Int.j.pathol.2019;17(1):213-216

Original Article

In-vitro Antimicrobial Activity of Fosfomycin Tromethamine against Urinary Extended Spectrum Beta Lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*

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ABSTRACT

Background: *Escherichia coli* and *Klebsiella pneumonia* are the most common infecting organisms in patients with uncomplicated Urinary tract infections. Resistance against most of the commonly used antibiotics is increasing. Fosfomycin tromethamine is still effective in UTIs especially in extended spectrum beta lactamase (ESBL) producing bacteria.

Objective: To determine in vitro antimicrobial activity of fosfomycin tromethamine in extended spectrum beta lactamase producing *E coli* and *Klebsiella pneumoniae* causing urinary tract infections.

Methods: This was a descriptive cross-sectional study carried out at Abbas Institute of Medical Sciences, AIMS, Muzaffarabad from January 2017 to December 2017.

Urine specimens from suspected cases of UTI were inoculated on cysteine lactose electrolyte deficient (CLED) agar and incubated aerobically at 35^{o}C ± 2 for 16-18 hours. After identification of Gram-negative rods; the isolates were screened for ESBL with cefotaxime 30 µg disc by Kirby-Bauer disc diffusion technique. The isolates with cefotaxime zone diameter equal to or less than 27 mm were further confirmed for ESBL by phenotypic confirmatory test applying cefotaxime and clavulanic acid 30/10 µg combination disc (double disc synergy). The inoculums of bacterial suspensions were plated on Mueller-Hinton agar with subsequent application of Fosfomycin tromethamine disc 200 µg. Plates were incubated overnight aerobically. Subsequently, resulting zone diameters were interpreted according to Clinical &Laboratory Standards Institute guidelines.

Results: Out of 84 ESBL producing Gram negative isolates, 81% (n=68) were identified as *E coli* and 19% (n= 16) as *Klebsiella peumoniae*. The age of the patients in ESBL producing urinary isolates ranged from 1 to 80 years, with larger numbers around 60 years of age. According to the study results, 82% (n=79) of E. coli and 75% (n =18) of *Klebsiella peumoniae* were susceptible to Fosfomycin tromethamine.

Conclusion: Fosfomycin has shown good sensitivity against ESBL producing *E coli* and *Klebsiella pneumoniae* emphasizing its role to be used in empirical therapy for simple lower urinary tract infections caused by these uropathogens.

Keywords: *Escherichia coli,* Extended-spectrum beta-lactamase, ESBL, Fosfomycin, *Klebsiella pneumoniae*

Introduction

Urinary tract infections (UTIs) are very common infectious diseases in clinical practice. UTIs caused by extended spectrum beta lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumonia*e have become a major concern because of limited therapeutic options.[1]

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ESBL producing isolates are resistant to all penicillins, cephalosporins, and aztreonam.[2]

These organisms can also develop co-resistance to other antimicrobials such as fluoroquinolones, co-trimoxazole, and aminoglycosides commonly used for the treatment urinary tract infections. Fosfomycin tromethamine is a stable salt of fosfomycin which is effective against various Gram positive and Gram-negative bacteria causing simple lower urinary tract infections (UTIs) in both sexes.[3]

The rationale of the study was to establish a susceptibility pattern of Fosfomycin against ESBL

producing *Escherichia coli* and *Klebsiella pneumoniae* in our set up as there is limited data available in Pakistan regarding the susceptibility of such uropathogens to Fosfomycin tromethamine.

Methods

This descriptive cross- sectional Study was carried out in Abbas Institute of Medical Sciences, AIMS, Muzaffarabad for the period of one year from January 2017 to December 2017.

Urine specimens from suspected cases of UTI were inoculated on cysteine lactose electrolyte deficient (CLED) agar and incubated aerobically at 35^{o}C ± 2 for 16-18 hours. After identification of Gram-negative rods; the isolates were screened for ESBL with cefotaxime 30 µg disc by Kirby-Bauer disc diffusion technique. The isolates with cefotaxime zone diameter equal to or less than 27 mm were further confirmed for ESBL by phenotypic confirmatory test applying cefotaxime and clavulanic acid 30/10 µg combination disc (double disc synergy). The inoculums of bacterial suspensions were plated on Mueller-Hinton agar with subsequent application of fosfomycin tromethamine disc 200 µg. Plates were incubated overnight aerobically. Subsequently, resulting zone diameters were interpreted according to Clinical &Laboratory Standards Institute (CLSI) guidelines

Results

In this study, a total of 84 ESBL producing gram negative bacteria were identified and tested against fosfomycin. All these isolates were obtained from 84 patients, one isolate from each patient. Among the 84 patients, 52 % (n=44) were males and 48 % (n=40) of the patients were females. The age of the patients in ESBL producing urinary isolates ranged from 1 to 80 years, with larger numbers around 60 years of age. From 84 ESBL producing Gram negative isolates, 81% (n=68) were identified as *E coli* and 19% (n= 16) as *Klebsiella pneumoniae* (Fig 1).

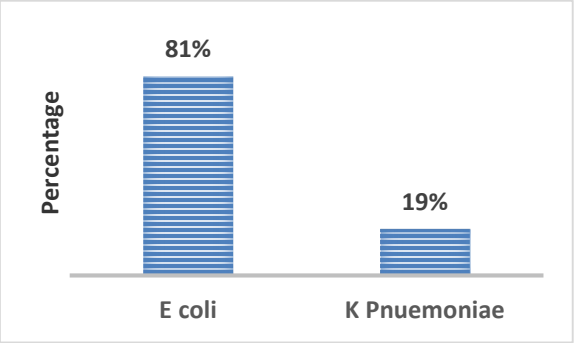

Figure 1. Distribution of common ESBL Producing uropathogens (n= 84)

The frequency of ESBL producing *E. coli* and *Klebsiella pneumoniae* in outdoor and indoor patient's urinary samples was 64% and 36%, respectively.

In our study 82% (n=79) of *E. coli* and 75% (n =18) of *Klebsiella pneumoniae* were found to be susceptible to fosfomycin tromethamine (Table 1).

Table 1. Sensitivity pattern of urinary ESBL *E coli* & *K pnuemoniae* against Fosfomycin

Types of ESBL Producers	No. of isolates	No. of susceptible isolates	Percentage susceptibility
E coli	68	56	82%
K pneumoniae	16	12	75%

ESBL, extended spectrum beta lactamase; No., number

Discussion

Urinary tract infections (UTIs) are very common among general population and they are also one of the commonest healthcare associated infections caused by various gram positive and gram-negative bacteria.[4] Inappropriate and irrational use of antibiotics has led to emergence of multidrug resistance in uropathogens. Members of Enterobacteriaceae especially *Escherichia coli* and *Klebsiella pneumoniae* are the commonest bacterial isolates in UTIs.[5] This finding is in complete agreement with our study results where these two isolates being the most common ones i.e., *E coli* (81%) with n=68 followed by *K. pneumoniae* (19%) with n=16.

Extended spectrum beta lactamases (ESBL) are the enzymes mainly produced by Enterobacteriaceae and other gram-negative bacteria. ESBLs confer resistance to all the penicillins, cephalosporins and aztreonam but they are sensitive to carbapenems.[6] Our study included 84 of the ESBL producing gram negative isolates.

Infections involving ESBL producing Enterobacteriaceae are associated with significant

morbidity and mortality. Fosfomycin has bactericidal properties against various Gram positive (staphylococcus including methicillin resistant *Staphylococcus aureus* MRSA, *Staphylococcus saprophyticus*, vancomycin resistant *enterococci* VRE) and Gram negative (*Escherichia coli, Klebsiella* spp) bacteria causing UTIs.[7]

In our study, frequency of ESBL among in-patients and out-patients was 64% (n= 54) and 36%, respectively. The occurrence of ESBL in in-patients is less than out-patients contrary to the findings of Kumar et al.[8] This is because ESBL producers are indeed more problem in the hospitals than in communities. Frequent use of urinary catheters and poor infection control practices may be the contributory factors for such infections.

ESBL producing *E. coli* isolates were wide spread among both in-patients and out-patients. Our study results have shown the higher cases of ESBL producers in indoor patients than the OPD. Our findings are comparable to the findings of K Danish et al.[9]

According to our study results, 82% (n=79) of E. coli and 75% (n =18) of *Klebsiella peumoniae* were susceptible to Fosfomycin tromethamine. In the review by Falagas et al, fosfomycinhas shown good activity against *E coli* and the susceptibilities of ESBL-KP isolates to fosfomycin were 76.7% to 100%.[10]Our study showed the comparable susceptibilities, and the activity of fosfomycin against ESBL-*E coli* and ESBL-*Klebsiella pneumonia* isolates remained equally satisfactory.

Fosfomycin is an effective for the treatment of lower UTIs due to ESBL-producing members of the Enterobacteriaceae.[11]

The present study was undertaken to determine the pattern of antibiotic susceptibility of those gram negative bacteria which produce a special type of beta lactamase, ESBL i.e., extended spectrum beta lactamase against fosfomycin tromethamine. As the gram negative bacteria are the most common culprits in causation of UTIs, the tendency of the infections due to these resistant bacteria is rising alarmingly. As these ESBL producing isolates are resistant to commonly prescribed antibiotics some changes in empirical therapy of UTIs is needed.

According to literature review, fosfomycin is an effective, safe and convenient therapeutic option for the treatment of uncomplicated UTIs during pregnancy.[12,13,14]

According to the latest guidelines endorsed by the Infectious Diseases Society of America (IDSA) and the European Society for Clinical Microbiology and Infectious Diseases (ESCMID); fosfomycin is recommended as one of the first-line agents for treatment of urinary tract infections caused by ESBL producing uropathogens.[15]

Due to oral dosage, least side effects, good tissue penetration and safety in pregnancy, fosfomycin can be considered as an effective empirical treatment option for lower UTIs. This study has only one limitation. In this study, the susceptibility of fosfomycin was determined only by phenotypic disk diffusion method. We did not process for MICs (agar dilution or broth microdilution). De Cuetoet alreported greater resistance to fosfomycin for *K pneumoniae* by disk diffusion method than agar dilution method, whereas no significant difference has been found for *E coli*.[16]

Conclusion

Fosfomycin has shown good sensitivity against ESBL producing E coli and *Klebsiella pneumoniae* emphasizing its role to be used in empirical therapy for simple lower urinary tract infections caused by these uropathogens.

Conflict of Interest: Authors declare no conflict of interest.

Grant Support & Financial Disclosures: None.

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HISTORY	
Date Received:	01-03-2019
Date sent for Reviewer:	14-03-2019
Date Received Reviewer's Comments:	08-05-2019
Date Received Revised Manuscript:	15-05-2019
Date Accepted:	16-05-2019

Authors' Contribution

MAK: Study conception, study conduction, data analysis and manuscript writing

MS & RR: Data analysis and manuscript writing