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IN-VITRO EFFICACY OF FOSFOMYCIN AGAINST E. COLI & ENTEROCOCCUS SPECIES ISOLATED FROM URINE SAMPLES

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Abstract

Background: Fosfomycin is one of the most prescribed antibiotics for treatment of urinary tract infections among outpatients. It is one of the most effective antibiotics against multi drug resistant pathogen causing UTIs. There is a need of more data for minimal inhibitory concentration of fosfomycin against most common uropathogens. **Materials and Methods:** Midstream urine was collected from 2725 outpatients suspected for UTIs and streaked semi-quantitatively of Cystine Lactose Electrolyte Deficient agar and incubated overnight. The significant growth of uropathogens was tested for identification and antibiotic susceptibility by disc diffusion method. Agar dilution was performed to determine the MIC of fosfomycin trometamol against isolated pathogens. **Result:** Out of 2725 samples, significant uropathogens were isolated in 365 samples. The most common isolate was E. coli (n=263) followed by Enterococcus species (n=38). Fosfomycin was recorded as most susceptible antibiotic in-vitro. Only four E. coli were found to be resistant to fosfomycin. Among 264 E. coli strains, 205 (77.65%) had a MIC value under MIC 16 µg/ml, 132 µg/ml was observed in three and >256 µg/ml in one strain. All the Enterococcus species were found to have a MIC value under 32µg/ml. **Conclusion:** Fosfomycin is the most effective antibiotic against most uropathogens with a low MIC value. Considering its in-vitro effectiveness against common uropathogens, fosfomycin deserves to be used in the empirical treatment of urinary tract infections in outpatients.

INTRODUCTION

Urinary tract Infections (UTIs) are the leading microbial disease which involves people of all age groups.[1] It is caused by wide range of pathogens among which Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus are most common bacteria. UTIs afflict around 150 million people worldwide every year.[2] E. coli accounts for approximately 80% of community-acquired uncomplicated UTIs while Enterococcus species cause around 15% of healthcare-associated UTIs, therefore these pathogens should be targeted when choosing empirical antibiotics.[3,4] Fosfomycin remains a reliable treatment choice for uncomplicated UTIs due to several advantages, including wide spectrum nature, single dose administration and persistent urine concentrations that rapidly kill bacteria, reducing the possibility of mutant selection. Fosfomycin, along with other antibiotics, is used intravenously to treat nosocomial infections caused by MDR bacteria. Intravenous fosfomycin has been available in India since 2016.[5] There are not many published data on minimal inhibitory concentration of Fosfomycin against uropathogens from India. Thus, the present prospective cross-sectional investigation was carried out to determine the Minimal Inhibitory Concentration of Fosfomycin against uropathogens.

MATERIALS AND METHODS

Study Setting: The current study was conducted in a tertiary care hospital situated in Haldwani, foothills of Kumaun region of Uttarakhand. Written permission was obtained from Institutional Human Ethical Committee. All the patients present with sign and symptoms suggestive for lower UTI, who were above 16 years of age, visiting OPDs of Sushila Tiwari Government Hospital, Haldwani were included in the study after getting the consent and filled proforma.

Sample Collection & Transportation: Midstream clean-catch urine samples were collected in sterile urine containers. Samples were transported to Microbiology laboratory and processed without any delay. In case of delay, samples were kept at 4-8°C.

Sample processing: Wet mount preparation was made directly from the samples and observed under light microscope for presence on pus cells, epithelial cells, bacteria, etc. Sample were streaked semi-quantitatively on cystine lactose electrolyte deficient (CLED) agar and incubated at 37°C for overnight. An uropathogen was defined as a known single type of etiological agent growth with a colony count of $\geq 10^5$ colony forming units per ml of urine and responsible for sign and symptoms of UTIs. Any suggestive growth was further tested for Gram's Staining and Biochemical identifications as per standard laboratory protocol. They were further subjected to Antibiotic susceptibility testing by the Kirby-Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines, 2016.[6]

Agar Dilution Method: The isolates were subjected to minimal inhibitory concentration (MIC) testing against Fosfomycin trometamol by agar dilution method on Muller Hinton agar (HiMedia, Mumbai) supplemented with 25 µg/ml of glucose-6- phosphate to reduce the rate of false resistance as per CLSI guidelines 2016. Fosfomycin trometamol was used as fosirol powder (Cipla Ltd.). Muller Hinton Agar with different concentrations of fosfomycin (2, 4, 8, 16, 32, 64, 128, 256 µg/ml) was used. After adjusting the turbidity with 0.5 McFarland standards, 10 µl of bacterial culture of test organism was spot inoculated on MHA plate with different concentrations of fosfomycin. Plates were incubated overnight at 37°C and examined for growth. The MIC values obtained were interpreted according to the following criteria: [Susceptible (S) ≤64µg/ml, Intermediate (I)-128µg/ml, Resistant (R) ≥256µg/ml [6]. The control strains used were *E. coli* ATCC 25922 and *E. faecalis* ATCC 51299.

Statistical Analysis: The data collected throughout the study was recorded and stored in a Microsoft Excel spreadsheet, and the results were analyzed using Epi Info software version 7.2.3.1. The Chi square test was performed to examine the different variables and determine any statistical significance. A p-value of 0.5 or below was regarded as statistically significant.

RESULTS

A total of 2725 patients volunteered in the study out of which 1027 had no uropathogens isolated from their urine. Insignificant numbers of colonies were recorded in 828 urine specimens while multiple organisms grown in 506 specimens. Significant uropathogens grown in 365 samples in which a total of 327 Gram- negative bacilli and 38 Enterococcus species were isolated. The maximum cases were of E. coli (n=263) followed by Enterococcus species (n=38), Klebsiella species (n=29) and Proteus species (n=11). (Table 1.)

Fosfomycin, followed by Nitrofurantoin were recorded as most sensitive antibiotics in- vitro against E. coli by Kirby-Bauer's Disc Diffusion test. There were only two E. coli strains (1.35%) which were recorded as resistant against Fosfomycin with zone ≤12 mm in diameter. Nitrofurantoin resistance was observed in eight E. coli strains (5.37%). Fluroquinolones exhibited the highest prevalence of resistance (67.79%), followed by cefazolin (65.78%) and folate inhibitors (64.43%). (Table 2).

By Fosfomycin Agar dilution method MIC were obtained of each E. coli strain. There were two E. coli strains which had an MIC of 128 µg/ ml while one with MIC ≥256 µg/ ml. (Graph 1) The MIC of 128 µg/ ml was interpreted as Intermediate and the MIC of ≥256 µg/ ml as Resistance (as per CLSI guidelines 2016).

Enterococcus spp. showed 100 % sensitivity towards fosfomycin & nitrofurantoin and maximum resistance against fluoroquinolones. (Table 3)

The MIC of Fosfomycin was recorded between 4 to 32 by agar dilution method. More than 40% (n=10) of Enterococcal isolates exhibited MIC of 32 µg/ml. [Graph 1]

Table 1: Organism isolated from UTI patients.

Organisms	Total No. (n=365)	Percentage
E. coli	264	72.32%
Enterococcus spp.	38	10.43%
Klebsiella spp.	29	7.96%
Proteus spp.	11	3.02%
Citrobacter spp.	07	1.92%
Enterobacter spp.	07	1.92%
Pseudomonas spp.	07	1.92%
Acinetobacter spp.	02	0.54%

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Table 2: Organism- wise antibiotic sensitivity pattern by Kirby- Bauer disc-diffusion method among Gram-negative uropathogens [n =327]

Organisms [n = 327]	FOS n [%]	NIT n [%]	COT n [%]	CZ n [%]	FQ n [%]
E. coli [n = 264]	260 [98.48]	250 [94.69]	90 [34]	77 [29.16]	76 [28.78]
Klebsiella spp. [n = 29]	26 [89.65]	20 [68.96]	16 [55.17]	15 [51.72]	15 [51.72]
Proteus spp. [n = 11]	05 [45.45]	*NA	02 [18.18]	02 [18.18]	07 [63.63]
Citrobacter spp. [n = 07]	07 [100]	06 [85.71]	05 [71.42]	04 [57.14]	04 [57.14]
Enterobacter spp. [n = 07]	07 [100]	05 [71.42]	03 [42.85]	01 [14.28]	05 [71.42]
Pseudomonas spp. [n = 07]	06 [85.71]	02 [28.57]	02 [28.57]	02 [28.57]	05 [71.42]
Acinetobacter spp. [n = 02]	02 [100]	00 [00]	02 [100]	00 [00]	01 [50]

*NA – Not Applied

FOS- Fosfomycin, NIT- Nitrofurantoin, COT- Co-trimoxazole, CZ- Cefazoline, FQ-Fluoroquinolones

Table 3: Antibiotic sensitivity pattern by Kirby- Bauer disc-diffusion method in Enterococcus spp. [n =38]

Antibiotics	Sensitive	Intermediate	Resistant
FOS n(%)	38 (100)	00 (00)	00 (00)
NIT n(%)	38 (100)	00 (00)	00 (00)
AMP n(%)	26 (68)	00 (00)	05 (13)
FQ n(%)	10 (26)	00 (00)	28 (73)
MI n(%)	8 (21)	09 (23)	21 (55)
LZ n(%)	35 (92)	00 (00)	03 (07)
E n(%)	4 (10)	00 (00)	06 (15)

FOS- Fosfomycin, NIT- Nitrofurantoin, AMP- Ampicillin, FQ- Fluoroquinolones, MI- Minocycline, LZ- Linezolid, E- Erythromycin

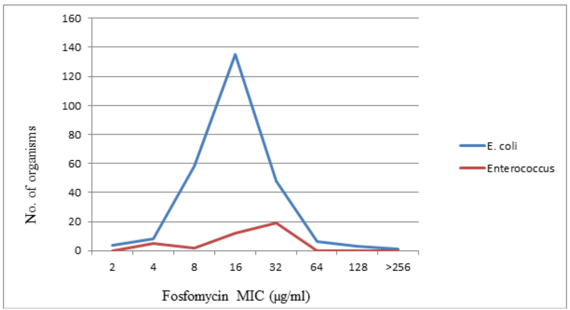

Figure 1: Distribution of fosfomycin MIC by agar dilution method among uropathogens

DISCUSSION

Urinary tract infections remain one of the most prevalent bacterial infection and second most common infectious disease in community setting. In present study, a total of 365 clinical urinary isolates from OPD were included from adult patients (>16 years age). The 365 tested urinary isolates represented 327 (89.58 %) Gram- negative isolates and 38 (10.41 %) Gram-positive isolates. The 327 isolates represented 318 (97.24 %) members of Enterobacteriaceae & 09 (2.75 %) non-fermenters. [Table 1]

Although the antibiotic susceptibility pattern has shifted in recent years, the spectrum of causative agents causing community-acquired UTI has remained generally stable.[7,8] E. coli remains the predominant causative agent of UTIs, accounting for 75% to 90% of infections.[9] In present study E. coli was identified as primary causative agent in most cases which is in concordance with previous findings.[8-11] Enterococcus is reported as emerging common causative agent of UTIs in recent studies similar to current study.[12-14]

In the present study, out of 327-gram negative isolates, 207 strains (63.3%) were found to be resistant towards trimethoprim/sulphamethoxazole. There were 64% of E. coli strains resistant to co-trimoxazole which similar to the reports of other authors.[15,16] The gradual but widespread use of co-trimoxazole owing to their added advantage like availability in oral formulations, potent broad-spectrum activity and comparatively cost-effective drug may be the reasons for increasing resistance towards it.[17] It is recommended drug for the treatment of UTIs in settings where the prevalence of resistance is <20% according to Infectious Disease Society of America (IDSA) guidelines. However, another disadvantage of co-trimoxazole is Enterococcus which is among the commonest pathogen other than E. coli causing UTIs is inherently resistant to it.[6]

In the present study, fluoroquinolone resistance was noted less among most of the uropathogens (28-50%) except E. coli (71.22%) and Enterococcal isolates (73%). Similarly, high rate of ciprofloxacin resistance in E. coli has been reported by other authors.[15,18] There are multiple factors associated with increase in fluoroquinolones resistance.[19,20]

High rate of Cefazolin resistance was noted among the gram- negative uropathogens except Klebsiella and Citrobacter species. Increasing resistance against cephalosporins among the uropathogens has also been suggested by many authors.[12,21]

Irrational use of higher antibiotics has led to increase in multi drug resistance in bacterial isolates which have thrown up a lot of challenges in the treatment of UTI over the years.[22]

Nitrofurantoin is preferred drug for the management of cystitis. It is eminently effective E. coli with 0.9% resistance in female patients.[9,23] In current study the resistance rate against E. coli was recorded 5.31% while all the Enterococcus species were sensitive towards it. The results are in well support of other studies.[2,15,17]

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Nitrofurantoin is used in UTI treatment for its broad-spectrum activity against gram-negative as well as gram-positive bacteria with exception of some Klebsiella species, Pseudomonas aeruginosa and tribe Proteeae which carry intrinsic resistance.[24]

Fosfomycin is an old broad-spectrum antibiotic having a good in-vitro activity against the common uropathogens, notably towards members of Enterobacteriaceae family. It is chiefly used in treatment of UTIs where the etiological agents are E. coli and Enterococcus species.[25] It has unique mechanism of action which may provide a synergistic effect to other classes of antibiotics including beta-lactams, aminoglycosides, and fluoroquinolones.[26] It shows low level of resistance as compared to other antibiotics. It also shows antimicrobial activity against MDR pathogens. In present study very low level of fosfomycin resistance (0-14.29%) was noted against all the pathogens except Proteus species (54.55%) by disc diffusion method. A sensitivity rate of 98-99.6% of E. coli towards fosfomycin has been reported by other studies which are similar to current study where the sensitivity of fosfomycin to E. coli was recorded 98.48%.[27,28]

Out of four E. coli strains which were interpreted as resistant, only one strain was found to have a value of MIC more than 256 µg/ml. A MIC value of 132 µg/ml was observed in three E. coli strains. There were only one strain each of Klebsiella species and Proteus species which were also found to be resistant by agar dilution method. Out of 327 gram-negative uropathogens, 298 (91.13%) strains have a MIC value under 32 µg/ml. Among 264 E. coli strains, 205 (77.65%) had a MIC value under MIC 16 µg/ml. All the Enterococcus species were found to have a MIC value under 32µg/ml. [Figure 1]

CONCLUSION

Nitrofurantoin, co-trimoxazole and fluoroquinolones has been recommended as an empirical therapy for lower UTI by National treatment guidelines for antimicrobial but in the current study more than 60% resistance was observed against co-trimoxazole and fluoroquinolone especially in E. coli and Enterococcus species. Hence, these drugs should not be used as an empirical therapy in UTI in the study area. Fosfomycin and nitrofurantoin were found to be the drug of choice in lower UTIs in out-patients and can be used for empirical treatment of UTI in our setup. However, continuous monitoring of nitrofurantoin & fosfomycin is needed.

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