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ORIGINAL ARTICLE

Fosfomycin, interesting alternative drug for treatment of urinary tract infections created by multiple drug resistant and extended spectrum β-lactamase producing strains

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ABSTRACT

Background and Objectives: The emergence and spread of multidrug resistant (MDR) and extended spectrum β-lactamase (ESBL) producing strains reduces the number of effective drugs that can be used for treatment. The aim of this study was to evaluate the susceptibility profile of Enterobacteriaceae isolated from UTIs, specifically MDR and ESBL producing strains, to fosfomycin and other antibiotics.
Materials and Methods: The study was performed during a 6 month period (February 2014 to August 2015). A total of 219 non-duplicate urinary isolates of Enterobacteriaceae were collected. Identification and susceptibility testing was done according to standard microbiological procedures and the Kirby- Bauer test, respectively. Based on the results obtained from susceptibility testing, MDR bacteria were recovered and identification of ESBL production was done according to CLSI recommendation.
Results: Isolates of *E. coli* and *Klebsiella* spp.were responsible for 80.8% and 12.8% of patients with UTIs respectively. The rates of resistance to ampicillin, cefazolin, nalidixic acid, trimethoprim-sulfamethoxazole were 86.3%, 79.4%, 68.5% and 63.9% respectively. In contrast, high sensitivity rates were detected to fosfomycin, amikacin and amoxicillin-clavulanic acid with 97.3%, 91.8% and 80.8%, respectively. Of all isolates, 167 (76.3%) were detected as MDR and 75 (34.2%) as ESBL producing strains.
Conclusion: The rate of antibiotic resistance among uropathogens Enterobacteriaceae is remarkably high. The most effective antibiotic was fosfomycin. Moreover, susceptibility to fosfomycin is over 90% for MDR and ESBL producer isolates. Therefore, fosfomycin can be a good option for treating UTIs.

Keywords: Enterobacteriaceae, ESBL, Fosfomycin, MDR, UTIs

INTRODUCTION

Urinary tract infections (UTIs) are the most common type of human bacterial infections (1). Over 150 million cases of UTIs occur annually in the world (2). The main cause of infection in 80-90% of uncomplicated UTIs is *E. coli* (3, 4). Today, an increase in antimicrobial resistance of UTI pathogens is observed (5). Additionally, the emergence and spread of MDR Gram-negative bacteria associated with UTIs, in the community and hospitals, is increasing in the world (6). MDR was defined as resistance to

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at least one agent of three or more antibiotic classes (7). The number of effective drugs that can be used for treatment of UTIs, through increase of MDR, is reduced (8). Uropathogen bacteria, particularly *E. coli*, exhibit high rates of resistance through production of extended-spectrum β-lactamases (ESBL) (1). Clinically, ESBL confers considerable resistance to broad-spectrum penicillins, monobactams and cephalosporins (except cephamycins), and are often associated with co-resistance to unrelated antibiotics in MDR pathogens (5). Trimethoprim-sulfamethoxazole previously has been used to treat UTIs, due to the increasing occurrence of resistance among uropathogens. Currently, fluoroquinolone are being used frequently as first-line therapy. However, the frequency of ciprofloxacin resistance in *E. coli* has been rapidly increased worldwide (9).

Recently, fosfomycin has been introduced for the treatment of UTIs and systemic infections caused by MDR Gram-negative bacteria, especially Enterobacteriaceae that are resistant to traditional antimicrobial agents (10). Fosfomycin is a broad spectrum bactericidal antibiotic that interferes with cell wall synthesis in both Gram-negative and Gram-positive bacteria (11). There are a few studies on the *in vitro* activity of fosfomycin against commonly encountered bacteria isolated from the urine, except for *E. coli* and *E. faecalis* (12). The aim of this study was to investigate the susceptibility of isolates to eight genera belonging to Enterobacteriaceae family cultured from patients with UTIs, in particular MDR and ESBL producers, to fosfomycin.

MATERIALS AND METHODS

Bacterial isolates. A total of 219 non-duplicate urinary isolates of Enterobacteriaceae were collected during February 2014 through August 2015 from two cities of Iran; Tabriz (117 Sample) and Uremia (102 Sample). Isolates were identified using biochemical tests in the Department of Microbiology, Tabriz University of Medical Sciences, Iran.

Antimicrobial susceptibility testing. The antimicrobial susceptibility testing was performed on Mueller – Hinton agar using Kirby-Bauer's technique according to the Clinical and Laboratory Standards Institute's (CLSI) guidelines (12). The antibiotic discs used for antimicrobial susceptibility testing were as follows: amoxicillin-clavulanic acid (20/10µg), ampicillin (10µg), cefotaxime (30µg), ceftazidime (30µg), cefepime (30µg), cefuroxime (30µg), aztreonam (30µg), gentamicin (10 µg), amikacin (30µg), ciprofloxacin (5µg), nalidixic acid (30µg), trimethoprim-sulfamethoxazole (30µg), nitrofurantoin (300µg) and fosfomycin (200µg). All antibiotics, except fosfomycin (Rosco, Taastrup, Denmark), were obtained from MAST (MAST Chemical Co, UK). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 strains were used for quality control. Isolates that were resistant to at least one agent of three or more classes of antibiotic were considered as MDR.

ESBL detection. The screening test for the identification of ESBL production was done according to CLSI recommendations. An inhibition zone of 22≥ mm for ceftazidime and 27≥ mm for cefotaxime indicated the possibility of ESBL producing isolate. The confirmatory test for ESBL was performed using double disc synergy between amoxicillin-clavulanic acid (20/10µg), cefotaxime (30µg) and ceftazidime (30µg). Amoxicillin-clavulanic acid was placed in the center of the plate, which was already inoculated with the test organism, and cefotaxime and ceftazidime discs were placed 20mm (center to center) apart from the amoxicillin-clavulanic acid on the same plate. Organisms that showed synergy effects (extension zone of inhibition around cephalosporin discs on the side nearest to the amoxicillin-clavulanic acid) were considered as positive for ESBL phenotype (13). *E. coli* ATCC 25922 (ESBL negative) and *K. pneumoniae* ATCC 700603 (ESBL positive) were used as quality control strains in the phenotypic testing of ESBL production.

Statistical analyses. The results were analyzed using SPSS software for Windows (version 19 SPSS Inc., Chicago, IL, USA). In this study, pv≤0.05 was regarded as statistically significant.

RESULTS

In the current study, a total of 219 Enterobacteriaceae from urine samples were collected. *E. coli* was the most frequently isolated bacteria. The frequency of bacterial isolates were *E. coli* (n= 177, 80.8%), *K. pneumoniae* (n= 28, 12.8%), *E. cloacae* (n= 7, 3.2%), *P. mirabilis (n*= 2, 0.9%), *M. morgani* (n= 2, 0.9%), *K. oxytoca* (n= 1, 0.5%), *C. freundii* (n= 1, 0.5%), and *P. vulgaris* (n= 1, 0.5%). Out of 219 sample, 141 (64.4%) and 78 (35.6%) cases were related to females and males samples, respectively. There was a significant relationship between the UTIs and gender (Pv≤0.05). Patient ages ranged from 1 to 93 years. The age distribution of patients was 7.3% for children (1-15), 53% for middle aged patients (16-60) and 39.7% for old aged patients (61-93 years). The resistance rates was different between ages and gender but there was no significant relationship between antimicrobial resistance and age groups and gender (pv>0.05).

The rates of antimicrobial resistance are shown in Table 1 and Fig. 1. According to the results, the highest rate of resistance was in the penicillin group (ampicillin) with 86.3%, followed by the cephems group (cefazolin) with 79.4%, the quinolones group (nalidixic acid) with 68.5% and the folate pathway inhibitors with 63.9%. In contrast, higher sensitivity rates were discovered in fosfomycin with 97.3%, the aminoglycoside group (amikacin) with 91.8% and in the β-lactam/β-lactamase inhibitors group (amoxicillin/clavulanic acid) with 80.8%. The rate of MDR recovered bacteria was 76.3% (n=167). The most prevalent MDR patterns belonged to the isolates showing resistance to ampicillin, cefazolin, nalidixic acid, trimethoprim-sulfamethoxazole, cefuroxime and ciprofloxacin. Furthermore, 34.2% of isolates (n=75) were found as ESBL producers. Of all ESBL producing isolates, 60 (80%) and 15 (20%) were *E. coli* and *K. pneumoniae*, respectively. The antimicrobial susceptibility patterns of MDR and ESBL producing isolates are shown in Figs. 2 and 3. According to the results, fosfomycin was also the most effective antibiotic against all MDR and ESBL producing isolates followed by amikacin, amoxicillin-clavulanic acid and nitrofurantoin.

DISCUSSION

In the present study, we investigated the susceptibility profile of Enterobacteriaceae isolated from urinary tract, specifically MDR and ESBL producing isolates,

Table 1. Distribution of resistance patterns of Enterobacteriaceae isolates to various antimicrobial agents

Antimicrobial Class	Antimicrobial agents	177 E.coli, n(%)	28 K. pnumonia n(%)	7 E.cloacae n(%)	2 P.mirabilis n(%)	2 M.morganii n(%)	1 K.Oxytoa n(%)	1 C.freundi n(%)	1 P.vulgris n(%)
Fosfomycin	FO	2(1.1)	1 (3.6)	1 (14.3)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)
β-lactam/β-lactamase inhibitor	AMC	17 (9.6)	16 (57.1)	6 (85.7)	1 (50)	0 (0.0)	1 (100)	1 (100)	0 (0.0)
Nitrofuran	NI	29 (16.4)	18 (64.3)	6 (85.7)	2 (100)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)
Aminoglycosi des	AK	12(6.8)	6 (21.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	GM	60(33.9)	13 (46.4)	3 (42.8)	1 (50)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cephems	CZ	141 (79.7)	21 (75)	6 (85.7)	1 (50)	2 (100)	1 (100)	1 (100)	1 (100)
	CPM	58 (32.8)	18 (64.3)	3 (42.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	CXM	93 (52.5)	17 (60.7)	4 (57.1)	1 (50)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)
	CFO	46 (26)	12 (42.8)	7 (100)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)
Monobactams	ATM	83 (46.9)	17 (60.7)	3 (42.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Quinolones	NA	128 (72.3)	16 (57.1)	4 (57.1)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)
	CIP	110 (62.1)	15 (53.6)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Folate pathway inhibitors	TS	115 (65)	19 (67.8)	4 (57.1)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Penicillin	AP	152 (85.9)	27 (96.4)	6 (85.7)	1 (50)	0 (0.0)	1 (100)	1 (100)	1 (100)

Abbreviations: n(number), fosfomycin (FO), amoxicillin-clavulanic acid (AMC), nitrofurantoin (NI), amikacin (AK), gentamicin (GM), cefazolin (CZ), cefepime (CPM), cefuroxime (CXM), cefotaxime (CFO), aztreonam (ATM), nalidixic acid (NA), ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (TS), ampicilin (AP).

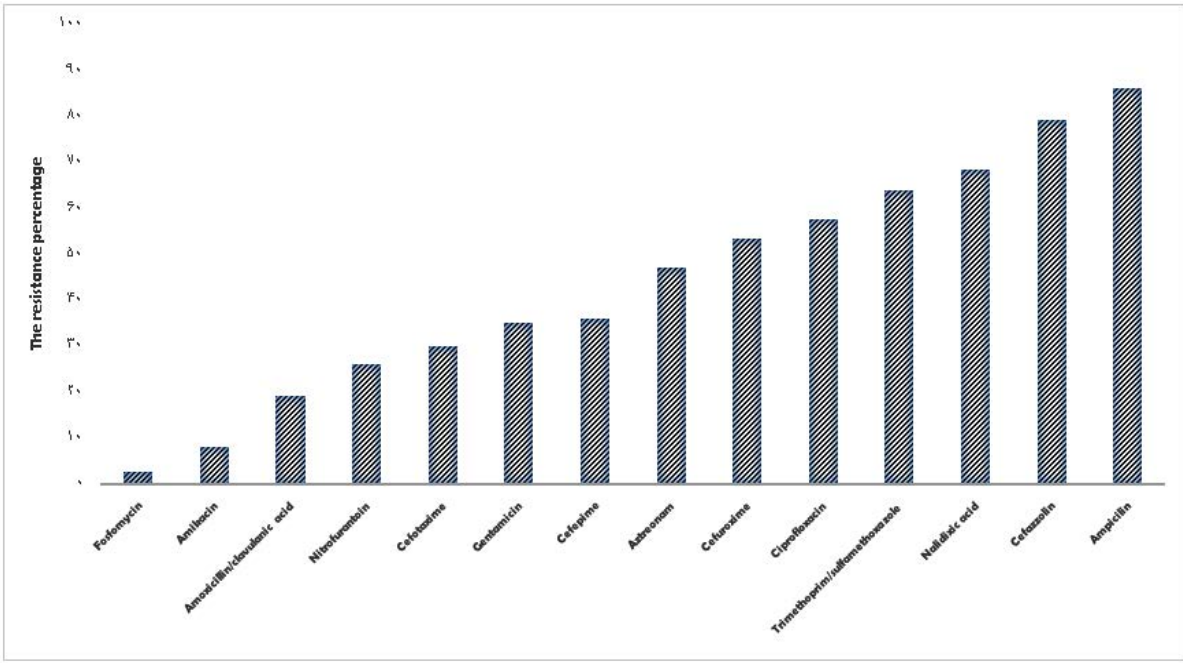

Fig. 1. Distribution of resistance rates of Enterobacteriaceae isolates to different antibiotics

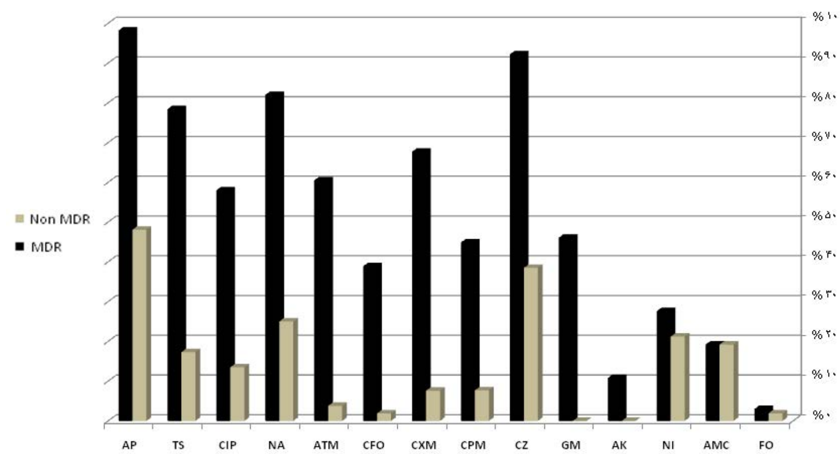

Fig. 2. Antimicrobial resistance patterns of MDR and non-MDR isolates

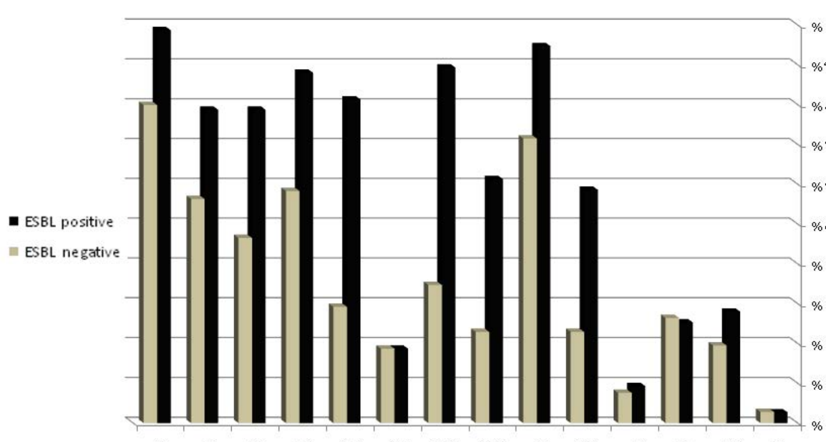

Fig. 3. Antimicrobial resistance patterns of ESBL positive and ESBL negative isolates

to fosfomycin and other antibiotics. Moreover, we compared the results resistant profile at different gender and age categories.

Urinary tract infections are the most common infectious diseases. Almost 40-50% of women will suffer from UTIs during their lifetime and one-third of women before the age of 24 will require antibiotic therapy for UTIs (14). The high infection rates among females are related to differences between the female and male genitourinary systems in anatomy and micro-flora (15). Females are more prone to UTIs due to anatomical structure i.e. short urethra, close proximity of urinary tract with anal canal (16). In contrast males are less susceptible to UTIs, due to a longer urethra and the presence of antimicrobial substance in prostatic fluid (17). The sex distribution of patients in this study showed females statistically predominant over males; this result is consistent with previous reports (15, 18).

Among age groups, middle aged patients contributed over 50% of UTIs which concurs with previous studies (18). Although no significant difference was found between antibiotic resistance patterns and gender of patients, males showed a high resistance rate to antibiotics.

The most common pathogens recovered were *E. coli* (80.8%) and *Klebsiella* spp. (12.8%), which is similar to previous reports (2, 13-14, 19).

This *in vitro* susceptibility study showed that fosfomycin was highly active against the majority of isolates. In total, 2.7% of the isolates were resistant to fosfomycin. In comparison to *E. coli* (1.1%), *Klebsiella* spp. (3.6%) showed higher rates of resistance to fosfomycin. This is consistent with the findings of the Demir et al. (20). The low level of resistance to fosfomycin among *E. coli* strains probably is due to limited use of fosfomycin for the treatment of uncomplicated UTIs. 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%, and for *E. cloacae* and *M. morgani* was detected in 85.7% and 0%, respectively. These results are partly in concordance with previous reports, which indicated that all *M. morgani* were resistant to fosfomycin (20).

In comparison to tested antibiotics, apart from fosfomycin, amikacin and amoxicillin-clavulanic acid showed relatively high activity against bacterial isolates. In contrast, high rates of resistance to ampicillin, cefazolin, nalidixic acid and trimethoprim-sulfamethoxazole were detected. However, limited options of oral antibiotics are available for treatment of UTIs. Trimethoprim-sulfamethoxazole is one of the oral antimicrobial agents that have been used in the treatment of UTIs for a long period of time and the recommended drug for the treatment of UTIs in settings where resistance is lower than 10-20%. If the resistance rate to trimethoprim-sulfamethoxazole is higher than 20%, the drugs of choice is quinolons (21). Because of the wide spread resistance rates to quinolons, its use has declined. Our study indicated that resistance to trimethoprim-sulfamethoxazole was remarkable (63.9%). These rates are lower than other studies performed in Iran (91.7%) (22) and Pakistan (81 %) (18) but are higher than a study conducted by Garau et al. 43% (23). In contrast to several reports that indicated high sensitivity rates to ciprofloxacin 90.2% (19) and 84.7% (23), in the present study the rate of resistance was found to be 57.5%. Long term exposure to low antibiotic levels and use in animal feeds is a risk factor for increased resistance of uropathogens to quinolons (24). The rate of susceptibility to nitrofurantoin (74%) obtained in our study was lower than previous study (94.75%) in Iran (23). This reduction in sensitivity may be due to inappropriate- and overuse of nitrofurantoin, easy access, incomplete treatment, and poorly controlled infection.

One of the important mechanisms in antibiotic resistance among bacteria is the production of ESBLs (19). The spread of ESBLs among isolates of Enterobacteriaceae is a growing concern and confers resistance to third and fourth generation cephalosporins, monobactams and is related to co-resistance with other antimicrobial classes such as fluoroquinolonnes, cotrimoxazol, tetracyclines and aminoglycosides (25). 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 trimethoprim-sulfamethoxazole, quinolones and aminoglycosides at the same time (19). In the present study, the overall ESBL production rate was determined in 75 (34.2%) of isolates. Similar findings were also reported from Turkey (34.1% and 14.1% for inpatient and outpatient, respectively) (26), and India (32.1%) (2). In a study carried out in Iran, prevalence of ESBL producing among Enterobacteriaceae was reported as 53.8% (27). ESBL producing *E. coli* is one of the most serious problems in the clinical setting (9). In our study, 33.9% of *E. coli* and 53.6% of *K. pnomoniae* isolates produced ESBL. The prevalence of ESBL producing *E. coli* isolates in our study was lower than a study performed by Ullah et al. (56.9%). (18), and higher than a study conducted by Ko KS et al. (7.8%) (9). The prevalence and distribution of ESBL among microorganisms significantly varies with bacterial species, time and geographical location (27). Antimicrobial resistance to ESBL producing bacteria showed that all bacteria (except 2 *E. coli* isolated) have emerged as MDR pathogens. This is similar to a reported data from Pakistan (5). The most of ESBL producing isolates were resistant to ampicillin, cefazolin, cefuroxime, nalidixic acid and trimethoprim-sulfamethoxazole.

On the other hand, very low resistance rate was detected among ESBLs producer's isolates to fosfomycin (2.7%) and amikacin (9.3%). However, in the current study, ESBL producing strains showed 97.3% sensitivity to fosfomycin which concur with reports of previous studies (2, 26). Due to the increasing prevalence of infections, caused by resistant bacteria and especially MDR strains, availability of alternative effective antibiotics is restricted. This study, like previous studies (19), showed that fosfomycin can be a suitable drug to treat infections caused by ESBL producers and MDR strains. 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 β-lactams, aminoglycosides and fluoroquinolone (11). Moreover, fosfomycin is a good option in the treatment of uncomplicated UTIs because of the administration of a single dose per day, a fine safety profile, availability during pregnancy and the effectiveness on the anaerobic gut flora (6).

As a result, for the effective clinical appropriate treatment of UTIs, knowledge on local epidemiology and antibiotic resistance patterns is essential. We recommend annual studies like this research be conducted for determining local trends of resistance antibiotic, and proper and accurate application of drugs can decrease the resistance rates to antibiotics.

In conclusion, we observed high levels of resistance to commonly used antibiotics. The data presented in this study shows that the most effective antimicrobial agent is fosfomycin when compared with other antibiotics against uropathogens Enterobacteriaceae isolates.

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