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Bacterial Isolation and Antibiotic Resistance Patterns in Urinary Tract Infections: A Focus on Fosfomycin Resistance in *E. coli*

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ABSTRACT: ***Background:*** Urinary tract infections (UTIs) are one of the most common bacterial infections globally, with increasing antimicrobial resistance (AMR) posing a significant challenge to effective treatment. ***Objective*:** This study aims to isolate and identify bacterial uropathies, assess their antimicrobial susceptibility patterns, and determine the presence of Fosfomycin-resistant E. coli strains in a tertiary care hospital in Rajshahi, Bangladesh. ***Methods*:** A descriptive cross-sectional study was conducted at Rajshahi Medical College Hospital, Bangladesh, from January to December 2019. A total of 285 urine samples were collected from clinically suspected UTI patients. Bacterial identification was performed using culture, Gram staining, and biochemical tests. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method, following Clinical and Laboratory Standards Institute (CLSI) guidelines. Fosfomycin-resistant E. coli isolates were subjected to polymerase chain reaction (PCR) for the detection of the fosA3 gene. Data analysis was performed using SPSS version 24.0. ***Result*:** Among 285 participants, 53.68% (153/285) were culture positive, with E. coli (60.49%) being the most prevalent isolate. Gram-negative bacteria accounted for 83.95% of isolates. E. coli exhibited high resistance to Amoxiclav (79.59%) and Ciprofloxacin (56.12%), while showing highest sensitivity to Imipenem (88.78%). Fosfomycin resistance was detected in only 2 E. coli isolates. Other uropathogens such as Klebsiella spp., Pseudomonas spp., and Proteus spp. exhibited high resistance to cephalosporins but remained sensitive to carbapenems. Among Gram-positive bacteria, Staphylococcus aureus was fully susceptible to Levofloxacin, while Coagulase-negative Staphylococci (CoNS) showed 100% sensitivity to Linezolid. PCR analysis confirmed the presence of the fosA3 gene in the two Fosfomycin-resistant *E. coli* isolates. ***Conclusion*:** The study highlights E. coli as the dominant UTI pathogen with significant resistance to β-lactams and fluoroquinolones, reinforcing the importance of carbapenems and Fosfomycin as viable treatment options.

Keywords: Urinary Tract Infection, Antimicrobial Resistance, *E. Coli*, Fosfomycin, Fosa3 Gene, Multidrug Resistance.

Article at a glance:

Study Purpose*: To isolate UTI-causing bacteria and assess antibiotic resistance, with a focus on Fosfomycin-resistant E. coli strains*

Key findings: *E. coli was the most common UTI pathogen, showing high resistance to Amoxiclav and Ciprofloxacin, but sensitivity to Imipenem.*

Newer findings: *Fosfomycin resistance in E. coli was rare, and the fosA3 gene was detected, suggesting continued usefulness of Fosfomycin in MDR cases.*

Abbreviations: *UTI – Urinary Tract Infection, AMR – Antimicrobial Resistance, MDR – Multidrug-Resistant, PCR – Polymerase Chain Reaction*

INRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections encountered in clinical practice, affecting millions of individuals worldwide. They range from uncomplicated cystitis to severe cases involving bacteremia, leading to significant morbidity and healthcare burden.[1] UTIs are prevalent in both community and hospital settings, often associated with recurrent infections that impact patients' quality of life.[2, 3] According to global prevalence study, 404.61 million cases and 236,790 deaths were estimated in 2019. 2.4 times growth in deaths from 1990 to 2019 was observed. [4] The prevalence of UTI varies across regions: 42% in Bangladesh, 34.6% in India and 22.7% in Ethiopia.[5,6,7] Bacteria are the primary etiological agents of UTIs, with Gram-negative bacteria accounting for 80-85% and Gram-positive bacteria for 15-20% of cases. [8] Among these, *Escherichia coli* remains the most frequently isolated uropathogen, responsible for 85% of community-acquired and 50% of hospital-acquired infections.[9] Other significant pathogens include *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter spp., Enterococcus spp., and Staphylococcus aureus*.[9] The risk factors influencing UTI prevalence include gender, age, diabetes, urinary catheterization, previous antibiotic therapy, and congenital urinary tract abnormalities.[10] UTIs are significantly more common in females, with reported prevalence rates of 63.39% in Bangladesh and 65% in India.[11,12] The widespread misuse of antibiotics—including indiscriminate prescriptions, improper dosing, self-medication, and over-the-counter sales—has contributed to rising resistance levels, particularly in developing countries where inadequate healthcare infrastructure and poor hygiene practices exacerbate the problem.[13] The World Health Organization has declared AMR a global threat, emphasizing the need for strict antimicrobial surveillance and antibiotic stewardship programs.[14] Due to the increasing resistance against commonly used antibiotics like Penicillin, Cephalosporins (Cefotaxime, Ceftriaxone, Ceftazidime, Cefuroxime, Cefepime), Fluoroquinolones (Ciprofloxacin, Levofloxacin), and Macrolides (Azithromycin), treatment options for multidrug-resistant (MDR) uropathogens have become limited. Carbapenems, Colistin, and Fosfomycin are often the last resort for treating these infections.[15] Fosfomycin, a phosphonic acid derivative first discovered in 1969, has resurfaced as an effective treatment option, particularly for uncomplicated UTIs.[16] Studies indicate that 98–99% of ESBL-producing uropathogens remain susceptible to Fosfomycin.[17] The prevalence of Fosfomycin resistance varies geographically, with limited data available, especially in Bangladesh and other developing nations. Given the limited availability of novel antimicrobials, re-evaluating older antibiotics like Fosfomycin presents an opportunity to counter rising drug resistance. Despite its broad-spectrum activity, single-dose efficacy, low resistance rates, and cost-effectiveness, Fosfomycin remains underutilized in many regions, including Bangladesh. This study aims to isolate and identify bacterial uropathogens and assess their antimicrobial susceptibility to better understand Fosfomycin resistance trends.

Aims and Objectives

To isolate and identify bacterial uropathogens along with their susceptibility pattern and determination of Fosfomycin resistant *E. coli* strain in a tertiary care hospital in Rajshahi.

MATERIALS AND METHODS

A descriptive type of cross-sectional study was conducted at the Department of Microbiology, Rajshahi Medical College, Rajshahi, Bangladesh, with molecular analysis performed at the Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi. The study was carried out from January 2019 to December 2019, including adult patients of both sexes with clinically suspected urinary tract infections (UTIs) attending inpatient and outpatient departments of Rajshahi Medical College Hospital. Ethical clearance was obtained from the Institutional Review Board (IRB) of Rajshahi Medical College, and additional approvals were secured from the hospital authorities before study initiation. The sample size was determined using Cochran's formula, and 285 urine samples were collected using a purposive sampling technique. Midstream urine samples were obtained from non- catheterized patients, while urine from catheterized patients were collected aseptically using a sterile syringe. Patients were included in the study if they presented with UTI symptoms such as dysuria, urgency, and frequency. Exclusion criteria included patients on antibiotic therapy at the time of sample collection, those with evidence of hematuria or chyluria, and those unwilling to participate in the study. Informed written consent was obtained from all participants before sample collection. A structured, pre-tested questionnaire was used to collect demographic and clinical information from the participants. Microscopic examination of urine was performed by centrifuging 5 ml of each sample at 3000 rpm for 5 minutes, followed by wet mount examination of the sediment under a microscope at 10X and 40X magnifications. Urine samples were cultured on chromogenic UTI agar, MacConkey agar, blood agar, and nutrient agar using a calibrated loop method and incubated at 37°C for 18-20 hours. Bacterial identification was performed based on colony morphology, Gram staining, and standard biochemical tests. Antimicrobial susceptibility testing was conducted using the Modified Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines. Gram-positive bacteria were tested against amoxiclav, oxacillin, vancomycin, cefuroxime, gentamicin, amikacin, ciprofloxacin, azithromycin, linezolid, ceftriaxone, nitrofurantoin, levofloxacin, imipenem, meropenem, and fosfomycin. Gram-negative bacteria were tested against amoxiclav, cefuroxime, ceftazidime, gentamicin, amikacin, ciprofloxacin, azithromycin, fosfomycin, ceftriaxone, nitrofurantoin, meropenem, and imipenem. Zone sizes were measured and categorized as sensitive, intermediate, or resistant according to CLSI (2017) guidelines. Fosfomycin-resistant *E. coli* isolates were preserved at -20°C in trypticase soy broth with 20% glycerol. Molecular detection of the *fosA3* gene was performed using conventional polymerase chain reaction (PCR). Plasmid DNA extraction was carried out using a Promega plasmid DNA isolation kit, followed by amplification using specific primers. PCR products were analyzed via agarose gel electrophoresis and visualized under a UV illuminator. Data were analyzed using SPSS version 24.0, and results were presented in tables, charts, and descriptive statistics.

RESULTS

Sociodemographic Characteristics of Participants

The study included 285 participants, with the majority (30.18%) aged between 21-30 years, followed by 26.6% in the 31-40 years category. A smaller proportion (5.2%) were aged 51 years or older. The participants were predominantly female (65.26%). Most of the participants were married (75.8%), suggesting that the study population had a significant proportion of adults who might be engaged in household or occupational responsibilities that could influence bacterial infections (Table 1).

Table I: Distribution Of the Participants According to Sociodemographic Characteristics (n=285)

Variables	Frequency	Percentage
Age		
≤20	62	21.75
21-30	86	30.18
31-40	76	26.6
41-50	46	16.1
≥51	15	5.2
Sex		
Female	186	65.26
Marital status		
Married	216	75.8

Distribution of Bacterial Isolates

The bacterial isolates were distributed as culture negative (132, 46.32%) and culture positive (153, 53.68%). Among the culture positive isolates, 144 (94.1%) were single isolates and 9 (5.88%) were multiple isolates. (Figure 1).

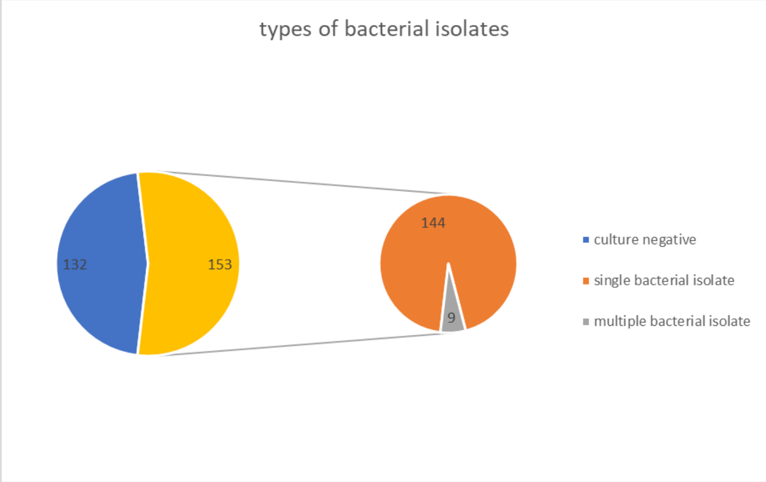

Figure 1: Distribution of the Participants According to Types of Bacterial Isolates (n=285)

Gram Staining of the Organisms

The distribution of bacterial isolates according to Gram staining showed higher prevalence of gram negative (136, 83.95%) organisms. (Figure 2)

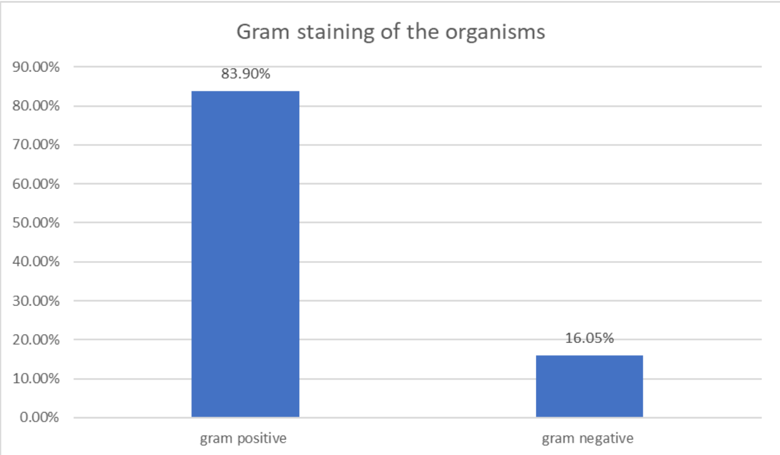

Figure 2: Distribution of the Participants According to Gram Staining of the Organisms (n=285)

Antimicrobial sensitivity and resistant pattern

E coli showed the highest sensitivity towards Imipenem (88.78%) and highest resistance to Amoxiclav (79.59%). Fosfomycin resistance was shown in only 2 isolates of E. coli. But nearly 100% sensitivity against rest of the pathogens. *Klebsiella* had the highest sensitivity to Meropenem (85.71%) and highest resistance to ceftriaxone (71.43%). *Pseudomonas spp.* and *Proteus spp.* had highest resistance against amoxiclav (83.3%, 66.7%) and both showed sensitivity against carbapenems. Although *Enterobacter spp.* had resistance (83.3%) towards Amoxiclav but remains 100% sensitive towards Amikacin. *Coagulase-negative Staphylococci* showed 100% sensitivity towards Linezolid. All *Staphylococcus aureus* isolates were sensitive to Levofloxacin and complete resistance to Gentamycin was observed. All *Enterococcus spp.* isolates were fully susceptible to Meropenem (table 2).

Table 2: Distribution of the Participants According to Antimicrobial Susceptibility and Resistance Pattern

Antibiotics	E. coli (98)	Klebsiella spp. (14)	Pseudomonas spp. (12)	Proteus (6)	Enterobacter spp. (6)	CoNS spp. (7)	Staph aureus (6)	Enterococcus (9)
Amikacin								
Sen						3	4	6

Int						1		
Res						3	2	3
Amoxiclav								
Sen	16	3	2	2	1	2	3	5
Int	4	3				1		2
Res	78	8	10	4	5	4	3	2
Azithromycin								
Sen	64	10		4	4	2	4	6
Int	4	1						1
Res	30	3		2	2	5	2	2
Cefepime								
Sen	79							
Int	1							
Res	18							
Ceftazidime								
Sen	40	5		2	3			8
Int	2	1						
Res	56	8		4	3			1
Ceftriaxone								
Sen	37	3	6	3	4	2	3	8
Int	5	1	2			1	3	
Res	56	10	4	3	2	4		1
Cefuroxime								
Sen		3	7		4			
Int		1	2		1			
Res		10	3		1			
Cefotaxime								
Sen	32			2	3			
Int	1							
Res	65			4	3			
Ciprofloxacin								
Sen	38	5	7	4	5	2	1	3
Int	3	2	2			1	1	
Res	57	7	3	2	1	4	4	6
Co-trimoxazole								
Ses	22	5	4	4	1			
Int	7	2	2		1			
Res	72	7	7	2	4			
Gentamycin								
Sen	63	10	9	4	4	4		6
Int	2	1	1		1	1		
Res	33	3	2	2	1	2	6	3
Levofloxacin								
Sen	49	10	6	4		5	6	
Int	3	2	2					
Res	46	2	4	2		2		
Linezolid								
ses						7	6	
Meropenem								
Sen	85	12	9	6	6			
Inter	4							

Res	9	2	3					
Imipenem								
Sen	87	12	10	6	6			9
Int	4							
Res	7	2	2					
Piperacillin/Tazobactam								
Sen			10					
Int			1					
Res			1					
Vancomycin								
Sen						7	6	8
Int								
Res								1
Oxacilin								
Ses						5	6	6
Int						1		
Res						1		3
Nitrofurantoin								
Sen	70	10	10	6	5		5	6
inter	7	1						
Res	21	3	2		1		1	3
Fosfomycin								
Sen	96	13	11	6	6	6	6	9
Int		1	1				1	
Res	2							

DISCUSSION

Antimicrobial resistance poses a major challenge to effective treatment, especially for UTIs, leading to increased mortality, morbidity, and healthcare costs. Key contributors in developing countries include irrational antibiotic use, lack of awareness, unauthorized sales, limited healthcare access, weak surveillance, and antimicrobial use in livestock and agriculture.[18] A total of 285 urine samples were collected from clinically suspected urinary tract infections cases in the present study; where majority of the participants (86, 30.18%) were within the age group 21-30 years with female predominance (65.26%). Even though age prevalence of UTI varies greatly depending on hygiene practice, sexual activity, reproductive changes and hormonal changes, several literatures found similar age range to be prevalent among UTI patients.[19-21]Female predominance in UTI was supported by a couple of national and international studies.[11,21-23] The reason behind this high prevalence of UTI in female is due to short urethra, close proximity of the urethra with anus, deferred voiding habit, wearing tight undergarments, use of diaphragms and spermicides, long duration tampons use, hormonal effect, host factor such as changes in normal vaginal flora, cleaning perineum forward from the anus to the vulva. Like the present study, UTI among married persons were more common; possibly because lack of interest hygiene after and before sexual activity and use of certain contraceptives.[20,24-26] In present study 191 urine samples 153 (53.68%) samples were culture positive, whereas 132 (46.32%) samples were culture negative. Among all culture positive cases mono microbial growth were 144 (94.12%) and poly microbial growth were 9 (5.88%) which were supported by a couple of original researches.[27,28] Higher prevalence of gram negative organisms also showed similarity with several articles.[29-33] *E. coli* were found to be the most predominant bacteria (60.49%) both in the present study and others supporting the hypothesis that *E. coli* have an adherence factor called P fimbriae or pilli, hemolysin, mannose resistant fimbria, sederophore and P-1 blood group phenotype receptor which mediate the attachment of *E. coli* to uroepithelial cells.[34,36-38] *E. coli* showed high resistance to commonly used β-lactam antibiotics such as Amoxiclav, indicating extended-spectrum beta-lactamase (ESBL) production. Carbapenems (Imipenem and Meropenem) remain the most effective choices for treating infections caused by this pathogen.[12,39,40] In present study, higher rate of resistance exhibited by *E. coli* towards amoxiclav, cotrimoxazole and ciprofloxacin, 77.55%, 71.43% and 56.12% respectively. Near similar observations were also true for several studies.[41-43] Fosfomycin showed the highest sensitivity to highest number of organisms. Fosfomycin showed high level of susceptibility against all of the uropathogens. 100% susceptibility was seen for *Proteus spp*., *Enterobacter*, *S. aureus* and *Enterococci. E. coli only* had 2 instances of resistance. Highest susceptibility towards Fosfomycin for almost all organism is found invariably in multiple literatures also.[44-49] The cross-sectional nature of the study is the limitation of the present study. This study has no conflict of interest among the involved parties.

CONCLUSION

E. coli was the most prevalent bacterial isolate and exhibited high resistance to commonly used antibiotics like Amoxiclav, Ciprofloxacin, and Ceftriaxone. Carbapenems and Fosfomycin remained effective. *Klebsiella spp. and Pseudomonas spp.* showed resistance to cephalosporins but were highly sensitive to carbapenems and aminoglycosides. Gram-positive organisms (*Staphylococcus aureus, CoNS, and Enterococcus*) showed high sensitivity to Vancomycin and Linezolid, confirming the importance of these drugs in treating resistant Gram-positive infections. Fosfomycin resistance was minimal, reinforcing its role in treating multidrug-resistant UTIs.

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