REVIEW / DERLEME

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Legionnaires' Disease

Lejyoner Hastalığı

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Abstract

Legionnaires' disease is a severe form of pneumonia caused by *Legionella* species which are ubiquitous in both natural and man-made environments. It causes high morbidity and case fatality rates. The disease is acquired by aspiration of water or inhalation of aerosols containing the bacteria from environmental sources. Early diagnosis and appropriate treatment are important factors in determining the prognosis. Clinical and laboratory predictors will not reliably identify cases of Legionnaires' disease; therefore, the diagnosis requires specific laboratory tests. Legionnaires' disease is diagnosed by culture, *Legionella* urinary antigenuria, polymerase chain reaction, or serologic analyses. Isolation of *Legionella* from clinical samples is the gold standard. *Legionella* urinary antigen tests are easy and useful for early diagnosis of Legionnaires' disease. Fluoroquinolones, macrolides, and doxycycline are drugs of choice. Legionnaires' disease is considered to be preventable illness since it is possible to control and remove the bacteria in reservoirs. In Turkey, travel-associated Legionnaires' disease has a specific surveillance program since 1996. After the year of 2015, it became mandatory to take annual water cultures for *Legionella* from hospital water systems. The aim of this review is to raise awareness of legionellosis and to summarize the current literature.
Keywords: Legionnaires' disease, *Legionella pneumophila, Legionella* spp.

Öz

Lejyoner hastalığı *Legionella* türü bakterilerin neden olduğu ciddi morbidite ile seyreden ve tedavisiz bırakılan hastalarda yüksek olgu fatalite hızına sahip bir pnömoni tablosudur. *Legionella* türü bakterilerin habitatı doğal su kaynaklarıdır ve uygun şartlarda bina su sistemlerinde kolonize olabilirler. Sularda kolonize olmuş bakterinin inhalasyonu veya aspirasyonu Lejyoner hastalığının temel bulaş yoludur. Erken tanı ve uygun tedavi prognozu belirleyen en önemli faktördür. Hastalığın tanısında klinik ve laboratuvar özellikleri güvenilir değildir ve özgül laboratuvar testlere ihtiyaç vardır. Lejyoner hastalığının tanısı kültür, idrar antijen testi, polimeraz zincir reaksiyonu ve serolojik testlerle konmaktadır. Tanıda altın standart klinik örneklerden bakterinin izolasyonudur. İdrar antijen testi kolay ve kullanışlı olması nedeniyle erken tanıda değerli bir testtir. Lejyoner hastalığının tedavisinde florokinolonlar, makrolidler ve doksisiklin tercih edilecek ilaçlardır. Olası kaynağın tespiti ve dekolonizasyonu sonucu yeni olguların önlenebilmesi nedeniyle epidemiyolojik önemi de olan bir hastalıktır. Ülkemizde 1996 yılından beri seyahat ilişkili Lejyoner hastalığı için özel bir sürveyans yöntemi yürütülmektedir ve 2015 yılından itibaren de hastane su sistemlerinde *Legionella* için yıllık su kültürlerinin alınması zorunlu kılınmıştır. Bu derlemede Lejyoner hastalığına karşı farkındalığı artırmak ve ilgili güncel literatürü gözden geçirmek amaçlanmıştır.
Anahtar Kelimeler: Lejyoner hastalığı, *Legionella pneumophila, Legionella* spp.

Introduction

Infections caused by *Legionella* bacteria are referred to as legionellosis. There are two main forms of legionellosis: Legionnaires' disease, a multisystemic infection predominantly affecting the lungs, and Pontiac fever, which manifests as an influenza-like illness and resolves spontaneously without treatment. In rare cases, *Legionella* bacteria may spread from the respiratory system to involve other systems and organs (e.g.: the heart, liver, spleen, brain, skin, and subcutaneous tissues) or may directly cause extrapulmonary infections without pulmonary involvement[1,2].

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Legionnaires' disease first came to the attention of the medical community in 1976 due to an outbreak in a hotel in Philadelphia. That epidemic affected 182 people and caused 29 deaths[3]. In 1977, a previously unidentified bacterium was isolated from the deceased patients and named *Legionella pneumophila*[4]. Shortly after this epidemic, an outbreak of hospital-acquired Legionnaires' disease was reported[5]. This was followed by the discovery of *Legionella* bacterial colonization of water systems used in the homes of patients with community-acquired Legionnaires' disease, demonstrating what a widespread and significant health problem *Legionella* infections were[6,7]. In Turkey, Legionnaires' disease was added to the list of notifiable diseases in 1996 and special surveillance for travel-associated cases was initiated with the Legionnaires' Disease Control Program[8,9].

The Regulation on Legionnaires' Disease Control Procedures and Principles was published in the Official Gazette of Turkey with number 29354 and went into effect on May 13, 2015 to regulate the procedures and principles of reporting the disease[10]. In this article, current literature on Legionnaires' disease and precautions that should be taken in hospitals to prevent Legionnaires' disease are reviewed.

Microbiology

Legionella bacteria are nonspore-forming, uncapsulated, aerobic bacilli measuring 0.3-0.9 µm wide and 2-20 µm in length. The cytoplasmic membrane consists of an inner cytoplasmic membrane, a thin peptidoglycan layer, and an outer membrane that contains the heat-stable lipopolysaccharides. The life cycle of *Legionella* bacteria has two phenotypically distinct phases: a nonmotile, replicative phase and a virulent, motile transmissive phase. Despite being Gram-negative, they are difficult to stain due to the predominance of branched-chain fatty acids in their cell wall. Using 0.1% basic fuchsin is preferable to safranin as the contrasting dye in Gram stain to increase the likelihood of staining. In clinical samples, *Legionella* can appear as Gram-negative small coccobacilli or short bacilli, and in culture media they can form filamentous structures.

The *Legionella* genus includes more than 59 species and 70 serotypes, 30 of which have been shown to infect humans[11]. In Europe and United States, *L. pneumophila* is responsible for 90-95% of Legionnaires' disease and nonpneumophila strains cause 5-10%. Of the 16 serotypes of *L. pneumophila*, serogroup 1 is the most frequently isolated. In Australia and New Zealand, *L. longbeachae* is commonly isolated as the etiological agent of Legionnaires' disease. *L. micdadei*, *L. bozemanii*, and *L. dumoffii* are other frequently isolated nonpneumophila species[1,2,12].

Legionella inhabits natural water resources such as rivers, lakes, and thermal waters. Water stagnation, temperature, commensal microflora, sediment accumulation, and biofilm layers are important factors in the bacterial colonization of water systems. The ideal temperature for proliferation of *Legionella* bacteria in water systems is 35-45 ˚C while they cannot reproduce below 20 ˚C.

Free-living amoebae in water, air, and soil feed by phagocytosing bacteria, fungi, and algae. Virulent *Legionella* are resistant to phagocytes and continue to multiply. These amoebae are important reservoirs for *Legionella* bacteria. It has been shown that at least 20 species of free-living amoebae and 2 ciliated protozoans act as *Legionella* hosts. Encystation of *Legionella* protects them from unfavorable conditions and the effects of chlorine (Cl) and other biocides, thus playing a key role in the bacteria's long-term viability[13,14].

The formation of a biofilm layer makes it difficult to eliminate *Legionella* from a system. Factors that promote biofilm formation include the presence of organic substances, other microorganisms, water stagnation or reduced water flow, and corrosion. The biofilm layer serves as an important source of food for *Legionella* as well as a refuge from adverse external factors. Various studies have also shown that *Legionella* in a biofilm layer are more virulent and more resistant to biocides than *Legionella* living free in water[13,14].

Legionella bacteria have also been isolated in soil[15]. *L. longbeachae* is found in soil and is the only species transmitted to humans through dust or garden work but has not been shown to transmit to people through contaminated water[16].

Pathogenesis: Both virulent and nonvirulent *Legionella* strains are phagocytosed by the alveolar macrophages, after which only the virulent strains inhibit phagosome fusion with lysosomes and multiply intracellularly. They do this by inhibiting oxidative burst after phagocytosis, reducing phagosomal acidification, blocking phagosomal maturation, and altering traffic between organelles. The macrophage subsequently dies, releasing a large number of bacteria that then infect new cells. As with other intracellular pathogens, the host's primary defense system is cellular immunity. Humoral immunity has a secondary role in protection. Specific IgM and IgG antibodies developed after infection neither prevent cell death by complement activation nor inhibit intracellular proliferation through promoter activity[1,2,13,14,17].

Isolation and identification: Culturing *Legionella* spp. *in vitro* requires special growth media. The most commonly used medium is buffered charcoal yeast extract (BCYE-a) agar, which contains yeast extract as the nutrient source, activated charcoal for purification of toxic byproducts, L-cysteine as an essential component for growth, and iron salt and alpha-ketoglutarate to accelerate growth. Selective media have also been developed by adding antimicrobial drugs to inhibit the growth of other microorganisms. Plates should be incubated at 35 ˚C in high

humidity. Incubation in 2-5% CO_2 may facilitate the growth of some nonpneumophila *Legionella* spp. Colonies usually form in 3-5 days on specific media and are typically 1-2 mm in diameter with a smooth surface, gray-white center, and green- or blue-tinted frosted glass appearance around the edges (Figure 1). Suspected colonies with faint Gram-negative staining can be subcultured in parallel on media with and without cysteine. Colonies that grow on the cysteine medium are subjected to advanced *Legionella* identification methods.

L. pneumophila and its serogroups are identified using specific antisera that contain monoclonal antibodies produced against *Legionella* cell surface liposaccharides. More sophisticated tests are required to identify species other than *L. pneumophila*. Today, *Legionella* spp. can also be identified based to 16S ribosomal RNA or 'macrophage infectivity potentiator' (*mip*) genes. Gene banks have been established in the United Kingdom or United States for this purpose. Molecular sequence-based typing can be performed directly on clinical samples or with isolated strains according to the data in these gene banks.

There have also been reports of *Legionella*-like amebal pathogens shown to cause Legionnaires' disease which primarily obligate intracellular parasites of amoebae but do not growth on current laboratory media[1,2,13,14].

Epidemiology

Legionnaires' disease can be acquired by inhalation of droplets or aerosol containing *Legionella* or by aspiration of water contaminated with *Legionella*. There is no evidence of transmission by microaspiration of oropharyngeal secretions colonized with *Legionella* bacteria[18]. The use of water colonized with *Legionella* in patient care (nasogastric irrigation, nebulization, etc.) is another important route of transmission. *L. longbeachae* is believed to have a different transmission route; risk is increased by gardening and any other exposure to soil and soil products[15,16].

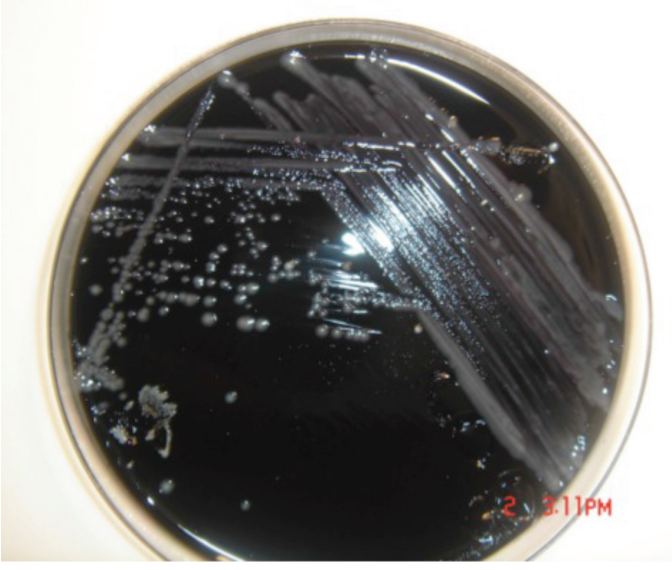

Figure 1. Cultured on buffered charcoal yeast extract-a agar, typical *Legionella* colonies are 1-2 mm in diameter with smooth, gray-white, blue-tinted ground glass appearance

Only 0.01-6.4% of individuals exposed to *Legionella* bacteria develop Legionnaires' disease. This is largely dependent on the bacterial load in the colonized source, differences in virulence among *Legionella* species, the droplet type and spread pattern, and the intensity of the exposure. Risk factors for Legionnaires' disease include advanced age; smoking; chronic pulmonary diseases such as emphysema or chronic obstructive pulmonary disease; underlying chronic diseases such as diabetes, renal or hepatic failure, and presence of conditions or use of drugs associated with immunosuppression (e.g.: solid organ transplant, long-term steroid use, biologic agents). Neutropenia has not been shown to increase risk[1,2,17]. Legionnaires' disease is rare in children, and is usually reported in immunosuppressed children or infants born in water.

Only one case of person-to-person transmission has been reported to date. During an outbreak in Vila Franca de Xira in Portugal, a 48-year-old male patient who worked in cooling tower maintenance visited his mother 300 km away in Porto. His mother cared for him for eight hours in a small, nonventilated room. Both the son and his mother, who had never been to Vila Franca de Xira, were diagnosed with Legionnaires' disease. The same *Legionella* strain was isolated in both cases. It was believed that the outbreak strain, which was not previously seen in Porto, passed to the mother through close contact with her son[19].

Prevalence: Legionnaires' disease accounts for 2-10% of community-acquired pneumonia cases requiring hospitalization. New cases may be seen throughout the year, with peaks in summer and early fall. The true incidence of Legionnaires' disease is difficult to determine, mainly because diagnosis requires specific laboratory tests which are available in few hospitals, and awareness of Legionnaires' disease among clinicians is low. In Europe and North America, the prevalence of Legionnaires' disease is reported to be 9 to 11.5 per million on average[1,2]. Approximately 20-30% of Legionnaires' disease is travel-associated and 5-10% is hospital-acquired. Legionnaires' disease has been reported in Turkey as sporadic cases[20-26]. Erdoğan and Arslan[27] and Ozerol et al.[28] reported two small clusters, one in a newly opened hotel in Alanya and the other in a hospital in Malatya, respectively. However, according to data from the European Working Group for *Legionella* Infections, Turkey is one of the countries in which travel-associated legionnaires' disease is most often diagnosed[29,30]. Seventeen Legionnaires' disease cases from a hotel in Kuşadası in July/August 1994 and 16 cases from a hotel in İstanbul in September/October 1997 were reported

in persons who were diagnosed after returning to their home after Turkey visit [31].

In the few studies that have been conducted in Turkey, *Legionella* colonization rates have been reported as 10-76.2% in hotel water systems[32-35] and 7-27.2% in hospital water systems[36-38]. In other studies, Burak and Zeybek[39] reported *Legionella* colonization in 21.3% of household water systems and Türetgen et al.[40] in 26% of water systems in cooling towers. Erdogan and Arslan[41] reported a 13.3% rate of colonization in the water systems of Turkish baths in hotels in Alanya, and Alim et al.[42] reported that 11.5% of the thermal pools in the central Anatolian district were colonized with *Legionella*. The rates of *Legionella* bacterial colonization observed in environmental samples in Turkey are summarized in Table 1.

Clinical Signs and Symptoms

Legionnaires' disease may manifest with mild symptoms or with severe pneumonia requiring treatment in an intensive care unit. It tends to follow a severe course if left untreated. Reported mortality rates are 5-10% in community-acquired and 30-50% in hospital-acquired Legionnaires' disease. The incubation period is between 2-10 (mean 6-7) days. This period may be longer than 10 days (up to 19 days has been reported). Pulmonary symptoms may be absent or very mild at onset. Cough is mild, with 25-78% of patients exhibiting dry cough. There is usually high fever[1,17,43,44]. It should be kept in mind that physical examination of the lungs may also be normal early in the disease. Pneumococci and *Legionella* are the most common agents of community-acquired pneumonia leading to intensive care admissions. Various studies have shown that Legionnaires' disease has similar clinical and radiological findings to pneumococcal pneumonia. Therefore, it should be included in the differential diagnosis. One of the most important features distinguishing atypical pneumonia from typical pneumonia is the presence of extrapulmonary symptoms and signs. Gastrointestinal symptoms are common in patients with Legionnaires' disease, detected in approximately 20-50% of cases. Abdominal pain, diarrhea, nausea, and vomiting are the most common gastrointestinal symptoms. Splenomegaly should raise suspicion of other atypical pneumonia agents (e.g.: Q fever, psittacosis). A substantial proportion of patients experience neurological symptoms, headache being a common complaint. Unlike confusion secondary to fever in

Table 1. Studies investigating *Legionella* in environmental water samples in Turkey

Author	Year	Location of sample analysis	Source of sample	Number of samples (n)	*Legionella*-producing samples, n (%)	*Legionella* species
Burak and Zeybek[39]	-	İstanbul	Home	61	13 (21.3%)	Lp SG 2-14 (87.5%) Lp SG 1 (12.5%)
Sepin Özen et al.[32]	2010	Antalya	Hotel	1403	142 (10.1%)	Lp SG 2-14 (85.2%) Lp SG 1 (14.8%)
Akkaya and Özbal[37]	2008	Kayseri	Hospital, hotel, residence, school	120	8 (6.7%)	Lp SG 2-14 (62.5%) Lp SG 1 (37.5%)
Uzel et al.[35]	2000	İzmir	Hotel	168	128 (76.2%)	Lp SG 2-14 (6.3%) Lp SG 1 (85.9%) Nonpneumophila (7.8%)
İğnak and Gürler[36]	2006-2007	İstanbul	Hospital	100	7 (7%)	Lp SG 1 (42.9%), Nonpneumophila (57.1%)
Türetgen et al.[40]	1996-2000	İstanbul	Cooling tower	103	27 (26%)	Lp SG 1 (44%)
Erdogan and Arslan[33]	2003-2005	Alanya	Hotel	491	93 (18.9%)	Lp SG 6 (63.5%) Lp SG 1 (21.5%)
Erdogan and Arslan[41]	2003-2013	Alanya	Turkish bath	135	18 (13.3%)	Lp SG 6 (55.6%) Lp SG 1 (22.2%)
Alim et al.[42]	2001	Central Anatolia Region	Thermal pools of hot springs	209	24 (11.5%)	Lp SG 2-14 (83.4%) Lp SG 1 (8.3%)
Erdogan et al.[38]	2006	Ankara, İzmir, Konya, Alanya	Hospital	125	34 (27.2%)	Lp SG 1 (58.8%) Lp SG 6 (35.3%)
Akbas et al.[34]	1995-1997	Aegean and Mediterranean Region	Hotel	592	92 (15.5%)	Lp SG 2-14 (89.4%) Lp SG 1 (6.3%) Nonpneumophila (4.2%)

Lp: *Legionella pneumophila*, SG: Serogroup

typical pneumonia, Legionnaires' disease patients may present with encephalopathy. Focal neurological findings, epileptic seizures, and walking and speech disorders associated with cerebellar involvement have been reported[43-46]. Relative bradycardia is another important finding that supports a diagnosis of Legionnaires' disease. Other cardiac arrhythmia types may also be seen.

Extrapulmonary involvement: Extrapulmonary legionellosis is very rare and more common in immunosuppressed patients. Reported manifestations include myocarditis, pericarditis, and infective endocarditis due to heart involvement; cerebral abscess, meningoencephalitis, aseptic meningitis, cerebellitis, and peripheral neuropathy due to neurological involvement; rhabdomyolysis due to muscle involvement; acute kidney injury and pyelonephritis due to renal involvement; abscess and cellulitis due to skin and subcutaneous tissue involvement; and perirectal abscess, pancreatitis, and peritonitis due to gastrointestinal involvement[1,2,17,23,25,47].

Laboratory results: Neutrophil-dominated leukocytosis is a common finding. The presence of leukopenia or predominance of lymphocytes suggests other diagnoses. Another common finding is abnormal liver enzymes, such as mildly elevated

Table 2. The demographic, clinical, and laboratory characteristics of patients in some of the reported Legionnaires' disease cases

	Kirby et al.[50] (n=65)	Dias et al.[44] (n=43)	Isenman et al.[49] (n=107)	Erdogan et al. [21] (n=17)
Source	**Hospital-acquired**	**Community-acquired**	**Community-acquired***	**Travel-associated**
Mean age (years)	59	56	65	61
Gender, M/F (%)	95/5	61/40	63/37	47/53
Comorbid diseases (%)				
COPD/asthma	22	21	19	12
Diabetes mellitus	9	16	13	41
Cancer	29	2	10	-
Immunosuppression	42	0	8	-
Smoking	72	77	16	35
Signs and symptoms (%)				
Dry cough	92	63	38	65
Productive cough	54	16	46	11
Chest pain	33	33	-	24
Respiratory distress	36	40	-	41
Diarrhea	47	21	18	47
Nausea/vomiting	25	21	28	29
Headache	28	35	41	47
Disturbances in the consciousness	38	35	9	35
Fever ≥39.4 °C	79	63	-	47
Relative bradycardia	60	-	-	53
Laboratory findings (%)				
Leukocytosis (>12,000/mm^3)	78	49	-	94
Elevated CRP (>200 mg/dl)	-	-	69	77
Hyponatremia (Na <130 mEq/l)	54	21	12	41
Abnormal liver enzymes (ALT or AST)	49	55	62	59
Hypophosphatemia (≤2.7 mg/dl)	51	43	-	58
Elevated CK (>168 IU/l)	2	29	-	40
Elevated creatinine (>1.3 mg/dl)	-	51	28	35
ICU requirement (%)	32	16	25	65
Mortality (%)	25	0	5	24

*Legionnaires' disease caused by *Legionella longbeache*, M: Male, F: Female, COPD: Chronic obstructive pulmonary disease, Na: Sodium, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C-reactive protein, ICU: Intensive care unit, CK: Creatine kinase

aspartate aminotransferase and alanine aminotransferase. Hypophosphatemia, hyponatremia, elevated serum ferritin, elevated creatinine levels, elevated C-reactive protein, high erythrocyte sedimentation rate, microscopic hematuria, proteinuria, and creatine kinase elevation are other laboratory findings frequently seen in Legionnaires' disease[1,2,17,21,24,43-50]. The demographic, clinical, and laboratory characteristics of Legionnaire's disease patients in some previous studies are presented in Table 2.

Radiologic findings: Despite being referred to as atypical pneumonia, typical alveolar infiltration is a feature of Legionnaires' disease. Radiologic imaging often shows patchy infiltration which usually progresses to lobular infiltration. Lower lobe involvement is more common. Approximately one in four patients has mild pleural effusion. The interstitial involvement seen in atypical pneumonia is very rare. Nodular involvement, cavitation, and abscess formation have also been reported, particularly in immunosuppressed patients. Even under appropriate treatment, progression of the infiltration may be evident on chest X-ray (Figures 2, 3)[49-52].

Laboratory Diagnosis

Isolation of *Legionella* bacteria is the gold standard for diagnosing Legionnaires' disease. Urine antigen test, direct fluorescent antibody staining, and polymerase chain reaction are rapid diagnostic tests. A combination of urinary antigen test and culture of respiratory tract specimens is recommended for diagnosis of Legionnaires' disease. Serological testing for *Legionella* infection has little impact on making early clinical decision. The sensitivity, specificity, advantages, and disadvantages of the tests used to diagnose Legionnaires' disease are shown in Table 3[53-56]. The definite and presumed laboratory diagnostic criteria for patients with clinical and/or radiological findings consistent with pneumonia, according to the criteria in the Infectious Diseases Notification System, Standard Diagnosis, Surveillance and Laboratory Guidelines in Turkey are shown in Table 4[57]. These diagnostic criteria are the same as those used in Europe and the United States. Using the Legionnaires' Disease Analysis Request Form, samples obtained from hospitals with limited laboratory capacity for diagnosis or which require further examination can be processed in the Public Health Laboratory of their district or the National Reference Laboratory. In such cases, it is recommended to act in accordance with the guidelines and contact the relevant public health authorities.

Culture: This is the gold standard diagnostic test for Legionnaires' disease. The major advantage of culturing is that all strains of *Legionella* can be identified, and the isolated strain may provide important epidemiological data. *Legionella* bacteria can be isolated from lower respiratory samples as well as from other nonrespiratory samples (pleural fluid, abscess, wound site, etc.). Less than half of Legionnaires' patients produce sputum, which is typically nonpurulent (low-neutrophil) and watery. Ingram and Plouffe demonstrated that 47-84% of *L. pneumophila* positive samples were obtained from sputum that were considered to be poor quality to test[53]. Therefore, *Legionella* culture should be

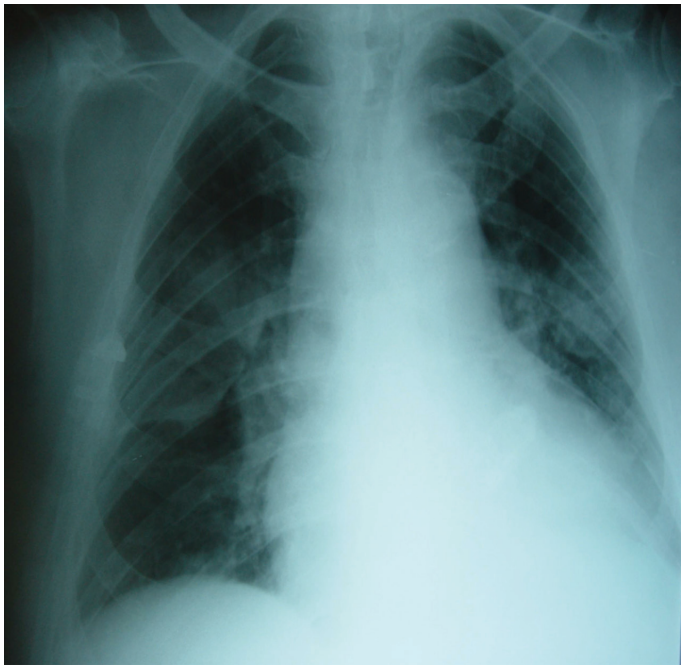

Figure 2. Posterior-anterior chest x-ray taken on the first day of hospitalization shows patchy infiltration in the middle and lower zones bilaterally in a patient diagnosed in our clinic with Legionnaires' disease

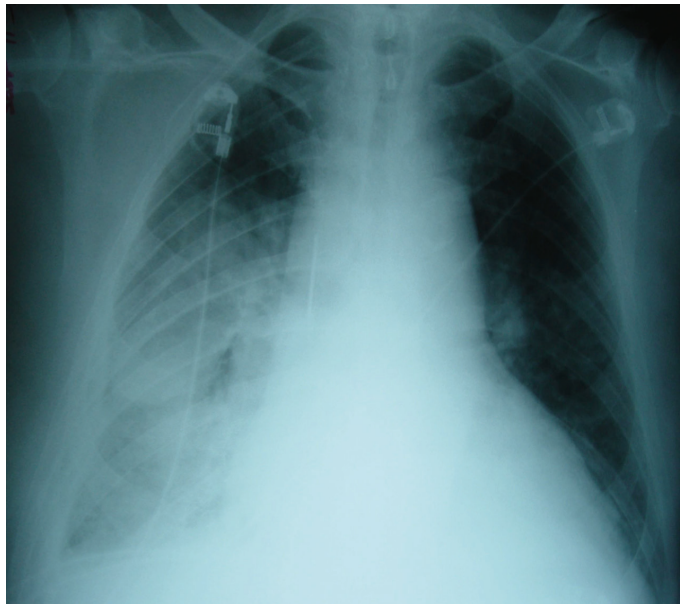

Figure 3. On the third day of hospitalization, infiltration progressed in the lower and middle zones of the right lung despite appropriate antibiotic therapy and clinical improvement

done regardless of the quality of sputum specimens. Samples for culture should be taken before antibiotic therapy is initiated and should be transferred to the laboratory quickly. The main disadvantage of culture is that the results usually become available in 3-5 days. The sensitivity of sputum cultures varies from 25% to 81%, and the experience of laboratory personnel is important. The rate of positive culture is higher in severe cases due to higher bacterial load. It should be kept in mind that species other than *L. pneumophila* may grow more slowly and require at least 10 days of incubation to reach detectable levels[55,56].

***Legionella* urine antigen test:** This test is based on the detection of lipopolysaccharide antigen on the cell wall of *Legionella* bacteria. It is a valuable test because i)it is easy to apply ii) results become available within 15 minutes for the card test and 90 minutes for ELISA iii) it is usually positive 48-72 hours after symptom onset iv) results are unaffected by antibiotic usage v) specificity of the test is approaching to 100%. The sensitivity of the urine antigen test is correlated with disease severity.

Epitopes associated with the virulence of *L. pneumophila* have been identified in the cell wall lipopolysaccharides. Strains carrying these virulence-associated epitopes can be detected using monoclonal antibodies (Dresden panel 3/1 or MAb 2 international panel). The test has highest sensitivity (up to 95%) for MAb 3/1-positive *L. pneumophila* serogroup 1 strains, which are the most common cause of community-acquired or travel-

Table 3. Sensitivity, specificity, advantages, and disadvantages of diagnostic tests used in Legionnaires' disease

Diagnostic tests	Sensitivity (%)	Specificity (%)	Advantages	Disadvantages
Urine antigen test	40-95	≥99	- Early positivity - Easy to apply - Provides rapid results - Not affected by antibiotic use	- Only reliable for *L. pneumophila* SG 1 - Can stay positive long-term
Culture	25-81	100	- Is the gold standard - Can identify all *Legionella* species - Gives very important information for detecting source	- Fewer than one half of patients with Legionnaires' disease produce sputum - *Legionella* does not grow in routinely used media - Requires specific growth medium - At least 3-5 days is required to develop colony morphology
Serologic tests	41-75	96-99	- Valuable in epidemiological studies	- Low reliability for species other than *L. pneumophila* - Seroconversion takes 4-12 weeks - Limited use in early diagnosis
PCR	40-99	95-100	- Can be applied to a wide range of samples (sputum, blood, urine, etc.) - Can identify *L. pneumophila* serogroups and nonpneumophila species - Useful in early diagnosis	- Has not been standardized - Requires laboratory equipment - Expensive
DFA staining	25-70	96-99*	- Provides rapid results - Useful in early diagnosis	- Requires experienced personnel - Less sensitive than culture

*For staining with monoclonal antibodies, DFA: Direct fluorescent antibody, PCR: Polymerase chain reaction

Table 4. Diagnostic criteria of Legionnaires' disease in patients with clinical and/or radiological signs of pneumonia

Confirmed case:

1. Isolation of *Legionella* bacteria in cultures of sputum, lung tissue, pleural fluid, or other clinical samples,
2. Detection of *L. pneumophila* SG 1 antigen in urine,
3. Demonstration of ≥4-fold increase in serum antibody titer against *L. pneumophila* SG 1 (by IFA or ELISA),

Probable case:

1. Detection of *Legionella* antigen in respiratory secretions or lung tissue by DFA staining using monoclonal antibodies,
2. Demonstration of ≥4-fold increase in serum antibody titer against *Legionella* spp. other than *L. pneumophila* SG 1 (by IFA or ELISA),
3. Presence of antibody titers against L*egionella* spp. ≥1/256 in a single serum sample (by IFA or ELISA),
4. Detection of *Legionella* nucleic acids by PCR in clinical specimens such as respiratory tract secretions, lung tissue, or sterile body fluid.

SG: Serogroup, IFA: Indirect fluorescent antibody, DFA: Direct fluorescent antibody, PCR: Polymerase chain reaction

associated Legionnaires' disease. Sensitivity decreases to 40% in MAb 3/1-negative *L. pneumophila* serogroup 1 strains, which are less virulent and commonly isolated in hospital-acquired Legionnaires' disease. The ELISA and card test used for urine antigen testing have comparable sensitivity and specificity rates. About 70-90% of Legionnaires' disease reported in Europe and United States are diagnosed with urine antigen tests. The main disadvantage of this test is low reliability for nonpneumophila species and serogroups other than *L. pneumophila* serogroup 1. However, clinicians should keep in mind that urine antigen tests may still be positive for these other serogroups and nonpneumophila species, especially in patients with high bacterial load. Approximately 8% of Legionnaires' patients do not excrete antigen in their urine[1,29,30,54]. Kim et al.[58] reported that the peptidoglycan-associated lipoprotein, which they previously demonstrated in experimental animal studies to be common to all *Legionella* spp., can be detected in urine with high sensitivity and specificity. This is a promising development toward urine antigen tests that can recognize any *Legionella* bacterium.

Direct fluorescent antibody staining: Sensitivity varies between 25-70%, and the test is less sensitive than bacteriological culture. A high specificity of 96-99% was achieved with the use of monoclonal antibodies. The test is suitable for rapid diagnosis, particularly during outbreaks. However, it requires experienced personnel and technical equipment[55,56].

Polymerase chain reaction: Polymerase chain reaction-based tests can detect all *Legionella* spp. using the ribosomal RNA gene or all serogroups of *L. pneumophila* using the *mip* gene. Sputum, lower respiratory tract samples obtained by invasive procedures, nasopharyngeal specimens, urine, blood, sterile body fluids, and tissue specimens can be tested. Reported sensitivity rates are 40-99% in sputum, 91-99% in lower respiratory tract samples taken invasively, 38-60% in blood samples, and 26-70% in urine samples. Specificity is high[2,59,60]. However, polymerase chain reaction-based tests may yield false positives due to contamination of the kits used. Disadvantages of PCR include lack of standardization, high cost, and requirement of experienced personnel and laboratory equipment.

Serology: Serology provides important information for epidemiological studies, but has limited use in early detection. The definitive diagnostic criterion is a 4-fold increase in antibody titers against *L. pneumophila* serogroup 1 in indirect fluorescent antibody tests, which is the standard reference test. Sensitivity varies between 41-75%. In some patients, antibody response time may be over four weeks. Furthermore, antibody response may not occur in patients who receive early antibiotic therapy or are immunosuppressed. Specificity is low for strains other than *L. pneumophila* serogroup 1. Due to the possibility of cross-reactivity, the high false-positive rate, and low incidence, a 4-fold increase in specific antibody titers to serogroups other than *L. pneumophila* serogroup 1 and other nonpneumophila species is diagnosed as presumed Legionnaires' disease. In about one-third of patients, the specific antibody response may be detected for more than two years. Therefore, high antibody titers against *Legionella* spp. in a single serum sample may also indicate past infection. Evaluation of antibody subtypes such as IgM and IgG is of limited benefit in diagnosis of acute infection[55,56].

Treatment

Early and appropriate treatment is the most important factor in decreasing the high case-fatality rate. Antibiotics that have good lung and intracellular ppenetration (particularly in alveolar macrophages), and have *in vitro* activity against *Legionella* should be used in treatment. Fluoroquinolones, macrolides, tetracycline, tigecycline, trimethoprim-sulfamethoxazole, and rifampicin are drugs that can be used in therapy. Rifampicin

Table 5. Antimicrobial drugs and doses used in the treatment of Legionnaires' disease

Ciprofloxacin	400 mg IV three times a day 750 mg PO twice a day
Levofloxacin	500 mg IV or PO twice a day 750 mg IV or PO once a day
Moxifloxacin	400 mg PO once a day
Clarithromycin	500 mg IV or PO twice a day
Tigecycline	100 mg initial dose, followed by 50 mg IV twice a day
Azithromycin*	1 gr initial dose, 500 mg IV or PO once a day
Erythromycin*	500 mg IV or PO four times a day
Doxycycline*	100 mg IV or PO twice a day
Trimethoprim-sulfamethoxazole**	160 mg (trimetoprim) IV or PO three times a day
Rifampicin**	300 mg PO twice a day

IV: Intravenous, PO: Per oral, *IV form not available in Turkey, **can be used as a component of combination therapy in severe cases

is not recommended as monotherapy due to its high potential for resistance development. Fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin), new macrolides (azithromycin, clarithromycin), and doxycycline may be preferred for treatment. Fluoroquinolones should be used to treat patients receiving chemotherapy due to drug interactions with macrolides[61-64]. Although in some studies quinolones did not show a significant reduction in mortality compared to macrolides, treatment with quinolones was associated with earlier fever response, fewer side effects, and shorter hospital stay[63]. However, most of these studies included few serious cases and mostly patients with low case-fatality rate. In a recent study, Cecchini et al.[64] retrospectively evaluated 211 Legionnaires' patients who required treatment in intensive care units, 69% of whom had acute respiratory distress syndrome. They reported that fluoroquinolone-based treatments significantly reduced mortality. However, their study had several limitations, such as the retrospective design and 10-year span of the study, and the fact that they did not take into account recent developments in intensive care or differentiate between old and new macrolides.

Since antimicrobial susceptibility tests are not standardized, the results are difficult to interpret and do not guide treatment. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) report no antibiotic breakpoints for *Legionella*. Drawbacks of conventional methods using agar and broth dilution are that charcoal binds some antibiotics and reduces their effect, and they do not show intracellular bactericidal action. *In vitro* intracellular models and animal studies have higher accuracy rates[1,2]. We previously conducted susceptibility testing of *Legionella* strains isolated from environmental specimens obtained from different regions in Turkey to rifampicin, clarithromycin, azithromycin, ciprofloxacin, and levofloxacin by broth dilution method, and we detected no resistance to the tested antibiotics[65]. However, there are also reports of resistant strains in environmental samples[66]. To date, only one *Legionella* strain isolated from clinical specimens has been reported as ciprofloxacin-resistant[67].

Empirical therapy for severe community-acquired pneumonia should include antimicrobial drugs effective against Legionnaires' disease. Treatment response is usually seen in 2-3 days, though the febrile response sometimes lasts as long as 5-7 days. Patients should be reevaluated within 48-72 hours of starting treatment. Treatment failure can be presumed for patients whose condition deteriorates or does not improve, and treatment may be changed to another drug group. Although the results of *in vivo* studies suggest combination therapies may be effective, clinical trials have not corroborated these findings[1,2,61,64]. Clinicians tend to prefer combination therapies (macrolide + fluoroquinolone or rifampicin) in severe cases and patients with extrapulmonary involvement. Switching to monotherapy when the patient's clinical condition improves is crucial to reduce side effects. Though very rare, the possibility of coinfection or superinfection with other microorganisms must also be considered, especially in immunosuppressed patients[68].

Treatment duration is 5-14 days. In severe cases and immunosuppressed patients, treatment may be extended to 21 days based on the patient's clinical response and laboratory parameters. Extrapulmonary organ involvement such as endocarditis requires long-term treatment. Parenteral treatment is a good initial choice in severe cases due to the frequency of gastrointestinal involvement in Legionnaires' disease and since this may affect oral absorption of the antibiotic. The agents used in the treatment of Legionnaires' disease have excellent oral absorption. Therefore, patients who show clinical improvement and have no problem with oral intake should be switched to oral administration as early as possible. Oral therapy is the route of choice because it is cost-effective, causes fewer side effects, and shortens the length of hospital stay[1,2,61-64,69]. The doses and routes of administration of drugs used to treat Legionnaires' disease are summarized in Table 5.

Prevention Studies

A water system management plan should be implemented routinely to prevent *Legionella* colonization in the water systems of buildings such as hospitals and hotels. One individual should be appointed responsible for the water system management plan, and a team knowledgeable and aware of Legionnaires' disease should be established to implement it. Checklists of routine preventive measures should be created. Procedures and checklists for internal and external audit should be documented in writing.

The basic strategy in the water system management plan should be "to keep water temperature above 50 °C in the entire hot water systems and lower than 20 °C in the cold water systems". Biofilm in the water system should be prevented. Storage tanks should be cleaned regularly and areas of stagnation that prevent water flow should be eliminated from the system. Water should be run through unused showerheads and faucets at regular intervals. If a water system is out of service for even a short time due to system maintenance or interruptions in water service, it should be disinfected before being used again. Use of the municipal water supply in patient care should be limited In healthcare facilities[70-72].

Early detection of Legionnaires' cases is one of the most important prevention strategies. Hospital laboratories should be equipped to perform the specific tests (urine antigen test, culture, etc.) used in the diagnosis of hospital-acquired Legionnaires' disease.

Environmental surveillance: When a case of Legionnaires' disease is identified, environmental water systems should be cultured for detection and decolonization of the probable source. According to the United States Centers for Disease Control, water systems should be investigated for *Legionella* bacteria in the following situations i)hospitals treating patients with high risk for Legionnaires' disease ii)buildings with water systems that are difficult to maintain within control limits iii) hospitals that have reported nosocomial Legionnaires' infections[71]. Although *Legionella* testing cannot be considered to be a control measure it can be used for validation of actions taken to prevent *Legionella* colonization in hospital water systems[72].

The challenges of culturing *Legionella* and variations in the amount of bacteria when culturing make it difficult to give cut-off values for water cultures. The infective dose that causes Legionnaires' disease is also unknown[73]. According to the European technical guidelines for the prevention, control, and investigation of infections caused by *Legionella* species, actionable levels vary for samples taken from cooling towers, spas, and cold/hot water systems. In case of positive samples from hot and cold water systems with 1000-10,000 colony-forming units per liter (CFU/L), the proportion of positive samples should be considered. Resampling is recommended if a small proportion of samples show growth (10-20%). If similar growth is observed in resampling, it is recommended that control measures be reviewed and risk assessment carried out to identify any remedial action required. A large proportion of positive samples, even with very low levels of *Legionella* growth, indicates colonization, and disinfection of the water system is recommended. If there is >10,000 CFU/L in samples, the guidelines recommend resampling of the system with immediate review of control measures, risk assessment, and necessary remedial actions including disinfection of the water system[70]. The WHO *Legionella* and Legionellosis prevention manual specifies target levels of <1000 CFU/L for healthcare facilities where there are patients with classic risk factors, and <50 CFU/L where there are high-risk patients[72].

A National Legionnaires' disease laboratory network was established in Turkey with the Regulation on Legionnaires' Disease Control Procedures and Principles. Only these laboratories are authorized to study *Legionella* in samples from hospital water systems. In terms of routine preventive measures, water samples should be taken at least once a year from hospital and healthcare institutions even in the absence of Legionnaires' cases. In hospitals with units considered high-risk, such as tissue/organ transplantation, hematology, or oncology, samples representing these units must be taken twice a year at equal intervals. In the event of a case of disease or positive culture of a sample taken during routine studies from hospital and health care institutions, it is recommended that active surveillance studies be initiated within the scope of case surveillance[10,57].

Decontamination Methods

Decontamination methods applicable in building hot and cold water systems are briefly described below. The decontamination methods in Turkey should comply with the disinfection technical instructions published in the official gazette of Turkey and the regulation on Waters for Human Consumption in Turkey[74,75]. Biocides or other disinfectant products cannot take the place of a good water source and water system with regular flow while they cannot eliminate the shortcomings of a poorly engineered system. *Legionella*-negative water samples obtained from areas where biocides are used do not show that a system is safe[70].

Thermal shock: The water temperature in hot water tanks is briefly raised to 70-80 °C, with temperatures reaching at least 65 °C at end use points within in buildings and hot water is run through all taps and appliances for at least 5 minutes. This high temperature can be applied for up to three days. The most important advantage of this method is that it requires no special equipment. It can be used as emergency disinfection or part of a long-term control program. It is not suitable for large buildings because it requires high energy and human power. Its use is limited in buildings with thermostatic mixers. It should be noted that recolonization may occur a few weeks after this procedure. Measures should be taken to prevent boiling[57,70,76].

Constant maintenance of the temperature between 55°C and 60 °C: *Legionella* can survive for 80-120 minutes at 50 °C and 2 minutes at 60 °C. Maintaining water temperature above 50 °C at taps and appliances reduces the likelihood of hot water system colonization with *Legionella*. Although it does not completely eliminate *Legionella* colonization from the system, its greatest advantage is the ability to prevent further cases. It is easy to implement and control. Disadvantages include high energy expenditure and risk of boiling[70,57,76].

Chlorination: Chlorine (Cl) is a halogen element that is a gas in normal conditions. It becomes a liquid when compressed under high pressure. It is in liquid state as sodium hypochlorite (NaClO) and a solid as calcium hypochlorite ($Ca(ClO)_2$). Chlorine gas dissolves in water to form hypochlorite (OCl^-) and hypochlorous acid (HOCl). Hypochlorous acid has high oxidation potential and a stronger disinfectant effect than hypochlorite. Hypochlorous acid dominates at low pH (6-7), whereas hypochlorite becomes dominant at high pH (above 8.5). For this reason, chlorine has reduced effectiveness at high pH. Its greatest advantages are that it is inexpensive and levels can be monitored with simple chemical tests. The disadvantages are that chlorine is corrosive and reacts with organic substances to form by-products suspected to be carcinogenic, such as trihalomethanes and

haloacetic acid. Free-living *Legionella* bacteria in the water were found to survive for 3 minutes at 2 mg/l chlorine, while bacteria within cysts could survive for up to 18 hours at 50 mg/l chlorine level. Chlorine levels of 1-2 mg/l are required for disinfection of *Legionella*. Shock hyperchlorination is implementing a chlorine level of 20-50 mg/l throughout whole water system at least 1-2 hours. Water temperature should be below 30 °C during shock chlorination[57,70,74-76].

Bromide: It is a halogen element, like chlorine. Hypobromous acid or hypobromite formed when bromide dissolves in water have an oxidating effect. A water level of 2-3 mg/l is required[70].

Monochloramine: It is obtained by adding chlorine to water containing ammonia or by adding ammonia to chlorinated water. The desired concentration in drinking water is 1-2 mg/l. Despite being a weak disinfectant, it has the advantage of remaining stable for longer and being more effective against biofilm layers compared to chlorine[77]. Marchesi et al.[78] reported that monochloramine was more effective than heating, chlorine dioxide, and hydrogen peroxide in preventing *Legionella* colonization.

Chlorine dioxide: A chemical compound with molecular formula ClO_2. It takes electrons from the cell wall of microorganisms, thereby causing oxidative damage. It is usually produced in a generator from the reaction of hydrochloric acid (HCl) or chlorine gas with sodium chloride ($NaClO_2$). Chlorine dioxide is extremely volatile and unstable at high concentrations, and is therefore produced on the location where it will be used. A concentration of 0.3-0.4 mg/l is required for disinfectant effect. Advantages include efficacy in a broad pH range and less volatility at high temperatures compared to chlorine. Disadvantages are that it is corrosive and forms harmful by-products such as chlorite and chlorate. The United States Environmental Protection Agency has reported maximum values of 0.8 mg/l for chlorine dioxide and 1 mg/l for chlorite or chlorate. Chlorine dioxide has long been used successfully for the prevention of *Legionella* colonization[78-80].

Copper (Cu)-Silver (Ag) ions: Electrodes connected to a device produce Cu and Ag ions that are used for water disinfection. The ions interact with cell walls of the microorganism, causing altered cell permeability, protein denaturation, and ultimately lysis and cell death. Manufacturers of the devices recommend Cu-Ag ionization in the range of 0.2-0.8 mg/l. Considerations to bear in mind are that it may be difficult to reach desired concentrations of Ag ions in hard water due to deposits on the electrodes, and ionization is pH-dependent. The high cost and difficulties monitoring this method are its disadvantages. However, it has been in use for many years, especially in the United States and Spain. Stout and Yu[81] conducted a study of 16 hospitals using the Cu-Ag ionization method in the United States. All of the hospitals had previous cases of nosocomial Legionnaires' disease and 65% had used heat shock and flushing, ultraviolet, and hyperchlorination disinfection methods. In approximately 47% of the hospitals, more than 30% of samples taken prior to installing the new system were positive for *Legionella* colonization. No positivity was detected in 50% of the hospitals 5 years after implementation of Cu-Ag ionization, and in 47% after 10 years. They also reported that no Legionnaires' disease were diagnosed during this period. However, in 2015 an epidemic of Legionnaires' disease was reported in a hospital using Cu-Ag ionization[82].

Hydrogen peroxide (H_2O_2): Hydrogen peroxide is a powerful oxidizing disinfectant that has no toxic, mutagenic, or carcinogenic effect. Silver-stabilized forms of H_2O_2 have been developed to benefit from the bactericidal action and synergy of Ag. Hydrogen peroxide is recommended for use at 15-20 parts per million in routine applications. It should not be used in water systems that support dialysis units. Further studies are needed regarding the effect of H_2O_2 on prevention of *Legionella* colonization[70,83].

Ozonization: Ozone is a strong oxidizing agent and has stronger biocidal activity than chlorine. Concentrations in drinking and domestic water supplies should be 1-2 mg/l[57,84].

Ultraviolet light: Ultraviolet light (254 nm) induces the formation of thymine dimers in DNA, thus disrupting DNA replication and killing bacteria. It has minimal effect on *Legionella* in biofilm layers, dead spaces, and blind spots. There is no residual effect. For these reasons, using ultraviolet light alone is not recommended for *Legionella* control. However, there is evidence that it is effective in *Legionella* control when in close proximity to risky units. Advantages include being easily implemented, having no detrimental effect on pipes, and having no effect on the taste and potability of water[70,85,86].

Terminal filtration: Bacterial filters are placed to prevent *Legionella* and other bacteria from passing through faucets and shower heads. This method is used in hospitals to prevent infection, especially in high-risk areas[70].

Conclusion

Legionnaires' disease is a serious form of pneumonia caused by *Legionella* bacteria. The main route of transmission of Legionnaires' disease is inhalation or microaspiration of water colonized with *Legionella*. However, there are also a substantial number of cases of Legionnaires' disease transmitted through soil and soil products, primarily due to *L. longbeachae*. Early and appropriate treatment can be life-saving. Clinical and laboratory features are not reliable in the diagnosis of Legionnaires' disease, so specific laboratory tests are required.

The real incidence of Legionnaires' disease in Turkey is unknown. Therefore, there is a need for multicenter studies to determine the incidence of Legionnaires' disease among community-acquired pneumonia cases, the predominant serogroups and subgroups, and regional distribution patterns. There are very few hospitals capable of performing the specific laboratory tests for the diagnosis of Legionnaires' disease. Hospitals should be equipped at least to do *Legionella* urine antigen testing and *Legionella* culture, especially those hospitals with high-risk patients. For suspected cases it should be noted that samples obtained in hospitals with limited laboratory capacity or which require further testing to diagnose Legionnaires' disease may be processed in provincial Public Health Laboratories or the National Reference Laboratory using the Legionnaires' Disease Analysis Request Form.

Legionnaires' disease is of epidemiological importance because identifying and decolonizing the potential sources are key for prevention of new cases. In Turkey, the Regulation on Legionnaires' Disease Control Procedures and Principles has mandated annual water cultures for *Legionella* in hospital water systems, even in hospitals with no reported cases. Any growth of *Legionella* in samples taken during routine controls is considered to be an actionable level, without exception. This may cause unnecessary and excessive use of biocides and other disinfectant products against *Legionella* in hospitals.

Ethics

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