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Original Research Article

Fosfomycin Susceptibility Among Urinary Isolates of *Escherichia Coli* at a Tertiary Care Teaching Hospital

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ABSTRACT

Background: Urinary Tract Infection (UTI) is a prevalent infectious disease caused by Enterobacteriaceae, with *Escherichia coli* being the predominant etiological agent. The rise in multidrug resistance (MDR) and dearth of effective oral antibiotics have constrained the therapeutic option for UTIs. Fosfomycin, an orally administrated bactericidal broad-spectrum antibiotic exhibits activity against MDR pathogens. The aim of this study was to assess in vitro efficacy of Fosfomycin against *Escherichia coli* isolates.

Materials and Methods: This descriptive cross-sectional study spanned a duration of 6 months. A total of 3782 samples were collected from suspected UTI patients and processed. *Escherichia coli* isolates were identified by conventional method and subjected to in vitro susceptibility testing to Fosfomycin and other commonly utilized antibiotics by Modified Kirby-Bauer disk diffusion method.

Results: Among 3782 samples, 929 (24.56%) exhibited significant colony growth, with *Escherichia coli* accounting for 378 (40.6%) of the positive isolates. Higher rates of resistance were observed for Nalidixic acid (92.60%), Norfloxacin (78.58%) and Doxycycline (76.99%). Of the *Escherichia coli* isolates, 269 (71.76%) isolates were identified as MDR isolates. The susceptibility of *Escherichia coli* isolates to Fosfomycin was 95.23%, while that of MDR *Escherichia coli* isolates was 95.54%.

Conclusion
Fosfomycin exhibits excellent in vitro susceptibility against MDR *Escherichia coli* isolates in UTIs, suggesting its potential as a promising alternative oral agent for outpatient therapy of UTI.

Keywords: Urinary Tract Infection, *Escherichia coli*, Fosfomycin

INTRODUCTION

Urinary Tract Infection (UTI) is a prevalent condition encountered in routine clinical practice in both community and healthcare settings, affecting individuals of all age groups and constituting 25% of all infections.[1] Globally, around 150 million individuals are diagnosed with Urinary Tract Infection annually.[2] Gram-negative bacteria, particularly Enterobacteriaceae, are responsible for the majority of Urinary Tract Infection cases, despite variations in risk factors and age groups in different settings. These organisms are known to possess various mechanisms of multiple drug resistance (MDR), whether inherited or transmissible, and chromosomal or extrachromosomal, against commonly utilized oral antimicrobial agents such as fluoroquinolones, trimethoprim-sulfamethoxazole, nitrofurantoin, and second and third generation cephalosporins.[3] Gram-negative organisms have developed significant resistance, particularly in developing countries like India, due to the excessive use and misuse of these drugs, rendering oral therapy increasingly challenging.

Fosfomycin, originally referred to as phosphonomycin, is a phosphonic acid derivative with bactericidal effects on the

cell walls of both gram-positive and gram-negative bacteria by inhibiting the initial step involving phosphoenolpyruvate synthase. It hinders the synthesis of peptidoglycan by impeding the formation of N-acetylmuramic acid.[4] Notably, Fosfomycin does not share structural similarities or cross-resistance with other antimicrobial agents. It reaches a high concentration of 2000 µg/ml in urine and maintains this level for over 24 hours.[5] Fosfomycin is primarily used in treating Urinary Tract Infections, especially those caused by *Escherichia coli* and Enterococcus faecalis, and in combination with other antibiotics to address nosocomial infections caused by resistant Gram-positive and Gram-negative organisms.[6,7]

The objective of this study was to assess the in vitro susceptibility of Fosfomycin against *Escherichia coli* isolates, as the Clinical and Laboratory Standards Institute (CLSI) breakpoint is only available for this bacterium among the Enterobacteriaceae, and to determine the resistance pattern against commonly prescribed antimicrobials.

MATERIALS AND METHODS

Study Design and Duration

This descriptive cross-sectional study was carried out over a period of 6 months, spanning from September 2023 to February 2024, within the Bacteriology section of the Diagnostic Laboratory at the Department of Microbiology in the Medical College Baroda &S.S.G. Hospital, Vadodara.

Study Population

Inclusion Criteria: Urine specimens from patients of all age groups exhibiting clinical symptoms indicative of Urinary Tract Infections in both outpatient and inpatient settings were considered for inclusion in this study.

Exclusion Criteria: Samples obtained from the same patient on multiple occasions were excluded from the study.

Collection of samples: Midstream urine samples from non-catheterized patients were obtained using a clean catch method. Suprapubic aspirates were collected from paediatrics patients and certain adult patients as indicated. For catheterized patients, urine samples were gathered from the catheter using aseptic techniques.

Processing of sample: Urine samples were immediately processed within 30 minutes of collection. Initial microscopic examination of uncentrifuged urine samples was conducted to identify pus cells and bacteria. Centrifuged deposits were further analyzed under a microscope to detect casts and crystals. The urine samples were then cultured on CLED (Cystine lactose electrolyte deficient) agar using a semi-quantitative approach and were left to incubate at 37°C for 16-18 hours. The following day, any significant growth was identified and colony counts were measured. Isolates of Escherichia coli were identified based on colony characteristics, gram staining, and standard biochemical reactions. Antimicrobial susceptibility testing was carried out on Mueller-Hinton agar using the Modified Kirby-Bauer disc diffusion method. Discs containing Fosfomycin (200µg) with 50 µg of glucose-6-phosphate, as well as other commonly used antibiotics such as Norfloxacin (10 µg), Doxycycline (10 µg), Piperacillin-Tazobactam (100/10 µg), Co-trimoxazole (1.25/23.75 µg), Nalidixic Acid (30µg), and Nitrofurantoin (300 µg) were utilized in this process (HiMedia Laboratories). The interpretation of results was based on the CLSI M100-Ed33 (2023) guidelines.[8] Additionally, the ATCC 25922 strain of *Escherichia coli* was employed as a quality control measure. Multi-drug resistance (MDR) was defined as resistance to antibiotics from three distinct classes.[9]

Statistical Analysis: The data gathered was organized and analyzed in Microsoft Excel spreadsheet. Additional computation was conducted utilizing relevant statistical analyses such as frequency tables accompanied by corresponding percentages.

RESULTS

A total of 3,782 urine samples were collected from individuals suspected of having urinary tract infections in both the outpatient and inpatient departments during the study period. Among these, 929 samples (24.56%) exhibited a significant colony count. Of the positive samples, 378 (40.6%) were identified as *Escherichia coli* isolates. Within this group of 378 isolates, 146 (38.62%) were males and 232 (61.38%) were females. Furthermore, of the 378 isolates, 81 (21.42%) were from the outpatient department, 256 (67.74%) were from the hospital wards, and 41 (10.84%) were from various intensive care units. A higher prevalence of female patients was observed in the outpatient department, hospital wards, and intensive care units. Highest number of isolates among wards was obtained from the medicine wards, accounting for 131(51.17%) of the total, followed by the paediatrics ward at 16.40%. In the ICUs, the highest number of isolates were found in the Medical Intensive Care Unit (MICU) with 16 (39.02%), followed by the Cardiac Intensive Care Unit (ICCU) with 10 (24.39%) (Tables 1 & 2).

A total of 99 isolates (26.19%) were identified in individuals aged between 21-40 years and 41-60 years, with a higher prevalence among females at 62 (16.4%) and 61 (16.13%) respectively. The lowest number of isolates, 38 (10.05%), were found in the age group of less than 1 year, with a female predominance of 23 (6.08%). Male predominance was observed in the age group of over 60 years, with 32 (8.46%) isolates (Figure-1).

Table-1: Gender and location wise distribution of patients

	OPD (n=81)	Wards (n=256)	ICUs (n=41)
Male	34 (8.99%)	95 (25.13%)	17 (4.49%)
Female	47 (12.43%)	161 (42.59%)	24(6.37%)

Table-2: Distribution of patients in various wards and ICUs

Wards	Total (n=256)	ICUs	Total (n=41)
Medical	131 (51.17%)	**MICU**	16 (39.02%)
Paediatrics	42 (16.40%)	**ICCU**	10 (24.39%)
Surgical including Orthopedics	34 (13.28%)	**PICU**	9 (21.95%)
Gynecology	29 (11.32%)	**EMNICU**	6 (14.64%)
Others (TBCD, skin etc.)	20 (7.83%)	**Others**	0 (0%)

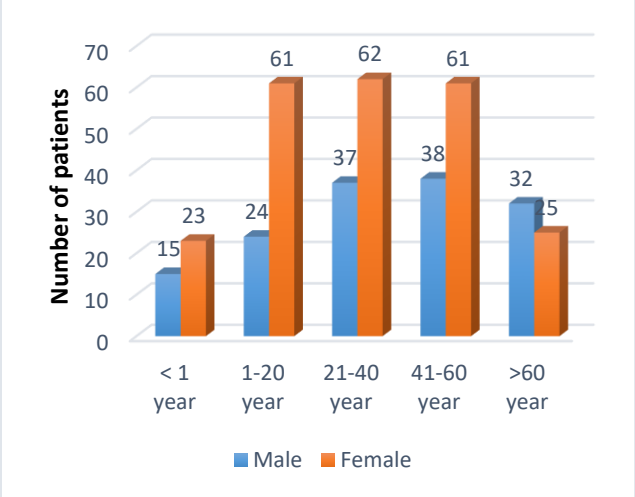

Figure-1: Age-wise distribution of patients

Out of a total of 378 isolates, 269 (71.16%) were found to be multidrug-resistant (MDR). A greater proportion of MDR isolates of *Escherichia coli* were identified in the intensive care units (ICUs) at 85.36%, with 72.65% being found in the general wards (Table-3).

Table-3: Distribution of MDR isolates in OPD, wards and ICUs

	OPD	Wards	ICUs
Total	81	256	41
MDR	48	186	35
Percentage	59.25%	72.65%	85.36%

Out of 378 isolates, 360 (95.23%) were found to be susceptible to Fosfomycin. Among the 269 isolates classified as multidrug-resistant (MDR), Fosfomycin demonstrated susceptibility in 257 (95.54%) cases. Conversely, Nalidixic acid, Norfloxacin, and Doxycycline exhibited elevated resistance rates of 92.60%, 78.58%, and 76.99% respectively. The majority of drugs displayed notable resistance levels within inpatient departments, with the exception of Nitrofurantoin, which showed a high resistance rate of 53.08% in outpatient settings (Table-4 and Figure-2).

DISCUSSION

Urinary tract infections (UTIs) are prevalent bacterial infections on a global scale, manifesting in both community and healthcare environments. UTIs are typically categorized as uncomplicated or complicated. The most frequent cause of uncomplicated UTIs, in both community and healthcare settings, as well as complicated UTIs, is uro-pathogenic Escherichia coli (UPEC). Gram-negative bacteria are primarily responsible for UTIs and pose a growing public health concern due to their capacity to acquire genes encoding extended-spectrum β-lactamases (ESBLs) on transferable plasmids.[10] ESBLs are problematic because they are typically found on plasmids that also carry resistance genes against various classes of antibiotics (e.g., aminoglycosides, sulfonamides, and quinolones).[11,12] Consequently, the reassessment of underutilized antibacterial medications like Fosfomycin is crucial. Fosfomycin possesses qualities that make it a compelling option for UTI treatment, including prompt absorption post oral administration, biofilm activity,[13,14] and efficacy against numerous multi-drug resistant organisms, including extended-spectrum beta-lactamase (ESBL) and AmpC-producing Enterobacteriaceae.[15]

In this current study, we assessed the in vitro activity of Fosfomycin and other frequently used antimicrobial drugs against *Escherichia coli* strains isolated from suspected UTI patients. Female patients were more commonly affected, except in the ≥ 60 age group, where male patients were more prevalent, likely due to obstruction from prostatic hypertrophy.

In this study, 92.60% of *Escherichia coli* isolates exhibited resistance to Nalidixic acid, a significantly higher rate compared to other antibiotics. Sreenivasan et al. also reported a 91.4% resistance to Nalidixic acid in their study.[16] A high resistance rate of 78.58% was observed against Norfloxacin in this study, similar to the findings of Ahmed Sardar, who reported a 79% resistance to Norfloxacin.[17] Kumar et al. documented an 88% resistance against Norfloxacin in their study, which is notably higher.[18]

Table-4: Antibiotic Susceptibility Pattern of E. coli in outpatient and inpatient

	Outpatient (n=81)			Inpatient (n=297)			Total (n=378)		
Antibiotics	**Susceptible**	**Intermediate or Susceptible Dose Dependent**	**Resistant**	**Susceptible**	**Intermediate or Susceptible Dose Dependent**	**Resistant**	**Susceptible**	**Intermediate or Susceptible Dose Dependent**	**Resistant**
Fosfomycin	79 (97.53%)	0	2 (2.47%)	281 (94.61%)	2 (0.67%)	14 (4.72%)	360 (95.23%)	2 (0.52%)	16 (4.25%)
Nitrofurantoin	43 (53.08%)	15 (18.51%)	23 (28.41%)	155 (52.18%)	63 (21.21%)	79 (26.61%)	198 (52.38%)	78 (20.63%)	102 (26.99%)
Nalidixic Acid	4 (4.93%)	4 (4.93%)	73 (90.14%)	10 (3.36%)	10 (3.36%)	277 (93.28%)	14 (3.70%)	14 (3.70%)	350 (92.60%)
Norfloxacin	24 (29.62%)	4 (4.93%)	53 (65.45%)	45 (15.15%)	8 (2.69%)	244 (82.16%)	69 (18.25%)	12 (3.17%)	297 (78.58%)
Piperacillin Tazobactam	7 (8.64%)	29 (35.80%)	45 (55.56%)	19 (6.39%)	61 (20.53%)	217 (73.08%)	26 (6.87%)	90 (23.80%)	262 (69.33%)
Cotrimoxazole	25 (30.86%)	4 (4.93%)	52 (64.21%)	87 (29.29%)	10 (3.36%)	200 (67.35%)	112 (29.62%)	14 (3.70%)	252 (66.68%)
Doxycycline	12 (14.81%)	7 (8.64%)	62 (76.55%)	31 (10.43%)	14 (4.71%)	252 (84.84%)	43 (15.46%)	21 (7.55%)	314 (76.99%)

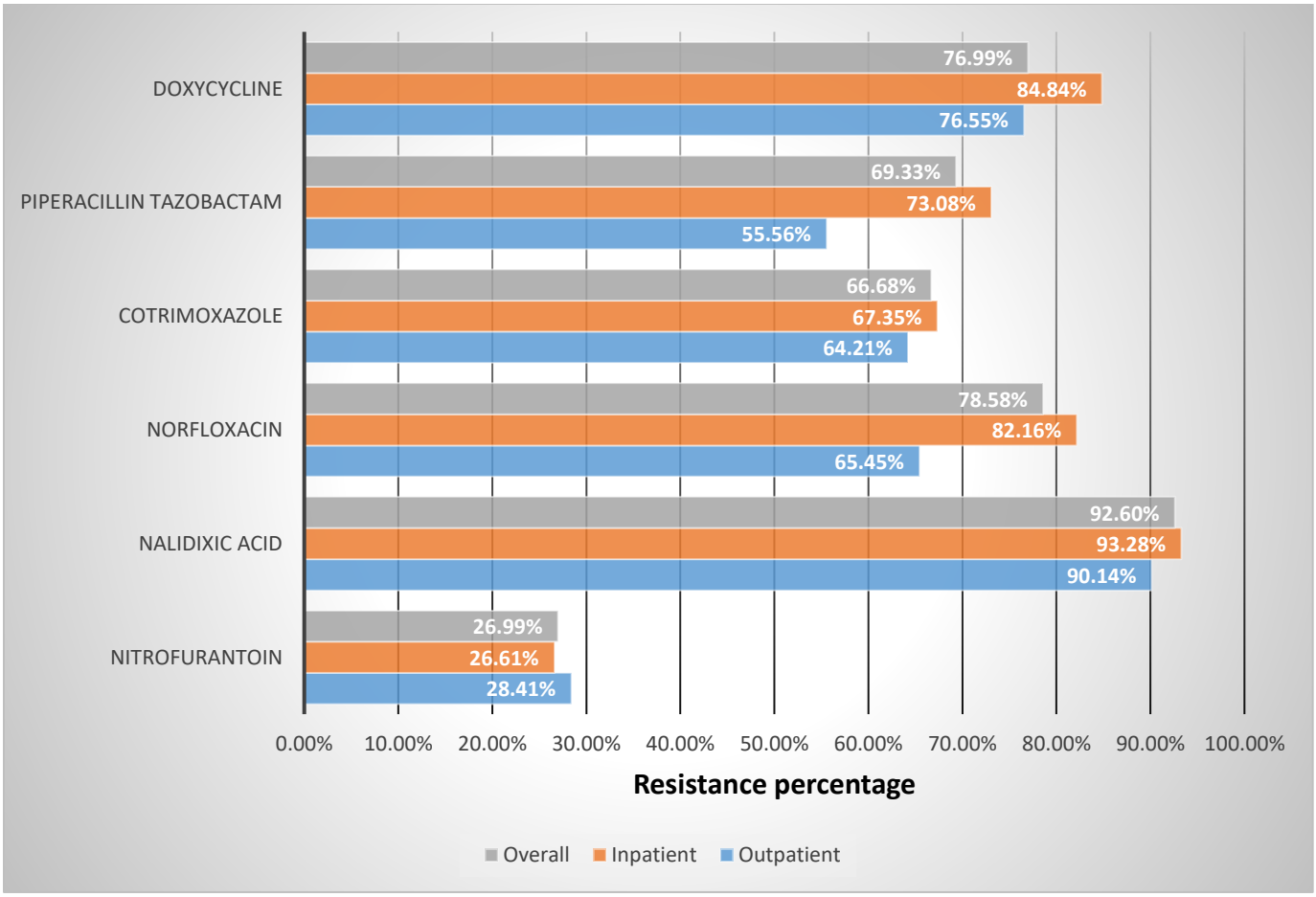

Figure-2: Resistance pattern of commonly used drugs against E. coli isolates

Cotrimoxazole susceptibility was only 29.62% against E. coli isolates in this study. Likewise, Simon et al. and Niranjan et al. reported 27% and 35.8% susceptibility, respectively, mirroring our results.[19,20]

The in vitro susceptibility of Fosfomycin against E. coli was 95.23% in this study. A similar outcome was reported by Banerjee et al[21] at 98.12%, while Ahmed Sardar et al[17] and Kumar et al[18] documented 100% susceptibility against E. coli isolates. In this study, 71.16% of *Escherichia coli* isolates were multidrug-resistant (MDR), a figure comparable to that of Niranjan et al,[20] who reported 76.5% MDR isolates, but higher than other studies like Hasan et al., who reported 52.9% MDR isolates. The susceptibility of Fosfomycin against MDR *Escherichia coli* was 95.54% in our investigation, aligning closely with other studies.[18,21,22] The low resistance to Fosfomycin may be attributed to its unique target action and chromosomal resistance rather than plasmid-mediated resistance.

Limitation of study
ESBL detection was not accounted for in this study, which is an important aspect of nosocomial infections.

CONCLUSIONS

This study has identified an increase in drug resistance of E. coli against commonly used antibiotics. The high susceptibility against MDR pathogens and its unique properties make Fosfomycin a promising and safe alternative oral agent for both outpatient and inpatient UTI therapy.

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