Fosfomycin, a therapeutic option for infections produced by multiple drug-resistant *Enterobacteriaceae*

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Abstract

Due to the increasing prevalence of infections caused by resistant bacteria and especially multiple drug resistance *Enterobacteriaceae*, availability of alternative effective antibiotics is restricted. The goal of this study was to investigate the susceptibility profile of multiple drug resistance and extensively drug resistance *Enterobacteriaceae* isolated from various clinical samples to fosfomycin. A total of 303 non-duplicate *Enterobacteriaceae* isolates were collected. Identification and susceptibility testing were done according to standard microbiological procedures and the Kirby-Bauer test, respectively. Of all isolates, 272 (89.8%) and 26 (8.6%) were detected as multiple drug resistance and extensively drug resistance strains, respectively. The most effective antibiotic (98%) was fosfomycin, when compared with other antibiotics against multiple drug resistance and extensively drug resistance *Enterobacteriaceae* isolates. In this study, we find high levels of resistance to commonly used antibiotics. However, fosfomycin can be a good option for treating multiple drug resistance *Enterobacteriaceae*.

Introduction

The *Enterobacteriaceae* is the largest, most diverse group of medically important Gram-negative bacilli and cause a variety of human infections, including septicemia, urinary tract, wound and gastrointestinal infections.[1] As rapidly as novel antimicrobial agents are introduced, *Enterobacteriaceae* can develop resistance to antibiotics. Nowadays, a significant increase in antimicrobial resistance of *Enterobacteriaceae* is observed.[2] Moreover, the emergence and spread of MDR (multiple drug resistance) and XDR (extensively drug resistance) *Enterobacteriaceae*, in the community and hospitals, is increasing in the world.[3] Due to the high rate of antibiotic resistance, selection of antibiotics against MDR bacteria has been limited.[4] MDR bacteria can transfer the gene to other clinical strains, so the detection of this strain is important. In an era of MDR and XDR, emphasis should be given not only to the development of new antibiotics but also to the reassessment of older and forgotten antimicrobial agents.[5] Recently, there has been renewed interest in the use of fosfomycin for the management of infections caused by MDR Gram-negative bacteria, mainly *Enterobacteriaceae* that are resistant to usually used agents.[6] Fosfomycin is a natural, forgotten antibiotic, known for nearly four decades, broad spectrum and a bactericidal antibacterial agent that inhibits cell wall synthesis in bacteria by inactivating the UDP-*N*-acetyl-glucosamine-3-*o*-enolpyruvultransferase.[6,7] There are a few studies on the *in vitro* activity of fosfomycin against commonly encountered bacteria, except for *Escherichia coli* and *Enterococcus faecalis*.[8] In this regard, the aim of this study was to evaluate the antimicrobial activity of fosfomycin against MDR and XDR *Enterobacteriaceae* that are resistant to traditional antibiotics.

Materials and Methods

Bacterial isolates

A total of 303 non-duplicates *Enterobacteriaceae* isolates were collected in tertiary care hospitals during February 2014 through August 2015 from 3 cities of Iran; Tabriz, Khoy, and Uremia. Identification of isolates was done by using biochemical tests in the Department of Microbiology, Tabriz University of Medical Sciences, Iran.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was done on Mueller-Hinton agar (Merck, Germany) using Kirby-Bauer's technique according to the Clinical and Laboratory Standards Institute's (CLSI) guidelines.[8,9] The antibiotic discs that were used for determining antimicrobial susceptibility testing were including: ampicillin (10 µg), cefazolin (30 µg), ceftazidime (30 µg), cefepime (30 µg), imipenem (30 µg), cefuroxime (30 µg), aztreonam (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), piperacillin/tazobactam (100/10), cotrimoxazole (30 µg) and fosfomycin (200/50 µg) (Mast, Chemical Co, UK). EUCAST has defined fosfomycin zone breakpoints for *Enterobacteriaceae* (susceptible ≥ 14mm and resistant ≤13 mm).[10] *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27,853 strains we used as quality control. In this study, isolates that are resistant to three or more classes of antibiotics are considered MDR. Of these isolates, those that were resistant to entirely but one or two classes of effective antimicrobial agents (not considering fosfomycin) were categorized as extensively drug-resistant (XDR).[11]

Phenotypic screening and confirmatory tests for extended spectrum beta-lactamase (ESBL), OXA48, class C cephalosporinases (AmpC), *Klebsiella pneumoniae* Carbapenemase (KPC), and Methalo Beta lactamase (MBL)-production

First screening for ESBL, AmpC and carbapenemase production was carried out based on the disc diffusion agar using ceftazidime (30 µg), cefoxitin (30 µg), imipenem (10 µg) and meropenem (10 µg) (Mast, Chemical Co, England) according to the Clinical Laboratory Standards Institute (CLSI) screening criteria for -lactamase production.[9] Cefoxitin non-susceptible isolates were considered presumptive AmpC-producers. Suspected isolates for β-lac-

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tamase production were further confirmed using total ESBL/AmpC confirms kit and KPC/MBL and OXA-48 confirmation kit (Rosco Diagnostica, Denmark). Since the detection of ESBLs can be obscured by chromosomal AmpC producers, ESBL confirmation kit (Rosco Diagnostica, Denmark) was used to distinguish ESBLs in such isolates. Production of KPC and MBL was detected if inhibition zones around meropenem discs containing phenylboronic acid (KPC inhibitor) or dipicolinic acid (MBL inhibitor) were extended by more than 4 and 5 mm, respectively when compared with meropenem disc without inhibitor. Carbapenem resistance related to AmpC production couple to decreased permeability was characterized by a ≥5 mm, difference in zones between meropenem and meropenem/cloxacillin discs along with at least a 4mm difference between meropenem and meropenem/phenylboronic acid discs. Temocillin non-susceptible and susceptible isolates, showing negative synergy tests, were identified as OXA-48 and porin-deficient ESBL producers, respectively. *E. coli* ATCC 25922 and *Klebsiella pneumonia* (*K. pneumonia*) ATCC 700603 were used as quality control strains in each set of susceptibility tests.

Statistical analyses

The results were analyzed using SPSS software for Windows (version 17SPSS Inc., Chicago, IL, USA). In this study, P≤0.05 was regarded statistically significant.

Results

The mean age of patients was 52 years old, ranging from 1 to 90 years old, including 125 males and 178 females. The median hospitalization of patients was 6 days. *E. coli* was the

Table 1. Antimicrobial resistance patterns of *Enterobacteriaceae* to fosfomycin according to source, bacterial isolates, wards, cities, hospitals and other tested antibiotics.

Variables, total (%)	Susceptible to fosfomycin, n (%)	Non susceptible to fosfomycin, n (%)
Type of infection		
Urinary, 219 (72.3)	213 (97.3)	6 (2.7)
Bacteremia, 43 (14.2)	43 (100)	0
Burn, 11 (3.6)	11 (100)	0
Respiratory, 12 (4)	7 (58.3)	5 (41.7)
eritoneal, 3 (1)	3 (100)	0
Meningitis, 2 (0.7)	2 (100)	0
Bacteria		
E. coli, 219 (72.3)	217 (99.1)	2 (0.9)
K. pneumonia, 57 (18.8)	56 (98.2)	1 (1.8)
E. cloacae, 14 (4.6)	13 (92.9)	1 (7.1)
P. mirabilis, 5 (1.7)	5 (100)	0
M. morganii, 2 (0.7)	0	2 (100)
K. oxytoa, 2 (0.7)	2 (100)	0
C. freundi, 1 (0.3)	1 (100)	0
P. vulgris, 2 (0.7)	2 (100)	0
Wards		
Internal, 180 (59.4)	179 (99.4)	1 (0.6)
Intensive care unit, 37 (12.2)	35 (94.6)	2 (5.4)
Surgery, 55 (18.2)	52 (94.5)	3 (5.5)
Children, 18 (5.9)	18 (100)	0
Burn, 15 (5)	15 (100)	0
Cities		
Tabriz, 179 (89.1)	176 (98.3)	3 (1.7)
Uremia, 100 (33)	97 (97)	3 (3)
Khoy, 24 (7.9)	24 (100)	0
Hospitals		
Emmam Reza, 108 (35.6)	105 (97.2)	3 (2.8)
Sina, 71 (23.4)	71 (100)	0
Emam Khomeini, 100 (33)	97 (97)	3 (3)
Qamar, 9 (3)	9 (100)	0
Madani, 15 (5)	15 (100)	0
Antibiotics resistant		
Cefazolin, 244	238 (97.5)	6 (2.5)
Imipenem, 16	15 (93.7)	1 (6.3)
Cefepime, 112	110 (98.2)	2 (1.8)
Ampicillin, 265	261 (98.5)	4 (1.5)
Gentamicin, 110	107 (97.3)	3 (2.7)
Cefuroxime, 170	166 (97.6)	4 (2.4)
Ciprofloxacin, 172	169 (98.3)	3 (1.7)
Cotriomoxazol, 195	192 (98.5)	3 (1.5)
Aztreonam, 146	144 (98.6)	2 (1.4)
Ceftazidime, 121	119 (98.3)	2 (1.7)
Tetracycline, 118	115 (97.5)	3 (2.5)
Piperacillin/tazobactam, 98	95 (96.9)	3 (3.1)

most frequently isolated bacteria (Table 1). According to the results, the highest rate of resistance was in the penicillin group (ampicillin) with 87.5%, followed by the cehems group (cefazolin) with 80.5%, and the folate pathway inhibitors with 64.4% (Table 2). In contrast, the highest sensitivity rates were discovered in fosfomycin with 98% and the carbapnem group (imipenem) with 94.7% (Figure 1). Out of 6 fosfomycin resistant isolates, 5 isolates were isolated from males and 1 was from a female. None of the isolates were sensitive to all antibiotics. The frequency of ESBL, AmpC, KPC and MBL-producing isolates were 112 (36.9%), 28 (9.2%), 4 (1.3%) and 9 (2.9%) (Table 2). Based on results obtained from susceptibility testing, MDR bacteria was recovered which was 272 out of 303 (89.8%) of the total isolates. Frequency of MDR to three, four, five, six and seven antimicrobial agents were 41 (15.1%), 52 (19.1%), 58 (21.3%), 51 (18.7%) and 44 (16.2%), respectively. The XDR was observed in 26 cases (8.6%). The most prevalent MDR patterns were resistance to ampicillin, cefazolin, trimethoprim-sulfamethoxazole, cefuroxime and ciprofloxacin. According to results, fosfomycin was also the most effective antibiotic against MDR, XDR, ESBL, AmpC, KPC and MBL-producing isolates.

Discussion

In the present study, we investigated the susceptibility profile of MDR *Enterobacteriaceae* isolates to fosfomycin. The main finding of this study is that fosfomycin showed a high *in vitro* susceptibility to MDR, XDR, CRE, KPC, OXA48 and ESBL producing isolates. In total, 2% of the MDR and the XDR isolates were resistant to fosfomycin. Numerous studies have distinguished good activity of fosfomycin against MDR *Enterobacteriaceae*.[5,10,12] Fairly few studies have assessed the antibacterial activity of fosfomycin against *Enterobacteriaceae* isolates with XDR, and have provided favorable findings concerning the potential worth of fosfomycin in this regard.[5,11,13]

In comparison to *E. coli* (0.9%), *K. pneumonia* (1.8%) showed higher rates of resistance to fosfomycin. The low level of resistance to fosfomycin probably is due to limited use of fosfomycin for the treatment of infections in this area. The susceptibility to fosfomycin has not been widely studied for other *Enterobacteriaceae*. In our study, the majority of isolates were susceptible to fosfomycin; susceptibility rates for *P. mirabilis, C. freundii, K. oxytoca* and *P. vulgaris* were 100%, except for *E. cloacae* and *M. morganii* which was detected in 92.9% and zero, respectively. These results are partly in concordance with previous reports, which indicated that low rate of *E. coli*, *P. mirabilis* and *P. vulgaris* were non-susceptible to fosfomycin.[3,14] Unfortunately, the spread of ESBL significantly limits the treatment options. The gene encoding the ESBL is located in plasmids and is transmitted among bacteria. These plasmids can carry MDR genes against cotrimoxazole, quinolones and aminoglycosides at the same time.[15] In the previous studies, 90% or more of the isolates of *Enterobacteriaceae* with advanced resistance

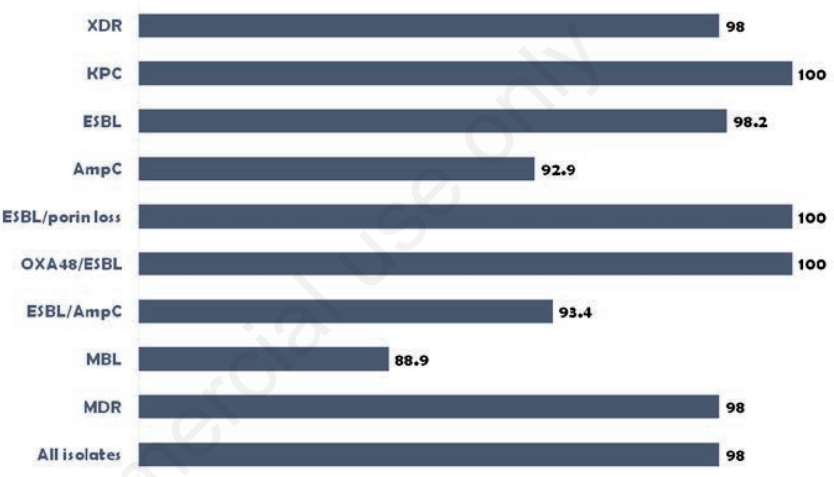

Figure 1. Susceptibility rates (%) of multiple drug resistance, extensively drug resistance, extended spectrum beta-lactamase, class C cephal*osporinases (AmpC), OXA48, mannan-binding lectin and* Klebsiella pneumoniae carbapenemase (KPC)-producing *Enterobacteriaceae* to fosfomycin.

Table 2. *In vitro* resistance rates of multiple drug resistance (MDR), extensively drug resistance (XDR), extended spectrum beta-lactamase (ESBLs), class C cephalosporinases (AmpC), mannan-binding lectin (MBL) and Klebsiella pneumoniae Carbapenemase (KPC) producing *Enterobacteriaceae i*solates to different antibiotics.

Antibiotics	All isolates	MDR	XDR	ESBL	AmpC	KPC	MBL
Fosfomycin	2	2	2	1.8	7.1	0	11.1
Cefazolin	80.5	91.7	100	93.7	89.3	100	100
Ceftazidim	39.9	41.1	100	81.3	50	100	100
Cefepime	37	44.46	98	58.9	14.3	100	100
Cefotaxime	56.1	67.5	100	87.5	71.4	100	100
Aztreonam	48.2	59.3	100	80.4	39.3	75	77.8
Imipenem	5.3	3.7	25	10.7	7.1	100	100
Ampicillin	87.5	98	100	100	78.6	100	100
Gentamicin	36.3	45.7	100	58.9	17.9	75	100
Ciprofloxacin	56.8	69.5	100	73.2	57.1	25	88.9
Cotrimoxazol	64.4	77	100	75.9	42.9	50	100
Tetracycline	38.9	45.7	100	42	39.3	25	44.4
Piperacillin/Tazobactam	32.3	37	75	28.6	35.7	0	33.3

to antimicrobial drugs were susceptible to fosfomycin.[5,16] By contrast, in two studies,[10,14] fewer than 60% of the isolates (which were isolates of *E. aerogenes* and *K. pneumoniae*) were susceptible to fosfomycin. *E. coli* seem to be the most susceptible to fosfomycin of the *Enterobacteriaceae* that create ESBL that in concordance with previous reports.[10,14,16] However, in the current study, ESBL producing strains showed 98.2% sensitivity to fosfomycin which concur with reports of previous studies.[17,18] However fosfomycin is chemically unrelated to other anti-bacterial agents, due to the unique mechanism of action it may provide a synergistic effect with other antibiotics including beta-lactams, aminoglycosides and fluoroquinolone.[7]

While ESBLs are the chief reason of resistance to cephalosporins between *Enterobacteriaceae* in the world, AmpC beta-lactamases are emerging as a probable risk to the activity of cephalosporins in many regions.[19] Cefepime is believed a fourth-generation and frequently effective to AmpC beta-lactamases. A new worldwide review of 23,918 isolates from ICUs demonstrated that 74–100% of isolates were sensitive to cefepime.[20] In the current study, the most effective antibiotics against AmpC producer *Enterobacteriaceae* were fosfomycin and imipenem (92.9%), followed cefepime (85.7%). Furthermore, fosfomycin and carbapenems are considered extremely effective treatment for AmpC producing infections.[21]

In comparison to tested antibiotics, apart from fosfomycin, imipenem (94.7%) showed high activity against bacterial isolates. Fosfomycin seems to have retained antibacterial activity against *Enterobacteriaceae* with progressive resistance patterns, even carbapnem-resistant *K. pneumoniae*. However, CRE is the main threat, and CRE is increasingly common in various parts of the world.[14] Though, among beta-lactamases, KPC and OXA48 are all carried on plasmids and with no trouble transferred to other isolates. OXA48 enzymes are rising predominantly in the Middle Eastern and European regions and management options are limited.[14] In the present study, all KPC, AmpC-porin loss, and OXA48 strains were sensitive to fosfomycin. One of the MBL strains was resistant, and 8 were susceptible to fosfomycin. According to results, fosfomycin seems to be a choice antibacterial agent for the management of such difficult to treat infections. Several studies have reported excellent *in vitro* activity of fosfomycin against CRE isolates.[6]

One important consideration for the clinical use of fosfomycin is the possible for the emergence of resistance during management and for the choice of resistant mutants.[6] While the natural mutation rate of fosfomycin resistance in *Enterobacteriaceae* appears to be reasonably high *in vitro*, this has not commonly been related to the increase of clinically very important fosfomycin resistance in clinical practice.[11] Nowadays, the oral form of fosfomycin has mainly been used in the management of uncomplicated urinary tract infections in the United States, the United Kingdom, and other countries. However, the intravenous form has been used for indications beyond urinary tract infections in some countries such as Germany, France, Spain and Japan.[5] The resistance rate to fosfomycin was zero in *Enterobacteriaceae* isolated from blood, CSF, peritoneal fluid, and burn samples. However, the frequency of resistance to fosfomycin was high (58.3%) in *Enterobacteriaceae* isolated from respiratory samples. Current data recommend that fosfomycin might be considered as an option in the management of MDR bacterial infections other than of UTIs, except respiratory infections. The activity of fosfomycin seems to be prone by the site from which the pathogen is isolated. The absence of cross-resistance to fosfomycin with other antibiotics may be attributed to the exclusive mechanism of action of this antimicrobial agent, which includes inhibition of a primary step in bacterial cell wall synthesis. Furthermore, fosfomycin does not seem to be a substrate for common resistance mechanisms of XDR and MDR such as efflux pumps. Moreover, the main type of fosfomycin resistance seems to be chromosomal rather than via plasmid, which reduces the likelihood of co-transmission of resistance to fosfomycin along with resistance to other antibiotics.[11]

In the current study, the disk diffusion assay was used for fosfomycin susceptibility testing. The agar dilution assay is an appropriate method for fosfomycin susceptibility testing, whereas broth dilution tests might give conflicting findings.[5] Considering that MIC is a time-consuming assay, that several automated systems have not yet included fosfomycin, and that the E-test has shown incompatible results, disc diffusion appears to be the most useful option in routine laboratories to assess susceptibility to fosfomycin.[22]

Conclusions

In this study, we find high levels of resistance to commonly used antibiotics. The most effective antibiotic is fosfomycin. So, fosfomycin can potentially be considered in the management of infections caused by MDR and XDR *Enterobacteriaceae* if recognized therapeutic options are not obtainable. Furthermore, fosfomycin can be included in the routine panel of antibiotics for susceptibility testing by disc diffusion to provide fast and reliable information for the selection of treatment alternatives for MDR and XDR strains.

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