

## INCIDENCE OF FOSFOMYCIN ROMETHAMINE AGAINST EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING URINARY TRACT BACTERIA

Dr. Krishna Kumar Patel,<sup>1</sup> Dr. Sarita Patel,<sup>2</sup> Dr. Chandrakishor Chandrawanshi,<sup>3</sup> Vishal Kahre,<sup>4</sup> Medha Singh<sup>5</sup>

<sup>1</sup>Department of Microbiology, Government Science College, Ambikapur (C. G.), India  
Email-drkrishnabsp@gmail.com

<sup>2</sup>Department of Zoology, DLS (PG) College Bilaspur, Bilaspur (C. G.) India

<sup>3</sup>Department of Chemistry, Government Science College, Ambikapur (C. G.), India

<sup>4</sup>C.G. Bio-fuel Development Authority (CBDA) Department of Energy, Raipur (C. G.), India

<sup>5</sup>Department of Agriculture, Government of Chhattisgarh, Raipur (C. G.), India

### ABSTRACT

To determine the incidence of Fosfomycin tromethamine against extended spectrum beta-lactamase producing uro-pathogens at Microbiology Laboratory at Chhattisgarh Institute of Medical Sciences, Bilaspur, Chhattisgarh, India from May 2014 to May 2015. 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). 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% *E. gergoviae*. 100% *Enterobacter aerogenes* and 50% *Stenotrophomonas maltophilia* were susceptible to this chemical compound. Fosfomycin showed excellent effectiveness to most of the common ESBL producing bacteria such as *E. coli*, *Klebsiella*, *Enterobacter* and *Proteus* spp.

### INTRODUCTION

Beta-lactam antimicrobial agents are used profusely in the treatment of bacterial infections. Resistance to  $\beta$ -lactam antibiotics among clinical isolates, especially among gram-negative bacteria is most often due to the production of  $\beta$ -lactamases [1]. These enzymes are numerous and they mutate continuously in response to heavy pressure of antibiotic use and have lead to the development of extended spectrum  $\beta$ -lactamases (ESBLs). Many of these ESBLs have evolved from the  $\beta$ -lactamases that are widely distributed among the Enterobacteriaceae [2-4].

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

	Isolates (n)	Fosfomycin	
		Susceptibility	Percentage
<i>Escherichia coli</i>	272	252	93%
<i>Klebsiella</i> spp.	56	36	64%
<i>Proteus</i> spp.	32	16	50%
<i>Enterobacter cloacae</i>	8	6	75%
<i>Citrobacter</i> spp.	6	6	100%
<i>Enterobacter aerogenes</i>	1	1	100%
<i>Enterobacter sakazakii</i>	2	0	0%
<i>Enterobacter gergoviae</i>	2	2	100%
<i>Stenotrophomonas</i> spp.	2	1	50%
<b>Total</b>	<b>381</b>	<b>320</b>	<b>84%</b>

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 [5]. 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[6]. 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 [7]. Fosfomycin is gaining importance to treat ESBL producing urinary isolates, because resistance against commonly used oral antimicrobials is increasing [8]. There is also an evidence that antimicrobial activity of Fosfomycin against ESBLs may be accompanied by an immunomodulating activity [9]. 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.

### MATERIAL AND METHODS

A total of 381 ESBL producing Gram negative bacilli isolated from urine specimens received at the Clinical Microbiology Laboratory at Chhattisgarh Institute of Medical Sciences, Bilaspur, Chhattisgarh, India. Urine samples were collected from patients suspected to have urinary tract infection at the discretion of the provider. These included clean catch midstream urine, catheter, suprapubic and nephrostomy samples. Urine (10 µl) was inoculated onto Mac Conkey Agar medium (M 081B, Hi-Media Laboratories, Mumbai, India) for culture and sensitivity were included in this study. Permission was taken from the Institutional © K. K. Patel et al. J. Sci. Res. Adv. Vol. 3, No. 1, 2016, 175-179| 176

Ethical and Research Committee for research purpose. Nonprobability 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. Urine specimens were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar (Hi-Media Laboratories, Mumbai, India) and incubated aerobically at 37°C 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 (Hi-Media, Mumbai) by Kirby-Bauer disc diffusion technique according to Clinical Laboratory Standards Institute (CLSI) guidelines.<sup>10</sup> 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) [10]. Prepared Mueller-Hinton (Hi-Media Laboratories, Mumbai, India) were inoculated with the test organism (0.5McFarland 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 37°C, an increased zone diameter of 35 mm for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirmed the isolate as an ESBL producer[10].

According to CLSI guidelines, inoculum of bacterial suspension (0.5 McFarland standard) was inoculated on Mueller-Hinton agar (Hi-Media Laboratories, Mumbai, India) followed by application of Fosfomycin tromethamine disc of 200µg (Hi-Media Laboratories, Mumbai, India). The plates were incubated aerobically at 37°C for 18-24 hours. Zone of inhibition around the discs were interpreted as per CLSI guidelines [10].

## 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%). Out of the total ESBL producing urinary isolates, 320 (84%) isolates were susceptible to Fosfomycin. Of the two most common uro-pathogens, 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 [11]. 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 [12]. 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 [13]. 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 antimicrobials 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 uro-pathogen 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[14-15]. 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*. [16-17] A study conducted in my institute also revealed that *E. coli* was isolated from 63% of all culture positive urine samples in 2011[18]. 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 neighboring countries showed the prevalence of ESBL producing organisms in the range of 6.6 to 68% in India and 40% in Bangladesh[19]. 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 [20-21]. 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 [22]. 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 [23]. In another study, de Cueto *et al*. evaluated *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 [24]. 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 uro-pathogens 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.

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