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ORIGINAL ARTICLE

In Vitro Susceptibility of *Escherichia coli* and *Enterococcus faecalis* Isolates from Patients with Urinary Tract Infections to Fosfomycin in North India: A Retrospective Study in a Tertiary Care Center

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ABSTRACT

There has been a rapid rise in multidrug-resistant uropathogenic organisms. A retrospective study was carried out in our Institution, which is a Tertiary Care Center in North India, to find out susceptibility of *Escherichia coli* (*E. coli*) and *Enterococcus faecalis (E. faecalis)* isolates to an older generation antibiotic fosfomycin. A total of 3270 isolates from urinary samples were tested. 1701 (52.02%) were *E. coli* and 500 (15.29%) *Enterococcus faecalis* isolates. 704 (41.39%) of *E. coli* were found to be extended spectrum β-lactamase (ESBL) producers and 183 (10.76%) metallo β-lactamase (MBL) producers. Out of 500 *E. faecalis* isolates, 247 (47.4%) were high-level aminoglycoside resistant (HLAR) and 5 (1%) vancomycin-resistant enterococci (VRE). On testing sensitivity to fosfomycin, 94.89% ESBL producing *E. coli*, 89.16% MBL producing *E. coli*, 97.17% HLAR and 80% VRE were found susceptible. Overall only 1.09% of *E. Coli* and 1.33% of *Enterococcus faecalis* isolates were fosfomycin resistant. From this study, we conclude that fosfomycin may be considered as a useful oral antibiotic for the treatment of urinary tract infections with drug-resistant *E. coli* and/or *E. faecalis*.

Keywords: Fosfomycin, Susceptibility, Urinary tract infection (UTI).

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INTRODUCTION

Urinary tract infections (UTIs) are one of the common infective diseases for which antibiotics are prescribed.[1] Over the last few years, the uropathogenic micro-organisms have developed resistance to antibiotics.[2,3] Clinicians have to resort the using newer and costlier antibiotics for treatment. We conducted a retrospective study to re-visit drug sensitivity of two common organisms, namely *E. coli* (*E. coli*) and *E. faecalis*, which cause urinary tract infections and find out their susceptibility to an older generation antibiotic namely fosfomycin. Fosfomycin is a bactericidal broad-spectrum antibiotic that acts against a bacterial cell wall. It can be given orally and has a low incidence of severe side effects. Its major drawback is the development of bacterial resistance often prolonged use. However, for urinary tract infections, it is usually given in a single oral dose. It has not been found to exhibit teratogenic effects on the fetus. Therefore it could be administered to pregnant women also. Because of the limited data on the sensitivity of *E. coli* and *E. faecalis* to fosfomycin in North India isolates, the present study was undertaken to determine the susceptibility of these two uropathogens in our institution which is a tertiary care centre.

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MATERIALS AND METHODS

Ethical Approval

The present study was approved by the institutional ethical committee by letter no. 536/RMLIMS/2018, Dated 02/05/2018.

Study Design

The study was conducted retrospectively in the Department of Microbiology, at an apex tertiary care center in Lucknow, during a two year period from January 2016 to December 2017 after receiving ethical clearance from the institutional review board. In-patients and out-patients with clinical evidence of UTI were included in the study. We included all the consecutive, non-repeated and clinically significant uropathogenic isolates, obtained from all the patients, diagnosed with UTI. Patients were asked to collect fresh, midstream urine samples aseptically in sterile universal containers and were submitted to the clinical microbiology laboratory.

The samples received were inoculated onto chromogenic agar (HiChrome UTI, Hi-Media Laboratories Pvt Ltd., Mumbai, India). After an aerobic incubation at 37° C, for 24 hours, the plates showing significant growth were processed further, and the isolates were identified up to the species level by using standard biochemical tests. The antibiotic sensitivity pattern was determined using Mueller Hilton agar by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[4,5] The following antibiotic discs (drug concentrations in µg, Hi-Media Laboratories Pvt Ltd., Mumbai, India) were used: Norfloxacin (10), levofloxacin (5), gentamicin (10,120), tetracycline (30), doxycycline (30), penicillin (10 U), nitrofurantoin (300), fosfomycin (200), vancomycin (30), piperacillin-tazobactam (100/10), cefotaxime (30), ceftazidime (30), ceftriaxone (30), ceftazidime/clavulanic acid (30/10), nitrofurantoin (300) and imipenem (10). As interpretive criteria for fosfomycin susceptibility from the CLSI are not available for all bacteria other than *E. coli* and *E. faecalis.* Therefore, results were interpreted accordingly.

The isolates were also tested for the ESBL production according to the CLSI guidelines.[6] Cefotaxime (30), ceftazidime (30) and ceftriaxone (30) discs were used to screen for the ESBL production. The isolates which tested positive by the screening test were subjected to the confirmatory test. Ceftazidime (30) and Ceftazidime/clavulanic acid (30/10) discs were used for the confirmatory test. The results were interpreted according to the recent CLSI guidelines. "Metallo β-lactamase detection was performed by using imipenem (10), and imipenem–ethyldiaminetetraacetic acid (EDTA)(10/750) combined disk test. A zone diameter difference between imipenem and imipenem+EDTA of 7 mm was interpreted as a positive result for MBL production".[4,5]

In *E. faecalis*, high-level aminoglycoside resistance was determined by disc diffusion method using high-level gentamicin (120) disc. Vancomycin-resistant *Enterococcus faecalis* isolates were collected based on disc diffusion results as per CLSI guidelines using vancomycin disc (30) and E test strips. All culture media, antibiotics discs and standard strains of bacteria used in this study were procured from Hi-media Laboratories Pvt. Ltd., Mumbai, India. Control strains included were *E. faecalis* ATCC 51299 and *E. faecalis* ATCC 29212. MIC values were interpreted according to the CLSI guidelines.

Statistical Analysis

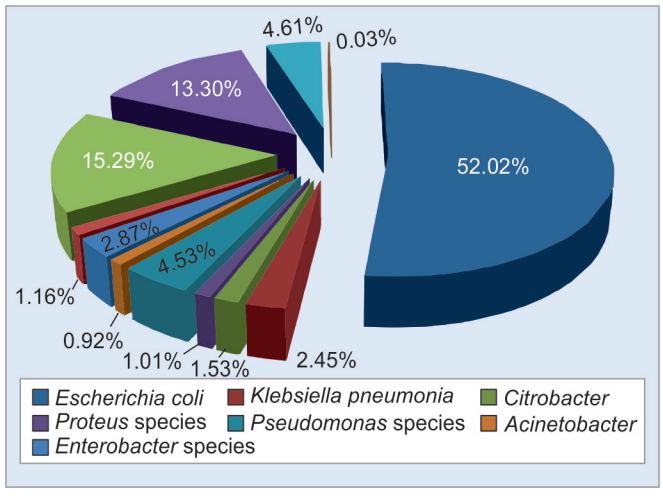
The Statistical Package for Social Sciences (SPSS version 20.0, IBM Corp., Armonk, NY, USA) and Microsoft office excel 2010 was used for analyzing the data. A p-value of <0.05 was considered statistically significant.

RESULTS

The study was performed on 3270 isolates from the urinary samples of the patients with a clinical diagnosis of urinary tract infection. There were 960 male and 2310 female patients. Overall, 52.02% (1701) *E. coli* and 15.29% (500) *E. faecalis* were isolated from 3270 culture positive urinary samples (Graph 1). About 41.39% (704/1701) *E. coli,* were extended ESBL producers and 10.76% (183/1701) were MBL producers. While 49.4% (247/500) isolates of *E. faecalis*, were HLAR and 1% (5/500) were Vancomycin-resistant *E. faecalis* (VRE) (Table 1).

About 94.89% (668/704) ESBL and 89.16% (164/183) MBL producing *E.coli* were susceptible to fosfomycin. (Table 1) The associated p-value equals 0.009 (z-statistic = 2.58), showing that fosfomycin was found to be more resistant in MBL producing *E.coli* than ESBL producing *E.coli* and the difference was found to be statistically significant.

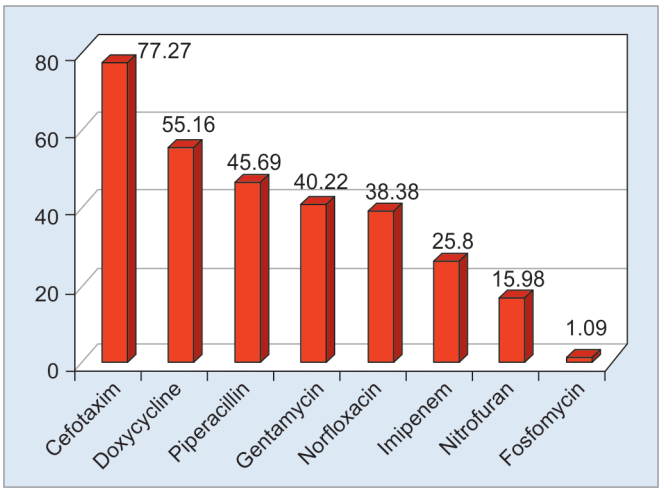
About 97.17% (240/247) HLAR, and 80% (4/5) VRE were susceptible to fosfomycin. The associated p-value equals 0.07 (z-statistic is 1.8), so the difference was not statistically significant. Inward wise study, it was found that 5.18% (31/598) ESBL producing *E. coli* isolates were resistant in outpatient department (OPD) patients, 2.10% (2/95) in inpatient department (IPD) patients, however 27.27% (3/11) were resistant in intensive care unit (ICU) patients, which was found to be statistically significant (p = 0.004, z-statistics = 2.87). 10.34% (15/145) MBL producing *E. coli* isolates were resistant in OPD patients, 3.44% (1/29) were resistant in IPD patients, while 33.33% (3/9) were resistant in ICU patients, which was found to be statistically significant (p = 0.03, z-statistics = 2.11) (Table 2). 1.10% (2/181) HLAR *E. faecalis* were resistant to Fosfomycin in OPD, 1.69% (1/59) in IPD, whereas 42.86% (3/7) in ICU, which was found to be statistically significant ($p < 0.0001$, z-statistics = 4.25). Only 20% (1/5) vancomycin-resistant *E. faecalis* was resistant to Fosfomycin, and this isolate was from ICU (Table 2).



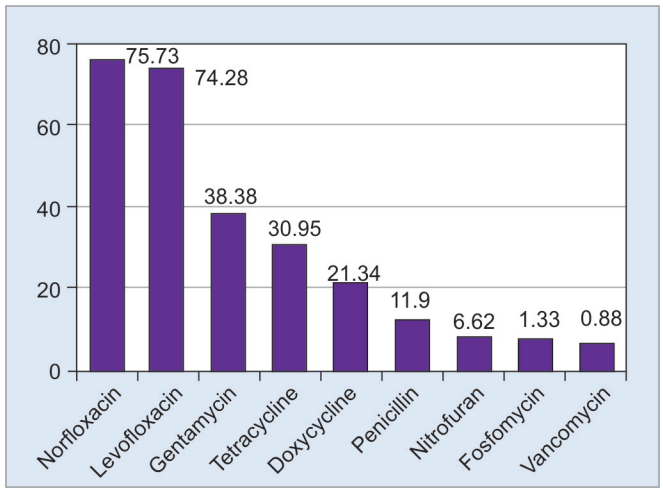
Graph 1: Distribution of various urinary pathogens (n = 3270)

Table 1: Susceptibility to fosfomycin as per CLSI guidelines 2016 and 2017

Total no. of isolates	*E. coli* 1701 (57.2%)						
	ESBL 704 (41.39%)			MBL 183 (10.76%)			
Fosfomycin	S	I	R	S	I	R	p <0.05
	668 (94.89%)	0	36 (5.11%)	164 (90.90%)	0	19 (10.38%)	
	Enterococcus faecalis 226 (19.1%)						
	HLAR 247 (49.4%)			VRE 5 (1%)			p >0.05
Fosfomycin	S	I	R	S	I	R	
	240 (97.17%)	0	7 (2.83%)	4 (80%)	0	1 (20%)	



Graph 2: Overall percentage of resistance of *E. coli* to different antimicrobials in UTI



Graph 3: Overall percentage of resistance of *E. faecalis* to different antimicrobials in UTI

The susceptibility profiles of uropathogenic *E. coli* and *E. faecalis* have been depicted in Graphs 2 and 3. In our study, fosfomycin has been found to have high-quality *in vitro* activity in comparison to other antimicrobials already tested. Overall only 1.09% of *E. coli* were resistant to fosfomycin, ($p < 0.05$) (Graph 2). and 1.33% of *E. faecalis* were resistant to fosfomycin ($p < 0.05$). However, the isolates were least resistant to vancomycin (0.88%) (Graph 3).

DISCUSSION

Reconsideration of current antibiotic treatment regimes for bacterial infections is being done because of a gradual increase in drug resistance in bacteria worldwide, in last few decades. UTIs being one of the most common infections amongst all bacterial infections.[7] With the rise of drug resistance, we are left with limited antimicrobial options to treat drug-resistant infections including urinary tract infections, and there is a need to evaluate older antibiotics scientifically for their efficacy against present-day drug-resistant isolates.[8] In our study, we evaluated *in vitro* activity of fosfomycin, an oral drug, which has the advantage of being administered in a single dose, particularly useful for treating UTI patients on OPD basis in view of compliance. Apart from these fosfomycin serves as a good option because of its broad-spectrum activity against both Gram-positive and Gram-negative bacteria.

Table 2: Susceptibility of isolates of *E.coli* and *E. faecalis* to fosfomycin using CLSI guidelines 2016 and 2017

	E. coli						*E. faecalis*					
Fosfomycin	*ESBL*			*MBL*			*HLAR*			*VRE*		
No. of isolates	OPD 598	IPD 95	ICU 11	OPD 145	IPD 29	ICU 9	OPD 181	IPD 59	ICU 7	OPD 0	IPD 1	ICU 4
Susceptible	567 (94.81%)	93 (97.89%)	8 (72.73%)	130 (89.66%)	28 (96.55%)	6 (66.66%)	178 (98.34%)	58 (98.31%)	4 (57.14%)	0	1 (100%)	3 (75%)
Intermediate	0	0	0	0	0	0	0	0	0	0	0	0
Resistant	31 (5.19%)	2 (2.11%)	3 (27.27%)	15 (10.34%)	1 (3.45%)	3 (33.33%)	2 (1.66%)	1 (1.69%)	3 (42.86%)	0	0	1 (25%)

Efforts have been made to evaluate the *in vitro* activity of Fosfomycin against *E. coli,* and *E. faecalis* isolates from patients with urinary tract infection and compared its *in vitro* activity with other antibiotics.

Extended spectrum β-lactamase (ESBL) production among Enterobacteriaceae is a growing concern worldwide. In this study, more than 40% of the strains of *E. coli* were ESBL-producers. Regarding ESBL production, a marked difference in the fosfomycin resistance rate was not detected among *E. coli* strains (0.9% *vs.* 5.11%). In previous studies on ESBL-producers, fosfomycin resistance rates of 0 to 9.1% for *E. coli* have been reported.[9] The present study revealed good *in vitro* susceptibility of fosfomycin against multidrug resistant (MDR) organisms, similar to earlier performed studies.[10] Fosfomycin was found to be susceptible in 94.89% (668/704) ESBL and 89.16% (164/183) MBL producing *E. coli.* These data are in concordance with other studies.[11-13]

The resistance rate of fosfomycin was higher among indoor strains than among outpatient strains: 27.27% *vs.* 5.18% ($p = 0.004$, z-statistics = 2.87) for ESBL and 10.34% *vs*. 33.33% for MBL producing *E.coli* ($p = 0.03$, z-statistics = 2.11). In addition, higher resistance rates were also detected among indoor strains compared to community strains: 1.10% *vs.* 42.86% respectively, among HLAR *E. faecalis*, which was similar to the other studies,[13,14] however, the difference was not statistically significant in vancomycin-resistant *E. faecalis,* this may be because less number of isolates of vancomycin-resistant *E. faecalis* detected in our study.

In our study, the *in vitro* activity of fosfomycin was found to be much superior to other oral antimicrobials tested against all the isolates of *E. coli* and *E. faecalis* ($p < 0.05$). Maraki et al.[9] reported that fosfomycin was found to be active *in vitro* against a considerable percentage of urinary isolates, which exhibited high antimicrobial resistance against the conventionally used antimicrobial agents for the treatment of UTIs.[9] Our results of high susceptibility to fosfomycin against various urinary isolates are supported by many other recent studies from different parts of the world.[13,15-17] The most important finding of our study was the susceptibility profiles of ESBL and MBL-producing *E. coli* and *Klebsiella* sp to fosfomycin, which was found to be 97.8, 92.1 and 95.2%, 92.8% respectively. Notably, Maraki et al.[9] in their recent study have demonstrated 100% fosfomycin susceptibility to ESBL-producing *E. coli* and *K. pneumoniae*.[9] Similarly, in many other studies from different places, it has been reported that fosfomycin has a superior *in vitro* activity against ESBL producing *E. coli*, as was also seen in our study.[18,19]

One of the vital findings of the present study is the excellent *in vitro* activity of fosfomycin against MDR urinary isolates of *E.coli* and *E. faecalis*. Its activity was found to be superior to other oral antimicrobials tested against these MDR isolates ($p < 0.05$). Neuner et al.[20] in their recent study, observed that 92% of urinary isolates were fosfomycin susceptible. There are other comparable efficacy reports of fosfomycin against various MDR organisms also support our findings.[8,18] One of the recent systematic review and meta-analysis performed, had concluded that fosfomycin has retained efficacy against various MDR organisms and therefore it has got a special place in the treatment of infections caused by MDR bacteria.[21] However, a recent study also analyzed fosfomycin's clinical efficacy in difficult-to-treat chronic bacterial prostatitis.[22] In our study, fosfomycin was found to be effective even among the Gram-positive urinary isolate, i.e., *E. faecalis*. A total of 97.17% and 80% of HLAR and VRE isolates respectively, of *Enterococcus* sp., were found to be fosfomycin susceptible. Similarly, 99% *in vitro* susceptibility to fosfomycin in *Enterococci* was reported in a recent study from South India,[23] whereas in a study from, Jaipur-India, 100% Enterococcal isolates tested were fosfomycin susceptible.[18]

Strengths and limitations of the study

This is a 2 years retrospective study performed in an apex tertiary-care institute in which a large number of patients were recruited. Though all the clinical and demographic characteristics of the patients were included in the study, the genetic evaluation of drug resistance was not implemented. Also, *in vivo* or clinical follow-up efficacy of the Fosfomycin could not be evaluated in this study.

CONCLUSION

In conclusion, a considerable proportion of the MDR uropathogenic *E. coli* and *E. faecalis* with diverse resistance mechanisms, including ESBL, MBL, HLAR, and VRE were found susceptible to fosfomycin. Fosfomycin was found to have good *in vitro* activity in both admitted patients and OPD patients. Its in vitro activity was promising in complicated as well as uncomplicated UTI. We observed that Fosfomycin appears to be an efficient oral antibiotic against *E.coli* and *E. faecalis* in this era of antimicrobial resistance among uropathogens

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