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Increasing antimicrobial resistance for uropathogens: Fosfomycin

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Abstract
Urinary tract infection (UTI) is one of the choice of antibiotics for the treatment of UTI an urgent need to discover new effective treatment solutions. Fosfomycin may be an interesting alternative to the currently used treatments of UTIs. The study was conducted over 6 months per Microbiology, Chhattisgarh Institute of Medical Science, Bilaspur. A total of 1240 done as per standard microbiological procedures. Methicillin- resistant *Staphylococcus aureus* (MRSA), high-aminoglycoside resistance (HLAR), extended spectrum (MBL) production was detected. Culture was positive in 339 (27.4%) cases. Gram production was detected in 154 (37.1%) while 82 (21 bacteria, 68 (51.5%) were MRSA. Fosfomycin was effective to fosfomycin in AmpC producers was extremely high effective as only three isolates were sensitive to cotrimoxazole. *Pseudomonas* species showed 65% and 75% susceptiblee. Fosfomycin has emerged as a promising option, pathogens in which previous antibiotics have failed to cure the infection.

Keywords: *Enterobacteriaceae*, fosfomycin, muti- drug resistant

Introduction
Over 150 million of urinary tract infections (UTIs) occurs annually in the world [1]. It accounts for a large proportion of antibiotic consumption and has a large socioeconomic impact and may contribute to bacterial resistance [2]. Clinicians often face problems in choosing appropriate antibiotic therapy for treating UTIs caused by multi-drug resistant (MDR) bacteria [3]. The emergence of extended spectrum beta-lactamases (ESBL), Amp C production by Gram negative.
Fosfomycin trometamol may be an interesting alternative to the currently used treatments of UTIs. It is a well-tolerated drug and has a broad spectrum of activity. The aim of this study was to assess the susceptibility profile of uro-pathogens against fosfomycin, norfloxacin, cotrimoxazole, polymyxin B and colistin apart from the other routine antibiotics.

Materials and Methods
Sample collection and analysis
The study was conducted over a period of 6 months (August 2020 to January 2021) in the Department of Microbiology Chhattisgarh Institute of Medical Science, Bilaspur. Total 1240 freshly voided midstream specimens of urine were submitted to the Clinical Microbiology Laboratory of CIMS, Bilaspur for processing. Semi quantitative urine culture using a calibrated loop was used to inoculate blood agar and MacConkey plates [4]. Following the recommendations of Kass [5] in distinguishing genuine infection from contamination, significant monomicrobial bacteriuria was defined as culture of a single bacterial species from the urine sample at a concentration of $>10^5$ cfu/ml. Inadequate urine samples (<10 ml urine), urine bag collected samples, specimens collected more than 2 h before submission, specimens submitted in leaking, or dirty unsterile containers and specimens revealing growth of more than two types of bacteria on culture were excluded from the study. The significant pathogens were identified by standard biochemical procedures [6].

Antibiotic susceptibility testing
Antimicrobial susceptibility testing of all isolates was performed on Mueller Hinton agar by Kirby-Bauer disk diffusion method for fosfomycin (50 g) and norfloxacin (5 g). Along with these, the susceptibility to the following antimicrobial agents was also performed as per clinical laboratory standards institute guidelines [7]. All the disc were obtained from Hi-Media Laboratories, Mumbai, India.
Gram negative isolates: Cotrimoxazole (1.25/23.75 g), amikacin (30 g), gentamicin (10 g), ofloxacin (5 g), ceftriaxone (30 g), cefoperazone (CP) (75 g), cefoperazone sulbactam (CPS) (75 g, 1:1), cefixime (5 g) cefotaxime (30 g), cefepime (30 g) and ceftriaxone salbactam (30/15 g) as first line drugs. Pathogens resistant to these drugs were considered multi-drug resistant and were tested against second line drugs: Piperacillin (100 g), piperacillin tazobactam (100:10 g), tobramycin (10 g), imipenem (10 g), polymyxin B (300 g) and colistin (10 g) *Pseudomonas* spp.: Piperacillin (100 g), piperacillin tazobactam (100:10 g), tobramycin (10 g), imipenem (10 g), ticarcillin (75 g), polymyxin B (300 g), and colistin (10 g). Gram- positive isolates: Amikacin (30 g), gentamicin (10 g), levofloxacin (5 g), sparfloxacin (5 g), erythromycin (15 g), vancomycin (30 g), oxacillin (1 g), tobramycin (10 g), clindamycin (2 g), and amoxicillin (30 g).

Detection of extended spectrum and AmpC beta lactamase
Screening of possible ESBL production was done by using ceftriaxone (30 g) and CP (75 g). Isolates showing zone

diameter less than 25 mm for ceftriaxone and less than 19 mm for CP were subsequently confirmed by disc potentiating test using CP and CPS combination [8]. Organism sensitive to cefoxitin and resistant to cefoperazone- salbactam and piper acillin- tazobactam combination were considered to be Amp C producers [9].

Detection of metallo beta lactamases

Imipenem resistant isolates were tested for metallo- beta- lactamases (MBL) production by modified Hodge test and Double Disc synergy test using EDTA [7]. Screening for methicillin resistance in *Staphylococcus* species and high- level aminoglycoside resistance in enterococci Test was performed on Muller Hilton agar with 4% NaCl using oxacillin 1 g disc. Isolates showing a reduction in zone size <13 mm were considered resistant. In case of enterococci, high- level aminoglycoside resistance (HLAR) was detected using high content gentamycin (120 g) and streptomycin (300 g).

Results

Of 1240 urine samples, 339 (27.4%) were culture positive. Majority were females (*n* = 942) 76% and the female to male ratio was 4:1. Gram- negative bacilli of which 371 (73.8%) belonged to *Enterobacteriaceae* family. In the *Enterobacteriaceae* group, the frequency of *Escherichia coli* and *Klebsiella pneumoniae* were 90% and 6%, respectively. Etiological profile is shown in Tables 1. In addition, 4% of total isolates were non-enterobacteriaceae Gram- negative organisms, among which *Pseudomonas aeruginosa* (3.4%). Tables 2 The frequency of Gram- positive pathogens was 65 (13%) for *Staphylococcus* spp. *Streptococcus* species and 2 (0.4%) for *Corynebacterium* species. Antibiotic Susceptibility patterns of most frequent uropathogens to different antibiotics are shown in Tables 3.

Among Gram- positive bacteria, the highest level of susceptibility was observed for vancomycin (96%) followed by nitrofurantoin (85.7%). Erythromycin and fluoroquinolones were effective in 58.9% and 44.6% of Gram- positive isolates, respectively. *Staphylococcus* species showed 96% susceptibility to both amikacin and gentamycin. Isolates of *Corynebacterium* spp., (*n* = 2) were resistant to oxacillin, nitrofurantoin, and levofloxacin.

All the Gram- negative bacteria were sensitive to imipenem. Amikacin showed good results being effective in 96.39% isolates while CPS and piperacillin- tazobactam were effective in 74% of isolates. 69% and 40% isolates were sensitive to gentamicin and ofloxacin, respectively. *Pseudomonas* species showed 65% and 75% susceptibility to colistin and polymixin B, respectively.

On further analyzing the MDR isolates, 154 (37.1%) were ESBL producers, 82 (21.6%) were Amp C. No, MBL was detected. Among Gram-positive bacteria, 68 (51.5%) were methicillin- resistant *Staphylococcus aureus* (MRSA) while 4 (13.3%) were vancomycin resistant enterococci (VRE). HLAR was seen in 53.3% of enterococci. Other two drugs norfloxacin and cotrimoxazole were not proved effective as only three isolates were sensitive to norfloxacin, while all Gram- negative isolates were resistant to cotrimoxazole. Fosfomycin was effective in 100% of MRSA, VRE, ESBL, HLAR, and overall, susceptibility to fosfomycin in AmpC producing isolates was extremely high (99%).

Table 1: Distribution of various urinary pathogens (*n*=339)

Species	Number
Escherichia coli	251
Klebsiella pneumonia	20
Citrobacter	10
Proteus species	08
Pseudomonas species	08
Acinetobacter	1
Staphylococcus aureus	20
Staphylococcus epidermidis	5
Enterococcus faecalis	10
Streptococcus species	05
Corynebacterium species	1
Total	339

Table 2: Sensitivity pattern of Gram- positive isolates

Antibiotics	*Staphylococcus aureus*		Coagulase negative *Staphylococcus* (*n*=10) (%)	
	MRSA (*n*=29)	MSSA (*n*=26)	Methicillin resistant (*n*=4)	Methicillin sensitive (*n*=6)
Amikacin	28 (93)	4 (100)	6 (100)	-
Ofloxacin	-	-	-	0 (0)
Norfloxacin	0 (0)	0 (0)	0 (0)	1 (10)
Vancomycin	30 (100)	26 (100)	4 (100)	6 (100)
Fosfomycin	30 (100)	26 (100)	4 (100)	6 (100)
Gentamycin	28 (93)	26 (100)	4 (100)	6 (100)
Cefazolin	0 (0)	24 (93)	0 (0)	6 (100)
Oxacillin	0 (0)	26 (100)	0 (0)	6 (100)
Erythromycin	12 (40)	22 (84.6)	2 (50)	2 (33.3)
Levofloxacin	14 (47)	22 (84.6)	2 (50)	6 (100)
gentamycin	-	-	0 (0)	2 (16)
streptomycin	-	-	0 (0)	4 (33)
Amoxicillin- clavulanate	-	-	3 (75)	10 (83)
Nitrofurantoin	24	(80)	6 (100)	4 (100)

Table 3: Sensitivity pattern of ESBL producing, non- ESBL and Amp C

Antibiotics	*Enterobacteriaceae* (%)			*Pseudomonas aeruginosa* (*n*=16)		
	ESBL (*n*=146)	Non- ESBL (*n*=133)	AmpC (*n*=92)	ESBL (*n*=4)	Non- ESBL (*n*=10)	AmpC (*n*=2)
Amikacin	146 (100)	132 (98)	84 (91)	4 (100)	10 (100)	2 (100)
Gentamycin	118 (80)	124 (92)	68	(73)	10 (100)	2 (100)
Ofloxacin	36(24)	96 (71)	12 (13)	-	-	-
Norfloxacin	0 (0)	3 (2.2)	0 (0)	0 (0)	0 (0)	0 (0)
Ceftriaxone	8 (5.4)	134 (100)	0 (0)	-	-	-
Cefoparazone	0 (0)	134 (100)	0 (0)	-	-	-
Cefixime	20 (13)	120 (89)	0 (0)	-	-	-
Ceftazidime	-	-	-	2 (50)	10 (100)	0 (0)
Cefepime	50(34)	134 (100)	14 (15)	-	-	-
Cefpodoxime	0 (0)	72 (53)	0 (0)	-	-	-
Cefoperazone- salbactum	146 (100)	134 (100)	0 (0)	-	-	-
Ceftriaxone- salbactam	146 (100)	134 (100)	-	-	-	-
Piperacillin	-	-	-	0 (0)	10 (100)	0(0)
Piperacillin- tazobactum	-	-	-	4 (100)	10 (100)	0 (0)
Ticarcillin	-	-	-	2 (50)	10 (100)	1 (50)
Nitrofurantoin	104 (71)	132 (98)	60(65)	4 (100)	6 (60)	2 (100)
Colistin	-	-	-	2 (50)	8 (80)	2 (100)
Polymyxin B	-	-	-	2 (50)	10 (100)	2 (100)
Cotrimoxazole	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
Fosfomycin	146 (100)	134 (100)	88(95)	4 (100)	10 (100)	2 (100)
Imipenem	146 (100)	134 (100)	88(95)	4 (100)	10 (100)	2 (100)

Discussion

This study was conducted to evaluate the potential of certain older antibiotics in the treatment of UTIs, especially against MDR pathogens. In our study, *E. coli* (65%) was the most common pathogen followed by *S. aureus* (11%). Okonko *et al* [10]. Also reported similar findings in their study.

Compared to other antibiotics, aminoglycosides, carbepenems, glycopeptides and colistin showed good results, but all these are parenteral antibiotics. Limited options of oral antibiotics are available for the treatment of UTI.

Significant resistance to cotrimoxazole and norfloxacin, which concur with reports of previous studies [11, 12]. The other two oral antibiotics, which were tested in this study were nitrofurantoin and fosfomycin, but nitrofurantoin showed decreased susceptibility against MDR bacteria. As high as 99% of the MDR isolates were sensitive to fosfomycin in our study.

Fosfomycin (FOS) is broad spectrum antibiotic which acts on proliferating bacteria by inhibiting cell wall and early murein/peptidoglycan synthesis. Other properties of this drug include inhibition of bacterial adhesion to epithelial cells, exopolysaccharide biofilm penetration, immunomodulatory effect, phagocytosis promotion and protection against the nephrotoxicity caused by other drugs.

Fosfomycin has emerged as a promising treatment option. It has rare adverse reactions which develop in 1-8% of all patients, with the most common ones being diarrhea, nausea, vomiting, skin rash, heartburn, vaginitis, headache, chills and asthenia [13]. Fosfomycin has a low molecular weight and a relatively long half- life (mean half- life-SD, 5.7 -2.8 h) and therefore, penetrates various tissues with ease, achieving the minimum inhibitory concentrations needed to inhibit the growth of most pathogens.[3] Resistance rate is low and most frequently acquire by chromosomal mutations that do not spread easily.[14] Clinical studies have shown fosfomycin to be effective for the treatment of lower UTIs due to ESBL- producing Members of the *Enterobacteriaceae* [15, 16]. Fosfomycin has been reported to have high activity against the majority of *Enterobacteriaceae*, but not toward the Gram- positive bacteria [17]. However, in our study 100% of VRE isolates showed susceptibility to fosfomycin. This finding is in concordance with study of Shrestha *et al* [18]. Who reported 98.7% of sensitivity among VRE isolates to fosfomycin.

In previous studies, around 10% of strains of *P. aeruginosa*, were resistant to fosfomycin [19]. Current studies on *P. aeruginosa* isolates demonstrated higher rates of resistance to fosfomycin *in vitro* [20]. However, our *P. aeruginosa* isolates showed 100% susceptibility to fosfomycin. This finding could be because most of *P. aeruginosa* isolates were sensitive strains. Polymyxin B and colistin also demonstrated good results against *Pseudomonas* spp.

Conclusion

Fosfomycin is a bactericidal agent showing low level of resistance as compared to other antibiotics. Antimicrobial activity of fosfomycin, especially against MDR pathogens, makes it an effective and safe drug in the treatment of UTIs due to Gram- positive and Gram- negative bacteria, especially in cases involving MDR pathogens in which previous antibiotics have failed to cure the infection or when patients are intolerant to the antibiotics considered as first- line treatment agents.

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