Original Article

Increasing antimicrobial resistance among uropathogens: Is fosfomycin the answer?

Asfia Sultan, Meher Rizvi, Fatima Khan, Hiba Sami, Indu Shukla, Haris M. Khan

Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Abstract

Introduction: Urinary tract infection (UTI) is one of the most common infectious diseases in clinical practice. The choice of antibiotics for the treatment of UTI is limited by the rising rates of antibiotic resistance. There is an urgent need to discover new effective treatment solutions. Fosfomycin may be an interesting alternative to the currently used treatments of UTIs.

Materials and Methods: The study was conducted over 6 months period (January to June 2013) in Department of Microbiology, JNMCH, AMU, Aligarh. A total of 1840 urine samples were submitted. Culture and sensitivity was done as per standard microbiological procedures. Methicillin-resistant *Staphylococcus aureus* (MRSA), high-level aminoglycoside resistance (HLAR), extended spectrum beta-lactamases (ESBL), AmpC and metallo-beta-lactamases (MBL) production was detected.

Results: Culture was positive in 504 (27.4%) cases. Gram-negative etiology was identified in 390 (73%) cases. ESBL production was detected in 154 (37.1%) while 82 (21.6%) were Amp C. No, MBL was detected. Among Gram-positive bacteria, 68 (51.5%) were MRSA, while 4 (13.3%) were vancomycin resistant enterococci (VRE). HLAR was seen in 53.3% of enterococci. Fosfomycin was effective in 100% of MRSA, VRE, ESBL, HLAR, and overall, susceptibility to fosfomycin in AmpC producers was extremely high (99%). Norfloxacin and cotrimoxazole were not proved effective as only three isolates were sensitive to norfloxacin, while all Gram-negative isolates were resistant to cotrimoxazole. *Pseudomonas* species showed 65% and 75% susceptibility to colistin and polymixin B, respectively.

Conclusion: Fosfomycin has emerged as a promising option, especially in cases involving multi-drug-resistant pathogens in which previous antibiotics have failed to cure the infection.

Key Words: *Enterobacteriaceae*, fosfomycin, muti-drug resistant, urinary tract infection

Address for correspondence:
Dr. Asfia Sultan, Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.
E-mail: drasfia@gmail.com

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INTRODUCTION

Over 150 million episodes 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), AmpC production by Gram-negative bacteria and methicillin resistant *Staphylococcus* species further limits the choice of antimicrobials.[4]

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

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study was to assess the susceptibility profile of uropathogens 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 (January 2013 to June 2013) in the Department of Microbiology JNMCH, AMU, Aligarh. Total 1840 freshly voided midstream specimens of urine were submitted to the Clinical Microbiology Laboratory of JNMCH, Aligarh for processing. Semi quantitative urine culture using a calibrated loop was used to inoculate blood agar and MacConkey plates.[5] Following the recommendations of Kass[6] 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.[7]

Antibiotic susceptibility testing

Antimicrobial susceptibility testing of all isolates was performed on Mueller Hinton agar by Kirby-Bauer disk diffusion method for fosfomycin (50 μg) and norfloxacin (5 μg). Along with these, the susceptibility to the following antimicrobial agents was also performed as per clinical laboratory standards institute guidelines.[8] All the disc were obtained from Hi-Media Laboratories, Mumbai, India.

Gram-negative isolates: Cotrimoxazole (1.25/23.75 μg), amikacin (30 μg), gentamicin (10 μg), ofloxacin (5 μg), ceftriaxone (30 μg), cefoperazone (CP) (75 μg), cefoperazone-sulbactam (CPS) (75 μg, 1:1), cefixime (5 μg) cefotaxime (30 μg), cefepime (30 μg) and ceftriaxone-salbactam (30/15 μg) as first line drugs. Pathogens resistant to these drugs were considered multi-drug-resistant and were tested against second line drugs: Piperacillin (100 μg), piperacillin-tazobactam (100:10 μg), tobramycin (10 μg), imipenem (10 μg), polymyxin B (300 μg) and colistin (10 μg).

Pseudomonas spp.: Piperacillin (100 μg), piperacillin-tazobactam (100:10 μg), tobramycin (10 μg), imipenem (10 μg), ticarcillin (75 μg), polymyxin B (300 μg), and colistin (10 μg).

Gram-positive isolates: Amikacin (30 μg), gentamicin (10 μg), levofloxacin (5 μg), sparfloxacin (5 μg), erythromycin (15 μg), vancomycin (30 μg), oxacillin (1 μg), tobramycin (10 μg), clindamycin (2 μg), and amoxicillin (30 μg).

Detection of extended spectrum and AmpC beta lactamase

Screening of possible ESBL production was done by using ceftriaxone (30 μg) and CP (75 μg). Isolates showing zone diameter less than 25 mm for ceftriaxone and less than 19 mm for CP were subsequently confirmed by disc potentiation test using CP and CPS combination.[9] Organism sensitive to cefoxitin and resistant to cefoperazone-salbactam and piperacillin-tazobactam combination were considered to be Amp C producers.[10]

Detection of metallo-beta-actamases

Imipenem resistant isolates were tested for metallo-beta-lactamases (MBL) production by modified Hodge test and Double Disc synergy test using EDTA.[8]

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 μg 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 μg) and streptomycin (300 μg).

RESULTS

Of 1840 urine samples, 504 (27.4%) were culture positive. Majority were females (n = 1474) 76% and the female to male ratio was 4:1. Among the isolated strains, 390 (77%) were Gram-negative bacilli of which 372 (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 Table 1. In addition, 4% of total isolates were nonenterobacteriaceae Gram-negative organisms, among which *Pseudomonas aeruginosa* (3.4%) predominated followed by *Acinetobacter* (0.4%).

Table 1: Distribution of various urinary pathogens (n=504)

Species	Number	Percentage
Escherichia coli	330	65.4
Klebsiella pneumoniae	22	4.3
Citrobacter	12	2.3
Proteus species	8	1.5
Pseudomonas species	16	3.1
Acinetobacter	2	0.4
Staphylococcus aureus	56	11
Staphylococcus epidermidis	10	1.9
Enterococcus faecalis	30	5.9
Streptococcus species	16	3.1
Corynebacterium species	2	0.4
Total	504	100

The frequency of Gram-positive pathogens was 66 (13%) for *Staphylococcus* spp., 30 (6%) for *Enterococcus* spp., 16 (3%) for *Streptococcus* species and 2 (0.4%) for *Corynebacterium* species.

Antibiotic Susceptibility patterns of most frequent uropathogens to different antibiotics are shown in Tables 2 and 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

Table 2: Sensitivity pattern of Gram-positive isolates

Antibiotics	*Staphylococcus aureus* (*n*=56) (%)		Coagulase negative *Staphylococcus* (*n*=10) (%)		*Enterococcus faecalis* (*n*=30)* (%)		*Streptococcus* species (*n*=16) (%)
	MRSA (*n*=30)	MSSA (*n*=26)	Methicillin resistant (*n*=4)	Methicillin sensitive (*n*=6)	VRE (*n*=4)	HLAR (*n*=12)	
Amikacin	28 (93)	26 (100)	4 (100)	6 (100)	-	-	-
Ofloxacin	-	-	-	-	0 (0)	8 (66)	12 (75)
Norfloxacin	0 (0)	0 (0)	0 (0)	1 (10)	0 (0)	0 (0)	2 (12)
Vancomycin	30 (100)	26 (100)	4 (100)	6 (100)	0 (0)	12 (100)	16 (100)
Fosfomycin	30 (100)	26 (100)	4 (100)	6 (100)	4 (100)	12 (100)	16 (100)
Gentamycin	28 (93)	26 (100)	4 (100)	6 (100)	-	-	-
Cefazolin	0 (0)	24 (93)	0 (0)	6 (100)	0 (0)	0 (0)	8 (50)
Oxacillin	0 (0)	26 (100)	0 (0)	6 (100)	-	-	-
Erythromycin	12 (40)	22 (84.6)	2 (50)	2 (33.3)	0 (0)	0 (0)	12 (75)
Levofloxacin	14 (47)	22 (84.6)	2 (50)	6 (100)	-	-	-
High content gentamycin	-	-	-	-	0 (0)	2 (16)	-
High content streptomycin	-	-	-	-	0 (0)	4 (33)	-
Amoxicillin-clavulanate	-	-	-	-	3 (75)	10 (83)	14 (87.5)
Nitrofurantoin	24 (80)	24 (93)	4 (100)	6 (100)	4 (100)	12 (100)	16 (100)

*Four isolates of *Enterococcus faecalis* were sensitive strains, MRSA: Methicillin resistant *Staphylococcus aureus*, MSSA: Methicillin sensitive *Staphylococcus aureus*, VRE: Vancomycin resistant *enterococci*, HLAR: High level aminoglycoside resistance

Table 3: Sensitivity pattern of ESBL producing, non-ESBL and AmpC producing strains

Antibiotics	*Enterobacteriaceae* (%)			*Pseudomonas aeruginosa* (*n*=16) (%)		
	ESBL (*n*=146)	Non-ESBL (*n*=134)	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)	4 (100)	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)	0 (0)	-	-	-
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)	84 (100)	4 (100)	10 (100)	2 (100)

ESBL: Extended spectrum beta-lactamase

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%).

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*.[11] also reported similar findings in their study.

Prevalence of ESBL (37.1%) and AmpC (21.6%) production was consistent with that reported by Taneja *et al*.[12] Among Gram-positive bacteria a high percentage of MRSA (51.5%), VRE (13.3%) and HLAR (53.3%) was observed. All these findings are higher than our previous reports[4] which points to exonerable increase in drug resistance.

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. The current study demonstrated significant resistance to cotrimoxazole and norfloxacin, which concur with reports of previous studies.[13,14] 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 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.[15] 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.[16] Clinical studies have shown fosfomycin to be effective for the treatment of lower UTIs due to ESBL-producing members of the *Enterobacteriaceae*.[17,18] In our study, all the sensitive and ESBL producing strains showed 100% sensitivity to fosfomycin while 98.7% of AmpC producers were sensitive to this drug. It has previously been reported by other authors that fosfomycin has good *in vitro* activity against ESBL producing *E. coli* and *K. Pneumoniae*.[17,19] Fosfomycin has been reported to have high activity against the majority of *Enterobacteriaceae*, but not toward the Gram-positive bacteria.[20] However, in our study 100% of VRE isolates showed susceptibility to fosfomycin. This finding is in concordance with study of Shrestha *et al*.[21] 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.[22] Current studies on *P. aeruginosa* isolates demonstrated higher rates of resistance to fosfomycin *in vitro*.[23] 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.

Further studies are needed, but fosfomycin appears to have an excellent potential as a possible oral option for the treatment of MDR Gram-positive as well as Gram-negative pathogens. However, increased usage has been shown to correlate with increasing resistance among ESBL-producing strains.[24]

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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