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Antibiotic Susceptibility Pattern of Urinary Isolates of *Escherichia coli* with focus on Fosfomycin and Nitrofurantoin

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Abstract

Urinary tract infections (UTIs) are primarily attributed to Gram-negative bacteria (GNB), especially *Escherichia coli*, which is the most prevalent pathogen. Since nearly all UTIs are treated empirically, either without sending the sample for culture or before the culture and sensitivity reports are obtained. Hence, this study aimed to find out the antibiotic susceptibility profile of urinary isolates of *E. coli*, specifically focusing on fosfomycin and nitrofurantoin. This cross-sectional study was conducted for the period of one year, from 1st July 2023 to 30th June 2024, at the Department of Microbiology, Geetanjali Medical College and Hospital (GMCH), Udaipur. A total of 600 *E. coli* showed a significant bacteriuria from both genders and all age groups, and both outdoor and indoor patients were included. The received urine samples were processed immediately as per the routine microbiological techniques with aseptic precautions. These samples were inoculated onto nutrient agar, blood agar, and MacConkey agar. *E. coli* were identified based on their colony morphology and routine biochemical tests. The modified Kirby-Bauer disc diffusion method was employed for antimicrobial susceptibility testing. Out of 600 *E. coli*, 326 (54.33%) were found in females and 274 (45.67%) in males. The age group most frequently afflicted was 61-70 years, 151 (25.16%). The majority of *E. coli* were from indoor patients, 336 (56%). ESBL production was seen in 396/600 (66.0%), while 454/600 (75.67%) were multidrug-resistant. Maximum susceptibility among antimicrobials was seen in fosfomycin (96.17%), nitrofurantoin (95.83%), and minocycline (93.17%). In the current situation with challenging treatment of UTIs caused by ESBL-producing and MDR *E. coli* and limited options of antibiotics, fosfomycin and nitrofurantoin are the promising and safe alternatives as oral agents for both outpatient and inpatient therapy.

Keywords: *Escherichia coli*, Fosfomycin, Multidrug-resistance, Nitrofurantoin, Urinary Tract Infections

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INTRODUCTION

Urinary tract infections (UTIs) continue to be one of the most common infections encountered in medical practice, despite the widespread availability of medications.[1] UTIs occur across all age demographics and affect both females and males; however, the incidence rate is elevated in females relative to males. About 50% of the female population encounters at least one UTI episode during their lifetime, and 20%-40% experience recurrent UTI episodes.[1] Female are 60% more likely than males to develop a UTI throughout their lifetime, while men are just 13% more likely to do so.[2]

UTIs are primarily attributed to GNB, especially *E. coli*, which is the most prevalent pathogen in both uncomplicated and complicated UTIs in the community and healthcare-associated settings.[3,4] Usually, empirical antibiotic treatment is started in almost all UTIs either without sending the sample for culture or before the culture and sensitivity reports of urine samples are obtained.[5] This may lead to the frequent inappropriate and injudicious use of most commonly empirically prescribed antibiotics, such as fluoroquinolones, cephalosporins, and other β-lactams, which has contributed to the rise of highly drug-resistant uropathogens, including those that produce carbapenemase, extended-spectrum beta-lactamase (ESBL), and multidrug-resistant (MDR) uropathogens.[6,7] Due to these highly drug-resistant uropathogens, antibiotics like fosfomycin and nitrofurantoin are gaining importance.[7] The increase rate of resistance in uropathogenic *E. coli* is ominous and hence requires the re-evaluation of these antibiotics.[8]

Fosfomycin, a synthetic organic phosphonic acid derivative, prevents Gram-positive and Gram-negative bacteria from synthesizing their cell walls. It is a safe and efficient bactericidal drug for both uncomplicated and complicated urinary tract infections, but its usage should be restricted to delay the rising of resistance. Nitrofurantoin belongs to the nitrofuran class, disrupts bacterial ribosomal proteins, and impedes protein synthesis. It is suitable only for an uncomplicated urinary tract infection. Bacterial resistance is unusual with nitrofurantoin. The advantages of these drugs over newer drugs include higher urinary drug concentrations and fewer side effects.[3,9]

The antibiotic susceptibility profile of UTI-causing *E. coli* is inconsistent, differing by location and temporal factors. As the majority of UTIs are treated empirically, the criteria for antibiotic selection should be based on the most probable pathogen and its anticipated resistance profile in the specific geographic region.[2] Therefore, to ensure appropriate management of UTIs and to curtail the development of antibiotic resistance, the antibiotic susceptibility profile of *E. coli*, the predominant bacterium responsible for UTIs, should be updated from time to time. This will aid in formulating an antibiotic policy that will help clinicians to select the most appropriate empirical antibiotic for UTIs caused by *E. coli* before culture and sensitivity reports are obtained; hence, this study aimed to find out the antibiotic susceptibility profile of urinary isolates of *E. coli*, specifically focusing on fosfomycin and nitrofurantoin.

MATERIALS AND METHODS

This cross-sectional study was conducted for the period of one year, from 1st July 2023 to 30th June 2024, at the Department of Microbiology, GMCH, Udaipur. The demographic details, such as date of sample collection, age, gender, indoor/outdoor location of patients, and clinical information like history of catheterization, etc., were obtained from the requisition form submitted along with the sample.

Inclusion criteria

All *E. coli* isolates showed a significant count ≥10^5 CFU/ml from midstream urine and ≥10^4 CFU/ml from catheterized samples from both genders, all age groups, and both outdoor and indoor patients.

Exclusion criteria

Uropathogens other than *E. coli*, duplicate isolates from the same patients, specimens revealing growth of more than two types of bacteria on culture (i.e., polymicrobial growth), and urine samples showing insignificant count or doubtful significant growth.

Study procedure

The received urine samples were processed immediately as per the routine microbiological techniques with aseptic precautions. These samples were inoculated on blood agar by the semiquantitative method with a calibrated loop (0.001 ml) to determine the colony-forming unit (CFU). The quadrant streaking was done on nutrient agar and MacConkey agar to get the isolated colonies for performing biochemical reactions and antimicrobial susceptibility testing. The inoculation plates were subsequently incubated aerobically at 37 °C and examined for bacterial growth after 24 and 48 hours. *E. coli* were identified based on their colony morphology and routine biochemical tests. Mueller-Hinton Agar (MHA) plates were subjected to antibiotic susceptibility testing (AST) using the modified Kirby-Bauer disc diffusion method in accordance with the preset panel of antibiotics specified in CLSI (Clinical and Laboratory Standards Institute) guidelines 2023.[10]

All isolates were evaluated for ESBL production utilizing the modified Kirby-Bauer disc diffusion method for susceptibility to cefotaxime (30 µg), ceftriaxone (30 µg), and ceftazidime (30 µg) discs (HiMedia, Mumbai), yielding zone diameters of ≤27 mm, ≤25 mm, and ≤22 mm, respectively, and were classified as presumptive ESBL producers. The result was interpreted based on the CLSI 2023 guidelines. All presumptive ESBL producers were confirmed by the phenotypic confirmatory disc diffusion test (double disc synergy test). In this test, a disc of ceftazidime (30 µg) and a disc of ceftazidime with clavulanic acid (10 µg) were positioned 20 mm apart on a Mueller-Hinton agar plate inoculated with the test isolate and thereafter incubated at 35-37 °C for 18-24 hours. An increase of ≥5 mm in zone diameter when testing the combination of ceftazidime and clavulanic acid compared to testing ceftazidime alone was deemed indicative of an ESBL producer.[10]

Isolates were labelled multidrug-resistant (MDR) based on the Centers for Disease Control and Prevention (CDC) guideline. Multidrug-resistance was defined as isolates that were not susceptible to at least one agent in three or more antimicrobial groups.[11]

Table 1. Age-wise and Gender-wise distribution of urinary isolates of *E. coli* (n = 600)

Age (In years)	Male	Female	Total n (%)
≤10	06 (1.0%)	06 (1.0%)	12 (2.0%)
11-20	10 (1.66%)	16 (2.66%)	26 (4.32%)
21-30	25 (4.16%)	31 (5.16%)	56 (9.32%)
31-40	34 (5.66%)	29 (4.83%)	63 (10.49%)
41-50	32 (5.33%)	23 (3.83%)	55 (9.16%)
51-60	59 (9.83%)	71 (11.83%)	130 (21.66%)
61-70	62 (10.33%)	89 (14.83%)	151 (25.16%)
71-80	30 (5.0%)	40 (6.66%)	70 (11.66%)
≥81	16 (2.66%)	21 (3.50%)	37 (6.16%)
Total	274 (45.67%)	326 (54.33%)	600 (100%)

Statistical analysis

Statistical analysis was conducted using GraphPad Prism 7. All the data gathered in the current study were expressed in proportions and percentages. The disparity in proportion was analyzed with a chi-square test (χ^2). The significance criterion for the tests was established at 95% ($p < 0.05$).

RESULTS

A total of 600 urinary isolates of *E. coli* with significant counts were included. Out of 600 *E. coli*, 326 (54.33%) were found in females and 274 (45.67%) in males (Table 1). The ratio of male to female was 1:1.8. The age group most frequently afflicted was 61-70 years, 151 (25.16%), followed by 51-60, 130 (21.66%) (Table 1). The maximum isolates of *E. coli* were from indoor patients (IP), 336 (56.0%), and 264 (44.0%) were from outdoor patients (OP) (Table 2). Out of 600 *E. coli*, 396 (66.0%) were ESBL producers, and 204 (34.0%) isolates were ESBL non-producers, whereas 454 (75.67%) were MDR and 146 (24.33%) isolates were non-MDR. All the ESBL producers in our study were MDR. Among 454 multidrug-resistant *E. coli*, resistance to ≥6 drug classes was seen in 173 (38.11%), followed by 3 drug classes, 117 (25.77%), 4 drug classes, 90 (19.82%), and 5 drug classes, 74 (16.30%) (Table 3). The antimicrobials with the highest susceptibility were fosfomycin (96.17%), nitrofurantoin (95.83%), and minocycline (93.17%) (Table 4). The association between ESBL producers and ESBL non-producers, as well as MDR and

Table 2. Distribution of *E. coli* isolates according to location of patients

Type of patients	Number of *E. coli* isolates (n = 600)	ESBL producer (n = 396)	MDR (n = 454)
Outdoor patients	264 (44.0%)	66 (16.67%)	107 (23.57%)
Indoor patients	336 (56.0%)	330 (83.33%)	347 (76.43%)
Total	600 (100%)	396 (100%)	454 (100%)

Table 3. Frequency of MDR *E. coli* according to its resistance to three or more antimicrobial drug classes (n = 454)

No.	Drug combination	No. of isolates (n)	Percentage
1.	Resistant to ≥6 drugs classes	173	38.11%
2.	Resistant to 5 drugs classes	74	16.30%
3.	Resistant to 4 drugs classes	90	19.82%
4.	Resistant to 3 drugs classes	117	25.77%
	Total	454	100%

non-MDR, with fosfomycin (P = 0.91 for both) and nitrofurantoin (P = 0.78 and P = 0.94, respectively) was found to be statistically insignificant (Table 4).

DISCUSSION

Antimicrobial resistance increasing continually and spreading globally is a cause of concern worldwide. It results in treatment failure and an increase in morbidity, mortality, and economic burden on patients. UTI due to *E. coli* is a common bacterial infection, and the emergence of multidrug-resistant *E. coli* further complicates the therapy by posing a significant therapeutic challenge to the clinician. Hence, the neglected, antiquated drugs like fosfomycin and nitrofurantoin are being reexplored and gaining importance for the treatment of UTIs, especially cystitis. The antimicrobial susceptibility data of UTIs is variable, differing by location and temporal factors. Thereby, continuous monitoring of the antibiotic resistance pattern is crucial and essential.

Out of 600 urinary isolates of *E. coli*, 326 (54.33%) were isolated from female patients compared to 274 (45.66%) from male patients, which was in agreement with studies conducted by Bhargava et al.,[12] Kaur et al.,[13] Sreenivasan et al.,[14] and Ahmed et al.[15] The higher incidence of UTIs in females attributable to *E. coli* may result from the shorter length of the urethra, the anatomical proximity of the urethral meatus to the anus, hormonal changes, and sexual activity.

E. coli was predominantly isolated in the elderly age group 61-70 years, 151 (25.16%), followed by the 51-60 years age group, 130 (21.66%). This is in concordance with the studies conducted by Pardeshi,[2] Farouk et al.,[16] and Sundaramurthy et al.,[17] who also reported higher isolation rates of *E. coli* in the elderly age group 49.14%, 56%, and 67.30%, respectively. Higher isolation rates in the elderly age group can be due to the presence of comorbid conditions like diabetes mellitus; additionally, in males, it can be due to prostatic enlargement and neurogenic bladder, and in females, it can be due to urethral stricture and stones.

Maximum isolation of *E. coli* was from indoor patients, 336 (56%), as compared to outdoor patients, 264 (44%), which was in agreement with studies conducted by Sardar et al.,[7] Kaur et al.,[13] and Dalai et al.[18] Higher isolation rates of *E. coli* from indoor patients can be attributed to the fact that they have been more likely to have factors that increase the risk of UTI, including the use of urinary catheters, prolonged bed rest, weakened immune systems due to underlying medical conditions, and increased exposure to antibiotic-resistant bacteria within the healthcare setting.

Out of 600 urinary *E. coli*, ESBL producers were observed in 396 (66%) isolates, and 204 (34%) were ESBL non-producers. Similarly, high ESBL producers were observed in studies done by Sreenivasan et al. (74.13%),[14] Sundaramurthy et al. (54.17%),[17] Dalai et al. (65.39%),[18] and Gupta et al. (52.67%).[19] The high ESBL producers in our

Table 4. Antibiotic susceptibility pattern of urinary isolates of *E. coli* (n = 600)

No.	Antibiotic discs	Total *E. coli* isolates (n = 600)	ESBL produces (n = 396)	ESBL non-producers (n = 204)	χ^2	P-value	MDR isolates (n = 454)	Non- MDR isolates (n = 146)	χ^2	P-value
1.	Amikacin	427 (71.17%)	255 (64.39%)	172 (84.31%)	4.24	0.039	303 (66.74%)	124 (84.93%)	2.85	0.09
2.	Cefuroxime	160 (26.67%)	0	160 (78.43%)	-	-	51 (11.23%)	109 (74.66%)	108.65	<0.0001
3.	Ceftriaxone	184 (30.67%)	0	184 (90.19%)	-	-	56 (12.33%)	128 (87.67%)	126.72	<0.0001
4.	Ceftazidime	170 (28.33%)	0	170 (83.33%)	-	-	50 (11.01%)	120 (82.19%)	125.34	<0.0001
5.	Cefepime	286 (47.67%)	138 (34.84%)	148 (72.54%)	25.48	<0.0001	149 (32.82%)	137 (93.83%)	49.49	<0.0001
6.	Amoxicillin+ clavulanic acid	325 (54.17%)	175 (44.19%)	150 (73.53%)	13.18	0.0002	191 (42.07%)	134 (91.78%)	28.52	<0.0001
7.	Ampicillin+ sulbactam	315 (52.50%)	146 (36.87%)	169 (82.84%)	33.03	<0.0001	185 (40.75%)	130 (89.04%)	28.13	<0.0001
8.	Piperacillin+ Tazobactam	336 (56.0%)	180 (45.45%)	156 (76.47%)	14.06	0.0002	235 (51.76%)	101 (69.18%)	3.64	0.057
9.	Ciprofloxacin	216 (36.0%)	101 (25.50%)	115 (56.37%)	24.69	<0.0001	101 (22.25%)	115 (78.76%)	61.01	<0.0001
10.	Levofloxacin	235 (39.17%)	112 (28.28%)	123 (60.29%)	23.84	<0.0001	115 (25.33%)	120 (82.19%)	55.58	<0.0001
11.	Imipenem	385 (64.17%)	202 (51.01%)	183 (89.71%)	18.0	<0.0001	245 (53.96%)	140 (95.89%)	16.47	<0.0001
12.	Meropenem	401 (66.83%)	215 (54.29%)	186 (91.17%)	15.50	<0.0001	263 (57.93%)	138 (94.52%)	12.02	0.0005
13.	Ertapenem	366 (61.0%)	179 (45.20%)	187 (91.66%)	27.57	<0.0001	224 (49.33%)	142 (97.60%)	22.73	<0.0001
14.	Doripenem	360 (60.0%)	180 (45.45%)	180 (88.24%)	24.0	<0.0001	219 (48.23%)	141 (96.58%)	23.62	<0.0001
15.	Co-trimoxazole	350 (58.33%)	179 (45.20%)	171 (83.82%)	20.42	<0.0001	230 (50.66%)	120 (82.19%)	10.86	0.0009
16.	Aztreonam	355 (59.17%)	185 (46.72%)	170 (83.33%)	18.05	<0.0001	234 (51.54%)	121 (82.88%)	10.53	0.001
17.	Minocycline	553 (93.17%)	363 (91.67%)	190 (93.13%)	0.02	0.90	407 (89.65%)	146 (100%)	0.65	0.42
18.	Nitrofurantoin	575 (95.83%)	384 (96.97%)	191 (93.63%)	0.08	0.78	434 (95.59%)	141 (96.57%)	0.006	0.94
19.	Fosfomycin	577 (96.17%)	379 (95.70%)	198 (97.05%)	0.01	0.91	435 (95.81%)	142 (97.26%)	0.01	0.91

study are in concordance with the other studies, suggesting that the prevalence of infection by ESBL-producing *E. coli* varies greatly geographically and rapidly changes over time due to injudicious antibiotic usages.

Higher multidrug-resistance was observed in 454 (75.67%) isolates, and 146 (24.33%) were labeled as non-MDR. This is in agreement with studies conducted by Jain et al.,[3] Sreenivasan et al.,[14] Shakya et al.,[20] and Gupta et al.,[21] who also reported that 85.52%, 86.28%, 71.03%, and 47.88%, respectively, of isolates were MDR. The variances arise from disparities in antibiotic utilization and infection control protocols among hospitals in different geographical areas.

The majority of *E. coli* were resistant to 6 or more drug classes, 173/454 (38.11%), followed by 3 drug classes, 117/454 (25.77%), 4 drug classes, 90/454 (19.82%), and 5 drug classes, 74/454 (16.30%), while 101/600 (16.83%) isolates were sensitive to all the drug classes. This is in concordance with the study done by Das B et al.,[22] who showed maximum isolates were resistant to 5 or more drug classes (50%), while 22.2% of isolates exhibited sensitivity to all drug classes. In our study, all the ESBL producers were MDR. This association of ESBL producers and MDR parallels the study conducted by Jain et al.,[3] Das et al.,[22] and Bakshi et al.[23]; however, Sahni et al.[24] reported 76.80% of ESBL producers were MDR.

In our study, the most susceptible (effective) antimicrobial agents for uropathogenic *E. coli* were fosfomycin and nitrofurantoin, with a percentage susceptibility of 96.17% and 95.83%, respectively. Similarly, high susceptibility to fosfomycin and nitrofurantoin was observed by Jain et al.,[3] Kaur et al.,[13] Sreenivasan et al.,[14] Sundaramurthy et al.,[17] Gupta et al.,[19] Gupta et al.,[21] Das et al.,[22] and Wagle et al.[25] In ESBL producers, maximum susceptibility was observed in nitrofurantoin (96.97%), followed by fosfomycin (95.70%). Similarly high susceptibility to fosfomycin and nitrofurantoin in ESBL producers was observed by Gupta et al.,[19] Das et al.,[22] and Tulara.[26] In MDR isolates, maximum susceptibility was seen in fosfomycin (95.81%), followed by nitrofurantoin (95.59%). Similarly high susceptibility to fosfomycin and nitrofurantoin in MDR isolates was observed by Jain et al.,[3] and Das et al.[22] The Antimicrobial Resistance (AMR) Surveillance Network, Indian Council of Medical Research (ICMR), 2023, reported 93.70% and 86.60% of the urinary isolates of *E. coli* exhibited sensitivity to fosfomycin and nitrofurantoin, respectively.

In addition to fosfomycin and nitrofurantoin, very good susceptibility was seen towards minocycline (93.17%), including ESBL producing (93.67%) and MDR isolates of *E. coli* (89.65%). However, due to its broad spectrum and activity against MDR bacteria and its potential side effects, its use in bacterial infections has been limited; therefore, this drug is reserved for use in cases where other antibiotics are not effective or when the benefits outweigh the risks.

The higher resistance was observed in most empirically prescribed oral drugs, such as cephalosporin (cefuroxime 73.33%, ceftazidime 71.67%, ceftriaxone 69.33%, and cefepime 52.33%) and fluoroquinolones (ciprofloxacin 64.0% and levofloxacin 60.83%). This finding has also been observed by Pardeshi,[2] Sardar et al.[7] Bhargava et al.,[12] Sundaramurthy et al.,[17] and Shakya et al.[20] The elevated resistance rates to cephalosporins and fluoroquinolones may result from the imprudent use of these medications and inadequate enforcement of antibiotic prescribing policies. Also, *E. coli* has a tendency to acquire multidrug-resistance genes and develop unpredictable susceptibility patterns, thus emphasizing the need for constant surveillance of antibiograms so appropriate empirical treatment can be chosen for patients.

With reference to urinary antibiotics, the high susceptibility to fosfomycin and nitrofurantoin observed in our study is in concordance with the other studies; this may be due to the fact that fosfomycin and nitrofurantoin were absent for a prolonged period from routine clinical use with the availability of more user-friendly drugs like cephalosporin and fluoroquinolone groups. The cessation of an antibiotic from routine use or an extended duration of therapeutic inactivity is more likely to alleviate the selective pressure on the antibiotic. As compared to other drugs, these have the advantage of being oral agents with minimal side effects. Thereby, these drugs may serve as a promising alternative to the existing empirical treatment for UTIs in this era of multidrug-resistance for both inpatients and outpatients.

Limitations of the study

The present study focused only on *E. coli* and did not include other uropathogens. We have not studied AmpC beta-lactamase and metallo-beta-lactamase (MBL) production in these isolates, which may be associated with antibiotic resistance.

CONCLUSION

The antibiotic pattern of urinary isolates of *Escherichia coli* may vary over time and across different locations. The excessive and improper use of antibiotics has resulted in the rise of beta-lactamase-producing and multidrug-resistant bacteria. Escalating rates of antimicrobial resistance among *E. coli* are a significant concern worldwide. The efficacy of antimicrobial drugs is diminishing due to the proliferation of resistant organisms, indiscriminate antibiotic usage, patient noncompliance, insufficient awareness, and unhygienic conditions. This is the reason why infection control practices of healthcare setups should be strengthened to limit the spread of infections caused by MDR pathogens, and an effective antibiotic policy should be formulated for judicious use of antibiotics. Therefore, it is vital to conduct regular surveillance and monitoring of antibiotic susceptibility patterns in a specific healthcare setting. This will help clinicians understand the reasons for treatment failures caused by UTIs due to *E. coli* and guide them in selecting appropriate empiric antimicrobial therapy. This will not only avert the development and proliferation of drug-resistance but also aid in conserving the limited antibiotics available for future utilization. In the current situation with challenging treatment of UTIs due to multidrug-resistant (MDR) isolates of *E. coli* and limited options of antibiotics, fosfomycin and nitrofurantoin may be the preferred antibiotics for empirical treatment. The significant susceptibility against MDR organisms and their distinctive characteristics make fosfomycin and nitrofurantoin safe and promising alternatives as oral medicines for UTI therapy in both outpatient and inpatient settings.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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None.

DATA AVAILABILITY

The datasets generated and/or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Geetanjali Medical College and Hospital, Udaipur, Rajasthan, India, with reference number GU/HREC/EC/2023/2237.

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