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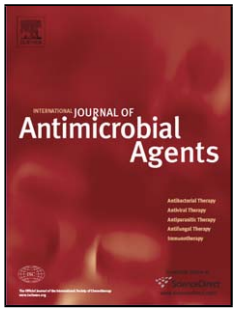

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Antimicrobial susceptibility of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Enterobacteriaceae isolates to fosfomycin

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ABSTRACT

The advancing antimicrobial drug resistance among Enterobacteriaceae renders the evaluation of potential novel therapeutic options necessary. We sought to evaluate the in vitro antimicrobial activity of fosfomycin against multidrug-resistant (MDR) Enterobacteriaceae isolates. Antimicrobial susceptibility to fosfomycin and 12 additional antibiotics of MDR Enterobacteriaceae isolates collected between November 2007 and April 2009 at the University Hospital of Heraklion, Crete, Greece, was examined using the Etest method. A total of 152 MDR Enterobacteriaceae isolates were studied, including *Klebsiella pneumoniae* (76.3%), *Escherichia coli* (17.1%), *Proteus mirabilis* (4.6%) and other species (2.0%). Antimicrobial susceptibility rates were highest for fosfomycin (92.8%), tigecycline (92.1%) and colistin (73.0%) followed by imipenem (35.5%), tetracycline (20.4%), gentamicin (19.7%), trimethoprim/sulfamethoxazole (12.5%) and ciprofloxacin (10.5%). Of the 152 isolates, 85 (55.9%) were extensively drug-resistant (XDR), of which 78 (91.8%) remained susceptible to fosfomycin. Susceptibility to fosfomycin of the 79 carbapenemase-producing, 34 extended-spectrum β-lactamase-producing and 24 metallo-β-lactamase-producing isolates was 94.9%, 94.1% and 83.3%, respectively. In conclusion, in this study fosfomycin exhibited good in vitro antimicrobial activity against MDR and XDR Enterobacteriaceae. We suggest further evaluation of the potential clinical utility of fosfomycin against infections caused by these pathogens.

1. Introduction

The evolution and spread of various mechanisms of antimicrobial drug resistance among common Enterobacteriaceae human pathogens, which translates into narrowing of the available therapeutic options, is of increasing concern. Such pathogens mainly include *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter* spp. [1]. Carbapenems, once considered the mainstay of therapy for systemic infections caused by Enterobacteriaceae isolates with multidrug resistance, may be found to be microbiologically inactive against contemporary isolates owing to the presence of various specific mechanisms of resistance. These include the expression of serine carbapenemases and metallo-β-lactamases (MBLs) or decreased porin expression in conjunction with extended-spectrum β-lactamase (ESBL) or AmpC β-lactamase production [2–4].

There is a need for the evaluation of available therapeutic options for infections caused by Enterobacteriaceae that are resistant to the traditionally used agents. Tigecycline and colistin have demonstrated good antimicrobial activity against such isolates, although the available specific relevant clinical data should be considered preliminary [5,6]. Furthermore, fosfomycin, which has been mainly used in the treatment of uncomplicated lower urinary tract infections [7], may exhibit good antimicrobial activity against Gram-negative isolates with multidrug resistance, including contemporary isolates [8,9].

In this regard, we sought to evaluate the antimicrobial activity of fosfomycin against multidrug-resistant (MDR) Enterobacteriaceae clinical isolates collected in a tertiary

care hospital in Greece, a country with very high rates of antimicrobial drug resistance among Gram-negative bacilli.

2. Materials and methods

MDR Enterobacteriaceae clinical isolates identified between November 2007 and April 2009 at the microbiological laboratory of the University Hospital of Heraklion (Crete, Greece) were selected. This hospital is a 700-bed tertiary care centre. Only the first isolate per patient was included in the study. Isolates resistant to at least three classes of potentially effective antimicrobial agents were considered as MDR. Of these isolates, those that were resistant to all except one or two classes of potentially effective antimicrobial agents (not considering fosfomycin) were subcategorised as extensively drug-resistant (XDR) [10].

Species identification of the studied isolates was performed by standard biochemical methods, the API 20E system or the Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France) [11]. Identification of ESBL production was performed by phenotypic testing based on demonstration of synergy between clavulanic acid and extended-spectrum cephalosporins [2]. Carbapenemase production was detected by the modified Hodge test, and MBL production was further detected by phenotypic methods based on the demonstration of synergy between imipenem and ethylene diamine tetra-acetic acid (EDTA) [specifically, a three-fold or greater decrease in the imipenem minimum inhibitory concentration (MIC) in the presence of EDTA] [3].

For all isolates studied, the MIC of fosfomycin and 12 other clinically relevant antimicrobial agents was determined by Etest (AB BIODISK, Solna, Sweden)

following the manufacturer's recommendations. All tests were performed in duplicate. The US Food and Drug Administration (FDA) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) MIC breakpoints were used to interpret susceptibility to tigecycline and colistin, respectively, whereas the Clinical and Laboratory Standards Institute (CLSI) MIC breakpoints were used to interpret susceptibility to fosfomycin and the remaining agents [12].

3. Results

A total of 152 MDR Enterobacteriaceae isolates were included in the study. Of these, 14 (9.2%) were isolated in 2007, 82 (53.9%) in 2008 and the remaining 56 (36.8%) in 2009. The majority of isolates (116; 76.3%) were *K. pneumoniae*, 26 (17.1%) were *E. coli*, 7 (4.6%) were *Proteus mirabilis*, 2 (1.3%) were *Enterobacter cloacae* and the remaining 1 (0.7%) was a *Klebsiella oxytoca* isolate. The majority of isolates originated from urine (67; 44.1%), whereas 29 isolates (19.1%) originated from lower respiratory tract specimens, 22 (14.5%) from blood, 12 (7.9%) from pus, 11 (7.2%) from normally sterile body fluids, 6 (3.9%) from intravascular catheter tips and the remaining 5 isolates (3.3%) originated from other types of specimens.

Table 1 presents data regarding the susceptibility of the studied isolates to the antibiotics tested. As shown in Table 1, fosfomycin was the most active antibiotic tested against all 152 MDR Enterobacteriaceae isolates studied (susceptibility rate 92.8%), followed by tigecycline (92.1%) and colistin (73.0%); the susceptibility rate to all other agents tested was <50%. The MIC_{50} and MIC_{90} values (MICs for 50% and 90% of the isolates, respectively) for fosfomycin were 16 mg/L and 48 mg/L, respectively (range 0.125–384 mg/L).

The majority (85; 55.9%) of the MDR isolates studied were further characterised as XDR, including 75 *K. pneumoniae*, 6 *P. mirabilis*, 3 *E. coli* and 1 *E. cloacae*. The 85 XDR isolates were susceptible to tigecycline (88.2% susceptibility), colistin (55.3%), imipenem (14.1%), tetracycline (5.9%), gentamicin (3.5%), ciprofloxacin (2.4%) and trimethoprim/sulfamethoxazole (1.2%). The great majority (78/85; 91.8%) of the XDR isolates remained susceptible to fosfomycin.

Regarding the expression of specific types of β-lactamases, 79 (52.0%) of the 152 isolates studied demonstrated expression of serine carbapenemases, whilst 34 (22.4%) produced ESBLs, 2 (1.3%) produced both serine carbapenemases and ESBLs and 24 (15.8%) produced MBLs. The remaining 13 (8.6%) of the 152 isolates studied did not demonstrate expression of any of the above types of β-lactamases. The susceptibility rates to fosfomycin for the carbapenemase-, ESBL- and MBL-producing isolates were 94.9%, 94.1% and 83.3%, respectively.

Table 2 presents data on the distribution of fosfomycin MICs for the studied isolates, by species, resistance pattern and specific resistance phenotype. The antimicrobial activity of fosfomycin was similar between MDR and XDR isolates. As can be inferred from Table 2, the use of a stricter MIC breakpoint of susceptibility to fosfomycin of ≤32 mg/L would result in characterisation of only 82.2% of the 152 isolates studied as susceptible to fosfomycin.

4. Discussion

The main finding of this study is that fosfomycin showed substantial antimicrobial activity against a collection of clinical Enterobacteriaceae with very high resistance rates to traditionally used antimicrobial agents. The antimicrobial activity of fosfomycin did not appear to be considerably influenced by the pattern of resistance of the studied isolates (either MDR or XDR) or the expression of specific resistance phenotypes (serine carbapenemases, MBLs or ESBLs).

The lack of cross-resistance to fosfomycin with other antimicrobial agents may be attributed to the unique mechanism of action of this agent, which comprises inhibition of an early step in bacterial cell wall synthesis [7]. Moreover, fosfomycin does not appear to be a substrate for common mechanisms of multidrug resistance such as multidrug efflux pumps [13,14]. In addition, the main type of resistance to fosfomycin appears to be chromosomal rather than plasmid-mediated [15], which diminishes the likelihood of co-transmission of resistance to fosfomycin along with resistance to other agents.

Several studies have noted good activity of fosfomycin against Enterobacteriaceae producing ESBLs or characterised as MDR [9]. However, relatively few studies have evaluated the antimicrobial activity of fosfomycin against Enterobacteriaceae isolates with extensive drug resistance (including carbapenem resistance), providing favourable findings regarding the potential value of fosfomycin in this regard [8].

A few clinical studies have evaluated the use of fosfomycin for the treatment of patients with infections caused by ESBL-producing Enterobacteriaceae. These

studies have mainly focused on patients with lower urinary tract infections and have demonstrated substantial clinical success with orally administered fosfomycin [16]. The accumulated clinical experience regarding the use of parenterally administered fosfomycin for various indications suggests that it may also be useful for the treatment of systemic infections. However, the appropriate dose and duration of fosfomycin therapy for such indications requires further evaluation [17].

Nevertheless, an important consideration in evaluating fosfomycin for clinical use refers to the potential of emergence of resistance during therapy. Although the spontaneous mutation rate of fosfomycin resistance in Enterobacteriaceae appears to be relatively high in vitro [18], this has not generally been related to the development of clinically apparent fosfomycin resistance in clinical practice [19]. The latter can be attributed to a biological cost associated with the development of resistance to fosfomycin or even loss of virulence [14].

5. Conclusion

This study shows that fosfomycin has substantial in vitro antimicrobial activity against a collection of clinical MDR and XDR Enterobacteriaceae isolates (mainly *K. pneumoniae*), the majority of which produced serine carbapenemases, ESBLs or MBLs. Since therapeutic options for these types of isolates have not been well established, the potential clinical utility of fosfomycin in this regard merits further evaluation.

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Competing interests

None declared.

Ethical approval

Not required.

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Table 1

Susceptibility rates of multidrug-resistant and extensively drug-resistant Enterobacteriaceae isolates to fosfomycin and other antibiotics

Enterobacteriaceae species	Susceptible [*n* (%)]	Intermediate [*n* (%)]
Klebsiella pneumoniae (*N* = 116)		
Aztreonam	0 (0)	0 (0)
Cefepime	0 (0)	0 (0)
Cefotaxime	0 (0)	0 (0)
Ceftazidime	0 (0)	0 (0)
Ciprofloxacin	6 (5.2)	0 (0)
Colistin	84 (72.4)	0 (0)
Fosfomycin	105 (90.5)	8 (6.9)
Gentamicin	22 (19.0)	16 (13.8)
Imipenem	23 (19.8)	39 (33.6)
PIP/TAZ	0 (0)	0 (0)
Tetracycline	22 (19.0)	32 (27.6)
Tigecycline	111 (95.7)	5 (4.3)
SXT	7 (6.0)	0 (0)
Escherichia coli (*N* = 26)		
Aztreonam	0 (0)	0 (0)
Cefepime	0 (0)	0 (0)
Cefotaxime	0 (0)	0 (0)
Ceftazidime	0 (0)	0 (0)
Ciprofloxacin	8 (30.8)	0 (0)
Colistin	23 (88.5)	0 (0)
Fosfomycin	26 (100)	0 (0)
Gentamicin	8 (30.8)	0 (0)
Imipenem	24 (92.3)	1 (3.8)
PIP/TAZ	0 (0)	0 (0)
Tetracycline	7 (26.9)	1 (3.8)
Tigecycline	26 (100)	0 (0)
SXT	12 (46.2)	0 (0)

Proteus mirabilis (*N* = 7)		
Aztreonam	0 (0)	0 (0)
Cefepime	0 (0)	0 (0)
Cefotaxime	0 (0)	0 (0)
Ceftazidime	0 (0)	0 (0)
Ciprofloxacin	1 (14.3)	0 (0)
Colistin	1 (14.3)	0 (0)
Fosfomycin	7 (100)	0 (0)
Gentamicin	0 (0)	0 (0)
Imipenem	6 (85.7)	0 (0)
PIP/TAZ	0 (0)	0 (0)
Tetracycline	1 (14.3)	0 (0)
Tigecycline	0 (0)	7 (100)
SXT	0 (0)	0 (0)
All Enterobacteriaceae species (*N* = 152) [a]		
Aztreonam	0 (0)	0 (0)
Cefepime	0 (0)	0 (0)
Cefotaxime	0 (0)	0 (0)
Ceftazidime	0 (0)	0 (0)
Ciprofloxacin	16 (10.5)	0 (0)
Colistin	111 (73.0)	0 (0)
Fosfomycin	141 (92.8)	8 (5.3)
Gentamicin	30 (19.7)	16 (10.5)
Imipenem	54 (35.5)	42 (27.6)
PIP/TAZ	0 (0)	0 (0)
Tetracycline	31 (20.4)	33 (21.7)
Tigecycline	140 (92.1)	12 (7.9)
SXT	19 (12.5)	0 (0)

PIP/TAZ, piperacillin/tazobactam; SXT, trimethoprim/sulfamethoxazole.

[a] Including in addition to the above isolates two *Enterobacter cloacae* and one *Klebsiella oxytoca*.

Table 2

Fosfomycin minimum inhibitory concentrations (MICs) for the studied Enterobacteriaceae isolates, by species, pattern of resistance and phenotypic expression of specific types of β-lactamases

Enterobacteriaceae species	MIC (mg/L)										MIC ≤ 32 mg/L (% of isolates)	MIC ≤ 64 mg/L (% of isolates)
	≤0.5	1	2	4	8	16	32	64	128	≥256		
Klebsiella pneumoniae												
All isolates (*N* = 116)	1	1	2	2	6	35	44	14	8	3	78.4	90.5
Resistance pattern												
XDR (*n* = 75)	0	1	1	0	4	25	28	9	5	2	78.7	90.7
MDR (*n* = 41)	1	0	1	2	2	10	16	5	3	1	78.0	90.2
Expression of specific β-lactamase												
MBL (*n* = 21)	0	0	1	1	0	4	7	4	3	1	61.9	81.0
Carbapenemase (*n* = 74)	1	0	1	1	5	28	26	8	3	1	83.8	94.6
Carbapenemase + ESBL (*n* = 2)	0	0	0	0	0	0	1	0	1	0	50.0	50.0
ESBL (*n* = 10)	0	0	0	0	1	2	3	2	1	1	60.0	80.0
Escherichia coli												
All isolates (*N* = 26)	2	12	5	5	0	1	1	0	0	0	100	100
Specific resistance phenotype												
ESBL (*n* = 24)	2	11	5	4	0	1	1	0	0	0	100	100
All Enterobacteriaceae [a]												
All isolates (*N* = 152)	5	14	7	10	7	36	46	16	8	3	82.2	92.8
Resistance pattern												
XDR (*n* = 85)	2	2	1	5	4	25	28	11	5	2	78.8	91.8
MDR (*n* = 67)	3	12	6	5	3	11	18	5	3	1	86.6	94.0

Expression of specific β-lactamase												
MBL (*n* = 24)	0	1	1	1	1	4	7	5	3	1	62.5	83.3
Carbapenemase (*n* = 79)	2	1	1	3	5	28	27	8	3	1	84.8	94.9
Carbapenemase + ESBL (*n* = 2)	0	0	0	0	0	0	1	0	1	0	50.0	50.0
ESBL (*n* = 34)	2	11	5	4	1	3	4	2	1	1	88.2	94.1

XDR, extensively drug-resistant; MDR multidrug-resistant; MBL, metallo-β-lactamase; ESBL, extended-spectrum β-lactamase.

[a] Including in addition to the above isolates seven *Proteus mirabilis*, two *Enterobacter cloacae* and one *Klebsiella oxytoca*.