ORIGINAL ARTICLE

In Vitro Activity of Fosfomycin Tromethamine against Extended Spectrum Beta-Lactamase Producing Urinary Tract Bacteria

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Abstract

Objective: To determine the *in vitro* activity of Fosfomycin tromethamine against extended spectrum beta-lactamase producing uropathogens.

Study Design: Experimental study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from October 2011 to October 2012.

Methodology: A total of 381 culture positive ESBL producing isolates from 2400 urine samples submitted over a period of one year were included in this study. Identification of isolates was done by standard biochemical profile of the organisms. The antimicrobial susceptibility of culture positive isolates was performed by disk diffusion method as recommended by Clinical Laboratory Standard Institute guidelines (CLSI).

Results: The antimicrobial activity of Fosfomycin to various isolates revealed that 93% of *E. coli*, 64% *Klebsiella* spp. 50% *Proteus* spp. 75% *Enterobacter cloacae*, 100% *Citrobacter freundii*, 100% *Burkholderia* spp. 100% *Serratia* spp. and 50% *Stenotrophomonas maltophilia* were susceptible to this chemical compound.

Conclusion: Fosfomycin showed excellent effectiveness to most of the common ESBL producing bacteria such as *E. coli*, *Klebsiella* and *Proteus* spp.

Key Words: *Antibiotic susceptibility. Fosfomycin. Extended Spectrum Beta-Lactamase producers (ESBLs). Urinary tract infection.*

INTRODUCTION

Urinary Tract Infections (UTIs), a very common disease among general practice patients that is caused by various Gram positive and Gram negative bacteria. A variety of antimicrobials are used to treat these infections such as beta-lactamases, co-trimoxazole, ciprofloxacin, norfloxacin and nitrofurantoin. An irrational use of antibiotics in our setup has immensely contributed to the antimicrobial resistance and emergence of multidrug-resistant urinary isolates.[1]

In the mid of 1980s, a new group of enzymes known as Extended Spectrum Beta-Lactamases (ESBL) was discovered, conferring resistance to penicillins, cephalosporins and monobactams. The ESBL producing organisms can also develop co-resistance to other antimicrobials such as fluoroquinolones, co-trimoxazole, and aminoglycosides frequently used to treat urinary tract infections.[2]

Fosfomycin a phosphonic acid derivative has bactericidal properties against various Gram positive and Gram negative bacteria causing UTIs. More than 90% of ESBL producing enterobacteriaceae have been found to be susceptible to Fosfomycin, whereas the cumulative susceptibility rate by the Clinical Laboratory Standards Institute (CLSI) criteria is 98.3% and 88.5% respectively.[3] Fosfomycin is gaining importance to treat ESBL producing urinary isolates, because resistance against commonly used oral antimicrobials is increasing.[4] There is also an evidence that antimicrobial activity of Fosfomycin against ESBLs may be accompanied by an immune-modulating activity.[5]

The rationale of the study was to establish a susceptibility pattern of Fosfomycin against ESBL producing urinary isolates in our set up as there is no current data available in Pakistan regarding the susceptibility of ESBL producing uropathogens to Fosfomycin tromethamine.

The objective of the study was to determine *in vitro* activity of Fosfomycin tromethamine against urinary tract infections caused by extended spectrum beta-lactamase producing bacteria.

METHODOLOGY

A total of 381 ESBL producing Gram negative rods isolated from urine specimens received at AFIP for culture and sensitivity were included in this study. Permission was taken from the Institutional Ethical and Research Committee for research purpose. Non-probability consecutive sampling was done. All non-ESBL producing urinary isolates as well as ESBL producing isolates from repeated samples of same patient were excluded from the study.

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Urine specimens were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar (Mast Diagnostics, UK) and incubated aerobically at 35°C ± 2 for 18 to 24 hours. After identification of Gram negative rods by colony morphology, Gram staining and biochemical reactions read from API 20E (bio-Merieux), the isolates were screened for ESBL with cefotaxime 30 µg disc (Oxoid, UK) by Kirby-Bauer disc diffusion technique according to Clinical Laboratory Standards Institute (CLSI) guidelines.[6] The isolates with cefotaxime zone diameter equal to or less than 25 mm were further confirmed for ESBL by phenotypic confirmatory test applying cefotaxime clavulanic acid 30/10 µg combination disc (double disc synergy).[6]

Prepared Mueller-Hinton agar (MAST Diagnostics, UK) were inoculated with the test organism (0.5 McFarland standard) to give a semi-confluent growth. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as control strains. A ceftazidime 30 µg disc or cefotaxime 30 µg disc along with ceftazidime-clavulanic acid 30/10 µg combination disc or cefotaxime-clavulanic acid 30/10 µg combination disc were then placed at 20 to 25 mm distance from each other. Following overnight incubation in air at 35°C ± 2, an increased zone diameter of ≥ 5 mm for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirmed the isolate as an ESBL producer.[6]

According to CLSI guidelines, inoculum of bacterial suspension (0.5 McFarland standard) was inoculated on Mueller-Hinton agar (MAST Diagnostics, UK) followed by application of Fosfomycin tromethamine disc of 200µg (Oxoid, UK). The plates were incubated aerobically at 35°C ± 2 for 18-24 hours. Zone of inhibition around the discs were interpreted as per CLSI guidelines.[6]

The data obtained was entered in SPSS-20 for statistical evaluation. Descriptive statistics was applied to calculate mean S.D. for age, frequencies and percentages for different variables like gender and *in vitro* activity of Fosfomycin in urinary isolates.

RESULTS

Three hundred and eighty one ESBL producing urinary isolates were tested against Fosfomycin tromethamine. Out of these, 300 (79%) were from samples of male patients while remaining 81 (21%) from female patients. The age of the patients in ESBL producing urinary isolates ranged from 1 to 85 years, with larger numbers around 50 years of age. Out of 381 ESBL producing Gram negative isolates, 272 (71%) were identified as *Escherichia coli* followed by *Klebsiella* spp. 56 (15%). The frequency of isolation of ESBL urinary isolates is shown in Figure 1.

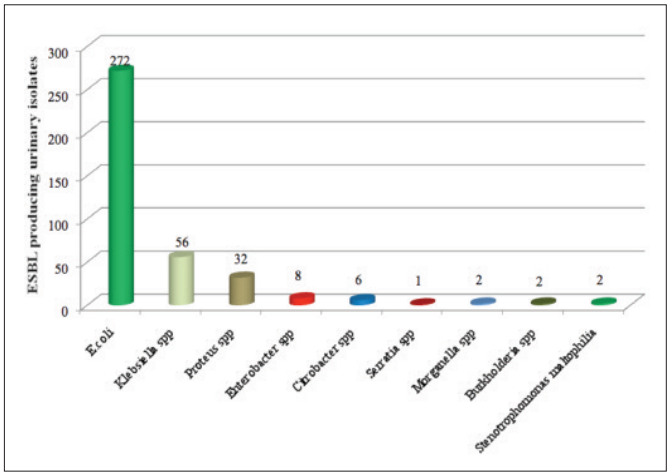

Figure 1: Frequency of ESBL producing urinary isolates (n=381).

Table I: Susceptibility of ESBL producing urinary isolates against Fosfomycin .

	Isolates (n)	Fosfomycin	
		Susceptible	Percentage susceptibility
Total	381	320	84%
E.coli	272	252	93%
Klebsiella spp.	56	36	64%
Proteus spp.	32	16	50%
Enterobacter spp.	8	6	75%
Citrobacter spp.	6	6	100%
Serratia spp.	1	1	100%
Morganella spp.	2	0	0%
Burkholderia spp.	2	2	100%
Stenotrophomonas spp.	2	1	50%

Out of the total ESBL producing urinary isolates, 320 (84%) isolates were susceptible to Fosfomycin. Of the two most common uropathogens, 93% of ESBL producing *E. coli* and 64% of *Klebsiella* spp. were susceptible to Fosfomycin. The susceptibility pattern of all ESBL producing urinary isolates to Fosfomycin is listed in Table I.

DISCUSSION

Urinary Tract Infections (UTIs) are one of the common bacterial infections encountered in clinical practice.[7] These infections are caused by various Gram positive and Gram negative bacteria. UTIs results in a significant morbidity and high medical cost in community. Females are usually more prone to UTIs, most probably because of the anatomical structure i.e. close proximity of urinary tract with anal canal and short urethra.

In this study, 79% of the total ESBL producing urinary isolates were from male patients and 21% from female patients. The most likely reason for this is the fact that the studied population group had male predominance because of the military setup. Majority of the urinary isolates were from patients between 30 to 70 years. The analysis of the data shows that ESBL producing urinary

isolates are encountered more frequently in older age group.

E. coli is the most common cause of community acquired urinary tract infection worldwide.[8] Extended spectrum beta-lactamase producing *E. coli* has emerged as a major cause of urinary tract infection in both communities as well as in hospitalized patients.[9] ESBL producing organisms are resistant to penicillin, cephalosporins and monobactam frequently used to treat most of the community and hospital acquired infections. Irrational and indiscriminate use of anti-microbials in our country as well as lack of effective antibiotic policies at all levels of treatment is the main contributory factors towards growing antimicrobial resistance.

Literature review of various studies has shown that frequency of *E. coli* as uropathogen varies in different regions of the world. *E. coli* has been reported to be as low as 25% in a study of Nigeria to as high as 81% in Nepal in 2012.[10,11] A study performed at Khyber Teaching Hospital, Peshawar, in 2002, reported *E. coli* to be 57% from the culture positive urinary specimens while a study conducted in India revealed 62% of total uropathogens as *E. coli*.[12,13] A study conducted in my institute also revealed that *E. coli* was isolated from 63% of all culture positive urine samples in 2011.[14]

ESBL producing *E. coli* was the most common uropathogen making about 72% of the culture positive urine samples in this study. It was followed by *Klebsiella* spp. 14%, and together these two microorganisms accounted for about 86% of the total ESBL producing urinary isolates. Similar studies from neighbouring countries showed the prevalence of ESBL producing organisms in the range of 6.6 to 68% in India and 40% in Bangladesh.[15] In a study conducted in Iran, the prevalence of ESBL producing *Klebsiella* spp. was 44.5%.[16] Since consecutive sampling was done, the most frequently isolated ESBL producing isolate was *Escherichia coli* followed by *Klebsiella* and *Proteus* spp. Similarly, a high percentage of *Escherichia coli* isolates being recovered from urine samples of outpatient department further raises the suspicion that such isolates may be frequently prevalent in community settings.[17,18]

In this study, majority of the urinary isolates i.e. 84% were susceptible to Fosfomycin. This shows that Fosfomycin is a better oral choice for the treatment of ESBL producing UTIs. The susceptibility results of this study were comparable to a study carried out in Spain in which 93% of the ESBL producing urinary isolates were sensitive to Fosfomycin.[19]

The antimicrobial susceptibility pattern of the two major isolates revealed that 93% of *E. coli* and 64% *Klebsiella* spp. were sensitive to Fosfomycin and these two results are in concordance with the study conducted in Taiwan by Liu *et al*. Their results revealed that 95% of *E. coli* and 57% *Klebsiella* spp. were susceptible to Fosfomycin.[20] In another study, de Cueto *et al*. evaluated 222 *E. coli* and *Klebsiella* isolates for the presence of resistance against Fosfomycin tromethamine. The results of this study revealed that 100% *E. coli* and 61% *Klebsiella* spp. were susceptible to Fosfomycin comparable to this study.[21]

Due to single oral dosage, least side effects, good tissue penetration and safety in pregnancy, Fosfomycin can be considered as an effective empirical treatment option for UTIs particularly against uropathogens resistant to routinely prescribed antimicrobials. The antimicrobial susceptibility results of Fosfomycin against various ESBL producing urinary isolates were very encouraging in this study. A limited data is available regarding the susceptibility of Fosfomycin against various isolates causing UTIs in our setup. Large scale studies should be carried out to check the susceptibility of this antibiotic against various isolates in our country, as this antibiotic can prove effective against MDR urinary isolates.

CONCLUSION

Fosfomycin revealed an excellent *in vitro* activity against ESBL producing urinary isolates. *E. coli* remained the most susceptible organism as 93% of the isolates were sensitive to Fosfomycin. Fosfomycin, with its limited side effects and single oral dosage, is a highly efficient antibiotic which can be considered for the treatment of UTIs. Fosfomycin could be considered as an important oral treatment option in UTIs caused by ESBL producing Gram negative bacteria.

REFERENCES

1. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum β-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. *Indian J Med Microbiol* 2006; **24**:208-11.
2. Paterson DL, Bonomo RA. Extended spectrum β-lactamases: a clinical update. *Clin Microbiol Rev* 2005; **18**:657-86.
3. Pullukcu H, Aydemir S, Isikgoz Tafibakan M, Cilli F, Tunger A, Ulusoy S. Susceptibility of extended spectrum beta-lactamase producing *Escherichia coli* urine isolates to Fosfomycin, Ciprofloxacin, Amikacin, and Trimethoprim-sulfamethoxazole. *Turkish J Med Sci* 2008; **38**:175-80.
4. Lortholary O, Martin C, Potel G, Plesiat P, Nordmann P. Addressing the challenge of extended-spectrum beta-lactamase. *Curr Opin Investig Drugs* 2009; **10**:172-80.
5. Tullio V, Cuffini AM, Banche G, Mandras N, Allizond V, Roana J. Role of Fosfomycin tromethamine in modulating non-specific defence mechanisms in chronic uremic patients towards ESBL-producing *Escherichia coli. Int J Immunopathol Pharmaco* 2008; **21**:153-60.
6. Clinical and Laboratory Standard Institute (CLSI). Performance standard for antimicrobial susceptibility testing; twenty-second informational supplement M100-S22. Wayne: *CLSI;* 2012.

7. Hooton TM. Clinical practice. Uncomplicated urinary tract infection. *N Engl J Med* 2012; **366**:1028-37.
8. Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME. Complicated catheter associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clin Microbiol Rev* 2008; **21**:26-59.
9. Rodrıguez-Bano J, Paterson DL. A change in the epidemiology of infections due to extended-spectrum beta-lactamase-producing organisms. *Clin Infect Dis* 2006; **42**:935-7.
10. Okesola AO, Aroundege DI. Antibiotic resistance pattern of uropathogenic *Esherichia coli* in South West Nigeria. *Afr J Med Sci* 2011; **40**:235-8.
11. Baral P, Neupane S, Marasini BP, Ghimire KR, Sharestha B. High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. *BMC Res Notes* 2012; **5**:38.
12. Gandapur AJ, Hameed A, Asghar AH. Etiology and clinical profile of UTI in children. *J Med Sci* 2003; **11**:59-61.
13. Sood S, Gupta R. Antibiotic resistance pattern of community acquired uropathogens at a tertiary care hospital in Jaipur, Rajasthan. *Indian J Community Med* 2012; **37**:39-44.
14. Amjad A, Mirza IA, Abbasi SA, Farwa U, Sattar A, Qureshi ZA. Spectrum and antimicrobial susceptibility pattern of pathogens causing urinary tract infection: experience in a tertiary care setting. *IDJP* 2011; **20**:297-301.
15. Rahman MM, Haq JA, Hossain MA, Sultana R, Islam F, Islam AH. Prevalence of extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in an urban hospital in Dhaka, Bangladesh. *Int J Antimicrob Agents* 2004; **24**:508-10.
16. Mehrgan H, Rahbar M, Halvaii ZA. High prevalence of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a tertiary care hospital in Tehran, Iran. *J Infect Dev Ctries* 2010; **4**:132-8.
17. Roshan M, Ikram A, Mirza IA, Malik N, Abbasi SA, Alizai SA. Susceptibility pattern of Extended Spectrum β-Lactamase (ESBL) producing Gram negative isolates from various clinical specimens. *J Coll Physicians Surg Pak* 2011; **21**:342-6.
18. Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. *J Antimicrob Chemother* 2005; **56**:52-9.
19. Ko KS, Suh JY, Peck KR, Lee MY, Oh WS, Kwon KT, *et al*. *In vitro* activity of Fosfomycin against ciprofloxacin-resistant or extended-spectrum β-lactamase-producing *Escherichia coli* isolated from urine and blood. *Diagn Microbiol Infect Dis* 2007; **58**:111-5.
20. Liu HY. Antimicrobial susceptibilities of urinary extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* to Fosfomycin and nitrofurantoin in a teaching hospital in Taiwan. *J Microbiol Immunol Infect* 2011; **44**:364-8.
21. de Cueto M, Lo´pez L, Herna´ndez JR, Morillo C, Pascual A. *In vitro* activity of Fosfomycin against extended spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae. Antimicrob Agents Chemother* 2006; **50**:368-70.