain combinations used for the same disease or condition, changes in bacterial taxonomy (such as the reclassification of genera and species) and strain identification, and the lack of global consensus on strain designations. Strain designations are often not reported in early clinical trial publications[83]. This may require tracing the development of the probiotic product used in the trials to identify the bacterial strain. Additionally, different strains and species may vary in their ability to restore the host's normal microbiota[101]. Some trials may also use probiotic products containing proprietary strains. This may limit our understanding of the specific mechanisms of action of a given strain or explain why the strain was included in the product[102]. Misidentification and misclassification of strains, as well as the presence of unlabeled species in products, may present additional challenges[103].

The doses of probiotics used across trials have also varied considerably. The bacterial dose, measured in CFUs, may affect the desired outcome of probiotic therapy. In a previous meta-analysis, doses of 5×10^9 CFU/day or higher appeared to be more effective, but strain-specific dose studies are still needed[91]. In the present review, most studies showing beneficial effects of probiotics (13 of 18 studies) used doses of $\geq 5 \times 10^9$ CFU. Interestingly, most studies showing no beneficial effects of probiotics for AAD (8 of 12 studies) also used doses of $\geq 5 \times 10^9$ CFU. This suggests that, in addition to probiotic dosage, the benefits of probiotics for AAD may depend on the combination of species in the preparation. Previous meta-analyses have suggested a possible dose–response relationship between probiotic use and AAD incidence. However, these analyses included various probiotic species rather than specific strains[82,92]. More studies are needed to validate this dose–response effect, particularly for individual strains.

The trials reviewed in this article had varying follow-up periods, but most lasted less than eight weeks after antibiotic therapy was discontinued. Therefore, the follow-up periods may have been insufficient to assess AAD, and cases may have been missed or undocumented after follow-up ended[86,89]. Additionally, adverse event reporting varied across trials. This may be because adverse events are often not predefined before trial participants are enrolled[90]. More standardized adverse event reporting across trials would enable a more detailed assessment of the risk–benefit profile to ensure the optimal use of LAB for AAD prevention.

Five studies involving pediatric patients and 25 studies involving adults were reviewed in this article. Gut colonization by microorganisms begins soon after birth, and processes affecting the abundance and species diversity of the gut microbiota lead to substantial changes in its composition within weeks or even days during the first 2–3 years of life. After this period, the rate of change becomes more stable as the gut microbiota matures. The relatively stable adult gut microbiota may recover better from interventions, such as antibiotic use, or other disturbances. However, when the same interventions or disturbances affect an infant's or child's gut microbiota, the effects may persist longer[104]. Thomas et al.[76] also highlighted that differences in gut microbiota composition between children and adults may affect the

effects of probiotic use. Patients' individual conditions and the timing of probiotic initiation also need to be considered. In a study by de Souza and Jorge,[69] probiotic administration was started 7–10 days after the initiation of antibiotic therapy. This raises the possibility that dysbiosis had already occurred before probiotics were started[69]. Alberda et al.[74] also noted that ICU patients often receive antibiotics for other conditions before ICU admission. This may complicate the assessment of probiotics as primary preventive agents for AAD or CDI[74]. Previous meta-analyses have highlighted the need to identify patients at higher risk for AAD to ensure the optimal benefits of probiotic use[84,104]. Both host and drug factors need to be considered when identifying patients at high risk for AAD. Age, underlying conditions, and nasogastric tube use, which is common among ICU patients, are among the host factors associated with AAD risk[85]. Additionally, complications from probiotic use tend to occur in certain populations, such as immunocompromised patients. Therefore, alongside adverse event reporting, identifying patients at risk for AAD will help ensure the safe use of probiotics for AAD prevention.

Most clinical trials reviewed in this article were conducted in European and North American populations, with only a small number of studies from Asia, South America, and Oceania. These results may not be applicable to populations in Asia, Africa, or South America without local validation studies. Lactobacillus spp. belong to the phylum *Firmicutes*, which makes up approximately 90% of a healthy person's gut microbiota. Meanwhile, *Bifidobacterium* spp. are the most abundant members of the phylum Actinobacteria in the human gut microbiota. However, Actinobacteria have a relatively low abundance in the human gut[105]. The abundance of these two genera in the human gut microbiota may be affected by geographic distribution, ethnicity, and dietary habits. For example, a metagenomic study focusing on *Lactobacillus* spp. found that Lactobacillus types (LbTypes) were more evenly distributed and more numerous in Europe and North America, where Delbrueckii and *Casei* LbTypes were prevalent. Europe had the highest prevalence of these two LbTypes, which may be attributed to yogurt consumption and the use of probiotic products. In contrast, these two LbTypes tend to be absent in Asia and nonindustrialized nations[106]. *Bifidobacterium* spp. are more commonly identified in the gut microbiota of Asian and European populations. The abundance of *Bifidobacterium* in Asian countries, such as Japan, China, and India, may be associated with the consumption of high-starch foods in these populations[107].

In addition to geographic factors, ethnicity may also affect the composition of the human gut microbiota. These differences remained observable after controlling for other relevant variables, including demographics and diet[108]. Dietary habits may also contribute to variations in gut microbiota diversity across populations. For example, gluten-free and Western diets were shown to decrease the abundance of Lactobacillus in the gut microbiota, whereas Mediterranean diets increased the abundance of both *Lactobacillus* and *Bifidobacterium*. In addition, dietary effects on the gut microbiota were observed across age groups in one study population[105]. In the present review, three trials explicitly instructed participants not to consume other probiotic or prebiotic products or fermented foods, apart from the intervention product, during the trials[55,67,68]. However, detailed information on participants' dietary habits was not recorded. This lack of dietary data may have confounded the results[102]. Consideration of participants' microbiomes and the factors influencing them (geography, ethnicity, and diet) is necessary because probiotics interact with the microbiota. However, baseline microbiome data are more widely available from Europe, North America, and China. In contrast, major gaps in microbiome data remain in other parts of the world, especially in low- and middle-income countries of the Global South[109]. Future clinical trials need to consider ethnogeographic and dietary variations between populations, as these factors influence gut microbiota dynamics and may affect the ability of LAB to prevent AAD. In addition, considering substantial microbiome variations within regions and among individuals in a trial may create opportunities to tailor probiotics by strain or species combination to achieve the greatest benefit in each population[109].

The forms of probiotic preparations used in trials also need to be considered, as they may help maintain LAB viability or provide additional effects. *Lactobacillus* spp. may be more suitable for non-gastroprotective probiotic preparations because of their ability to survive gastric pH. *Bifidobacterium* spp. are mostly found in the colon, where they play a role in the fermentation and digestion of complex carbohydrates from the host's diet. Therefore, the desired effects may be achieved if *Bifidobacterium* spp. in the probiotic preparation can reach and successfully colonize the colon[99]. Most trials reviewed in this article administered probiotics using conventional preparations, such as capsules, chewable tablets, and oral sticks or sachets. Conventional preparations are better characterized than nonconventional preparations, such as foods and drinks, for probiotic administration. Meanwhile, nonconventional probiotic preparations, such as cheese, yogurt, cream, and other food products, are easier to obtain and consume. However, both conventional and nonconventional products may differ in their effects because of the formulation process, bacterial viability, the varying ability of bacterial species to survive different host physiological processes, and their ability to adhere to the intestinal lumen[99].

Some trials used dairy-based food and beverage products, such as formula milk, fermented milk, and yogurt, as probiotic preparations. Dairy-based products are known to provide suitable media for probiotic growth. Probiotic bacteria obtain carbon and essential amino acids from lactose-hydrolyzing enzymes and a proteolytic system that digests casein[108,109]. The flavor, preservation, and presentation of dairy-based probiotic products are affected by compounds resulting from the metabolism of these nutrients[110]. Milk also has buffering capacity and fat content, which may help probiotic bacteria tolerate and survive conditions in the gastrointestinal tract[111]. Fermented dairy products require starter cultures to initiate fermentation. However, antagonistic interactions between the starter culture and added probiotic bacteria may occur because of the production of acidic compounds, peroxides, bacteriocins, and other metabolites. Therefore, when using dairy-based products and their fermented forms for probiotic preparations, compatibility between starter cultures and added probiotic bacteria also needs to be considered[110,111]. The addition of other ingredients, such as fruits, the introduction of oxygen during product preparation, and the fermentation process itself may further affect probiotic bacterial viability in dairy-based preparations[110].

Some trials used commercially available probiotic products as interventions. In addition to processing and preparation form, storage also plays a crucial role in maintaining LAB viability in probiotic products. Important factors to consider during storage include temperature, water activity or moisture, oxygen content, preparation composition, storage time, and pH. In general, probiotic products need to be stored at 4°C because of the temperature sensitivity of the bacteria. Storing products at room temperature may reduce their shelf life. In addition, LAB are anaerobic bacteria, so low-oxygen conditions and probiotic preparations made from low-water-activity matrices may improve their viability and maintain their physiological functions[112].

Another possible problem associated with probiotic use is the possibility that probiotic bacteria may serve as reservoirs of antimicrobial resistance genes. For example, resistance traits in *Lactobacillus* have two possible implications. Intrinsic resistance may be a desirable trait in probiotic bacteria, as it would allow them to survive antibiotic exposure. However, acquired resistance in probiotic bacteria may pose a risk of resistance spreading through mobile genetic elements, such as plasmids or integrated mobile genetic elements. The spread of resistance genes may affect nonpathogenic and facultative-pathogenic bacteria in both the gastrointestinal microbiota and the environment[111,112]. Studies have assessed the possibility of resistance spreading through mobile genetic elements in probiotic bacteria. Both phenotypic antibiotic resistance and antimicrobial resistance genes, such as the ESBL gene blaTEM and the tetracycline resistance gene tetK, have been found in isolates from commercially available products[113,114]. A bioinformatics study of LAB genomes from the orders Bifidobacteriales and Lactobacillales, obtained from food and probiotic supplements, also identified several antimicrobial resistance genes on mobile genetic elements[115]. Daniali et al. stated that horizontal transfer of resistance genes between probiotics and the microbiota may occur under dysbiotic conditions and may be influenced by target size, probiotic strain, and the ability to link to plasmids[116]. However, in addition to horizontal gene transfer, other mechanisms by which probiotics may confer resistance have been proposed, such as promoting the abundance of resistant bacteria or increasing resistome expansion in the lower gastrointestinal tract mucosa[117–119].

Several case reports have described bacteremia caused by bacteria found in probiotics. Some reported LAB, such as *Lacticaseibacillus* rhamnosus[120–122], and non-LAB bacteria, such as *Bacillus clausii*, as causative agents[123]. Bacteremia caused by *Lactobacillus* from probiotics has been reported in patients with immunosuppression, prolonged hospital stays, and a history of surgery[124]. These infections may occur when a damaged intestinal barrier allows bacteria such as *Lactobacillus* to enter the bloodstream. Therefore, probiotics are not recommended for patients with certain conditions, such as immunosuppression, and future studies should explore alternative strategies to prevent AAD in these vulnerable populations.

Several strategies may help address resistance modulation or transfer from probiotics. Strains intended for probiotic use may be selected or deliberately made resistant to multiple antibiotics to allow concurrent administration with antibiotic therapy and reduce gastrointestinal side effects. However, although selecting resistant traits may help ensure the effective colonization of probiotic bacteria in the gut mucosa, probiotic strains must be carefully selected to avoid those carrying resistance genes located on mobile genetic elements. This would help prevent the spread of resistance genes and further serious health consequences[112,115]. Another option in probiotic product development is to select strains carrying resistance genes that are not clinically significant and do not affect human antimicrobial therapy, or strains that carry no antimicrobial resistance genes[125].

Limitations and Future Directions

Several limitations remain in the present review. First, we included only articles published in English from two databases (PubMed and Scopus). As a result, the included articles were mostly from studies conducted in Europe and North America, with a small number of studies from Asia, South America, and

Oceania. This may limit the generalizability of our findings, as local studies published in other languages might have provided more data on other populations and a broader perspective on the use of LAB as probiotics for AAD. This review focused on Lactobacillaceae and *Bifidobacterium* because fewer studies have investigated LAB species outside these two groups (such as *Enterococcus* spp. or *Leuconostoc* spp.) as probiotics. Therefore, more studies are needed to provide a more complete picture of the benefits of LAB for AAD. We also noted that most of the included clinical trials (23 of 30 studies) were funded by probiotic manufacturers or pharmaceutical companies. Although we assessed the overall quality of all included clinical trials, sponsorship bias could not be ruled out.

Based on the findings of the present review, we recommend the following for future clinical studies.

1. The use of multispecies probiotic mixtures at a minimum dose of 5×10^9 CFU may be more beneficial. Additionally, although the use of *Lactobacillus acidophilus* consistently showed beneficial effects, the selection of species combinations in multispecies LAB preparations must consider interactions between species, such as possible inhibitory or synergistic effects, to ensure optimal benefits.

2. Preparation forms should be optimized in future studies to ensure effective probiotic delivery to the target site of action.

3. To reduce the possibility of missing AAD cases, the follow-up period should ideally extend to 8 weeks after antibiotics are discontinued, in accordance with the WHO definition of AAD.

4. Only a few trials further investigated whether AAD was caused by specific pathogens or by gut dysbiosis following antibiotic use. Future studies may need to identify the specific effects of probiotics on AAD caused by certain organisms. Additionally, studies on gut dysbiosis have mostly been limited to animal models. Further investigation is needed to determine how probiotics affect or alleviate dysbiosis in the human gut in relation to AAD.

5. To ensure probiotic safety and anticipate the risk of resistance gene transfer, future studies should ensure that the LAB used in trials are free of antimicrobial resistance genes.

Conclusion

The potential use of LAB as probiotics for AAD prevention and management may be explained by several mechanisms, including the prevention and alleviation of inflammatory responses, regulation of intestinal barrier integrity through SCFA production, competition with pathogenic species, and secretion of antimicrobial peptides, such as bacteriocins. However, these proposed mechanisms are based on *in vivo* studies and may not be directly extrapolated to humans because of biological differences in gut microbiota composition. Further studies are therefore needed.

Most clinical trials suggest that LAB have potential as probiotics for AAD prevention and management in adults. However, their clinical applicability remains challenging because of the heterogeneity of participating patients, LAB species and strains, use of single strains or combinations, doses and preparations, and follow-up durations. In addition, most trials have yet to consider other factors that may affect patients' gut microbiota, such as ethnogeographic background and dietary habits.

Further studies are needed to optimize LAB doses and preparation forms to maximize their benefits in AAD prevention and management. Factors that may influence baseline gut microbiota diversity in study populations, such as geographical distribution, ethnicity, and dietary habits, should also be considered. In addition, safety concerns, particularly the potential for adverse effects and probiotic-induced modulation of the gut resistome, warrant careful consideration in both clinical research and future probiotic product development.

Footnotes

Authorship Contributions

Concept: K.M., R.B., Design: K.M., R.B., Analysis or Interpretation: R.A.N., Literature Search: K.M., R.A.N., R.B., Writing: K.M., R.A.N., R.B.

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Deep Vein Thrombosis as a Complication of Pulmonary Tuberculosis: Clinical, Diagnostic, and Therapeutic Implications

Pulmoner Tüberkülozun Bir Komplikasyonu Olarak Derin Ven Trombozu: Klinik, Tanısal ve Terapötik Yansımalar

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Abstract

Tuberculosis (TB) is a multisystem infectious disease that can promote a hypercoagulable state through prolonged systemic inflammation or dysfunction of the endothelium and coagulation cascade. This report describes a case of deep vein thrombosis (DVT) involving the entire right iliofemoral venous axis and the infrarenal inferior vena cava, which developed 4 weeks after the initiation of anti-TB therapy in a young woman with pulmonary TB (PTB). The patient had no conventional risk factors for thromboembolism. Given the potential for drug interactions with rifampicin, she was successfully treated with fondaparinux for 10 months, with complete resolution and no complications during the 12-month follow-up. We also present a narrative review of the literature focusing on treatment options and risk factors. Clinicians should maintain a high level of clinical suspicion for DVT in patients with TB throughout the course of treatment.

Keywords: Tuberculosis, deep vein thrombosis, anticoagulant therapy

Öz

Tüberküloz (TB), uzun süreli sistemik enflamatuvar reaksiyon ya da endotel ve koagülasyon kaskadı disfonksiyonu sonucunda hiperkoagülabiliteye yol açabilen, multisistemik bir enfeksiyon hastalığıdır. Bu olgu sunumunun amacı, pulmoner TB tanısı alan genç bir kadında, anti-TB tedavisine başladıktan dört hafta sonra gelişen; sağ iliyak-femoral venöz aksın tamamını ve infrarenal inferior vena kavayı tutan bir derin ven trombozu (DVT) olgusunu sunmaktır. Hastada bilinen herhangi bir tromboembolik risk faktörü bulunmamaktaydı. Rifampisin ile olası ilaç etkileşimleri göz önünde bulundurularak, hasta on ay boyunca fondaparinuks ile başarılı bir şekilde tedavi edilmiş; 12 aylık takip süresince tam iyileşme sağlanmış ve herhangi bir komplikasyon gelişmemiştir. Ayrıca, tedavi seçenekleri ve risk faktörlerine odaklanan bir literatür derlemesine de yer verilmiştir. Klinisyenler, TB hastalarında tedavi süreci boyunca DVT gelişme ihtimalini her zaman akılda tutmalıdır.

Anahtar Kelimeler: Tüberküloz, derin ven trombozu, antikoagülan tedavi

Introduction

Tuberculosis (TB) is a multisystem infectious disease that can promote a hypercoagulable state as a result of prolonged systemic inflammation or endothelial dysfunction and coagulation cascade abnormalities[1-16]. Venous thrombosis and pulmonary thromboembolism can worsen the prognosis and clinical outcomes. The aim of this study was to review the literature on cases of deep vein thrombosis (DVT) in patients with TB, prompted by a recent case of extensive right iliofemoral venous axis in a young woman with pulmonary TB (PTB).

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Case Report

A 22-year-old woman of Romanian origin who had resided in Italy for 14 years was diagnosed with PTB in July 2023. The patient was immunocompetent and had no comorbidities other than mild iron deficiency anemia. She presented to the emergency department of our hospital with a persistent dry cough, night sweats, and fever lasting more than a month, despite taking various antibiotics at home. She was a non-smoker, was not obese, and had no history of recent surgery, trauma, prolonged immobilization, or previous thromboembolism. She denied using oral contraceptives or other hormonal therapies, and pregnancy was ruled out. Her family history was negative for cardiovascular or thrombotic diseases.

At that time, computed tomography (CT) revealed multiple bilateral centrilobular micronodular parenchymal consolidations, most prominent in the right lung and left lower lobe. Tuberculous cavities and partially obliterated bronchiectatic changes were also observed in the anterior and superior segments of the right upper lobe, the superior lingular segment, and the apical segment of the right lower lobe. The patient had multiple enlarged lymph nodes in the right hilar region and the bilateral upper and lower paratracheal regions. In addition, edematous thickening of the bowel wall was observed in the ascending colon, extending to the colonic flexure, cecum, and terminal ileum, accompanied by mild perivisceral fat stranding, reactive mesenteric lymph nodes, and a small amount of pelvic fluid. These findings strongly suggested severe active TB, with presumed concomitant intestinal involvement, given the subsequent complete resolution of these findings following targeted treatment. Mycobacteriological examination of sputum and bronchoalveolar lavage fluid was positive for acid-fast bacilli, and polymerase chain reaction testing for *Mycobacterium tuberculosis* DNA was positive. Drug susceptibility testing showed that the strain was fully susceptible to rifampicin and isoniazid.

The patient therefore began anti-TB treatment with rifampicin, isoniazid, pyrazinamide, and ethambutol on July 30, 2023, for 8 weeks. After completing the first 4 consecutive weeks of treatment, her clinical symptoms and microscopic examination findings gradually improved, and sputum samples tested negative for *Mycobacterium tuberculosis*. She was discharged home and continued to receive outpatient follow-up. However, after experiencing persistent hyperpyrexia and abdominal pain at home for approximately 5 days, she was readmitted to the hospital on September 4, 2023.

A chest and abdominal CT scan documented persistent grossly cavitated lesions at the right lung apex, the anterior segment of the right upper lobe, the lingula, and the apicobasal segments of the lower lobes, as well as an inferior pericardial effusion. It also revealed acute thrombosis involving the entire right iliofemoral venous axis, extending into the infrarenal inferior vena cava (IVC) to the saphenofemoral junction (Figure 1). A color Doppler ultrasound study performed on September 5, 2023, confirmed an occlusive right popliteal-femoral-iliac DVT without evidence of floating thrombi.

Laboratory tests showed the following results: prothrombin time, 12.2 seconds; international normalized ratio, 1.12 (normal values [NV], 0.85–1.15); activated partial thromboplastin time, 32 s (NV, 23–38); fibrinogen, 559 mg/dL (NV, 150–450); antithrombin III, 86.8% (80–120); factor V, 147% (70–140); factor VIII, 197.4% (70–150); protein C, 67% (70–140); free protein S, 74%; D-dimer, 4 mg/L; lupus anticoagulant screening



Figure 1. Acute thrombosis involving the entire right iliofemoral venous axis (computed tomography scan). An arrow has been overlaid on the final image to clearly indicate the occluded infrarenal segment of the inferior vena cava.

test (LA1), 45 s; confirmatory test (LA2), 35 s; LA1/LA2 ratio, 1.2 (NV, 1–1.3); C1 inhibitor, 31 mg/dL (NV, 21–39); and C-reactive protein, 77.2 mg/L (NV, 0–5). The elevated fibrinogen and factor VIII levels, combined with the mildly decreased protein C level were interpreted as consequences of the acute-phase inflammatory response rather than congenital thrombophilia.

Advanced interventions, such as systemic thrombolytic therapy or catheter-directed thrombolysis were carefully considered given the extensive proximal involvement. However, they were withheld because of an unfavorable risk–benefit ratio, specifically the high risk of bleeding associated with active cavitary pulmonary lesions and presumed intestinal TB lesions. Treatment with fondaparinux (7.5 mg/0.6 mL) was then initiated, gradually improving the extensive venous thrombosis. An initial venous Doppler ultrasound scan performed on September 19, 2023, revealed that the venous thrombosis persisted without any significant change. The patient completed the four-drug anti-TB therapy on September 30, 2023, and continued dual therapy with rifampicin and isoniazid for 16 consecutive weeks. A new venous Doppler ultrasound scan on October 17, 2023, confirmed right iliofemoral thrombosis but revealed initial recanalization. Fondaparinux was continued because oral anticoagulants (warfarin or direct oral anticoagulants) carry a high risk of treatment failure or toxicity due to rifampicin's potent CYP3A4- and P-glycoprotein-inducing effects. Ten months after starting fondaparinux therapy, a new venous Doppler ultrasound demonstrated complete resolution of the right iliofemoral DVT, from the infrarenal IVC to the saphenofemoral junction. After completing anti-TB therapy, the patient experienced no complications during a 12-month follow-up.

Discussion

To contextualize this case, we conducted a narrative literature review using the PubMed/MEDLINE databases covering publications through June 2026, with the search terms "tuberculosis," "deep vein thrombosis," and "venous thromboembolism." We included peer-reviewed case reports, case series, and retrospective cohort studies reporting individual patient data on DVT associated with active TB. A total of 69 unique cases of DVT in patients with PTB have been individually described in the literature, excluding duplicated registry cohorts (Table 1)[1-11,13-16]. Apart from the reports by White[1] (46 cases), El Fekih et al.[3] (a retrospective study of 14 cases), Ambrosetti et al.[2] (a retrospective study of 5 cases), and Shitrit et al.[4] (a retrospective study of 5 cases), all other reports described single cases or case series involving no more than three patients[5-11,13-16]. Clinical outcomes were favorable with complete recovery in most cases, although five patients died in the series reported by White[1], and one death was reported in another study[6]. A systematic review and meta-analysis by Danwang et al.[12] found that the incidence of DVT among 16,190 patients across nine studies was 1.3%, with a concomitant incidence of pulmonary thromboembolism of 5.8%. Based on our literature search using PubMed/MEDLINE databases, to our knowledge, this is the first case of extensive DVT involving the infrarenal IVC secondary to PTB reported in Italy, highlighting the educational value of managing extensive thrombosis during rifampicin therapy.

In our literature review, oral anticoagulants were used as medical treatment in most cases. Physicians should remain alert to the possibility of DVT in patients with TB, as TB should be considered a risk factor for DVT, particularly in malnourished, elderly, or seriously ill patients who may have poorer outcomes, a higher incidence of DVT, and impaired immune and coagulation functions. This association may be explained by inflammation-induced hypercoagulability, which leads to the release of cytokines (interleukin [IL]-1, IL-6, and tumor necrosis factor-alpha), and other factors that can promote endothelial damage, increase fibrinogen levels, disrupt fibrinolysis, and reduce antithrombin III levels. In some cases, enlarged lymph nodes associated with TB may form nodal masses that compress blood vessels, promote blood stasis, and thereby increase the risk of DVT. Importantly, our patient developed DVT one month after initiating anti-TB therapy. This timing raises the possibility that the early phase of treatment may coincide with a prothrombotic state, potentially due to transient coagulation imbalances or endothelial changes during the initial bacterial-killing phase. The therapeutic management highlights an important clinical consideration: rifampicin is a potent inducer of hepatic drug-metabolizing enzymes and transporters, including CYP3A4 and P-glycoprotein. Choosing fondaparinux over oral anticoagulants avoided potentially significant drug–drug interactions and provided stable anticoagulation for this extensive proximal thrombosis. Thrombolysis, although potentially effective for extensive proximal thrombosis, must be carefully weighed against the bleeding risks associated with cavitary PTB and mucosal TB lesions.

Study Limitations

As a narrative review and single case report, this work cannot establish a definitive epidemiological or causal association. Thrombophilia testing was performed during the acute phase, which may have affected baseline protein activity levels. Therefore, clinicians should maintain a high level of clinical suspicion for the possible association between DVT and TB, both during the initial stages of the disease and throughout

Table 1. Summary of primary case reports and case series of deep vein thrombosis associated with tuberculosis (1889–2025).

References, year	Cases	DVT site	PE	Concomitant risk factors	Anticoagulant treatment	Thrombolysis used?	Duration	Outcome
White[1], 1989	46	N.M.	N.M.	Malnutrition during the winter season	Heparin/oral Anticoagulants	No	N.M.	41 cured, 5 died
Ambrosetti et al.[2], 2006	5	N.M.	2	Active inflammation	N.M.	No	N.M.	Cured
El Fekih et al.[3], 2009	14	Lower limbs	2	Bed rest, inflammation	Oral anticoagulants	No	3–6 months	Cured
Goncalves et al.[5], 2009	2	Lower limbs	1	None	Oral anticoagulants	No	N.M.	Cured
Shitrit et al.[4], 2012	5	N.M.	1	Advanced age	Oral anticoagulants	No	Long-term	Cured
Kumarihamy et al.[6], 2015	1	Lower limb	Yes	Severe infection	Oral anticoagulants	No	N.M.	Died
Sangani et al.[7], 2015	1	Left femoral and IVC	No	Pediatric patient	Oral anticoagulants	No	6 months	Cured
Gupta et al.[8], 2017	3	Lower limbs	No	None	Oral anticoagulants/ LMWH	No	3 m/3 weeks	Cured
Azdaki et al.[10], 2018	1	Left popliteal to iliac	No	Hemoptysis	Oral anticoagulants	Yes (systemic)	N.M.	Cured
Mehra et al.[11], 2020	1	Left femoral	No	Celiac disease and disseminated TB	LMWH	No	N.M.	Cured
Subahi al.[13], 2021	1	Lower limb	Yes	Saddle PE	Oral anticoagulants	Yes (systemic)	N.M.	Cured
Shriwastav et al.[15], 2023	1	Left jugular and axillary	Yes	Upper extremity presentation	Oral anticoagulants	No	N.M.	Cured
Ahmad et al.[9], 2023	1	Bilateral lower limbs	No	Congenital protein S deficiency	Oral anticoagulants	No	N.M.	Cured
Anweri et al.[16], 2023	1	Left renal, IVC, and legs	No	Acute kidney injury	Oral anticoagulants	No	N.M.	Cured
Present case, 2026	1	Right iliofemoral and IVC	No	None (exclusion of conventional risks)	Fondaparinux (parenteral)	No bleeding risk	10 months	Cured

IVC, inferior vena cava; PE, pulmonary embolism; LMWH, low-molecular-weight heparin; N.M., not mentioned; DVT, deep vein thrombosis; TB, tuberculosis; m, months.

maintenance therapy.

Ethics

Informed Consent: The written informed consent for publication was obtained from the patient.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.G., Concept: V.V., Design: G.G., Data Collection or Processing: L.C., Literature Search: L.B., Writing: A.M.

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