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In vitro activity of fosfomycin tromethamine against ESBL: Producing enterobacteriaceae of urinary tract infections in India

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Abstract
The aim of this study was to evaluate the *in vitro* activities of antimicrobial agents including fosfomycin tromethamine against Gram-negative isolates recovered from urine samples. A total of 1205 strains (781 *Escherichia* coli, 255 *Klebsiella* spp, 43 Proteus spp, 38 *Pseudomonas* spp, 23 Enterobacter spp, 19 *Acinetobacter baumannii*, 4 *Citrobacter* spp, 3 *Morganella morganii*, and 3 *Serratia* spp) were identified by VITEK 2 during the study period, October 2014 to October 2016. Antimicrobial susceptibilities of the strains were also evaluated using the Kirby–Bauer disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute guidelines. Overall, 1080 (92.5%) of the isolates tested were susceptible to fosfomycin tromethamine. Higher resistance rates were observed among inpatients compared to outpatients. Resistance rates by strain were: 2.0% for E. coli, 4.4% for Enterobacter spp, 6.9% for *Klebsiella* spp, 9.4% for Proteus spp, 48.6% for A. baumannii, 56.0% for *Pseudomonas* spp, and 100% for *Morganella morganii*. All *Serratia* spp and *Citrobacter* spp strains were susceptible. The highest *in vitro* activity was detected for amikacin, piperacillin–tazobactam, and imipenem for all strains including ESBL-producers. Regardless of ESBL production, the excellent activity of fosfomycin against E. coli, Enterobacter spp, *Serratia* spp, and *Citrobacter* spp, indicates that the drug is a valuable therapeutic option for urinary tract infections, even those with co-trimoxazole- and ciprofloxacin-resistant isolates.

Keywords: UTI, Gram-negative bacteria, antimicrobial resistance

Introduction
Fosfomycin inhibits bacterial cell wall biogenesis by inactivat-ing the enzyme UDP-N- acetylglucosamine-3-enol-pyruvyltrans- ferase (MurA). It exhibits excellent tissue penetration and impairs adherence to the urogenital mucosa, and it is excreted unchanged in high concentrations in the urine [1, 3]. The emergence and spread of multidrug-resistant (MDR) Gram-negative bacteria related to urinary tract infections (UTIs) is increasing worldwide. The therapeutic option is a growing concern due to the production of extended-spectrum beta-lactamases (ESBLs) exhibiting resistance not only to cephalosporins but also quinolones and co-trimox-azole [1, 6]. With the advantages of administration as a single dose per day, a good safety profile, no effect on the anaerobic gut flora, and availability during pregnancy, this drug is a good option in the treatment of uncomplicated UTIs. [1, 3, 4, 6, 7, 8]. Fosfomycin tromethamine (FOF), a stable salt of fosfomycin, has been found to be effective for the treatment of UTIs related to *Escherichia* coli, *Citrobacter* spp, Enterobacter spp, *Klebsiella* spp, *Serratia* spp, and Enterococcus faecalis [3, 5, 7, 9]. Although it has been commonly prescribed in some countries in Europe and the USA for the treatment of uncomplicated UTIs for several years [3, 5, 8]. resistance rates have so far remained low [4, 10, 11]. Moreover, the drug was found to be effective against MDR and metallo – beta - lactamase (MBL) - producing Enterobacteriaceae strains, with sus-ceptibility rates over 83% [12, 13]. In the present study, we aimed to determine the *in vitro* FOF susceptibility of Gram-negative strains recovered from urine samples and to compare its activity with the other antimicrobial agents.

Materials and Methods
Urine samples of 5124 patients with clinical symptoms of UTI who were referred to the Department of Microbiology, Chhattisgarh Institute of Medical Sciences, Bilaspur, India. during the study period of July 2015 to Jun 2017, were evaluated. Significant bacteriuria is defined by counts of ≥10 [5]. cfu/ ml in the patient's mid-stream urine sample. A total of 1205 bacterial strains (781 E. coli, 255 *Klebsiella* spp, 43 Proteus spp, 38 *Pseudomonas* spp, 23 Enterobacter spp, 19 *Acinetobacter baumannii*, 4 *Citrobacter* spp, 3 *Morganella morganii*, and 3 *Serratia* spp).
Testing of susceptibility to ampicillin (AMP, 10 mg), amikacin (AMK, 30 mg), amoxicillin–clavulanic acid (AMC, 20/10 mg), aztreonam (ATM, 30 mg), cefepime (FEP, 30 mg), cefotaxime (CTX, 30 mg), ceftazidime (CAZ, 30 mg), ceftriaxone (CRO, 30 mg), cefuroxime (CXM, 30 mg), ciprofloxacin (CIP, 5 mg), co-trimoxazole (SXT, 1.25/23.75 mg), fosfomycin tromethamine (FOF, 200 mg), gentamicin (GEN, 10 mg), imipenem (IPM, 10 mg), and piperacil-lin–tazobactam (TZP, 100/10 mg) (Oxoid Ltd, Basingstoke, UK) was determined by Kirby–Bauer disk diffusion test method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. E. coli ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. ESBL screening of the isolates was performed by disk synergy test, and results were confirmed by cefotaxime, ceftazidime, cefotaxime–clavulanic acid (CTC, 30/10 mg),

and ceftazidime– clavulanic acid (CZC, 30/10 mg) disks, in accordance with CLSI guidelines [14].

E. coli ATCC 25922 (ESBL-negative) and *Klebsiella* pneumoniae ATCC 700603 (ESBL-positive) were used as quality control strains for the phenotypic testing of ESBL production. Several colonies from a 24-h culture plate were used to prepare the inoculum with a 0.5 McFarland standard density, and Mueller–Hinton agar plates were streaked using cotton swabs. The Etest MBL strips were then applied, and the plates were incubated at 35 °C in air for 16–20 h. A ratio of the MICs of the imipenem (IP) to imipenem plus ethylene diaminetetra acetic acid (EDTA) (IPI) of ≥8, or the presence of a phantom zone, i.e. an extra inhibition zone between the IP and IPI regions, or a deformation of the IP or IPI ellipses, was interpreted as being positive for MBL production

Results

A total of 1205 bacterial strains recovered from 5124 urine samples of 217 (18.6%) inpatients and 950 (81.4%) outpa were included in the study. The most commonly isolated pathogens were E. coli (66.9%) and *Klebsiella* spp (21.8%). Identification of the strains to the species level is shown in Table - 1. Antimicrobial resistance rates of the isolates belonging to the Enterobacteriaceae family (n = 1111) tested in this study were as follows: 71.6% to ampicillin, 38.7% to co-trimoxazole, 28.2% to cefuroxime, 25.4% to ciprofloxacin, 18.7% to gentamicin, 11.8% to amoxicillin–clavulanic acid, 5.5% to piperacillin–tazobactam, 3.7% to FOF, 2.3% to amikacin, and 0.04% to imipenem. Twenty-five percent of the strains were resistant to any of the third-generation cephalosporin group. Antimicrobial resistance rates in relation to species are shown in Table- 2.

Overall, the resistance rates of the isolates tested to co-trimoxazole and ciprofloxacin were 89.2% and 89.7%, respectively. Fosfomycin was found to be effective against strains resistant to co-trimoxazole and ciprofloxacin, displaying susceptibility rates of 94.6% and 93.3% for Enterobacteriaceae and 39.4% and 40.8% for non-fermenting Gram-negative bacilli. Among E. coli strains, the most active agents regardless of ESBL production were imipenem (99.9%) and fosfomycin (97.8%), followed by amikacin (96.9%) and piperacillin–tazobactam (94.6%). Comparison of the *in vitro* efficacy of the antimicrobials by strain type.

Table 1: Bacterial species distribution in this study

Bacterial strain	Number of strains	%
Escherichia coli	781	66.9%
Klebsiella spp	254	21.8%
Proteus spp	43	3.6%
Pseudomonas aeruginosa	75	3.2%
Enterobacter spp	23	1.9%
Acinetobacter baumannii	19	1.6%
Citrobacter spp	4	0.3%
Morganella morganii	3	0.3%
Serratia spp	3	0.3%
Total	1205	100

Table 2: Distribution of resistance rates of all isolates by strain type (n = 1205**)**

Antimicrobial	*Escherichia* coli	*Klebsiella* spp	*Enterobacter* spp	*Proteus* spp	*Morganella* spp	*Serratia* spp	*Citrobacter* spp	*Pseudomonas* spp	*Acinetobacter baumannii*
Ampicillin	69.5	79.6	84.4	55.3	85.7	50.0	87.5	98.7	100.0
Amoxicillin–clavulanic acid	10.3	13.9	42.2	4.7	71.4	16.7	12.5	90.7	70.3
Amikacin	2.1	2.9	4.4	-	-	-	12.5	12.0	73.0
Cefuroxime	28.9	28.3	44.4	7.1	57.1	-	25.0	92.0	97.3
Third-generation cephalosporin	26.1	24.4	35.6	3.5	28.6	-	25.0	80.0	97.3
Ciprofloxacin	29.5	18.7	6.7	3.5	28.6	-	-	24.0	81.1
Fosfomycin	2.0	6.9	4.4	9.4	100	-	-	56.0	48.6
Gentamicin	19.6	17.7	8.9	11.8	57.1	-	12.5	20.0	75.7
Imipenem	0.1	0.2	-	-	-	-	-	6.7	70.2
Co-trimoxazole	41.7	30.8	13.3	47.1	57.1	-	26.0	92.0	64.9
Piperacillin–tazobactam	4.7	9.4	2.2	1.2	-	-	-	12.0	81.1

Discussion

Fosfomycin is a cell wall active antimicrobial agent found to be effective against E. coli, *Citrobacter* spp, Enterobacter spp, *Klebsiella* spp, *Serratia* spp, and E. faecalis related UTIs. [7, 13 15, 16]. Although it has been used for several years, resistance has remained low, at 0.3– 2.8% in E. coli [4, 11, 17, 19]. And 7.2–28.6% in *Klebsiella* spp [17, 19] The CLSI recommends fosfomycin therapy only for the treatment of uncomplicated UTIs related to E. coli [14]. The explanation for this limitation is the reported discrepancies between disk diffusion and agar dilution tests observed for *Klebsiella* spp strains [19, 20]. In contrast to the good correlation in E. coli isolates [20, 21]. The present study compared the *in vitro* efficacy of FOF with that of other antimicrobials, against 1205 Gram-negative bacterial isolates representing nine species. The most common pathogens recovered from urine

were E. coli and *Klebsiella* spp. Overall, 6.1% of the isolates tested were resistant and 1.3% showed intermediate resistance to fosfomycin. Higher rates of resistance were detected among *Klebsiella* spp compared to E. coli strains (10.8% vs. 2.2%; $p < 0.05$), supporting the data published previously. [17,18,22-24] The low level of resistance among E. coli strains could be explained with the drug's limited use for the treatment of uncomplicated UTIs [4, 10, 11, 17, 19, 22]. Suggesting that fosfomycin is the drug of choice for the treatment of UTIs, especially those caused by E. coli? Co-trimoxazole is the recommended drug for the treatment of UTIs in settings where the resistance is <10–20%, 18 and quinolones are the drugs of choice if the co-trimoxazole resistance is higher than 20%. 25 Several studies have shown ciprofloxacin and co-trimoxazole to be highly active against E. coli, with susceptibility rates over 81–99%and 64–82% [17, 18, 20, 21, 25. 26, 27].

In this study, lower susceptibility rates for ciprofloxacin and co-trimoxazole were obtained: 70.5% and 38.3% in E. coli, and 81.3% and 69.2% in *Klebsiella* spp, respectively. The high levels of resistance to co-trimoxazole and ciprofloxacin reported in this study and previously[18] may indicate the misuse of these drugs for both inpatients and outpatients in our country, and it is clear that ciprofloxacin and co-trimoxazole therapy should be evaluated with caution in the treatment of UTI. In agreement with some reports [22]. fosfomycin appears to be an important treatment option for UTIs associated with E. coli and *Klebsiella* spp, even quinolone- and co-trimoxazole-resistant strains, with susceptibility rates of 93.3% and 94.6%, respectively. Several studies have shown that community-acquired ESBL-producing E. coli urinary isolates have high resistance rates to most of the currently used oral antimicrobial agents, with resistance rates of 84% for ciprofloxacin, 75% for co-trimoxazole, 15% for nitrofurantoin, and 0% for fosfomycin, [28] suggesting the use of fosfomycin and nitrofurantoin for the first-line empirical oral treatment of community-acquired uncomplicated UTIs. A single dose of FOF was found to be as effective as ciprofloxacin in the treatment of uncomplicated UTIs [29]. In a multicenter study, FOF, ciprofloxacin, and co-trimoxazole susceptibility rates were 99%, 98.3%, and 87.8%, respectively, among E. coli strains recovered from female patients with symptoms of uncomplicated cystitis; it was stated that co-trimoxazole and quinolones are not recommended as first-line drugs for the empiric treatment of uncomplicated cystitis because of the increasing resistance rates [30]. Four drugs, FOF, amikacin, piperacillin–tazobactam, and imipenem, were found to have maintained high activity against ESBL-producers in this study. For FOF the explanation lies in the decreased fitness of E. coli after acquiring a mutation that confers resistance to this drug [31].

Susceptibilities to fosfomycin of Enterobacteriaceae other than E. coli and *Klebsiella* spp were not extensively studied. In the limited number of studies available, susceptibility rates of Proteus mirabilis, Proteus vulgaris, M. morganii, and Enterobacter spp were 73.8–87.5%, 50%, 0%, and 82.9%, respectively [10, 17]. Additionally, more than 90% of the E. coli and *Citrobacter* spp, more than 70% of *Klebsiella* spp, Enterobacter spp, and P. mirabilis strains, 31.8% of P. aeruginosa, and 11.1% of Acinetobacter spp strains were reported to be susceptible to fosfomycin. Similar to previous reports, [17]. All *Morganella* spp were resistant to FOF, but resistance was not detected among *Serratia* spp and *Citrobacter* spp. However, the results should be evaluated with caution because of the limited numbers of strains. Resistance rates were higher for *Pseudomonas* spp and A. baumannii, at 56% and 48.6%, respectively. High activity was detected for Enterobacter spp, with a susceptibility rate of 4.4%, similar to the rate in a previous report [17]. In contrast to report FOF resistance rates of up to 40% for Proteus spp [11, 17]. A lower rate, 9.4%, was detected, indicating that the drug could be an alternative therapeutic option for UTIs related with these strains. Good *in vitro* activity against E. coli and *Klebsiella* spp was detected in several studies. However it is clear that further studies should be performed to determine and evaluate the drug efficacy in vivo for strains other than E. coli and *Klebsiella* spp.

In this study we could not classify complicated or uncomplicated UTIs due to the lack of information in the database concerning patients' previous treatment with antibiotics, previous hospitalization, and risk factors for UTIs. In conclusion, it is clear that FOF could be an alternative treatment option for UTIs related to E. coli and *Klebsiella* spp, but not for ESBL-producing *Klebsiella* spp. Although *in vitro* data seem to encourage the prescription of FOF, further clinical studies evaluating the clinical efficacy and safety profile of this drug should be conducted.

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