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

Selay Demirci-Duarte¹, Tugce Unalan-Altintop¹, Zeynep Gulay², Ayse Nur Sari Kaygisiz², Asli Cakar¹, Deniz Gur¹

¹ Department of Medical Microbiology, Hacettepe University Faculty of Medicine, Sihhiye, Ankara, Turkey  
² Department of Medical Microbiology, Dokuz Eylul University Faculty of Medicine, Balcova, Izmir, Turkey

**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 *blaOXA-48*, 15 isolates carried both *blaOXA-48* and *blaNDM*, three had both *blaOXA-48* and *blaVIM* and nine isolates had *blaNDM* alone. Four isolates carried only *blaVIM* and two isolates harbored *blaIMP* alone. One isolate co-harbored *blaVIM* and *blaNDM*. Among *E. coli* isolates, *blaOXA-48* and *blaNDM* 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 *blaOXA-48* carriers; 26.7% in isolates co-harboring *blaOXA-48* and *blaNDM* and 66.7% in *blaNDM* carriers. In *E. coli*, fosfomycin resistance was detected among 16.7% of the *blaOXA-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.

**Introduction**

Carbapenemase-producing Enterobacterales (CPE) are threatening 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 10⁴ 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 blaOXA-48 which was found in 123 isolates (75.5%). In 105 isolates, blaOXA-48 was found alone, whereas it was co-harboured with blaNDM and blaVIM in fifteen and three isolates, respectively. Eighteen of E. coli isolates (94.7%) harboured blaOXA-48; and one (5.3%) carried blaNDM alone.

In vitro activity of fosfomycin was evaluated against E. coli and Klebsiella spp. harboring blaOXA-48, blaNDM, blaVIM and blaIMP 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 blaOXA-48 gene carriers, four of fifteen blaOXA-48 +blaNDM co-carriers, six of nine blaNDM carriers, one of four blaVIM carriers and two of three blaVIM + blaOXA-48 co-carriers were resistant to fosfomycin. In contrast, only three of eighteen blaOXA-48 carriers and the only blaNDM carrier were resistant to fosfomycin among E. coli. (Table 1).

Resistance rate was significantly higher in blaOXA-48 carrying Klebsiella spp. compared to blaOXA-48 carrying E. coli. (p = 0.01). In Klebsiella spp., there was no significant difference for fosfomycin resistance between blaOXA-48 carriers and blaVIM, blaNDM and blaIMP carriers (p = 1).

**Discussion**
In this study, we evaluated in vitro activity of fosfomycin against carbapenemase producing E. coli and Klebsiella spp. harbouring blaOXA-48, blaNDM, blaVIM and blaIMP type...
carbapenemase genes. In Turkey, there is an endemicity of blaOXA-48 carbapenemase however, other types of carbapenemases have also been reported recently [3]. The high rates of blaOXA-48 carrying E. coli and Klebsiella spp. isolates (68.7%) in this study may be attributable to its endemic potential in Turkey.

In a study from China, the authors reported a high resistance rate (60.8%) against fosfomycin in KPC-producing Enterobacterales [9]. However, in America and Europe, resistance rates of fosfomycin remain 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 blaOXA-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 blaOXA-48 carriers which are endemic in Turkey.

In our study, in Klebsiella spp. harbouring blaOXA-48 alone or in combination, fosfomycin resistance was 47.2%. Among nine blaNDM harbouring Klebsiella spp. fosfomycin resistance was 66.7%. In E. coli however, 16.7% of the isolates carrying blaOXA-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 Enterobacteriales. 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 Enterobacteriales 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.

Acknowledgements

We would like to thank the following colleagues for their assistance with the collection of our data: Aslı Çakar, Yakut Akyön, Onur Karatuna, Dilara Öğüş, Betil Özşak Baysan, Nilay Çöplü, Mustafa Çağatay, Mehmet Baysallar, Zahir Bakici, Cem Çelik, Şohret Aydemir, Alper Aktaş, Çiğdem Kayacan, Gülçin Bayramoğlu, Faruk Aydın, Devrim Dündar, Ufuk Hasdemir, Ramazan Ayaş Keramettin Yanık, Murat Günaydın, Hüseyin Gündücüoğlu, Mehmet Parla.

References

1. Tsouvelakis LS, Markogiannakis A, Psychogiou M, Tassios PT, Daikos GL (2012) Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev 25: 682-707.
2. Van Duin D, Doi Y (2017) The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence 8: 460-469.
3. Labarca J, Poirol R, Ozdamar M, Turkoglu S, Hakko E, Nordmann P (2014). KPC-producing Klebsiella pneumoniae, finally targeting Turkey. New Microbes New Infect 2: 50-51.
4. Kaase M, Szabados F, Anders A, Gatermann SG (2014) Fosfomycin susceptibility in carbapenem-resistant Enterobacteriaceae from Germany. J Clin Microbiol 52: 1893-1897.
5. Çakar A, Akyön Y, Gür D, Karatuna O, Ögünc D, Öz hak Baysan B, Çöplü N, Çagatay M, Kılıç A, Baysallar M (2016) Investigation of the presence of carbapenemases in carbapenem-resistant Escherichia coli and Klebsiella pneumoniae isolates in Turkey in 2014. Mikrobiyol Bul: 21-33.
6. Clinical and Laboratory standard institute Performance standards for antimicrobial susceptibility testing, 28th informational supplement. CLSI document M100-S2810 (ISBN 1-56238-839-8).
7. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Available: http://www.eucast.org/clinical_breakpoints. Accessed in 27 January 2020.
8. Jiang Y, Shen P, Wei Z, Liu L, He F, Shi K, Wang Y, Wang H, Yu Y (2015) 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 45: 66-70.
9. Choudhury S, Yeng JL, Krishnan PU (2015) In vitro susceptibilities of clinical isolates of carbapenem-producing Enterobacteriaceae to fosfomycin and tigecycline. Clin Microbiol Infect 21: e75-76.
10. Endimiani A, Patel G, Hujer KM, Swaminathan M, Perez F, Rice LB, Jacobs MR, Bonomo RA (2010) In vitro activity of fosfomycin against blaKPC-containing Klebsiella pneumoniae isolates, including those nonsusceptible to tigecycline and/or colistin. Antimicrob Agents Chemother 54: 526-529.
11. Falagas ME, Maraki S, Karageorgopoulos DE, Kastoris AC, Mavromanolakis E, Samonis G (2010) Antimicrobial susceptibility of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Enterobacteriaceae isolates to fosfomycin. Int J Antimicrob Agents 35:240-243.
12. Suzuk Yildiz S, Kaskatepe B, Simsek H, Sarıguzel FM (2019) High rate of colistin and fosfomycin resistance among carbapenemase-producing Enterobacteriaceae in Turkey. Acta Microbiol Immunol Hung 66: 103-112.
13. Mendes AC, Rodrigues C, Pires J, Amorim J, Ramos MH, Novaís A, Peixe L (2016) Importation of Fosfomycin Resistance fosA3 Gene to Europe. Emerg Infect Dis 22: 346-348.
14. Silver LL (2017) Fosfomycin: Mechanism and Resistance. Cold Spring Harb Perspect Med 7.2:a025262.
15. Aslan, AT, Akova M (2019) Extended spectrum β-lactamase Producing Enterobacteriaceae: Carbapenem Sparing Options. Expert rev. anti-infect. ther 17: 969-981.

Corresponding author
Selay Demirci Dos Santos Duarte, MD
Hacettepe University Faculty of Medicine, Department of Medical Microbiology, Morphology Building, Floor 3, 06100, Sihhiye, Ankara, Turkey
Phone: +905512077232
E-mail: selayy@yahoo.com

Conflict of interests: No conflict of interests is declared.