Open Access 6 IMC Journal of Medical Science Original Article

Fosfomycin susceptibility among *Escherichia coli* causing urinary tract infection in a tertiary care centre in Western Maharashtra

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Abstract

Background and objective: Urinary tract infection (UTI) is one of the most common bacterial infections encountered in clinical practice. UTIs caused by extended-spectrum beta-lactamase (ESBL) AmpC and metallo-beta-lactamase (MBL) producing *Escherichia coli* (*E. coli*) are difficult to treat. Fosfomycin is an old antibiotic that has excellent bactericidal activity against a wide range of bacteria. This study aimed to determine the fosfomycin susceptibility of *E. coli* causing UTI in a tertiary care hospital in Western Maharashtra, India.

Material and methods: The study was conducted at a tertiary care center in Pune, a city of Western Maharashtra, India. Urine samples from UTI cases yielding significant (> 1x 10^5 cfu/ml) growth of *E. coli* were included in study. *E. coli* isolates were tested for susceptibility to fosfomycin and a panel of antimicrobial agents by Kirby Bauer disc diffusion method. All the isolates were tested for production of ESBL, AmpC and MBL.

Result: A total of 88 *E. coli* were isolated of which, 47 (53.40%) and 41 (46.59%) were from male and female patients respectively. Of the total *E. coli* isolates, 58 (65.9%) were from in-patient cases. Multi-drug resistance was found in 69 (78.40%) isolates and remaining 19 (21.6%) were resistant to different antimicrobials tested. All (100%) the MDR and non-MDR isolates were sensitive to fosfomycin. Highest resistance was present against nalidixic acid (93.8%) while resistance was least against nitrofurantoin (15.91%), piperacillin/tazobactam (17.1%) and meropenem (18.18%). Of the total, 35 (50.72%) isolates were both AmpC and ESBL producers while 11 (15.94%) and 8 (11.59%) were only AmpC and ESBL producers respectively. MBL was positive in 15 (21.73%) of *E. coli* isolates. All those isolates tested sensitive to fosfomycin.

Conclusion: The study revealed that fosfomycin had excellent activity against MDR *E. coli* causing UTI in our area.

IMC J Med Sci. 2023; 17(1): 008. DOI : https://doi.org/10.55010/imcjms.17.008

Introduction

Urinary tract infection (UTI) is a common bacterial infection of urinary system and requires antibiotics for treatment [1]. Beta-lactams, co-trimoxazole, fluoroquinolones and other antimicrobial agents have been used for many years in the treatment of UTI. But UTI caused by emerging multi-drug resistant and extended-spectrum beta-lactamases (ESBLs), AmpC and metallo-beta-lactamase (MBL) producing organisms has made treatment of UTI difficult and expensive. Fosfomycin is an old bactericidal agent which has a good *in vivo* and *in vitro* activity against a wide range of bacteria and thus making it a good option for the treatment of UTI [2-4]. Fosfomycin also shows very good activity in penetrating biofilms of Gram-negative bacteria

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in monotherapy as well as in combined therapy and has very good eradication activity [5]. The main mechanism by which fosfomycin acts is by irreversibly inhibiting the bacterial cell wall biosynthesis. After entering into cytoplasm of bacteria, fosfomycin binds with *MurA* enzyme and inhibits peptidoglycan biosynthesis [3,6]. Apart from being effective, fosfomycin formulations have less adverse effects than other antimicrobial agents. Mild gastro intestinal distress is the most commonly reported adverse effect [7]. Therefore, this study was undertaken to assess the fosfomycin susceptibility of *Escherichia coli* causing UTI in a tertiary care center in Western Maharashtra, India. Results of the study would help in guiding treatment of UTIs due to sensitive as well as multi-drug resistant (MDR) pathogens.

Material and Methods

The study was carried out at a tertiary care center based in Pune, a city of Western Maharashtra, India. It was approved by the Institutional Ethical Sub-committee (Letter number: IESC/30/2022 dated: 17 February 2022).

Urine samples from in and out patient departments having clinical features of UTI were collected and included in the study. Samples yielding significant (> 1x 10^5 cfu/ml) [8] growth of *E. coli* were included in study for further analysis. Any urine sample which yielded a non-significant count and organisms other than *E. coli* was excluded from the study. Relevant patient-related demographic information was collected in a pre-designed data sheet.

Standard procedures were followed for the collection, transport, processing, and culture of the urine samples. Samples once collected were sent to the laboratory immediately. From urine container, 0.01ml urine sample was inoculated immediately on a Cysteine Lactose Electrolyte Deficient (CLED) agar plate with the help of a calibrated double loop inoculator (Himedia, India). Plates were then incubated for 18-24 hours in an incubator at 37ºC. *E. coli* was identified by motility, sugar fermentation, methyl red, Voges Proskeuer, indole, citrate, urease, hydrogen sulfide formation, and oxidase tests [9]. *E. coli* isolates were tested for antibiotic susceptibility by Kirby Bauer disc diffusion method. Antibiotic discs used were gentamicin-10µg, amikacin-30µg, ampicillin-10µg, amoxicillin/clavulanic acid-20/10µg, ceftazidime-30µg, ceftriaxone-30µg, meropenem-10µg, piperacillin/tazobactam-100/10µg, nalidixic acid-30µg, norfloxacin-10µg, co-trimoxazole- 1.25/ 23.75µ, nitrofurantoin-300µg and fosfomycin-200 µg. The result was interpreted according to CLSI 2021 guidelines. For fosfomycin, the inhibition zone of >16mm, 13-15mm and <12mm was interpreted as sensitive, intermediate sensitive and resistant respectively according to CLSI 2021 guideline [10]. MDR was defined as resistance to a minimum one drug of three or more groups of antibiotics [11].

ESBL production in *E. coli* was detected by double disc synergy test (DDST) as described earlier [12]. Mueller Hinton agar was inoculated with standardized inoculums (corresponding to 0.5 McFarland tube) of test organism. An amoxicillin/clavulanic acid disc 20/10 µg was placed in the center of the plate and test discs of 3rd generation cephalosporins (ceftazidime- CAZ 30µg, ceftriaxone-CRO 30µg, cefotaxime-CTX 30µg) discs were placed at 20 mm distance (center to center) from the amoxicillin-clavulanic acid disc. The plate was incubated overnight at 35°C. Enhancement of the zone of inhibition of any one of the three drug discs toward amoxicillin-clavulanic acid suggested the presence of ESBLs. AmpC producers were detected by the cefoxitin-oxacillin disk diffusion test [13]. MBL detection was done by a combined disc test, in which imipenem and imipenem plus EDTA disc was used [14].

Results

During the study period, 88 *E. coli* were isolated. Out of them, 47 (53.40%) were from male patients and 41 (46.59%) were from female patients. Of the total samples, 30 (34.1%), 26 (29.55%) and 25 (28.41%) were from patients above 60, 18-40 and 41-60 years age group respectively. Out of 88 *E. coli* isolates, 58 (65.9%) were from in-patient cases (Table-1). Out of 58 urine samples from in-patient departments, only 4 were from intensive care unit (ICU).

Table-1: *Distribution of gender, age and source of study cases (N=88)*

Variable	Number (%)
Gender	
Male	47 (53.40%)
Female	41 (46.59%)
Age (years)	
Below 18	6 (6.82%)
18-40	26 (29.55%)
41-60	25 (28.41%)
Above 60	30 (34.1%)
Source of sample	
Out patient	30 (34.1)
In-patient	58 (65.9)

Susceptibility of isolated *E. coli* to different antimicrobial agents is shown in Table-2. Highest resistance of *E. coli* was noted against nalidixic acid (93.8%) followed by ampicillin (81.82%), cephalosporins (77.27%) and norfloxacin (72.73%). Rate of resistance was low for nitrofurantoin (15.91%), piperacillin/tazobactam (17.05%), meropenem (18.18%), amoxycillin+ clavulanic acid (25%) and amikacin (23.86%). All the 88 (100%) isolated *E. coli* was sensitive to fosfomycin. Out of total *E. coli*, 78.4% was MDR strains.

Table-3 shows that out of 88 *E. coli* tested, 35 (39.8%) isolates were both AmpC and ESBLs producers, while 11 (12.5%) and 8 (9.1%) were only ESBL and AmpC producers respectively. MBL was positive in 15 (17%) *E. coli* isolates. All 69 ESBL, AmpC and MBL positive *E. coli* isolates were sensitive to fosfomycin.

Discussion

Urinary tract infection is a common problem in clinical practice. The study was conducted in a tertiary care setting in western Maharashtra, India to find out the prevalence of fosfomycin resistance among *E. coli* isolated from patients with UTI. UTI caused by a multi-drug resistant strain pose a serious challenge for the physician and also is a burden on the patient. Multidrug-resistant (MDR) isolates have emerged worldwide with the widespread use of cephalosporins and fluoroquinolones [15,16]. As a result, use of carbapenems has increased over the last 20 years, resulting into dramatic spread of carbapenem resistance [17,18]. Fosfomycin, discovered more than 40 years ago, is active against a wide range of organisms, including MDR *Enterobacteriaceae* [3,19,20].

Table-2: *Susceptibility of isolated E. coli to fosfomycin and other antimicrobial agents (N=88)*

Antimicrobial agents	Out Patients (n=30)	In Patients (n=54)	ICU Patients (n=4)	Total (N=88)
	Resistant n (%)	**Resistant n (%)**	**Resistant n (%)**	**Resistant n (%)**
Gentamicin	6 (20)	25 (46.29)	3 (75)	34 (68.64
Amikacin	4 (13.33)	16 (29.62)	1 (25)	21 (23.86)
Ampicillin	21 (70)	48 (83.33)	4 (100)	72 (81.82)
Ceftazidime	20 (66.67)	44 (81.48)	4 (100)	68 (77.27)
Ceftriaxone	20 (66.67)	44 (81.48)	4 (100)	68 (77.27)
Amox/clav acid	03 (10)	17 (31.48)	2 (50)	22 (25)
Meropenem	0	14 (25.92)	2 (50)	16 (18.18)
Pip/Tazo	0	15 (27.77)	0	15 (17.05)
Cotrimoxazole	8 (26.66)	39 (72.23)	3 (75)	50 (56.82)
Norfloxacin	18 (60)	42 (77.77)	4 (100)	64 (72.73)
Nalidixic acid	25 (83.33)	53 (42.59)	4 (100)	82 (93.18)
Nitrofurantoin	03 (10)	8 (14.81)	3 (75)	14 (15.91)
Fosfomycin	**0**	**0**	**0**	**0**

Note: ICU: intensive care unit; Amox/Clav acid: amoxycillin/clavulanic acid; Pip/Tazo: Piperacillin/Tazobactam

Table 3: *Distribution of ESBL, AmpC and MBL positive E. coli and their susceptibility to fosfomycin*

Type	*E. coli* from (n%)			Total N=88 n (%)	Resistance to fosfomycin n (%)
	OPD n=30	IPD n=54	ICU n=4		
ESBL positive alone	5 (16.7)	6 (11.1)	0	11 (12.5)	0
AmpC positive alone	4 (13.3)	4 (7.4)	0	8 (9.1)	0
ESBL + AmpC positive	12 (40.0)	21(38.9)	2 (50)	35 (39.8)	0
MBL positive	0	13 (24.1)	2 (50)	15 (17.0)	0

Note: OPD: out-patient department; IPD: in-patient department; ICU: intensive care unit

Studies with fosfomycin are limited though, it is available for intravenous and oral use [19,21]. Recently, it has been shown to be non-inferior to piperacillin-tazobactam for the treatment of complicated urinary tract infections [22]. It is also been shown non-inferior to comparators for the treatment of bacteremic urinary tract infections due to MDR *E coli* [23].

In our study, a total of 88 isolates of *E. coli* were collected and analyzed. The majority of the urine samples were from male patients. Most isolates of *E. coli* were from UTI cases aged 60 and above and were from hospitalized patients (61%). About 78.4% of our *E. coli* isolates were MDR strains and positive for ESBL, AmpC or MBL. Niranjan et al found 38% of his *E. coli* isolates from UTIs were from in-patients and 76.5% of the isolates were MDR [24]. Hasan et al found 53% of *E. coli* from UTI cases as MDR strains [25]. Paul et al from Assam, India reported 26.2% ESBL and 12.6% carbapenemase producing *E. coli* from UTI cases [26]. All our *E. coli* isolates tested were sensitive to fosfomycin (100%). There was no difference in fosfomycin sensitivity between sensitive and MDR strains. Similar to our findings, Sabharwal *et al* in their study found 97% sensitivity to fosfomycin in *E. coli* isolated from UTI cases [27]. Our study has demonstrated that fosfomycin has excellent activity against MDR *E. coli* causing UTI in our area. Thus, the finding would help in formulating antibiotic treatment guideline for UTIs due to multi-resistant *E. coli*.

However, our study had some limitations. This study was conducted only for a short period of time with 88 *E. coli* isolates at a single center and minimum inhibitory concentration (MIC) of fosfomycin for those was not determined. Hence, multicenter studies with large sample size would provide a better perspective of the resistance pattern of uropathogenic *E. coli* to fosfomycin and other drugs in western part of Maharashtra.

Acknowledgement

The authors would also like to acknowledge the contributions of staff and laboratory personnel of the Department of Microbiology, Dr. D.Y. Patil Medical College, hospital and research center, Pimpri, Pune.

Conflict of interest: The authors have no conflict of interests to declare.

Fund: None

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Cite this article as:
Lohariwal Y, Das NK, Mirza S, Gandham N, Patil R, Mukhida, S, et al. Fosfomycin susceptibility among *Escherichia coli* causing urinary tract infection in a tertiary care centre in Western Maharashtra. IMC J Med Sci. 2023; 17(1): 008;
DOI: https://doi.org/10.55010/imcjms.17.008