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Original Article

Study of Urinary Tract Infection with isolation of uropathogen with their antibiotic susceptibility pattern and detection of dfrA1 gene in trimethoprim resistance Escherichia coli in Pregnant Women

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ABSTRACT

Background: Urinary tract infections (UTIs) frequently occur during pregnancy, primarily due to Escherichia coli and various other Gram-negative and Gram-positive bacteria. Excessive use of antibiotics has resulted in multidrug resistance (MDR), which includes resistance to trimethoprim through the dfrA1 gene. This research sought to identify pathogens causing UTIs, assess their antibiotic resistance, and find dfrA1 in E. coli. **Methods & Materials:** This cross-sectional research conducted at Dhaka Medical College (July 2023–June 2024) involved 152 pregnant individuals (≥28 weeks) with potential urinary tract infection (UTI). Aseptically collected urine samples were cultured, identified, and tested for antibiotic susceptibility, with chosen isolates undergoing further analysis for MIC and the dfrA1 resistance gene. Data were examined with SPSS v25, and ethical clearance was secured from the appropriate committees. **Results:** Out of 125 isolates from pregnant women, E. coli was the most prevalent (62.4%), succeeded by Pseudomonas spp (11.2%), Enterobacter spp (9.6%), Klebsiella spp (8.0%), Candida spp (4.8%), and Enterococcus spp (4.0%). Gram-negative bacteria exhibited significant resistance to beta-lactam antibiotics, whereas all Enterococcus spp were susceptible to linezolid, vancomycin, teicoplanin, and fosfomycin, but showed resistance to amoxicillin. MIC testing showed Pseudomonas spp colistin MIC 8–64 µg/ml and tigecycline MIC in gram-negatives 16–128 µg/ml. The dfrA1 gene was found in 23.3% of E. coli isolates resistant to trimethoprim. **Conclusion:** E. coli was the main uropathogen exhibiting significant antibiotic resistance, and the presence of the dfrA1 gene underscores increasing resistance, stressing the importance of prudent antibiotic use and regular susceptibility testing.

Keywords: *Urinary Tract Infection, Antibiotic susceptibility, dfrA1 gene, Trimethoprim resistance, Escherichia coli, Pregnant Women.*

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INTRODUCTION

The most common bacterial infection during pregnancy is urinary tract infection. It may be symptomatic or asymptomatic or may be complicated and uncomplicated [1]. *Escherichia coli* is the most common infecting organism in UTI. It causes about 85% of community acquired infections and approximately 50% of nosocomial infections. Other gram-negative organisms causing UTI include *Proteus*, *Klebsiella*, *Citrobacter*, *Enterobacter*, and *Pseudomonas* spp. Gram positive pathogens such as *Enterococcus* faecalis, Staphylococcus *saprophyticus* and *Group B streptocooci* also infect the tract [2,3].

UTI in pregnancy may start from 6 weeks of pregnancy and peaks during 22 to 24 weeks of pregnancy. Upto 70 % of pregnant women may develop glycosuria, which enhances bacterial growth in the urine [4]. UTI may manifest as asymptomatic bacteriuria (ASB) or symptomatic bacteriuria (SB). Symptomatic and asymptomatic bacteriuria has been reported among 17.9% and 13.0% of pregnant women, respectively [5].

The overuse and inappropriate use of antimicrobial medications have substantially increased the prevalence of microbial infections in recent decades, resulting in the emergence of resistance among various strains of microbes [6]. Multi-drug resistance is the insensitivity or resistance of a microorganism to antimicrobial drugs, despite its previous sensitivity to them [7]. The limited availability of effective treatments presents a significant challenge in healthcare contexts, as multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) bacterial strains, which are both pathogenic and opportunistic [8].

In a study it was found that *K. pneumoniae* were resistant to ampicillin (75.6%) followed by, nitrofurantoin and cefuroxime (73.1%) and least to chloramphenicol (12.1%), cotrimoxazole (65.2%) and lower resistance was (7.6%) to amikacin, observed. A higher resistance pattern to these two antibiotics was observed against ESBL non producing *K. pneumonia* but lowest to polymyxin B (13.3%) instead of amikacin (26.6%). All the isolates were found to be susceptible to imipenem [9]. Among Gram-positive bacteria tested all were sensitive to vancomycin (100%). However, more resistance was observed against ampicillin (100%) and gentamycin (66.7%). The possible explanation for different level of resistance may be due to frequent usage of antibiotic [10].

Various agents are used to treat UTIs and pyelonephritis according to international guidelines such as trimethoprim-sulfamethoxazole, fosfomycin, pivmecillinam, nitrofurantoin monohydrate, fluroquinolones and beta lactams [11]. At present indiscriminate and widespread use

of antibiotics increases antimicrobial resistance [12].

Trimethoprim is a common antibiotic used to treat bacterial infections, most notably urinary tract infection [13]. Treatment failure and increased burden in primary health care and first-line therapy result from the widespread use of trimethoprim antibiotics. Mutation in a dihydrofolate reductase (dfr) gene leads to trimethoprim resistance [14]. dfrA1 gene is one of the most commonly identified trimethoprim resistance genes in gram negative bacteria, particularly in member of Enterobacteriaceae family such as *E. coli* [15]. It is typically located on mobile genetic element, class 1 integrons, which facilitates horizontal gene transfer among bacterial populations [16].

E. coli with its multidrug-resistant strains is the most common cause of UTI among pregnant women [17]. Since asymptomatic bacteriuria (ASB) and obvious UTI has a close association, screening, and treatment of pregnant women with ASB may also help to reduce adverse outcomes of pregnancy like preterm birth and low birth weight baby [4]. The findings can help healthcare providers and policymakers devise appropriate preventive and therapeutic strategies to reduce the incidence of UTIs and improve maternal and neonatal outcomes [18].

The present study aimed to identify the organisms causing urinary tract infection (UTI) in pregnant women, determine the antibiotic susceptibility patterns of the isolates, and detect the *dfrA1* resistance gene among trimethoprim-resistant *Escherichia coli* isolates.

METHODS & MATERIALS

Study Design and Setting

This study was a cross-sectional observational study conducted from July 2023 to June 2024 at the Department of Microbiology, Dhaka Medical College, Bangladesh. The study aimed to identify uropathogens in pregnant women, determine their antibiotic susceptibility patterns, and detect the dfrA1 gene among trimethoprim-resistant Escherichia coli isolates.

Study Population

The study population included pregnant women (≥28 weeks gestation) attending inpatient and outpatient services of the Department of Obstetrics and Gynecology, Dhaka Medical College, who were clinically suspected of urinary tract infection (UTI).

Sample Size and Sampling Technique

A total of 152 pregnant women meeting the inclusion criteria were enrolled using a purposive sampling technique. Of these, 125 women yielded culture-positive urine samples, which were included in microbiological and molecular analyses.

Inclusion Criteria

- Single pregnancy
- Gestational age of 28 weeks or more
- Clinically suspected of having a UTI

Exclusion Criteria

- Twin pregnancy
- Chronic kidney disease
- Polyhydramnios
- Vaginal bleeding prior to delivery
- Pregnancy complicated with diabetes mellitus or hypertension
- Non-consenting patients
- Data Collection Procedure

 Sample **Collection:**

- Midstream urine samples were collected aseptically in sterile containers.

 Laboratory Analysis:

- **Microscopy and Culture:** Urine samples were examined microscopically and cultured on standard media (MacConkey agar, and Blood agar).
- **Identification of Isolates:** Bacterial isolates were identified using Gram staining, colony morphology, and standard biochemical tests.
- **Antimicrobial Susceptibility Testing (AST):** AST was performed by the Kirby-Bauer disc diffusion method according to CLSI 2024 guidelines.
- **Minimum Inhibitory Concentration (MIC):** Selected isolates were tested for MIC of colistin and tigecycline using the agar dilution method.
- **Molecular Detection:** Trimethoprim-resistant *E. coli* isolates were analyzed for the presence of the dfrA1 gene by polymerase chain reaction (PCR).

Data Recording: Demographic and clinical data, as well as laboratory results, were recorded in a pre-designed data collection sheet.

Statistical Analysis

Data were analyzed using SPSS version 25. Descriptive statistics were expressed as frequencies and percentages. Association between variables was assessed using Fisher's exact test, and odds ratios (OR) with 95% confidence intervals (CI) were calculated. A p-value <0.05 was considered statistically significant.

Ethical consideration: The Research Review Committee and Ethical Review Committee of Dhaka Medical College approved the study protocol, and data collection was authorized by the Department of Obstetrics & Gynecology, DMCH.

RESULTS

Frequency of Uropathogens

Out of 152 pregnant women enrolled, 125 (82.2%) yielded culture-positive urine isolates. *Table I* presents the distribution of bacterial uropathogens. E. coli was the most frequent isolate (62.4%), followed by Pseudomonas spp (11.2%), Enterobacter spp (9.6%), and Klebsiella spp (8.0%). Gram-positive isolates (Enterococcus spp and Candida spp) were less frequent.

Table I
Frequency of bacterial uropathogens isolated from pregnant women (*n*=125).

Variables	Frequency	Percentage (%)
Escherichia coli	78	62.4
Pseudomonas spp	14	11.2
Enterobacter spp	12	9.6
Klebsiella spp	10	8.0
Enterococcus spp	5	4.0
Candida spp	6	4.8
Total	125	100.00

Gram-Negative Bacteria

Table II shows antimicrobial resistance pattern of gram-negative bacteria isolated from urine culture of pregnant women. Almost all the isolated bacteria of the study were sensitive to colistin except *Pseudomonas* spp. Among the isolated *E. coli*, isolates exhibited the highest resistance to ceftazidime (84.61%), amoxiclav

(83.33%), cefixime (76.92%), and ceftriaxone (74%), while resistance to fosfomycin(6.41%) and colistin (0%) was low. *Pseudomonas* isolates demonstrated high resistance to amoxiclav (91.6%) ceftazidime (85.71%) and amikacin (78.51%), whereas resistance to netilmycin (42.85%) was minimal. *Enterobacter* isolates were completely resistant to cefixime (100%) and highly resistant to amoxiclav (91.67%) and ceftazidime (83,3%), but had lower resistance to fosfomycin (8.31%) and nitrofurantoin (16.67%). *Klebsiella* isolates displayed considerable resistance to amoxiclav (100%), cefixime (100%), and ceftazidime (80%), whereas resistance was lower for nitrofurantoin (50%) and fosfomycin (30%). *Pseudomonas* spp were observed resistance against colistin in (57.14%) cases.

Table II
Antimicrobial resistance pattern of gram-negative bacteria isolated from urine (*n* = 114 isolates).

Antimicrobial Drugs	E. coli (n= 78), n (%)	Pseudomonas spp (n= 14), n (%)	Enterobacter spp (n=12), n (%)	Klebsiella spp (n=10), n (%)
Amikacin	55 (70.51)	11 (78.51)	9(75.0)	8(80.0)
Amoxiclav	65(83.33)	----	11 (91.67)	10(100)
Aztreonam	61(78.21)	10(71.42)	9(75)	8(80.0)
Cefixime	60 (76.92)	----	12 (100)	10 (100)
Cefuroxime	57 (73.9)		9 (75.0)	7 (70.0)
Ceftazidime	66 (84.61)	12 (85.71)	10 (83.3)	8 (80.0)
Ciprofloxacin	50 (64.1)	9 (64.3)	9 (75.0)	7(70.0)
Ceftriaxone	57 (73.9)	----	8(66.6)	6 (60.00)
Cefepime	55(70.25)	8 (57.14)	7(58.33)	7(70.0)
Gentamicin	53 (68.5)	8 (57.14)	7 (58.33)	5(50.00)
Netilmicin	30 (38.46)	6(42.85)	5 (41.67)	7(70.0)
Cotrimoxazole	30 (38.46)	----	3(25.0)	8(80.00)
Doxycycline	25 (32.05)	----	10 (83.33)	5(50.0)
Fosfomycin	5 (6.41)	----	1 (8.3)	3(30.0)
Tigecycline*	38 (48.71)	----	7 (58.33)	6 (60.00)
Meropenem	20 (25.64)	7 (50.0)	5(41.67)	8(80.0)
Piperacillin Tazobactam	36 (46.15)	9 (64.3)	6 (50)	5(50.0)
Mecillinam	39 (50)	----	7(58.33)	8(80.0)
Colistin*	0	8(57.14)	0	0
Nitrofurantoin	6 (7.6)	0	2(16.67)	5(50.0)

*n= Total number of bacteria; *=Detected by MIC by agar dilution method.*

Gram-Positive Bacteria
Table III presents antimicrobial resistance pattern of gram-positive bacteria isolated from urine culture of pregnant women. All the isolated enterococci were sensitive to linezolid, vancomycin, teicoplanin and fosfomycin. Among the isolated enterococci, 100% were resistant to amoxicillin, 60% to ciprofloxacin and doxycycline and 40% to gentamycin.

Table III
Antimicrobial resistance pattern by disc diffusion method of gram-positive bacteria isolated from urine culture of pregnant women (*n*=5).

Antibiotics	Enterococcus spp. n(%)
Amoxicillin	5(100)
Ciprofloxacin	3(60)
Doxycycline	3(60)
Gentamicin (High)	2(40)
Nitrofurantoin	0(0.00)
Fosfomycin	0(0.00)
Teicoplanin	0(0.00)
Vancomycin*	0(0.00)
Linezolid	0(0.00)

*n= Total number of bacteria; *= Detected by MIC by agar dilution method.*

MIC Results
Table 4 presents MIC of colistin among gram-negative bacteria. Among them, out of 8 *Pseudomonas spp*, 4(50.00%) had MIC of 8µg/ml, 2 (25.00%) had MIC 16 µg/ml, and 2(25.00%) had MIC 64µg/ml.

Table IV
MIC of colistin among *Pseudomans* spp (*n*=8).

MIC of Colistin (µg/ml)	Number	Percentage
256	0	0
128	0	0
64	2	25
32	0	0
16	2	25
8	4	50
4	0	0
2	0	0
Total	8	100.00

Note: MIC interpretive criteria of colistin for Pseudomonas spp according to CLSI, 2024; Intermediate: ≤2µg/ml; Resistant: ≥4µg/ml.

Table V presents MIC of tigecycline among gram-negative bacteria. Out of 14 E. *coli 1*(7.14%) had MIC of 16µg/ml, 2 (14.29%) had of MIC 32 µg/ml, and 4(28.57%) had MIC of 64µg/ml and 7(50.00%) had MIC of 128µg/ml. Out of 1 *Enterobacter spp*, 1 (25%) had MIC of 64µg/ml. Out of 4 *Klebsiella spp*, 2 (40%) had MIC of 32µg/ml, 3(60%) had MIC of 64µg/ml.

Table V
MIC of tigecycline among gram negative bacteria (*n*=20).

Bacterial species	N	256 µg/ml	128 µg/ml	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml	4 µg/ml	2 µg/ml
Escherichia coli	14	0	7 (50.00)	4 (28.57)	2 (14.29)	1 (7.14)	0	0	0
Klebsiella spp.	5	0	0	3 (60.00)	2 (40.00)	0	0	0	0
Enterobacter spp.	1	0	0	1 (100.00)	0	0	0	0	0
Total	20	0	7 (35.00)	8 (40.00)	4 (20.00)	1 (5.00)	0	0	0

Note: FDA breakpoint of minimum inhibitory concentration (MIC) for Tigecycline (FDA,2024); *Sensitive: ≤2µg/ml; Intermediate: 4µg/ml; Resistant: ≥8µg/ml.*

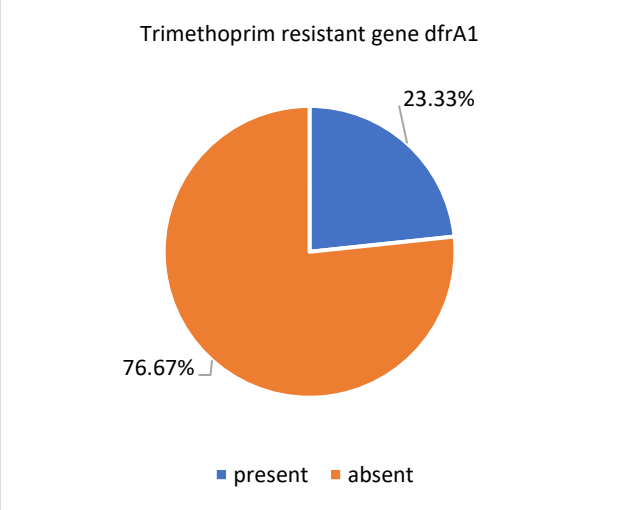

Figure 1 Distribution of the dfrA1 gene among trimethoprim resistant *E. coli* (*n*=30).

Detection of dfrA1 Gene
Figure 1 show, that the dfrA1 gene was detected in 7 out of 30 (23.33%) trimethoprim resistant *Escherichia coli* isolates, while it was absent in 23 (76.67%) cases.

DISCUSSION

In this study, Escherichia coli was identified as the predominant uropathogen (62.4%) among pregnant women, followed by Pseudomonas spp. (11.2%), Enterobacter spp. (9.6%), and Klebsiella spp. (8.0%). These results align with previous reports, such as Ejerssa et al. (2021), who documented E. coli (45.2%), Klebsiella spp. (16.1%), and Pseudomonas spp. (6.5%) as the most frequent urinary pathogens, emphasizing their global predominance in UTI cases [19]. The relatively low prevalence of Enterococcus spp. (4%) in this study contrasts with Ifrah et al. (2025), who reported 24.6%, likely reflecting differences in population characteristics, sample size, antibiotic usage, and infection control practices [20]. These findings underscore the regional and temporal variations in UTI pathogens, which are critical for local antibiotic stewardship programs.

Antimicrobial susceptibility testing revealed that E. coli exhibited high resistance to ceftazidime (84.6%), amoxiclav (83.3%), cefixime (76.9%), and ceftriaxone (74%), consistent with the findings of Abalkhail et al. (2022) and Haque et al. (2023), suggesting extensive beta-lactam exposure may drive resistance [21,22]. Pseudomonas spp. showed significant resistance to ceftazidime and amikacin, with lower resistance to netilmicin, while exhibiting colistin resistance in 57% of

isolates. These patterns reflect mechanisms such as beta-lactamase production and efflux pumps, as described by Abdulfatai et al. (2023) and Kamruzzaman et al. (2025) [23-25]. Klebsiella spp. demonstrated high resistance to amoxiclav and cefixime but remained relatively susceptible to nitrofurantoin, in line with prior reports [24]. Variations in resistance profiles across studies may be attributed to differences in local prescribing practices, population demographics, and laboratory methodologies.

MIC testing revealed elevated colistin levels among Pseudomonas spp., with 50% of isolates exhibiting MIC 8 µg/ml, highlighting the emerging threat of colistin resistance globally [25]. Tigecycline MICs were also high, with 50% of E. coli at 128 µg/ml, 60% of Klebsiella spp. at 64 µg/ml, and 100% of Enterobacter spp. at 64 µg/ml, reflecting the limited efficacy of this last-line antibiotic and potential mechanisms such as efflux pumps and ribosomal protection [26]. These results underscore the importance of routine susceptibility testing, particularly for last-resort antimicrobials.

The dfrA1 gene, a common marker for trimethoprim resistance, was detected in 23.3% of trimethoprim-resistant E. coli, consistent with studies by Yahiaoui et al. (2015) and AlZirjawi & Hamim (2016) [28,29]. This finding highlights the ongoing dissemination of resistance genes through mobile genetic elements like class 1 integrons, emphasizing the necessity for targeted antibiotic stewardship and infection control measures. Regular screening and management of asymptomatic bacteriuria during pregnancy remain essential to prevent adverse maternal and neonatal outcomes [30].

Overall, these findings reveal a concerning prevalence of multidrug-resistant uropathogens in pregnant women, highlighting the critical need for evidence-based antibiotic selection, judicious use of antimicrobials, and continued surveillance to mitigate resistance development.

CONCLUSION

This study demonstrates that Escherichia coli is the primary uropathogen in pregnant women, exhibiting significant resistance to beta-lactam antibiotics, while other gram-negative bacteria also display multidrug resistance. The detection of the dfrA1 gene in trimethoprim-resistant E. coli underscores the rising prevalence of antibiotic resistance. These findings emphasize the importance of regular susceptibility testing, prudent antibiotic use, and continuous surveillance to inform effective treatment strategies and reduce the burden of urinary tract infections in pregnancy.

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