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Fosfomycin: In vitro efficacy against multidrug-resistant isolates beyond urinary isolates

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ABSTRACT

Objectives: Fosfomycin (FOF) is a bactericidal antimicrobial agent active against a range of Gram-negative bacteria, including multidrug-resistant (MDR) and metallo-β-lactamase (MBL)-producing Enterobacteriaceae. However, data are scarce regarding use of the drug beyond urinary tract infections (UTIs).
Methods: In this study, susceptibility rates to FOF among 290 MDR Enterobacteriaceae isolates were analysed by gradient and disk diffusion tests and the results were compared with agar dilution according to the Clinical and Laboratory Standards Institute (CLSI). Minimum inhibitory concentrations (MICs) of imipenem (IPM) for isolates IPM-resistant/intermediate-susceptible isolates were determined by gradient test. In addition, the gradient test was used to determine MBL production.
Results: Of the 290 extended-spectrum β-lactamase (ESBL)-positive isolates, 60 (20.7%) were resistant to FOF, with rates of 9.5% for *Escherichia coli*, 28.0% for *Enterobacter* spp., 35.7% for *Klebsiella* spp. and 50.0% for *Morganella* spp. Among the 290 ESBL-positive isolates, 19 (6.6%) were resistant/intermediate-susceptible to IPM. In addition, 72.2% of extensively drug-resistant (XDR) and 61.1% of carbapenem-resistant isolates were resistant to FOF. In vitro FOF activity was higher among blood (86.9%) and genitourinary (91.7%) isolates. FOF showed excellent activity for a wide range of infections; however, further trials are necessary to evaluate its clinical efficacy.
Conclusions: FOF presented good activity even against carbapenem-resistant isolates and may be a treatment alternative for non-UTI isolates, but should be used with caution for infections related to ESBL-producing *Klebsiella* spp.

1. Introduction

Fosfomycin (FOF) is a bactericidal agent active against *Escherichia coli*,*Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp., *Serratia* spp. and *Enterococcus faecalis*. Limited therapeutic options for multidrug-resistant (MDR) Enterobacteriaceae is a growing concern worldwide and, in this era, FOF could be a valuable option for MDR Enterobacteriaceae isolates, displaying susceptibility rates of >83% [1–5]. In vitro studies showed that FOF reaches adequate concentrations in serum, prostate, lungs, inflamed tissues, bone, cerebrospinal fluid (CSF), abscess fluid and heart valves. The oral form of the drug is approved for urinary tract infections (UTIs) related to *E. coli* and *E. faecalis*, and the intravenous (i.v.) form is also available for the treatment of systemic diseases in some countries. In Turkey, only the oral form of the drug is licenced and in use for selected cases of UTI, displaying excellent in vitro activity in our previous study [6]. Clinical studies are limited and further trials are needed in order to evaluate the efficacy of this agent for the management of nosocomial infections other than UTIs [5,7]. In the present study, we aimed to evaluate in vitro FOF susceptibilities of MDR Enterobacteriaceae isolates of non-urinary origin.

2. Materials and methods

2.1. Study design and bacterial isolates

A total of 290 MDR Enterobacteriaceae recovered from various clinical samples of patients referred to a tertiary hospital in Turkey in the period 2013–2014 were included in the study. Species

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identification was performed using a VITEK®2 Compact system (bioMérieux, Marcy-l'Étoile, France). Non-susceptibility to at least one agent in three or more antimicrobial categories was defined as MDR; non-susceptibility to at least one agent in all but two or fewer antimicrobial categories was defined as extremely drug-resistant (XDR); and resistance to all antimicrobials was defined as pandrug-resistant (PDR). The first isolate recovered from each patient was included in the study.

2.2. Antimicrobial susceptibility

Susceptibilities to amikacin (30 μg), amoxicillin/clavulanic acid (20/10 μg), aztreonam (30 μg), cefepime (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), FOF (200 μg), gentamicin (10 μg), imipenem (IPM) (10 μg) and piperacillin/tazobactam (TZP) (100/10 μg) (Oxoid Ltd., Basingstoke, UK) were determined by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [8] as well as using the VITEK®2 Compact system. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control isolates in each batch. Disk synergy tests were performed for extended-spectrum β-lactamase (ESBL) screening and the results were confirmed with cefotaxime, ceftazidime, cefotaxime/clavulanic acid (30/10 μg) and ceftazidime/clavulanic acid (30/10 μg) disks in accordance with CLSI guidelines [9]. Minimum inhibitory concentrations (MICs) of IPM were determined by the gradient method (bioMérieux) for isolates that were IPM-resistant or intermediate-susceptible by disk diffusion test. In addition, the gradient test (bioMérieux) was used to determine metallo-β-lactamase (MBL) production in isolates resistant or intermediate-susceptible to any of the carbapenems. FOF MICs of the isolates were tested by gradient test and the results were compared with agar dilution supplemented with glucose-6-phosphate (25 mg/L) according to the recommendations of the CLSI [9]. In the interpretation of FOF disk diffusion testing result, zone diameters of ≥16 mm for susceptibility and ≤12 mm for resistance were used according to the CLSI [8]. MIC results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [10] (susceptible, ≤32 mg/L; resistant, ≥64 mg/L). In case of disagreement, the agar dilution method was used for reporting susceptibility.

2.3. Statistical analyses

Data were analysed using SPSS software v.15.0 (SPSS Inc., Chicago, IL) by χ^2 test, except when any of the data were scarce when Fisher's exact test was used. Significance was set at $P < 0.05$ using two-sided comparisons.

3. Results

Among a total of 770 isolates, 290 (37.7%) were ESBL-positive and were included in the study, including 158 (54.5%) *E. coli*, 79 (27.2%) *Klebsiella pneumoniae*, 22 (7.6%) *Enterobacter cloacae*, 19 (6.6%) *Klebsiella oxytoca*, 6 (2.1%) *Morganella morganii*, 3 (1.0%) *Serratia marcescens* and 3 (1.0%) *Enterobacter aerogenes*. ESBL-positive isolates were recovered from skin and soft-tissue samples ($n = 114$; 39.3%), sputum/tracheal aspirate ($n = 96$; 33.1%), blood ($n = 61$; 21.0%), genitourinary samples ($n = 12$; 4.1%) and sterile body fluids ($n = 7$; 2.4%). Among the 770 isolates tested, 18 (2.3%) were XDR, including 14 *K. pneumoniae* recovered from tracheal aspirate ($n = 8$) and skin and soft-tissue samples ($n = 6$), 3 *E. coli* recovered from aspirate ($n = 1$) and skin and soft-tissue samples ($n = 2$), and 1 *K. oxytoca*. One PDR isolate each of *K. pneumoniae* and *E. cloacae* were identified from tracheal aspirate samples. IPM resistance was detected in 17 isolates (14 *K. pneumoniae*, 2 *E. coli* and 1 *E. cloacae*) and IPM intermediate susceptibility in 1 isolate (*K. oxytoca*), all of which were MBL-producers, with MICs ranging between 2 mg/L and >32 mg/L. IPM and FOF were the most active agents, followed by amikacin and TZP. The distribution of resistance rates of the ESBL-positive isolates by type is shown in Table 1.

The FOF susceptibility of all of the isolates was evaluated by three methods, including disk diffusion, gradient strip test and agar dilution method. The same results were obtained by all tests, except for six isolates displaying a 1 log higher concentration by agar dilution compared with gradient test. The highest activity was observed for *E. coli* isolates, with a particularly low MIC_{90} (MIC for 90% of the isolates) of ≤2 mg/L. All *Serratia* spp. were susceptible to FOF, but 35/98 (35.7%) *Klebsiella* spp., 15/158 (9.5%) *E. coli*, 7/25 (28.0%) *Enterobacter* spp. and 3/6 (50.0%) *Morganella* spp. showed resistance. A higher resistance rate was detected among isolates recovered from inpatients compared with outpatients (22.1% vs. 14.5%; $P = 0.211$). In addition, 13/18 (72.2%) XDR and 11/18 (61.1%) carbapenem-resistant isolates were resistant to FOF, with MIC_{90} values of 1024 mg/L. According to the isolation site, the highest in vitro activity was observed for genitourinary discharges (11/12; 91.7%), followed by blood (53/61; 86.9%), sterile body fluids (4/7; 57.1%), skin and soft-tissue samples (89/114; 78.1%) and sputum/tracheal aspirate (73/96; 76.0%). The distribution of FOF susceptibility rates of bacterial isolates by isolation site is shown in Figs. 1 and 2.

Table 1
Antimicrobial resistance rates of extended-spectrum β-lactamase (ESBL)-positive Enterobacteriaceae isolates ($n = 290$) by disk diffusion test.

Antimicrobial agent	Resistance [*n* (%)]				
	Escherichia coli ($n = 158$)	*Klebsiella* spp. ($n = 98$)	*Enterobacter* spp. ($n = 25$)	*Morganella morganii* ($n = 6$)	*Serratia marcescens* ($n = 3$)
AMC	51 (32.3)	56 (57.1)	21 (84.0)	4 (66.7)	3 (100)
GEN	77 (48.7)	61 (62.2)	3 (12.0)	2 (33.3)	–
AMK	12 (7.6)	19 (19.4)	1 (4.0)	2 (33.3)	–
CRO	158 (100)	97 (99.0)	25 (100)	6 (100)	3 (100)
CAZ	154 (97.5)	98 (100)	25 (100)	6 (100)	3 (100)
CTX	154 (97.5)	97 (99.0)	25 (100)	6 (100)	3 (100)
FEP	151 (95.6)	96 (98.0)	23 (92.0)	6 (100)	3 (100)
ATM	153 (96.8)	97 (99.0)	21 (84.0)	6 (100)	3 (100)
CIP	112 (70.9)	60 (61.2)	2 (8.0)	1 (16.7)	–
FOF	15 (9.5)	35 (35.7)	7 (28.0)	3 (50.0)	–
IPM	2 (1.3)	14 (14.3)	1 (4.0)	–	–
SXT	98 (62.0)	59 (60.2)	5 (20.0)	3 (50.0)	–
TPZ	26 (16.5)	42 (42.9)	7 (28.0)	2 (33.3)	1 (33.3)

AMC, amoxicillin/clavulanic acid; GEN, gentamicin; AMK, amikacin; CRO, ceftriaxone; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; ATM, aztreonam; CIP, ciprofloxacin; FOF, fosfomycin; IPM, imipenem; SXT, trimethoprim/sulfamethoxazole; TPZ, piperacillin/tazobactam.

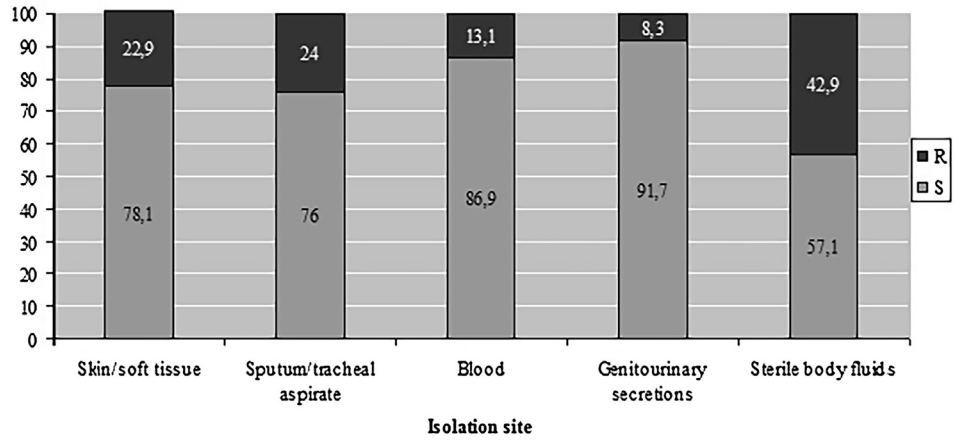

Fig. 1. Distribution of fosfomycin susceptibility rates of bacterial isolates by isolation site.

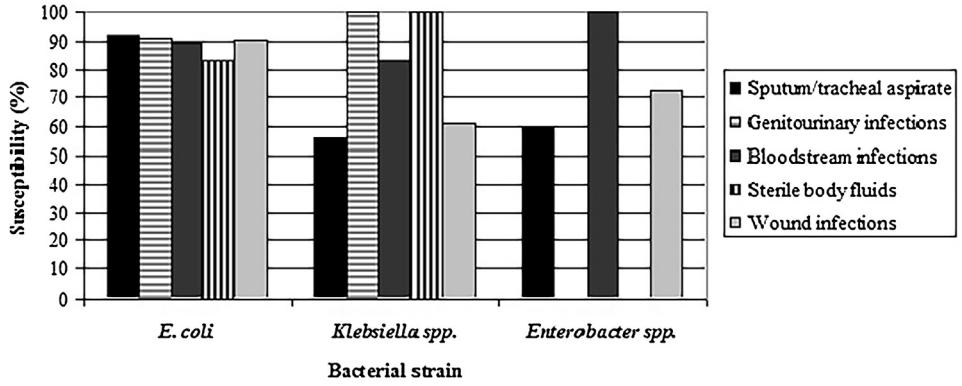

Fig. 2. Distribution of fosfomycin susceptibility rates of bacterial isolates by isolate type and isolation site.

4. Discussion

Multidrug resistance among Enterobacteriaceae is a growing concern owing to the limited therapeutic options, and old antimicrobials such as FOF and colistin are now being considered as alternative treatments. However, the therapeutic indication for FOF is currently limited only to *E. coli*- and *E. faecalis*-related UTIs [9], but the main interest is the assessment of the drug against systemic infections [2,11,12].

The present study aimed to compare the in vitro activity of FOF with that of other antimicrobials against 290 ESBL-positive Enterobacteriaceae recovered from non-urinary isolates. Among the 290 isolates tested, 60 (20.7%) were resistant to FOF, with a distribution of 77.9% vs. 85.5% for inpatient and outpatients, respectively. Agar dilution, gradient test and disk diffusion test were used in the detection of FOF susceptibility for all isolates and similar susceptibilities were detected, except for six isolates displaying a 1 log higher dilution by agar dilution. The highest in vitro activity of FOF was detected among *E. coli* isolates, which displayed a particularly low MIC_{90} of $\leq$2 mg/L. Similar to published data [6,13,14], *Klebsiella* spp. isolates showed significantly higher resistance rates compared with *E. coli* (35.7% vs. 9.5%; $P < 0.05$) indicating that the drug is a valuable option in the treatment of *E. coli*-related infections, and high in vitro activity (72.0%) was detected for *Enterobacter* spp. Among the isolates tested, the highest resistance rate was among *Morganella* spp. (50.0%), but the results should be evaluated carefully due to the small number of isolates ($n = 6$).

In previous studies, high in vitro FOF activity was reported for urinary isolates [3,15,16], but data are scarce for isolates other than *E. coli* urinary isolates. In these limited studies, FOF susceptibility rates of *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter* spp., *Citrobacter* spp., *P. aeruginosa* and *Acinetobacter* spp. were 73.8–100%, 50%, 82.9%, >90%, 31.8% and 11.1%, respectively [13,17–19]. Use of FOF beyond UTIs may be more prone to potential resistance development as higher resistance rates were observed among isolates recovered from respiratory tract infections and osteomyelitis [5,6,20,21].

In this study, IPM and FOF were the most active agents followed by amikacin, and high resistance rates were detected for *E. coli* and *Klebsiella* spp. isolates (48.7% vs. 62.2%) for gentamicin, suggesting that it should be used with caution in the treatment of ESBL-positive isolates.

FOF is approved in several European countries for the treatment of soft tissue infections and sepsis [22], but the i.v. formulation is available in only five countries in Europe (Spain, France, Germany, Austria and Greece) [7]. It is suggested that high-dose i.v. FOF could provide adequate concentrations even in the CSF and bone and joint infections, representing a valuable option even in carbapenem-resistant and MDR Enterobacteriaceae -related osteoarthritis, pneumonia and bacteraemia, with reported susceptibility rates of 64.8–76.8% [3,7,23–27]. Michalopoulos et al. [27] examined the effectiveness and safety of FOF in critically ill patients and concluded that the drug could be used in the treatment of carbapenem-resistant *K. pneumoniae*-related infections, especially in combination with other antibiotics.

Falagas et al. [3] reported that FOF resistance rates of carbapenemase-producing, ESBL-positive, MBL-positive and XDR isolates were 5.1%, 5.9%, 16.7% and 8.2%, respectively [3]. In the current study, high rates of FOF resistance were observed for carbapenemase-positive, ESBL-positive and XDR isolates (61.1%, 20.7% and 72.2%, respectively). Although FOF showed high in vitro activity against MDR Gram-negative bacteria [4,28], it is necessary to conduct further studies to confirm the clinical relevance of these findings as different susceptibility rates ranging from 46% to 92% for *Klebsiella* spp. [4,18,29], 100% for *E. coli* [29], 68–80% for *Enterobacter* spp. [18] and 80% for *Citrobacter* spp. [18] were detected.

Variation in the FOF resistance rate by isolation site was also reported. In this study, among the isolates tested the highest in vitro activity was observed for genitourinary infections (91.7%), bloodstream infections (86.9%), sterile body fluid (85.7%) and skin and soft-tissue infections (76.3%). It has been reported that the FOF susceptibility rate was 97–100% for *E. coli*, 100% for *K. pneumoniae* and 60% for *Proteus* spp. and *Morganella* spp. [16,28–31] among bloodstream isolates. In the current study, 89.5% of *E. coli*, 83.3% of *Klebsiella* spp. and 100% of *Enterobacter* spp. bloodstream isolates showed susceptibility, indicating the possibility that FOF should be considered as a valuable treatment option for bloodstream infections, soon after comprehensive clinical studies regarding FOF treatment.

In this study, 76.3% of all isolates recovered from wound samples were susceptible to FOF, with rates of 90.4% for *E. coli*, 61.4% for *Klebsiella* spp. and 72.7% for *Enterobacter* spp., similar to previously published data [28–31].

In addition, use of FOF in combination with tobramycin to treat lung infections in patients with cystic fibrosis has also been explored [32]. In the current study, the FOF resistance rate was 7.8% for *E. coli*, 40% for *Enterobacter* spp. and 44.1% for *Klebsiella* spp. respiratory system isolates.

In conclusion, FOF presented good activity even in carbapenem-resistant Gram-negative bacteria and may be an alternative in the treatment of infections related to Enterobacteriaceae, but should be used with caution in ESBL-producing *Klebsiella* spp. Although in vitro data appear to encourage the prescription of FOF, further clinical studies should be conducted evaluating the clinical efficacy of the drug.

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Competing interests

None declared.

Ethical approval

Not required.

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