RESEARCH ARTICLE / ARAŞTIRMA

DOI: 10.4274/mjima.galenos.2022.2022.40
Mediterr J Infect Microb Antimicrob 2022;11:40
Erişim: http://dx.doi.org/10.4274/mjima.galenos.2022.2022.40

Screening for Fosfomycin Resistance Genes in Carbapenem-Resistant Isolates of *Enterobacterales* from the Bloodstream of Liver Transplant Patients

Karaciğer Nakli Hastalarının Karbapenem Dirençli *Enterobacterales* Kan Dolaşımı İzolatlarında Fosfomisin Direnç Genlerinin Araştırılması

Yakup GEZER[1], Elif Seren TANRIVERDİ[2], Barış OTLU[2], Sezai YILMAZ[3], Yaşar BAYINDIR[4]

[1]*Konya City Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Konya, Turkey*
[2]*İnönü University Faculty of Medicine, Department of Medical Microbiology, Malatya, Turkey*
[3]*İnönü University Faculty of Medicine, Department of General Surgery, Liver Transplantation Institute, Malatya, Turkey*
[4]*İnönü University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Malatya, Turkey*

Abstract

Introduction: Interest in fosfomycin, an old antibiotic, has been reignited because of the use of its intravenous formulation (fosfomycin sodium) in resistant infections. The aim of this study was to screen for carbapenem resistance in *Enterobacterales* isolates from the bloodstream, and determine the frequency of carbapenamases types and fosfomycin resistance genes in carbapenem-resistant *Enterobacterales* (CRE) strains.

Materials and Methods: *Enterobacterales* isolates from the bloodstream of liver transplant patients aged 18 years and older were screened for carbapenem resistance between 2017 and 2019. In isolates that were resistant to at least one carbapenem antibiotic were further screened for fosfomycin susceptibility. Carbapenem susceptibility was tested for by the E-test, fosfomycin susceptibility was tested for by the agar dilution methods, and evaluated in accordance with European Committee on Antimicrobial Susceptibility Testing criteria. The frequency of OXA-48, NDM, KPC, VIM, and IMP type carbapenemases and *fosA*, *fosA3*, and *fosC2* fosfomycin resistance genes were screened for using the polymerase chain reaction method.

Results: A total of 115 *Enterobacterales* isolates from bloodstream infections were obtained. Carbapenem resistance was detected in 34 (29.3%) isolates, 41.2% of them were *Escherichia coli* and 58.8% of them were *Klebsiella pneumoniae*. Out of the 34 isolates, 61.8% produced carbapenemases, and OXA-48 was the most common type of carbapenemase. The fosfomycin resistance rate was 73.5%. Among the 34 carbapenem-resistant isolates, the frequency of the *fosA* gene was 5.9%. The genes *fosA3* and *fosC2* were not detected in any isolates. *FosA* + OXA-48 and *fosA* + NDM genes were detected in CRE isolates.

Conclusion: This is the first study reporting on the screening for fosfomycin resistance genes in blood isolates of liver transplant patients in Turkey. Despite high fosfomycin resistance, detection of only two resistance genes reveals that there may be fosfomycin resistance due to other resistance mechanisms.

Keywords: Bacteremia, *Enterobacteriaceae*, *fosA*, *fosA3*, *fosC2*, hepatic transplantation, phosphomycin resistance

Öz

Giriş: Eski bir antibiyotik olan fosfomisin, dirençli enfeksiyonlarda intravenöz formülasyonunun (fosfomisin sodyum) kullanılmasıyla tekrar ön plana çıkmıştır. Bu çalışmanın amacı *Enterobacterales* kan dolaşımı izolatlarında karbapenem direncini belirlemek ve karbapenem dirençli *Enterobacterales* (KDE) suşlarında karbapenem türleri ve fosfomisin direnç genlerinin sıklığını araştırmaktır.

Gereç ve Yöntem: *Enterobacterales* kan dolaşımı izolatları, 2017-2019 yılları arasında 18 yaş ve üzeri karaciğer nakli hastalarında değerlendirildi. Bu izolatlardan herhangi bir karbapenem antibiyotiğe dirençli olanlar tespit edilerek çalışmaya alındı. Karbapenem duyarlılığı E-test, fosfomisin

Cite this article as: Gezer Y, Tanrıverdi ES, Otlu B, Yılmaz S, Bayındır Y. Screening for Fosfomycin Resistance Genes in Carbapenem-Resistant Isolates of *Enterobacterales* from the Bloodstream of Liver Transplant Patients. Mediterr J Infect Microb Antimicrob. 2022;11:40.

Address for Correspondence/Yazışma Adresi: Yakup GEZER MD, Konya City Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Konya, Turkey
Phone: +90 505 885 43 76 **E-mail:** dryakupgezer@gmail.com **ORCID ID:** orcid.org/0000-0002-1582-7313
Received/Geliş Tarihi: 08.09.2022 **Accepted/Kabul Tarihi:** 08.11.2022

Published: 15 November 2022

Öz

duyarlılığı agar dilüsyon yöntemleri ile test edildi ve European Committee on Antimicrobial Susceptibility Testing kriterlerine göre değerlendirildi. OXA-48, NDM, KPC, VIM, IMP tipi karbapenemazlar ile *fosA, fosA3, fosC2* dirençli genlerin sıklığı polimeraz zincir reaksiyonu (PZR) yöntemi kullanılarak araştırıldı.

Bulgular: *Enterobacterales* kan dolaşımı enfeksiyonlarından toplam 115 izolat incelenerek herhangi bir karbapenem antibiyotiğe dirençli olan 34 (%29,3) izolat tespit edildi. Bu 34 izolatın %61,8'i karbapenemaz üretti ve OXA-48 en yaygın karbapenemaz tipiydi. İzolatların %41,2'si *Escherichia coli* ve %58,8'i *Klebsiella pneumoniae* idi. Izolatlarda fosfomisin direnç oranı %73,5 idi. *FosA* geni %5,9 olarak bulundu. Ancak hiçbir izolatta *fosA3* ve *fosC2* genleri yoktu. Karbapenem dirençli *Enterobacterales* izolatlarında *fosA*+OXA-48 ve *fosA*+NDM genleri tespit edildi.

Sonuç: Türkiye'de karaciğer nakli yapılan hastaların kan kültürü izolatlarında fosfomisin direnç genlerinin araştırıldığı ilk çalışmadır. Yüksek fosfomisin direncine rağmen sadece iki tane pozitif direnç geni saptanması, diğer direnç mekanizmalarına bağlı olarak fosfomisin direnci olabileceğini ortaya koymaktadır.

Anahtar Kelimeler: Bakteriyemi, *Enterobacteriaceae*, *fosA, fosA3, fosC2*, fosfomisin direnci, karaciğer transplantasyonu

Introduction

Sepsis and bacteremia, in particular, are complications causing high mortality in patients that have undergone liver transplantation and immunosuppressive treatments. In liver transplant patients, the most common microorganisms causing infections are enterococci, Gram-negative bacilli, and, rarely, other bacteria and candida strains[1]. Antibacterial resistance occurs in *Enterobacterales* by mutation, selection, or when sensitive bacteria gain resistance genes from other bacteria through conjugation or transduction[2]. The increase in multidrug-resistant (MDR) Gram-negative bacteria is causing difficulties in managing these patients.

Fosfomycin phosphoenolpyruvate stops the synthesis of peptidoglycan, one of the basic components of the cell wall, causing cell lysis and death of bacteria. Fosfomycin has been well-known as an oral antibiotic for the treatment of uncomplicated urinary tract infections. However, thanks to the intravenous formulation (fosfomycin sodium), it is now widely used for the treatment of vancomycin-resistant enterococcus, methicillin-resistant *Staphylococcus aureus*, and MDR Gram-negative bacterial infections[3]. Becuase of its antimicrobial, antioxidant, and anti-inflammatory effects, fosfomycin is a candidate for reducing sepsis-induced lung damage[4]. In *Enterobacterales* strains, genes that are mainly responsible for fosfomycin resistance are *fosA (1–10)*, *fosL1-2,* and *fosC2,* which codes for glutathione transferase[5]. These *fos* genes are currently of low to medium effectiveness in spreading fosfomycin resistance. Nevertheless, their occurrence in portable plasmids might render them one of the most effective agents in spreading fosfomycin resistance. Moreover, these genes co-occur with other genes that cause resistance to other groups of antibiotics, thereby enhancing the outbreak of MDR strains[6].

This study aimed to screen for fosfomycin susceptibility and *fos* resistance genes in carbapenem-resistant *Enterobacterales* (CRE) strains from positive blood cultures of liver transplant patients at the Liver Transplantation Institute, İnönü University. Currently, there are only a few studies on fosfomycin susceptibility and resistance genes in Turkey. To address the aim of this study, we used a gold standard method, the agar dilution method, to analyze for fosfomycin susceptibility and polymerase chain reaction (PCR) to detect *fosA, fosA3, fosC2* resistance genes. Finally, sequence analyses were performed to validate resulting isolates with resistant genes.

Materials and Methods

Patient Population and Bacteria

Between January 2017 and December 2019, we examined blood cultures of patients over the age of 18 who were hospitalized at the liver transplant institute and who had liver transplant. A total of 106 patients and 115 isolates with *Enterobacterales* blood infection were analyzed. Isolates that were resistant to at least one of the carbapenem antibiotics were included in the study. The Centers for Disease Control and Prevention defines CRE as *Enterobacterales* that are resistant to at least one of the carbapenem antibiotics or produce a carbapenemase. According to this definition, these isolates were considered CRE[7]. The strains were detected at different stages of bacteremia and only one of the strains with similar types and phenotypic resistance in the same attack were included in the study. Fosfomycin resistance genes and carbapenamase genes were screened for in 33 patients and 34 CRE isolates.

The blood culture analyses were performed in Render BC128 fully automated blood culture systems in compliance with the manufacturer's instructions (Shandong Huifa Electronics Technology Co., Jinan, Shandong, China). The isolates were typed according to "Matrix-Assisted Laser Desorption/Ionization Time of flight, Mass Spectrometry" (VITEK® MS, bioMérieux, Marcy l'Etoile, France)[8].

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) v12.0 guideline breakpoints were used to screen the isolates for antimicrobial susceptibility[9]. The gradient strip test

(E-test®, bioMérieux) was conducted to screen for carbapenem susceptibility[10]. Colistin susceptibility of isolates was screened for by broth microdilution method. Susceptibility to other antibiotics was investigated by the Kirby Bauer disk diffusion method[11].

The Preparation of Isolates

Bacterial isolates were stored at -80 °C until further analyzes. Before the study, subculturing was performed using blood agar with incubation of 18 to 24 h at 35 °C.

The Determination of Fosfomycin Susceptibility

The agar dilution method, which is the gold standard method according to EUCAST, was used to examine the susceptibility of the isolates to fosfomycin. The threshold minimum inhibitory concentration (MIC) for fosfomycin in EUCAST is 32 mg/l; thus, the strains with MIC ≤32 values were regarded as susceptible, and those with MIC of >32 values as resistant. The fosfomycin agar dilution test was carried out in Mueller-Hinton agar with a series of dilutions achieved by adding fosfomycin antibiotic powder (Sigma-Aldrich, St. Louis, MO, USA) and 25 mg/l glucose-6-phosphate (Sigma-Aldrich, St. Louis, MO, USA). The control strain, *E. coli* ATCC25922, was used in each study.

The DNA Extraction of Isolates and Screening for *fosA, fosA3, fosC2,* and Carbapenemase Genes by PCR

DNA isolation of bacteria isolates was performed using a QIAamp DNA midi kit (Qiagen, Hilden, Germany) on a QIAsymphony automated DNA extraction machine (Qiagen, Hilden, Germany). The DNA extracts were kept at -20 °C until they were used PCR. The *fosA3*, *fosA,* and *fosC2* resistance genes were examined by the in-house PCR method. To do so, the following primers were utilized: F-5'-ATC TGT GGG TCT GCC TGT CGT-3', R-5'-ATG CCC GCA TAG GGC TTCT-3' for *fosA*, F-5'-GGC ATT TTA TCA GCA GT-3', R-5'-AGA CCA TCC CCT TGT AG-3' for *fosA3* and F-5'-CGA GCC AAG ATT ACT GT-3', R-5'-AAC GAT TCC AAA CGA CT-3' for *fosC*[12]. The presence of blaIMP, blaVIM, blaOXA-48, blaKPC, and blaNDM genes of the isolates was determined by the in-house multiplex PCR method. For the carbapenemase genes, the primers used were those described by Poirel et al.[13].

DNA amplification was carried out by a thermocycler, GeneAmp PCR System 9700 (Applied Biosystems, Waltham, MA, USA). The amplification process for *fosA* was as follows: the first denaturation for 5 min at 94 °C, 30 cycles and denaturation for 30 s at 94 °C, annealing for 30 s at 59.5 °C, 1-min primer extension at 72 °C, and final extension for 10 min at 72 °C. Primer unification was set to 57.5 °C for *fosA3* and 50.5 °C for *fosC2*[12,14]. The amplification cycles for carbapenemase genes were as follows: 10 min at 94 °C and 36 cycles of amplification consisting of 30 s at 94 °C, 40 s at 52 °C, and 50 s at 72 °C, with 5 min at 72 °C for the final extension.

The amplicons were electrophoresed for 1 h at 100V in 1.5% agarose gel and stained by ethidium bromide before their UV images were taken using a Kodak Gel Logic 200 (1708x1280, Kodak Company, USA).

Molecular Typing of the CRE Strains

The clonal relationships between the isolates of the patients (n=34) were investigated by performing an arbitrary primer sequence-based PCR (AP-PCR). AP-PCR analysis was performed according to the protocol described by Grundmann et al.[15] with a minor modification. Briefly, AP-PCR reaction mixture (50 µl) contained 100 ng of genomic DNA, 100 pmol of M13 primer (5'-GAG GGT GGC GGT TCT-3'), 0.5 unit of Taq DNA polymerase (Vivantis Technologies, Malaysia), 200 µmol deoxynucleoside triphosphate mix, and 10X amplification buffer. Band profiles were analyzed using the GelCompar II software system (version 6.5; Applied Maths, Sint-Martens-Latem, Belgium). The dice correlation coefficient was used to calculate similarity for band analysis, and the UPGMA ("Unweighted Pairwise Grouping Mathematical Avenging" mathematical mean grouping of unweighted pairs) method was used for cluster analysis.

The Validation of Amplified Items by Sequence Analyses

The sequence analyzes for amplicons were made with Bigdye Terminator V3.1 cycle sequencing by ABI Prism 310 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). The obtained *fosA* gene sequences were aligned by the Ugene software (http://ugene.unipro.ru). In order to screen for *fosA* subtypes, GenBank and Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast) included in National Center for Biotechnology Information were consulted.

Statistical Analysis

All data obtained in the study were analyzed using the Statistical Packages for the Social Sciences (SPSS) version 26.0 (IBM SPSS®, Armonk, New York, USA). Mean values (±standard deviation) and median values for quantitative variables and numbers (percentages/rates) were used for qualitative variables. The Fisher's Exact test was used to compare categorical variables, and a p value of <0.05 (two-sided) was considered statistically significant.

Results

Table 1 shows the demographic and clinical characteristics of the 33 patients included in this study.

The median number of days (minimum-maximum) from transplantation to diagnosis of CRE bacteremia in the 33 patients was 64.5 (1-3469) days. The overall case fatality rate in the first month after liver transplantation was 15.2% (5/33) and the one year case fatality rate was 30.3% (10/33). The case

Table 1. Demographic and clinical characteristics of the liver transplant patients infected with carbapenem-resistant *Enterobacterales*

Demographic and clinical characteristics		(n)	(%)
Age, years	Mean±SD	50.58±11.38	
	Median (min-max)	55 (30-70)	
Sex	Male	24	72.7
	Female	9	27.3
Liver transplant type	Living	31	93.9
	Deceased	2	6.1
Liver transplant indication	Cryptogenic failure	9	27.3
	HBV	6	18.2
	HCV	4	12.1
	Budd-Chiari	3	9.1
	HBV + HCC	3	9.1
	Alcohol-related liver disease	2	6.1
	Primary sclerosing cholangitis	2	6.1
	HBV + HDV	1	3
	HBV + HDV + HCC	1	3
	Autoimmune hepatitis	1	3
	Acute liver failure	1	3

HBV: Hepatitis B virus, HCC: Hepatocellular cancer, HDV: Hepatitis D virus, min-max: Minimum-maximum, SD: Standard deviation

Table 2. Resistance rates of carbapenem-resistant *Enterobacterales* to antimicrobial agents (%)

Antibiotic	*Escherichia coli* (%)	*Klebsiella pneumoniae* (%)
Ampicillin	91.7	100
Amoxicillin/clavulanate	92.3	100
Amikacin	7.7	41.2
Gentamicin	36.4	50
Aztreonam	61.5	89.5
Ceftazidime	71.4	100
Ceftriaxone	81.8	100
Cefoxitin	58.3	87.5
Cefepime	71.4	89.4
Piperacillin/tazobactam	78.6	100
Ertapenem	100	100
Meropenem	14.3	75
Imipenem	14.3	45
Fosfomycin	35.7	100
Colistin	0	5.6
Levofloxacin	30	87.5
Ciprofloxacin	28.6	78.9
Trimethoprim/ sulfamethoxazole	92.9	89.5
Tigecycline	0	28.6

fatality rates in the first 28 days, one year, and two years after the diagnosis of bacteremia were 24.2% (8/33), 45.5% (15/33), and 48.5% (16/33), respectively.

The most common cause for bacteremia was an intraabdominal infection, which accounted for 85.3% (29/34) of the cases. Intraabdominal infections included biliary system infections, surgical infections, and peritonitis. Three cases were diagnosed with urinary tract infection, and two were diagnosed with pneumonia.

Results for Antibiotic Susceptibility of Isolates

The 34 isolates comprised *E. coli* (41.2%) and *K. pneumoniae* (58.8%), and all strains were ertapenem resistant. The prevalence of meropenem resistance was 14.3% in *E. coli* and 75% in *K. pneumoniae*. The prevalance of imipenem resistance in *E. coli* and *K. pneumoniae* was 14.3% and 45%, respectively. Table 2 shows *in vitro* susceptibility to antibiotics studied in here.

Of the 34 isolates, 21 (61.8%) produced carbapenemases. OXA-48 was the most common carbapenemase produced by *E. coli* (8/8, 100%) and *K. pneumoniae* (11/13, 84.6%). The distribution of carbapenemases among *E. coli* and *K. pneumoniae* isolates is presented in Table 3. The prevalence of carbapenemase-producing gene was 55.6% (5/9) in fosfomycin-susceptible isolates and 64% (16/25) in fosfomycin-resistant isolates. There was no significant difference between fosfomycin susceptibility and carbapenemase gene positivity (p=0.704). The AP-PCR results detected no dominant epidemic strain in the 34 CRE isolates.

Of all the isolates included in our study, 73.5% were resistant to fosfomycin. In the *E. coli* isolates, the rate of fosfomycin susceptibility was 64.3%, whereas no fosfomycin-susceptible isolate was found in the *K. pneumoniae* isolates (p<0.001). Figure 1 shows the resulting MIC values.

All isolates were screened for the *fosA, fosA3,* and *fosC2* resistance genes by PCR, and isolates that were positive for any of the resistant genes were validated by sequence analysis. The *fosA* gene was was detected in two (5.8%) isolates but *fosA3* and *fosC2* were not present in any isolate. The two *fosA* gene positive isolates were *K. pneumoniae* isolates, and the MIC values of fosfomycin for these isolates were 512 and >512. In addition, one of the isolates with the *fosA* gene was OXA-48 positive and the other was NDM positive. Figure 2 shows gel electrophoresis images of strains that were positive for *fosA*. The presence of the *fosA* resistance gene in these two isolates was confirmed by sequence analyses.

Table 3. Distribution of types of carbapenemases in fosfomycin-resistant and fosfomycin-susceptible isolates

	OXA-48	NDM	VIM	IMP	KPC	OXA-48, NDM
Escherichia coli						
Fosfomycin-susceptible	4	-	-	-	-	1
Fosfomycin-resistant	2	-	-	-	-	1
Klebsiella pneumoniae						
Fosfomycin-resistant	9	1	1	-	-	2
Total	**15**	**1**	**1**	**-**	**-**	**4**

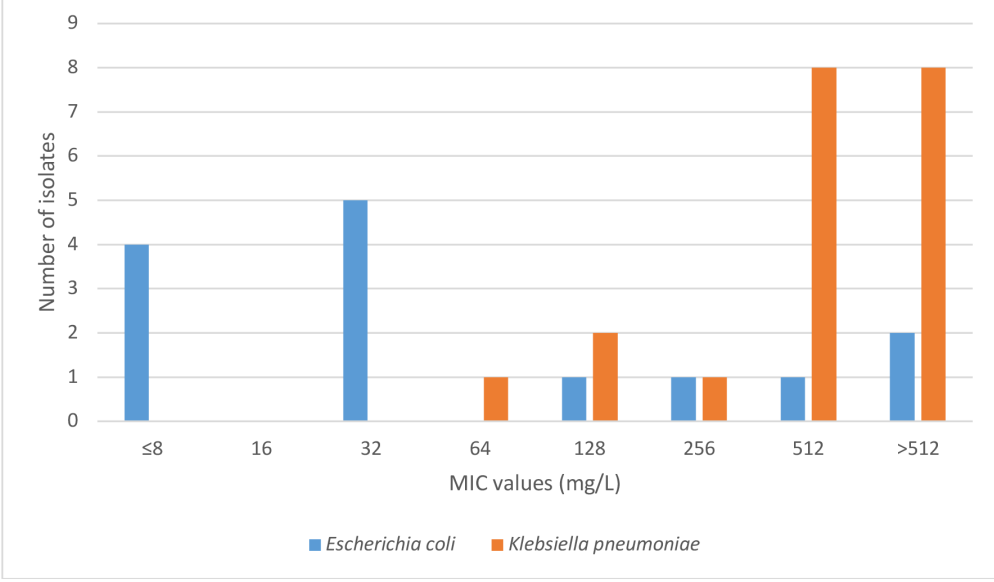

Figure 1. Distribution of isolates according to MIC values determined by fosfomycin agar dilution
MIC: 32 mg/L (cut-off fosfomycine in European Committee on Antimicrobial Susceptibility Testing).
MIC: Minimum inhibitory concentration

Discussion

Bacteremia is one of the critical complications that causes morbidity or mortality in solid organ transplanted patients. During the first year after liver transplantation, the rate of bacteremia is about 10-23%[16]. Carbapenem resistance in *Enterobacterales* isolates has recently increased, and most of *Enterobacterales* cause nosocomial infections. This kind of resistance raises challenges in treating the bacterial infection using antimicrobials and causes a high level of mortality. In a multi-centered cohort study focusing on CRE infections, 13% of the cases had bacteremia, *K. pneumoniae* was observed in 57%, and *E. coli* occurred in 12% of the isolates[17]. In our study, the distribution of the isolates was 58.8% *K. pneumoniae* and 41.2% *E. coli* in the liver transplant patients with bacteremia.

The rate of mortality is higher among patients with MDR bacterial infections than in those without[18]. Between 2015 and 2021, a total of 1206 patients over 18 years of age underwent liver transplantation in our hospital. The survival rate one month after the transplantation was 90% and it dropped to 79.4% after one year. One month and one year survival rates were lower in the group of patients with CRE bacteremia. Our findings suggest that circulatory infections caused by resistant bacteria like CRE bacteremia increase the rate of mortality. However, other causes such as unsolvable surgical complications, long-term stays in care units/hospitals, and long-term use of antibiotics can not be ruled out. Therefore, further studies are required to to investigate the causes of high mortality.

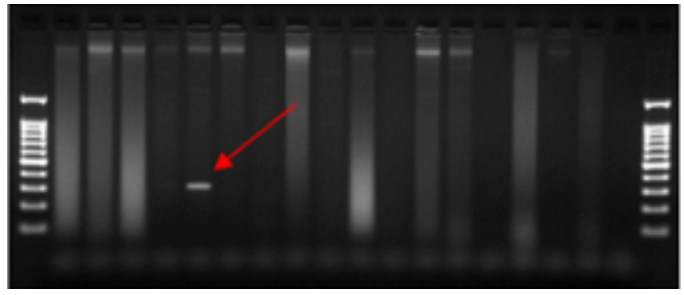

Figure 2. Gel electrophoresis image of isolates with *fosA* resistance gene

Jafarpour et al.[19] reported the occurrence of urinary infections (22%), intraabdominal and surgical infections (20.6%), pneumonia (20.6%), sepsis (19.1%), primary circulatory infection (2.2%) and other infections (8%) in liver transplant patients with bacterial infections. Another study reported aprevalence of 10.2% for pneumonia, 69.4% for intraabdominal and biliary system infections, and 20.4% for urinary infections in liver transplant patience with Entrobacterales infections[20]. Our findings indicate that the most common cause of infections was intraabdominal infections accounting for 85.4% of the cases.

A previous study reported antibiotic resistance rates of imipenem (97.2%), ertapenem (95.8%), meropenem (90.8%), and amikacin (24.1%) in *E. coli* isolates; and ertapenem (100%), imipenem (99.4%), meropenem (95.3%), and amikacin (41.8%) in *K. pneumoniae* isolates[21]. In a study on KPC positive *K. pneumoniae* isolates, meropenem, fosfomycin, amikacin, and colistin resistance rates were 100%, 41%, 76% and 65% respectively[22]. In our study, the rates of resistance in *K. pneumoniae* isolates were: fosfomycin (100%), ertapenem (100%), meropenem (75%), imipenem (45%), amikacin (41.2%), and colistin (5.6%). In *E. coli* isolates, the rates for resistance were: ertapenem (100%), fosfomycin (35.7%), meropenem (14.3%), imipenem (14.3%), amikacin (7.7%), and colistin (0%). Antibiotic resistance rates obtained in this study were similar to those of other studies, and resistance rates are higher in *K. pneumoniae* than in *E. coli*.

Antibiotic options to treat resistant bacterial infections are gradually becoming limited for critical patients, including solid organ transplant recipients. Intravenous fosfomycin (fosfomycin sodium) has a broad antibacterial effect on Gram-negative and positive bacteria, and when it is used with other antibiotics, such as beta-lactam, aminoglycoside, and glycopeptide, a synergistic effect might arise[23]. Clinical and microbiological responses have been reported when fosfomycin was used in combination treatment regimes[24,25]. It is now widely accepted that fosfomycin is most effective when used as a part of antibitoc combination regimes to treat extensive-drug resistance (XDR) *Enterobacterales* infections in critical patients[26].

In KPC-producing *K. pneumoniae* isolates, fosfomycin resistance rates of 36.4% and 60.8% have been reported[27,28]. A recent study by Zarakolu et al.[29] reported that from CRE from the blood cultures, fosfomycin susceptibility was 95.1% in *E. coli* and 69.4% in *K. pneumoniae*. Carbapenemase genes were detected in 88.1% of CRE isolates, and OXA-48 was the most common carbapenemase type. In another multi-centered study conducted in Turkey, in KPC-producing *Enterobacterales* isolates, the prevalence of fosfomycin resistance was 43.7%, and the rates for *Klebsiella* spp. and *E. coli* were 46.8% and 21.1%, respectively. OXA-48 is the most frequently detected type of carbapenemases[30]. In a study of *E. coli* isolates from Iran, OXA-48 and VIM were the most reported types of carbapenamases[31]. In another study, the most common VIM carbapenemase genes were detected in CRE isolates[32]. From the findings of these studies, we might conclude that fosfomycin resistance rates are higher in *Klebsiella* strains in CRE. Emerging fosfomycin resistance of carbapenem-resistant *K. pneumoniae* isolates is of concern. When all isolates were separately evaluated by agar dilution for fosfomycin resistance, fosfomycin resistance had a prevalence of 35.7% in *E. coli*, 100% in *K. pneumoniae*, and 73.5% in all the isolates combined. In our study, 61.8% of the isolates produced carbapenemases, and OXA-48 was the most common type. OXA-48 carbapenemase is endemc in Turkey. The higher prevalence of isolates with fosfomycin resistance than in previous studies might be due to our special study population, which included liver transplant patients with many risk factors like long-term hospital stays and intensive antimicrobial treatment exposure.

The resistance against fosfomycin, which is an important alternative treatment, has increased dramatically. The effect of *fos* genes synthesizing fosfomycin-inactivating enzymes on the resistance and spread of fosfomycin seems to be at a low to medium level. However, the occurrence of *fos* genes in mobile plasmids might be one of the effective means by which fosfomycin resistance spreads. Moreover, the co-occurrence of *fos* genes with other antibiotic resistance genes might increase XDR strains[6]. In Egypt, Elshimy et al.[33] examined *E. coli* isolates and detected the *fosA* resistance gene. In another study on the *fosA* gene in fosfomycin-resistant *K. pneumoniae* isolates, the rate of *fosA* was 40%[34]. Among the fosfomycin-resistant isolates in the carbapenem-resistant *K. pneumoniae*, no *fosA* and *fosC2* genes were detected apart from 15% *fosA3*[27]. In a similar study, it was reported that the *fosA3* gene had a prevalence of 77.2% and the *fosA* gene had a prevalence of 1.8%, but the *fosC2* gene was absent[35]. A previous study in Iran reported that the *fosA*, *fosA3,* and *fosC2* resistance genes were absent in all the isolates[36]. In other studies on fosfomycin-resistant *E. coli* isolates, Wachino et al.[37] reported that *fosA3* was present in 20% and *fosC2* in 10% of the isolates, Mueller et al.[38] reported that *fosA3* occurred in 23.5%, Loras et al.[39] reported that *fosA3* occurred in 5.1% of the isolates. In East Asian countries, the *fosA3* gene had a higher prevalence. It was concluded that this result was caused by the bacteria in the animals in that region carrying and spreading this resistance gene[40].

In Turkey, there are only a few studies so far on fosfomycin resistance genes. In a study by Nigiz et al., *fosA3* was detected in only one of 16 fosfomycin-resistant isolates, and *fosA* and *fosC2* genes were not detected[41]. Another study reported absence of *fosA, fosA3,* and *fosC2* resistance genes in bacterial isolates[42]. A recent study on *E. coli* and *K. pneumoniae* proliferating in blood cultures, reported that the *fosA3* gene was not detected

in the fosfomycin-resistant strains[29]. In this study, *fosA* was detected in 10% of all *K. pneumoniae* isolates and in 5.8% of total strains. No *fosA3* and *fosC2* genes were detected in any isolates. The isolates with the *fosA* gene were phenotypically fosfomycin-resistant, and had a high MIC value. In addition, one of the isolates with the *fosA* gene was OXA-48 positive and the other one was NDM positive. The recent detection of *fos* resistance genes in in Turkey suggests that plasmid-mediated fosfomycin resistance may increase in the near future in our country as well as in the whole world.

Study Limitations

The generalizability of the results is limited due to the fact that the study was conducted from a single center and to a specific patient group. In addition, the study was also limited by the small number of cases used.

Conclusion

Only two *fosA* resistance genes were detected in the carbapenem-resistant isolates studied but the prevalence of fosfomycin resistance was higher. These findings suggest that antibiotic resistance can be spread through other resistance mechanisms besides *fos* genes. This is the first study in Turkey of fosfomycin susceptibility and resistance genes on blood culture isolates of liver transplanted patients. Further studies are recommended to investigate treatment response and mortality rates after fosfomycin-based combined treatments for CRE-infected patients.

Ethics

Ethics Committee Approval: This study was retrospectively approved by Health Sciences, İnönü University, as a non-invasive clinical study and numbered 2020/1103, date: 13.10.2020.

Informed Consent: Informed consent was not obtained as only study isolates were tested, and electronic data of patients were used without identity information.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Romero FA, Razonable RR. Infections in liver transplant recipients. World J Hepatol. 2011;3:83–92.
2. Weiner-Lastinger LM, Abner S, Edwards JR, Kallen AJ, Karlsson M, Magill SS, Pollock D, See I, Soe MM, Walters MS, Dudeck MA. Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: Summary of data reported to the National Healthcare Safety Network, 2015-2017. Infect Control Hosp Epidemiol. 2020;41:1-18.
3. Falagas ME, Vouloumanou EK, Samonis G, Vardakasa KZ. Fosfomycin. Clin Microbiol Rev. 2016;29:321–47.
4. Yildiz IE, Topcu A, Bahceci I, Arpa M, Tumkaya L, Mercantepe T, Batcik S, Yildiz Y. The protective role of fosfomycin in lung injury due to oxidative stress and inflammation caused by sepsis. Life Sci. 2021;279:119662.
5. Zurfluh K, Treier A, Schmitt K, Stephan R. Mobile fosfomycin resistance genes in *Enterobacteriaceae*-An increasing threat. Microbiologyopen. 2020;9:e1135.
6. Falagas ME, Athanasaki F, Voulgaris GL, Triarides NA, Vardakas KZ. Resistance to fosfomycin: Mechanisms, Frequency and Clinical Consequences. Int J Antimicrob Agents. 2019;53:22-8.
7. Carbapenem Resistant *Enterobacterales* Technical Information. https://www.cdc.gov/hai/organisms/cre/technical-info.html. 25 July 2021
8. Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front Microbiol. 2015;6:791.
9. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. Available from: http://www.eucast.org
10. Marroki A, Bousmaha-Marroki L. Antibiotic Resistance Diagnostic Methods for Pathogenic Bacteria. Elsevier. 2022;4:320-34.
11. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology. 2009;15:55-63.
12. Sato N, Kawamura K, Nakane K, Wachino J, Arakawa Y. First detection of fosfomycin resistance gene fosA3 in CTX-M-producing *Escherichia coli* isolates from healthy individuals in Japan. Microb Drug Resist. 2013;19:477-82.
13. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011;70:119-23.
14. Hou J, Huang X, Deng Y, He L, Yang T, Zeng Z, Chen Z, Liu JH. Dissemination of the fosfomycin resistance gene fosA3 with CTX-M β-lactamase genes and rmtB carried on IncFII plasmids among *Escherichia coli* isolates from pets in China. Antimicrob Agents Chemother. 2012;56:2135-8.
15. Grundmann HJ, Towner KJ, Dijkshoorn L, Gerner-Smidt P, Maher M, Seifert H, Vaneechoutte M. Multicenter study using standardized protocols and reagents for evaluation of reproducibility of PCR-based fingerprinting of *Acinetobacter* spp. J Clin Microbiol. 1997;35:3071-7.
16. Singh N, Haidar G, Limaye AP. Infections in solid-organ transplant recipients. In: Bennet JE, Dolin R, Blaser MJ, edıtors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 9th ed. Canada, Elsevier. 2020. p.3672–97.
17. van Duin D, Arias CA, Komarow L, Chen L, Hanson BM, Weston G, Cober E, Garner OB, Jacob JT, Satlin MJ, Fries BC, Garcia-Diaz J, Doi Y, Dhar S, Kaye KS, Earley M, Hujer AM, Hujer KM, Domitrovic TN, Shropshire WC, Dinh A, Manca C, Luterbach CL, Wang M, Paterson DL, Banerjee R, Patel R, Evans S, Hill C, Arias R, Chambers HF, Fowler VG Jr, Kreiswirth BN, Bonomo RA; Multi-Drug Resistant Organism Network Investigators. Molecular and clinical epidemiology of carbapenem-resistant *Enterobacterales* in the USA (CRACKLE-2): a prospective cohort study. Lancet Infect Dis. 2020;20(6):731-41. Erratum in: Lancet Infect Dis. 2020.
18. Bodro M, Sabé N, Tubau F, Lladó L, Baliellas C, Roca J, Cruzado JM, Carratalà J. Risk factors and outcomes of bacteremia caused by drug-resistant

ESKAPE pathogens in solid-organ transplant recipients. Transplantation. 2013;96:843-9.

19. Jafarpour Z, Pouladfar G, Malek Hosseini SA, Firoozifar M, Jafari P. Bacterial infections in the early period after liver transplantation in adults: A prospective single-center cohort study. Microbiol Immunol. 2020;64:407–15.
20. Cinar G, Kalkan İA, Azap A, Kirimker OE, Balci D, Keskin O, Yuraydin C, Ormeci N, Dokmeci A. Carbapenemase-Producing Bacterial Infections in Patients With Liver Transplant. Transplant Proc. 2019;51:2461-5.
21. Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, Xie L, Yang C, Ma X, Li H, Li W, Zhang X, Liao K, Man S, Wang S, Wen H, Li B, Guo Z, Tian J, Pei F, Liu L, Zhang L, Zou C, Hu T, Cai J, Yang H, Huang J, Jia X, Huang W, Cao B, Wang H. Epidemiology of Carbapenem-Resistant *Enterobacteriaceae* Infections: Report from the China CRE Network. Antimicrob Agents Chemother. 2018;62:e01882-17.
22. Erturk Sengel B, Altinkanat Gelmez G, Soyletir G, Korten V. In vitro synergistic activity of fosfomycin in combination with meropenem, amikacin and colistin against OXA-48 and/or NDM-producing *Klebsiella pneumoniae*. J Chemother. 2020;32:237-43.
23. Barber GR. Unique Antibacterial Agents. In: Bennet JE, Dolin R, Blaser MJ, edıtors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 9th ed. Canada, Elsevier. 2020.p.449–60.
24. Machuca I, Gutiérrez-Gutiérrez B, Gracia-Ahufinger I, Rivera Espinar F, Cano Á, Guzmán-Puche J, Pérez-Nadales E, Natera C, Rodríguez M, León R, Castón JJ, Rodríguez-López F, Rodríguez-Baño J, Torre-Cisneros J. Mortality Associated with Bacteremia Due to Colistin-Resistant *Klebsiella pneumoniae* with High-Level Meropenem Resistance: Importance of Combination Therapy without Colistin and Carbapenems. Antimicrob Agents Chemother. 2017;61:e00406-17.
25. Simkins J, Fan J, Camargo JF, Aragon L, Frederick C. Intravenous Fosfomycin Treatment for Carbapenem-Resistant *Klebsiella pneumoniae* in the United States. Ann Pharmacother. 2015;49:1177-8. Erratum in: Ann Pharmacother. 2015;49:1362.
26. Mills JP, Wilck MB, Weikert BC, Porrett PM, Timko D, Alby K, Bonomo RA, Blumberg EA. Successful treatment of a disseminated infection with extensively drug-resistant *Klebsiella pneumoniae* in a liver transplant recipient with a fosfomycin-based multidrug regimen. Transpl Infect Dis. 2016;18:777-81.
27. Tseng SP, Wang SF, Ma L, Wang TY, Yang TY, Siu LK, Chuang YC, Lee PS, Wang JT, Wu TL, Lin JC, Lu PL. The plasmid-mediated fosfomycin resistance determinants and synergy of fosfomycin and meropenem in carbapenem-resistant *Klebsiella pneumoniae* isolates in Taiwan. J Microbiol Immunol Infect. 2017;50:653-61.
28. Jiang Y, Shen P, Wei Z, Liu L, He F, Shi K, Wang Y, Wang H, Yu Y. Dissemination of a clone carrying a fosA3-harbouring plasmid mediates high fosfomycin resistance rate of KPC-producing *Klebsiella pneumoniae* in China. Int J Antimicrob Agents. 2015;45:66-70.
29. Zarakolu P, Eser ÖK, Otlu B, Gürpınar Ö, Özakın C, Akalın H, Köksal İ, Ünal S. In-vitro activity of fosfomycin against Escherichiacoli and *Klebsiella pneumoniae* bloodstream isolates and frequency of OXA-48, NDM, KPC, VIM, IMP types of carbapenemases in the carbapenem-resistant groups. J Chemother. 2022;34:235-40.
30. Demirci-Duarte S, Unalan-Altintop T, Gulay Z, Sari Kaygisiz AN, Cakar A, Gur D. In vitro susceptibility of OXA-48, NDM, VIM and IMP enzyme- producing *Klebsiella* spp. and *Escherichia coli* to fosfomycin. J Infect Dev Ctries. 2020;14:394-7.
31. Deldar Abad Paskeh M, Mehdipour Moghaddam MJ, Salehi Z. Prevalence of plasmid-encoded carbapenemases in multi-drug resistant *Escherichia coli* from patients with urinary tract infection in northern Iran. Iran J Basic Med Sci. 2020;23:586-93.
32. Gajdács M, Ábrók M, Lázár A, Jánvári L, Tóth Á, Terhes G, Burián K. Detection of VIM, NDM and OXA-48 producing carbapenem resistant *Enterobacterales* among clinical isolates in Southern Hungary. Acta Microbiol Immunol Hung. 2020;67:209-215.
33. Elshimy R, Zedan H, Elmorsy TH, Khattab RA. A Study on Multidrug-Resistant *Escherichia coli* Clinical Isolates from Different Hospitals in Greater Cairo. Microb Drug Resist. 2021;27:1420-32.
34. Kashefieh M, Hosainzadegan H, Baghbanijavid S, Ghotaslou R. The Molecular Epidemiology of Resistance to Antibiotics among *Klebsiella pneumoniae* Isolates in Azerbaijan, Iran. J Trop Med. 2021;2021:9195184.
35. Xiang DR, Li JJ, Sheng ZK, Yu HY, Deng M, Bi S, Hu FS, Chen W, Xue XW, Zhou ZB, Doi Y, Sheng JF, Li LJ. Complete Sequence of a Novel IncR-F33:A-:B-Plasmid, pKP1034, Harboring fosA3, blaKPC-2, blaCTX-M-65, blaSHV-12, and rmtB from an Epidemic *Klebsiella pneumoniae* Sequence Type 11 Strain in China. Antimicrob Agents Chemother. 2015;60:1343-8.
36. Ghanavati R, Ohadi E, Kazemian H, Yazdani F, Torki A, Kalani BS, Irajian G. Evaluation of Fosfomycin Activity Against Extended Spectrum Beta Lactamase (ESBL) Producing *Enterobacteriaceae* Isolated from Three Centers of Tehran, Iran. Recent Pat Antiinfect Drug Discov. 2018;13:180-6.
37. Wachino J, Yamane K, Suzuki S, Kimura K, Arakawa Y. Prevalence of fosfomycin resistance among CTX-M-producing *Escherichia coli* clinical isolates in Japan and identification of novel plasmid-mediated fosfomycin-modifying enzymes. Antimicrob Agents Chemother. 2010;54:3061-4.
38. Mueller L, Cimen C, Poirel L, Descombes MC, Nordmann P. Prevalence of fosfomycin resistance among ESBL-producing *Escherichia coli* isolates in the community, Switzerland. Eur J Clin Microbiol Infect Dis. 2019;38:945-9.
39. Loras C, Mendes AC, Peixe L, Novais Â, Alós JI. *Escherichia coli* resistant to fosfomycin from urinary tract infections: Detection of the fosA3 gene in Spain. J Glob Antimicrob Resist. 2020;21:414-6.
40. Yang TY, Lu PL, Tseng SP. Update on fosfomycin-modified genes in *Enterobacteriaceae*. J Microbiol Immunol Infect. 2019;52:9-21.
41. Nigiz Ş, Hazırolan G, Köseoglu Eser Ö, Gür D. First Detection of *Klebsiella pneumoniae* Isolate Co-Harboring Fosfomycin Resistance Gene fosA3 and blactx-m Among Gram Negative Urine Isolates in a Turkish Hospital. Microb Drug Resist. 2022;28:317-21.
42. Demirci-Duarte S, Unalan-Altintop T, Eser OK, Cakar A, Altun B, Sancak B, Gur D. Prevalence of O25b-ST131 clone and fosfomycin resistance in urinary *Escherichia coli* isolates and their relation to CTX-M determinant. Diagn Microbiol Infect Dis. 2020;98:115098.