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Brief Original Article

In vitro susceptibility of OXA-48, NDM, VIM and IMP enzyme- producing *Klebsiella* spp. and *Escherichia coli* to fosfomycin

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Abstract

Introduction: Infections caused by Carbapenemase-producing Enterobacterales (CPE) are an important public health issue. Intravenous fosfomycin can be considered as an alternative for the treatment of serious infections caused by CPE. In this study, *in vitro* activity of fosfomycin was investigated among CPE isolates.

Methodology: Overall, 158 clinically relevant isolates obtained from 18 hospitals of 13 cities in Turkey with predetermined carbapenemase types were evaluated in the study, including *Escherichia coli* (n = 19) and *Klebsiella* spp. (n = 139). *In vitro* activity of fosfomycin was determined with agar dilution method. Among *Klebsiella* spp., 104 harbored bla_{OXA-48}, 15 isolates carried both bla_{OXA-48} and bla_{NDM}; three had both bla_{OXA-48} and bla_{VIM} and nine isolates had bla_{NDM} alone. Four isolates carried only bla_{VIM} and two isolates harbored bla_{IMP} alone. One isolate co-harbored bla_{VIM} and bla_{NDM}. Among *E. coli* isolates, bla_{OXA-48} and bla_{NDM} were carried by 18 and one isolates, respectively.

Results: Resistance to fosfomycin was detected in 43.7% of the isolates. Among *Klebsiella* spp. and *E. coli*, these rates were 46.8% and 21.1%, respectively. In *Klebsiella* spp. resistance to fosfomycin was 49.5% in bla_{OXA-48} carriers; 26.7% in isolates co-harbouring bla_{OXA-48} and bla_{NDM} and 66.7% in bla_{NDM} carriers. In *E. coli*, fosfomycin resistance was detected among 16.7% of the bla_{OXA-48} carriers.

Conclusions: High level of fosfomycin resistance in these isolates may be attributable to the fact that these isolates are multidrug resistant. The genetic background of resistance should also be investigated in order to understand the co-occurrence and transfer of resistance among the CPE.

Key words: fosfomycin; agar dilution; carbapenemase.

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Introduction

Carbapenemase-producing Enterobacterales (CPE) are threating problem and present global health issue, as they are frequently multidrug-resistant to available antibiotics. Infections due to CPE are mostly severe and related to poor outcomes [1]. Clinically and epidemiologically, three classes of carbapenemases are responsible for carbapenem resistance: Ambler class A enzyme (such as KPC type), zinc-dependent Ambler class B Metallo-ß-lactamases (MBLs) (such as VIM, IMP, and NDM types) and Ambler class D carbapenemases (such as OXA-48 type enzymes). Moreover, the prevalence of the types of carbapenemase varies with geographical location. In Turkey, OXA-48 enzyme is the most frequent type of carbapenemase, followed by NDM types [2]. The KPC enzyme is very rare in Turkey and has been detected very recently in a few isolates [3]. There are very few antimicrobial options for the treatment of infections caused by CPE and there is a great need for new alternatives.

Fosfomycin has been available for more than a decade and used orally for uncomplicated urinary tract infections. In some countries, intravenous form (IV) is available for gram-negative serious infections such as sepsis and pneumonia and considered to be effective in some infections caused by CPE [4]. The intravenous form of fosfomycin has just been introduced for clinical use in Turkey, however, studies evaluating *in vitro* activity of this drug against CPE isolates are lacking. The aim of this study was to determine the *in vitro* efficacy of fosfomycin against *Escherichia coli* and *Klebsiella* spp. with specific carbapenemase types.

Methodology

Isolates

A total of 158 clinically relevant isolates (one isolate per patient) which were shown to be resistant to

at least one carbapenem agent by microdilution method, were collected in 18 hospitals from 13 cities during 2014-2017 in Turkey. The types of carbapenemase enzymes were confirmed by phenotypic (KPC, MBL and OXA-48 carbapenemases confirm kit. Carbapenemases (Rosco Diagnostica, Taastrup, Denmark) and genotypic methods (multiplex PCR) in a previous study [5]. The isolates included in this study are: *E. coli* (n = 19), *Klebsiella pneumonia* (n = 138) and *K. oxytoca* (n = 1). They were isolated from blood (n = 55), lower respiratory tract secretions (n = 35), urine (n = 43), wound (n = 22) and puncture fluids (n = 3) and species identification was performed by MALDI-TOF MS (bio-Merieux, Crappone, France).

Antibiotic Susceptibility Testing

Minimum inhibitory concentration (MIC) of the isolates was determined with agar dilution method for fosfomycin (Abcam, Cambridge, USA) on Mueller-Hinton agar supplemented with glucose-6-phosphate (25 mg/L) (Sigma-Aldrich, St Louis, USA). Briefly, an inoculum of 10^4 CFU/mL was prepared and inoculated to the plates containing fosfomycin concentrations ranging from 0.25 to 128 mg/L using a multipoint inoculator and allowed to dry. Incubation was done at 35°C for 16–20 hours [6]. Results were interpreted following EUCAST v.10.0 guidelines. [7] ATCC 25922 *E. coli* was used as the quality control strain.

Statistical Analysis

All statistical analyses were performed using the SPSS software, version 23.0 for Windows (IBM Corp., New York, NY). A two-sided p value < 0.05 was considered as significant. Fisher's exact test and Pearson Chi-square were used to compare categorical variables as appropriate.

Results

In this study, CPE isolates from thirteen different cities of Turkey, collected during 2014-2017, were included to maintain geographic diversity. Among them, the most common carbapenemase producing gene in *Klebsiella* spp. was bla_{OXA-48} which was found in 123 isolates (75.5%). In 105 isolates, bla_{OXA-48} was found alone, whereas it was co-harboured with bla_{NDM} and bla_{VIM} in fifteen and three isolates, respectively. Eighteen of *E. coli* isolates (94.7%) harboured bla_{OXA-48}; and one (5.3%) carried bla_{NDM} alone.

In vitro activity of fosfomycin was evaluated against *E. coli* and *Klebsiella* spp. harboring bla_{OXA-48}, bla_{NDM}, bla_{VIM} and bla_{IMP} type carbapenemase genes in this study. Among all isolates, in-vitro resistance to fosfomycin was 43.7%. These rates were 46.8% and 21.1% in *Klebsiella* spp. and *E. coli*, respectively. As in other reports, fosfomycin resistance was significantly higher in *Klebsiella* spp. compared to *E. coli* (p = 0.047). Among 139 *Klebsiella* spp., 52 of 105 bla_{OXA-48} gene carriers, four of fifteen bla_{OXA-48} +bla_{NDM} co-carriers, six of nine bla_{NDM} carriers, one of four bla_{VIM} carriers and two of three bla_{VIM} + bla_{OXA-48} co-carriers were resistant to fosfomycin. In contrast, only three of eighteen bla_{OXA-48} carriers and the only bla_{NDM} carrier were resistant to fosfomycin among *E. coli*. (Table 1).

Resistance rate was significantly higher in bla_{OXA-48} carrying *Klebsiella* spp. compared to bla_{OXA-48} carrying *E. coli*. (p = 0,01). In *Klebsiella* spp., there was no significant difference for fosfomycin resistance between bla_{OXA-48} carriers and bla_{VIM}, bla_{NDM} and bla_{IMP} carriers (p = 1).

Discussion

In this study, we evaluated *in vitro* activity of fosfomycin against *E. coli* and *Klebsiella* spp. harbouring bla_{OXA-48}, bla_{NDM}, bla_{VIM} and bla_{IMP} type

Table 1. *In vitro* activity of fosfomycin against carbapenemase producing *E. coli* and *Klebsiella* spp. (n = 158).

Type of carbapenemase	n (%)[a]	Range[b]	MIC50[b]	MIC90[b]	Resistance to fosfomycin	
					n	%
Klebsiella spp. **(n=139)**						
OXA-48	105 (75.5)	0.5 -> 256	32	256	52	49.5
OXA-48+NDM	15	16-128	32	64	4	26.7
OXA-48+VIM	3	32 - < 256	256	> 256	2	*
NDM	9	0.5 - > 256	64	128	6	66.7
VIM	4	4-64	8	64	1	*
VIM+NDM	1	32	32	32	0	*
IMP	2	16-32	16	32	0	*
E.coli **(n=19)**						
OXA-48	18 (94.7)	0.25-128	16	64	3	16.7
NDM	1 (5.3)	64	64	64	1	*
All isolates	158	0,25 - > 256	32	256	69	43.7

* Insufficient number to give percentages; MIC: Minimum Inhibitory Concentration; [a] number; [b] mg/L (milligram/liter).

carbapenemase genes. In Turkey, there is an endemicity of *bla$_{OXA-48}$* carbapenemase however, other types of carbapenemases have also been reported recently [3]. The high rates of *bla$_{OXA-48}$* carrying *E. coli* and *Klebsiella* spp. isolates (%89.2) in this study may be attributable to its endemic potential in Turkey.

Intravenous form of fosfomycin was recently introduced into market for clinical use and started to be administered as an option for the treatment of infections caused by CPE in our country. To date, limited number of studies evaluated *in vitro* activity of fosfomycin against CPE. In Asia, fosfomycin resistance rates of CPE are relatively high. In a study from China, the authors reported a high resistance rate (60.8%) against fosfomycin in KPC-producing *K. pneumoniae* and an isolate harbouring both *blaKPC* and *fosA3* genes in the same plasmid were found [8]. In a study from Singapore, 1 of 3 bla$_{OXA-48}$ and 2 of 4 bla$_{IMP}$ harbouring *K. pneumoniae* isolates were resistant to fosfomycin [9]. However, in America and Europe, resistance rates of fosfomycin remains low among *Enterobacterales*. A study from USA, fosfomycin resistance was reported as 7% in KPC-producing *K. pneumonia* [10]. Similarly, fosfomycin resistance was detected in 5.1% of carbapenemase producers in a study from Greece [11]. A recent study from Turkey reported 76.1% fosfomycin resistance among CPE, which were slightly higher in bla$_{OXA-48}$ carriers (78.2%) [12]. In our study, resistance to fosfomycin was 46.8% and 21.1% in *Klebsiella* spp. and *E. coli*, respectively. These high rates are concerning for the treatment of CPE, especially in bla$_{OXA-48}$ carriers which are endemic in Turkey.

In our study, in *Klebsiella* spp, harbouring bla$_{OXA-48}$ alone or in combination, fosfomycin resistance was 47.2%. Among nine bla$_{NDM}$ harbouring *Klebsiella spp*. fosfomycin resistance was 66.7%. In *E. coli* however, 16.7% of the isolates carrying bla$_{OXA-48}$ had MIC values in the resistant range.

Fosfomycin resistance is usually obtained through two main genetic mechanisms: chromosomal mutations and plasmid-encoded resistance, inactivating the antibiotic molecule, which is due to drug modifying enzymes. The most concerning plasmid-mediated resistance determinants against fosfomycin have been characterized as three main types (*fosA, fosB, fosC)* and their subtypes in Enterobacterales. Plasmid mediated resistance determinants can be horizontally transferred and are usually related with multidrug resistance. Among them, *fosA3* is the most common and widely distributed type in Asia, and recently in Europe [13,14]. Co-carriage of fosfomycin resistance genes and carbapenemase coding genes has also been reported [14].

In our study, resistance to fosfomycin was significantly higher in carbapenemase-producing *Klebsiella* spp. compared to *E. coli* isolates. However, since the fosfomycin resistance mechanism is mostly related to chromosomal mutations in *Klebsiella* spp., the genetic background of resistance should also be investigated in order to understand the difference and the co-occurrence and transfer of resistance [15].

Conclusion

The OXA-48-type and Metallo-β-lactamase type carbapenemases are the most prevalent genes in carbapenemase-producing Enterobacterales in Turkey. In this study, we found a high level of *in vitro* resistance to fosfomycin among *Klebsiella* spp. isolates harbouring these carbapenemase genes. However, *in vitro* resistance to fosfomycin among *E. coli* was significantly lower than *Klebsiella* spp. isolates. We conclude that fosfomycin can be considered in combination therapies caused by carbapenemase-producing *E. coli* or *Klebsiella* spp. when MIC of fosfomycin is in the therapeutic range. Surveillance studies with fosfomycin should be performed in each center as the frequency of various carbapenemase genes may differ according to geographical location. Furthermore, plasmid-mediated fosfomycin resistance is reported from different geographical locations including Europe. This mechanism of resistance should be investigated in further studies.

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