

In Vitro Susceptibility of Fosfomycin against Extended-Spectrum β -Lactamase-producing *Escherichia coli* Isolated from Urine Specimens

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Abstract

Background: The production of extended-spectrum β -lactamases (ESBLs) brings challenge to the treatment of urinary infection caused by *Escherichia coli* with increasing resistance to common antibiotics. For the limited treatment option, fosfomycin as traditional antibiotic has been reevaluated. Yet there are scarce studies regarding the antibacterial activity of fosfomycin for urinary infection in China. With this regard, we investigated *in vitro* susceptibility of fosfomycin and other 7 agents against ESBLs-producing *Escherichia coli* isolated from urine specimens.

Methods: The minimum inhibitory concentrations (MICs) of fosfomycin and other comparative agents including meropenem, piperacillin-tazobactam, amikacin, cefepime, cefotaxime, ceftazidime and levofloxacin were determined by agar dilution method recommended by the Clinical Laboratory Standards Institute (CLSI) 2010.

Results: Of the 100 strains isolated from urine, none was found resistant to meropenem. The relatively high susceptibility was found to piperacillin-tazobactam (94%), amikacin (85%), fosfomycin (82%) and cefepime (60%). The susceptibility rate of fosfomycin did not differ significantly between the cefotaxime-resistant (CTX-R) and cefotaxime-susceptible (CTX-S) group (80.1% vs. 84.6%, $P=0.712$), ceftazidime-resistant (CAZ-R) and ceftazidime-susceptible (CAZ-S) group (78.1% vs. 84.8%, $P=0.552$), levofloxacin-resistant (LVX-R) and levofloxacin-susceptible (LVX-S) group (85.4% vs. 62.5%, $P=0.07$) respectively.

Conclusions: For the relatively high antibacterial activity *in vitro* against ESBLs-producing *Escherichia coli* isolated from urine specimens and the non-influenced by the pattern of resistance to other antimicrobials, fosfomycin is of great clinical value in the treatment of uncomplicated urinary infection caused by ESBLs-producing *Escherichia coli*.

Keywords: Extended-spectrum β -lactamases; *Escherichia coli*; Fosfomycin

Methods

100 non-replicate strains of *Escherichia coli* were isolated from urine samples of patients from the First Hospital of China Medical University from January to September, 2014. All the isolates were identified using the Vitek 2 system (bioMérieux S.A., Marcy l'Etoile, France). ESBLs production was confirmed phenotypically using double-disc diffusion tests with ceftazidime with/without clavulanic acid and cefotaxime with/without clavulanic acid. *Escherichia coli* ATCC 25922 was taken as quality control strain. This study was approved by the Ethics Committee of our hospital (Approval number, 2013114) and conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

The MICs of fosfomycin and other comparative agents including meropenem, amikacin, piperacillin-tazobactam, cefepime, cefotaxime, ceftazidime and levofloxacin (obtained from the National Institute for the Control of Pharmaceutical and Biological Products) were determined by agar dilution method recommended by CLSI (2010) [3]. Interpretation of the MIC was according to the CLSI (2010) recommendations. Data were analyzed with WHONET 5.3 software and processed by Chi-square test using SPSS16.0. P value of <0.05 were considered statistically significant.

Introduction

With the widespread use of antibiotics (especially third- and fourth-generation cephalosporins and fluoroquinolones), the production of ESBLs brings challenge to the treatment of urinary infection caused by *Escherichia coli* with increasing resistance to common antibiotics. For the limited treatment option, the value of fosfomycin as traditional antibiotic has been reevaluated, receiving increasing attention as the cure for ESBLs-producing *Escherichia coli*. High susceptibility of fosfomycin and no cross-resistance with other antibiotics to multidrug-resistant Gram-negative bacteria has been reported abroad [1,2]. Yet there are scarce studies regarding in China, a country with increasing rates of antimicrobial resistance to *Escherichia coli*. With this regard, we investigated the antibacterial activity *in vitro* of fosfomycin and other 7 agents against ESBLs-producing *Escherichia coli* isolated from urine specimens.

Results

Results of susceptibilities of 8 antimicrobial agents against ESBLs-producing *Escherichia coli* isolates were shown in Table 1.

Antimicrobial agent	Susceptibility (%)	MIC50 (μ g/ml)	MIC90 (μ g/ml)
Meropenem	100	0.03	0.03
Piperacillin/tazobactam	94	4	8
Amikacin	85	4	16
Fosfomycin	82	2	32
Cefepime	60	8	64
Cefotaxime	13	128	512
Ceftazidime	46	8	64
Levofloxacin	16	32	512

Table 1: Susceptibility of 100 ESBLs-producing *Escherichia coli* isolated from urine to 8 antimicrobial agents (%).

Of the 100 strains isolated from urine, none was found resistant to meropenem showing excellent activity of carbapenem. The relatively high susceptibility was found to piperacillin-tazobactam (94%), amikacin (85%), fosfomycin (82%) and cefepime (60%). The MIC50 and MIC90 for fosfomycin were 2 μ g/ml and 32 μ g/ml respectively, by contrast cefepime reached 8 μ g/ml and 64 μ g/ml for MIC50 and MIC90.

More than 50% of the isolates were not susceptible to cefotaxime, ceftazidime and levofloxacin. Table 2 showed the comparing of the susceptibility rates of fosfomycin between the cefotaxime-resistant (CTX-R) and cefotaxime-susceptible (CTX-S) isolates, ceftazidime-resistant (CAZ-R) and ceftazidime-susceptible (CAZ-S) isolates, levofloxacin-resistant (LVX-R) and levofloxacin-susceptible (LVX-S) isolates respectively.

Strain group	Susceptible of fosfomycin [n (%)]	P
CTX-Ra (n=76)	61(80.1)	0.712
CTX-S (n=13)	11(84.6)	
CAZ-R (n=32)	25(78.1)	0.552
CAZ-S (n=46)	39(84.8)	
LVX-R (n=82)	70(85.4)	0.07
LVX-S (n=16)	10(62.5)	

Table 2: Comparison of fosfomycin susceptibility according to cefotaxime-resistant, ceftazidime-resistant and levofloxacin-resistant in isolates.

The susceptibility rate did not differ significantly between the CTX-R and CTX-S group (80.1% vs. 84.6%, $P=0.712$), CAZ-R and CAZ-S group (78.1% vs. 84.8%, $P=0.552$), LVX-R and LVX-S group (85.4% vs. 62.5%, $P=0.07$).

Discussion

Urinary infection is a common infectious disease infecting more than 150 million people each year worldwide, with the primary pathogen of *Escherichia coli* [4]. However, with the widespread use of antimicrobial drugs (such as third- and fourth-generation cephalosporins and fluoroquinolones), the detection rate of ESBLs-producing *Escherichia coli* has been ever increasing. The susceptibility rates of cefepime, cefotaxime and ceftazidime to ESBLs-producing *Escherichia coli* in this study have shown lower than other agents. Moreover, in our previous study, all of these three agents were detected with inoculum effect, which means the MICs of cephalosporins increase as the inoculum increase [5]. For the urinary infection caused by ESBLs-producing *Escherichia coli*, cefepime, cefotaxime and ceftazidime are not reliable even though the *in vitro* tests susceptible.

Meropenem showed good activity against *Escherichia coli* (100%), followed by piperacillin-tazobactam (94%) and amikacin (85%). However, owing to not only high expenses but also the selection of resistant bacterial for the long time use of carbapenems and β -lactamase inhibitors, and the nephrotoxicity of aminoglycosides, it is necessary to consider other antimicrobial agent as therapy option.

Hence, fosfomycin has started to get attention as a traditional antibiotic. Fosfomycin is a kind of phosphoric acid derivatives separated from streptomycetes in 1969. It has a unique chemical structure and an unusual antibacterial mechanism of inhibiting the first step of bacterial cell wall synthesis. It can influence not only the reducing growth but also the property of adherence to uroepithelial cells, causing the defects of virulence [6]. This may explain the good activity of fosfomycin against *Escherichia coli*, especially for uropathogens. Recent researches have reported a high antibacterial activity of fosfomycin in the treatment of urinary infection [7]. In our study, 82% of ESBLs-producing *Escherichia coli* isolates being susceptible to fosfomycin was in accordance with that of Falaga's investigation [1], which also pointed out that a particularly high concentration of fosfomycin could be maintained for a long time in urine. And also Mazzei et al. proves the effective by pharmacokinetic parameter of fosfomycin, finding its high urinary concentration with C_{max} , 4415 mg/L [8]. Thus it could be considered in the treatment of uncomplicated urinary tract infection.

Moreover, few reports involved the cross-resistance to fosfomycin with other antimicrobial agents, it may be due to the unique mechanism of chromosomal-resistance other than plasmid-mediated-resistance and the no substrate for multidrug efflux pumps [9,10]. Our study showed the susceptibility of fosfomycin is nearly 80% in both CTX-R and CTX-S group (with no significance), also with the same results in CAZ-R and CAZ-S group. To our surprise, the susceptibility of fosfomycin was higher in the LVX-R group than the LVX-S group, but also with the P value of no significance. Further investigations are warranted to investigate the mechanism.

Conclusions

Consequently fosfomycin shows a relatively high antibacterial activity *in vitro* against ESBLs-producing *Escherichia coli* isolated from urine specimens and the non-influenced by the pattern of resistance to other antimicrobial agents. So with the increase of ESBLs, fosfomycin is of great clinical value in the treatment of uncomplicated urinary infection caused by *Escherichia coli*. However, whether there is synergistic effect with other antimicrobial agents in the treatment of severe cases remains to be investigated by further research.

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