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Evaluation of in Vitro Activity of Fosfomycin Against Uropathgens of Enterobacteriaceae Isolates in the Era of Multidrug Resistance

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ABSTRACT

The commonly isolated uropathogen are increasingly showing multidrug resistance against routinely used antimicrobial agents and limited therapeutic options for clinicians. Reevaluation of Fosfomycin for therapeutic management of UTI caused by MDR uropathogen is needed as Fosfomycin is broad spectrum antibiotic and has unique property of no cross resistance since it does not share structural similarity with other antibiotics. The present study was conducted to evaluate in vitro activity of Fosfomycin against Extended spectrum Beta–Lactamases (ESBL) producing isolates of Enterobacteriaceae isolated from urine samples. In this prospective study conducted over a period of 1 month , total 568 urine samples were processed. The Enterobacteriaceae isolates were subjected to Kirby – Bauer disk diffusion test to Fosfomycin along with other routinely used urinary antibiotics. . Isolates showing resistance to Cefotaxime (30 µg,) were then tested with phenotypic confirmatory combined disk test using Ceftazidime (30µg) and Ceftazidime -clavulanic acid disc (30/10 µg,) for ESBL production. Out of 568 urine samples, 131 (23.06%) showed significant growth of pathogen. Gram Negative isolates from Enterobacteriaceae were the predominant pathogen (n=95,) 72.51%.. Amongst Enterobacteriaceae. ESBL production was seen in 53.6% (n= 51). Reduced susceptibility was noted for cefotaxime (24.48%), ceftazidime (28.57%), Norfloxacin (30.61%) and Cotrimoxazole (18.36%) in susceptibility testing for E.coli.. Fosfomycin showed susceptibility against *Escherichia coli* 89.79% (n = 44). and Klebsiella pneumoniae Fosfomycin susceptibility was 65% (n=26). Nitrofurantoin showed susceptibility for Escherichia coli 71.22 % and Klebsiella pneumoniae 75 % of isolates. Fosfomycin has shown excellent in vitro susceptibility against uropathogenic Enterobacteriaceae isolates. These in vitro findings suggests that Fosfomycin as better therapeutic option for treating uncomplicated UTI.

INTRODUCTION

Urinary tract infections (UTI) are amongst the commonly occurring human infections. It is estimated that at least 50% of women experiences one UTI in their life time and at least 25 % experiences recurrent UTI[1]. The commonly isolated uropathogen are increasingly showing multidrug resistance against routinely used antimicrobial agents for therapeutic management of UTI like Nitrofurantoin, Trimethoprim–Sulfamethoxazole, Fluroquinolones, second and Third generation Cephalosporins has limited therapeutic options for clinicians[2]. Gram negative isolates especially Enterobacteriaceae are commonly responsible for majority of UTI episodes. Reevaluation of non-traditional antibiotics has as become even more necessary as the challenge of increase in multidrug resistance amongst uropathogen is on the rise[3]. Fosfomycin is a broad spectrum, bactericidal antibiotic. Initially identified and reported from various strains of Streptomyces in 1969 in Spain. It is member of epoxide group of antibiotics. Fosfomycin has been pointed out by various studies as potential alternative for the therapeutic management of multidrug resistant infection[4,5,9]. However, there are limited studies related to Indian scenario regarding in vitro activity of Fosfomycin against commonly isolated urinary tract pathogens. Also, Fosfomycin is not routinely used antibiotic in our hospital setting. Hence the study is conducted to study the microorganisms causing urinary tract infections and their antibiotic sensitivity pattern and also to evaluate the susceptibility pattern of Fosfomycin against urinary tract pathogens especially against ESBL producers Enterobacteriaceae isolates.

MATERIAL AND METHODS

The study was conducted in Index medical college Hospital and research centre, Indore. Institutional Ethics Committee (IEC) permission was taken before study (IEC reference number: IMCHRC/IEC/2017/75 dated 18.11.2017). The study was conducted from 1/12/2017 to 31/12/2017. The study included 568 Consecutive, non-duplicate Midstream Urine samples collected from the patients of both sexes irrespective of age groups with signs and symptoms of Urinary tract infection. For Semiquantitative Urine culture, 4 mm calibrated nichrome loop was used to inoculate Cystine- Lactose Electrolyte Deficient (CLED) Agar. Inoculated plates were incubated aerobically for 18-24 hours at 37°C. Growth on CLED Agar was assessed for significant bacteriuria with colony forming units $\geq 10^5$ /mL of pure growth of single isolate. Colonies showing only gram-negative bacilli on gram stain further identified by conventional biochemical tests as Enterobacteriaceae isolates were included in study[6,10].

Antimicrobial susceptibility testing of all isolates was performed as per Kirby – Bauer disk diffusion test on Mueller Hinton agar as per CLSI guidelines[7]. Antimicrobial agents included for testing were (Drug concentration in µg) for Gram negative bacilli :Cefotaxime(30), Ceftazidime (30), Amikacin(30), Gentamicin(10), , Norfloxacin(5), Nitrofurantoin (300), Cotrimoxazole (1.25/23.75), Fosfomycin (200) and ceftazidime/clavulanic acid (30/10), Imipenem(10) and Colistin (10).

Detection of Extended spectrum ß lactamases (ESBL) Organism showing resistance to Cefotaxime (30 µg,) zone size ≤ 27 mm were further tested for ESBL production. Identification of ESBL was done by Phenotypic confirmatory combined disk test using Ceftazidime (30µg) and Ceftazidime -clavulanic acid disc (30/10 µg,). The test was considered positive when there was an increase in growth inhibitory zone by 5 mm or greater around Ceftazidime-clavulanic acid disc (30/10 µg,) than that of the diameter around the disk containing Ceftazidime (30 µg,) alone[8].

Fosfomycin Zone Size Interpretation: Currently, CLSI-approved disk diffusion susceptibility breakpoints for Fosfomycin exist only for Escherichia coli and E. faecalis, with a zone diameter ≤12 mm considered resistant, > = 16 mm as sensitive, 13-15 mm as intermediate., and are approved only for testing isolates from urinary tract infections. CLSI zone diameter breakpoints for E.coli for disc diffusion test results were used to interpret Fosfomycin disc zone for other urinary isolates of enterobacteriaceae[8,11,12].

RESULTS AND DISCUSSIONS

A total of 568 urine samples with clinical suspicion of UTI were received in laboratory during study duration. Of 568 urine samples, 131 (23.06%) showed significant growth of pathogen and the rest were either sterile or showed contamination with growth of more than three different type of bacteria. Majority of the isolates were from female patients (n = 99), 76%. Gram Negative isolates from Enterobacteriaceae were the predominant pathogen (n = 95,) 72.51%. Distribution of isolates is shown in (Table 1). In Enterobacteriaceae family (n = 95) , Escherichia coli (n = 49) 37. 40 % was predominant pathogen followed Klebsiella pneumoniae (n = 40) 30.53%.

Antibiotic Susceptibility pattern among Escherichia coli and Klebsiella pneumoniae is shown in (Table 2) which shows none of the isolates in the present study was resistant to Imipenem, Colistin. For Escherichia coli reduced susceptibility was noted for cefotaxime (24.48%), ceftazidime (28.57%), Norfloxacin (30.61%) and Cotrimoxazole (18.36%). For Klebsiella pneumoniae reduced susceptibility was noted for cefotaxime (30%), ceftazidime (30%), Norfloxacin

Table 1: Distribution of urinary pathogens (n = 131)

Species	Number	Percentages
Escherichia coli	49	37.40
Klebsiella pneumoniae	40	30.53
Pseudomonas aeruginosa	18	13.74
Citrobacter species	4	3.05
Proteus species	2	1.52
Acinetobacter species	1	0.76
Staphylococcus aureus	12	9.16
Enterococcus faecalis	5	3.81

Table 2: Antibiotics susceptibility pattern amongst Escherichia coli and Klebsiella pneumoniae

Antibiotics	Escherichia coli (n= 49)	Klebsiella pneumonaie (n= 40)
Cefotaxime	12 (24.48 %)	12 (30%)
Ceftazidime	14(28.57%)	12 (30%)
Amikacin	32 (65.30%)	25 (62.5%)
Gentamicin	28 (57.14 %)	24 (60)
Norfloxacin	15 (30.61 %)	14 (35)
Nitrofurantoin	35 (71.22%)	30 (75%)
Cotrimoxazole	9 (18.36%)	9 (22.5 %)
Fosfomycin	44 (89.79%)	26 (65 %)
Ceftazidime/ clavulanic acid	26 (53.06)	12 (30 %)
Imipenem	49 (100%)	40 (100%)
Colistin	49 (100%)	40 (100%)

Table 3: Shows Fosfomycin susceptibility amongst ESBL producers

Fosfomycin	ESBL Escherichia coli (n=32)	ESBL Klebsiella pneumoniae (n=19)
Sensitive	29 (90.62 %)	16 (84.21 %)
Resistant	03 (9.37%)	03 (15.78 %)

(35%), Ceftazidime /Clavulanic acid (30%) and Cotrimoxazole (22.5%).

Fosfomycin showed better susceptibility against Escherichia coli 89.79% (n = 44). In Klebsiella pneumoniae Fosfomycin susceptibility was 65% (n=26). Nitrofurantoin showed susceptibility for Escherichia coli 71.22 % (n=35) and Klebsiella pneumoniae 75 % (n = 30) of isolates.

Amongst Enterobacteriaceae ESBL production was seen in 53.6% (n= 51) Which included ESBL E.coli 62.74% (n= 32) and ESBL Klebsiella pneumoniae 37.25% (n= 19). Fosfomycin susceptibility was seen in 90.62% (n=29) in ESBL Escherichia coli and to lesser extent in ESBL klebsiella pneumoniae 84.21% (n = 16) Table 3.

Increasing trends of ESBL producers amongst Enterobacteriaceae has limited therapeutic options. There is an urgent need to for a new antimicrobial agent or to review a old existing antimicrobial agent. Fosfomycin has broad spectrum of action and also oral dosing regimen which makes it suitable for the treatment of multidrug resistant UTI isolates from OPD patients. In the present study we tried to evaluate the sensitivity pattern of certain old antibiotics like Fosfomycin against Uropathogen especially with respect to ESBL producing MDR uropathogens. In our study E.coli 37.40% (n = 49) was the most common pathogen followed by Klebsiella pneumoniae 30.53% (n = 40) and Pseudomonas aeruginosa 13.74% (n = 18) , Chinmoy Sahu *et al.*[13] has reported similar findings previously. In our study none of the isolates of Enterobacteriaceae were resistant to Imipenem and Colistin , good invitro activity of Carbapenems and colistin has also been reported by Sultan *et al.*[14] and Chinmoy Sahu *et al.*[13]. Since Carbapenems and Colistin are Parenteral antibiotics hence choice of oral antibiotics for the treatment of UTI needs to be evaluated. In the present we found significantly lower susceptibility amongst Escherichia coli isolates for oral antimicrobial agents Cotrimoxazole (18.36%) and Norfloxacin (30.61%) which concur with the results of previous studies[14,15]. while other oral antibiotics Nitrofurantoin (71.22%) showed better susceptibility, similar findings has also been reported by Sonali Bhattar *et al.*[2] where they have reported 71% susceptibility for Nitrofurantoin amongst ESBL producing Enterobacteriaceae strain. In our study ESBL Production was seen in 53% of isolates. Variation from 21.8% to 64.8% in prevalence of ESBL producing isolates has been reported by previous authors[5,8], one the reasons for variation in prevalence of ESBL producing isolates is due to difference of usage of antibiotics in different areas. Fosfomycin susceptibility 90.62% (n= 29) amongst ESBL E.coli producing strains observed in our study was similar to Patel B *et al*[17] which has reported Fosfomycin susceptibility 92% amongst ESBL producing Enterobacteriaceae. However, Sultan *et al*[13] had reported 100 % susceptibility amongst ESBL producing Enterobacteriaceae. In present study, Fosfomycin susceptibility against ESBL Klebsiella pneumoniae was 84.21% similar view has been expressed by Patwardhan V and Singh S[19] they had reported susceptibility against ESBL Klebsiella 92.1%. Demonstration of high in- vitro activity by Fosfomycin against drug resistant E. coli and K. pneumoniae has reported by Gupta V *et al* and De Cueto M *et al.*[16,20]. Fosfomycin can be good treatment option, as is available orally hence less hospitalization required, and it can be used in OPD patients. Also, it has rare adverse reactions which may develop in 1-8% of all patients like diarrhea, nausea, vomiting, skin rash, vaginitis, headache, chills and asthenia[22]. Also it has been reported that Fosfomycin penetrates various tissues and achieves minimum inhibitory concentration needed to inhibit the growth of most pathogen due to its low molecular weight and relatively long half – life (mean half -SD, 5.7 -2.8h)[23]. The low resistance to Fosfomycin observed in our study suggests that Fosfomycin can be used in treatment of MDR Gram Negative uropathigens of enterobacteriaceae. However further studies are needed as increasing resistance among ESBL producing Enterobacteriaceae isolates due to Fosfomycin resistance determinants are been reported[21,24].

CONCLUSIONS

In our study Fosfomycin was the only oral antibiotic which had shown substantial in vitro antimicrobial activity against Enterobacteriaceae

isolates. Fosfomycin was sensitive against ESBL producing isolates. Therefore, it can be used as possible alternative to currently used first line antibiotics for the treatment of uncomplicated UTI.

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