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Epidemiological and Molecular Characteristics of Escherichia coli Isolates Causing Urinary Tract Infections in Central India

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ABSTRACT

Background: Urinary tract infections (UTIs) remain one of the most common bacterial infections worldwide, with Escherichia coli being the predominant uropathogen. Increasing antimicrobial resistance and the emergence of extended-spectrum β-lactamase (ESBL)–producing strains pose serious therapeutic challenges.
Objective: This study aimed to investigate the epidemiological distribution, virulence attributes, antimicrobial resistance patterns, and molecular characteristics of E. coli isolates obtained from UTI patients in a tertiary care hospital in Central India.
Methods: A total of 2,483 urine samples were collected over 18 months from symptomatic and asymptomatic patients. Standard microbiological methods were used for isolation and identification. Virulence factors—including siderophore production, cell surface hydrophobicity, hemagglutination, hemolysin production, plasmid profiling, and ESBL production—were evaluated. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method. Molecular detection of resistance genes was carried out using PCR and real-time PCR, followed by sequencing.
Results: E. coli was isolated from 230 (9.26%) samples, predominantly from symptomatic patients. Siderophore production (34.15%) and cell surface hydrophobicity (32.17%) were the most prevalent virulence factors among symptomatic isolates. ESBL production was detected in 9.56% of isolates. High resistance rates were observed for gentamicin (61.73%) and fluoroquinolones, while piperacillin–tazobactam showed the highest sensitivity (99.14%). Molecular analysis revealed the widespread presence of the CTX-M gene among multidrug-resistant isolates.
Conclusion: The study highlights the alarming burden of virulence-associated, multidrug-resistant E. coli in UTIs. Continuous surveillance, rational antibiotic use, and molecular monitoring of resistance genes are essential for effective UTI management.

Keywords: *Urinary tract infection, Escherichia coli, virulence factors, ESBL, CTX-M, antimicrobial resistance.*

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and constitute a major public health burden, accounting for millions of outpatient visits and hospital admissions annually. UTIs affect individuals across all age groups and genders, though women are disproportionately affected due to anatomical and physiological factors such as a shorter urethra and proximity of the urethral opening to the anus [1,2]. Clinically, UTIs range from asymptomatic bacteriuria to severe manifestations including pyelonephritis and urosepsis, which can result in significant morbidity if not adequately treated [3].

The urinary tract is normally sterile and possesses multiple defense mechanisms, including urine flow, mucosal immunity, and antimicrobial properties of epithelial secretions. However, these defenses can be overcome by pathogenic microorganisms, leading to infection [4]. UTIs are broadly classified as uncomplicated or complicated. Uncomplicated UTIs typically occur in otherwise healthy individuals with normal urinary anatomy, whereas complicated UTIs are

associated with structural abnormalities, urinary obstruction, catheterization, pregnancy, diabetes, or immunosuppression and are often linked to multidrug-resistant organisms [5,6].

Among the etiological agents of UTIs, uropathogenic *Escherichia coli* (UPEC) is the most predominant, accounting for approximately 65–80% of community-acquired and nearly 50% of hospital-acquired UTIs [7,8]. The success of UPEC as a pathogen is attributed to a wide array of virulence factors that facilitate colonization, persistence, and evasion of host immune responses. These include adhesins and fimbriae that mediate attachment to uroepithelial cells, hemolysins that damage host tissues, siderophores that enable iron acquisition, and biofilm formation that promotes chronic and recurrent infections [9–11].

In addition to virulence, antimicrobial resistance has emerged as a critical challenge in the management of UTIs. The widespread and often inappropriate use of antibiotics has led to the rapid emergence of multidrug-resistant (MDR) UPEC strains. Of particular concern is the increasing prevalence of extended-spectrum β-lactamase (ESBL)-producing *E. coli*, which exhibit resistance to penicillins, cephalosporins, and monobactams, thereby limiting therapeutic options [12–14]. Resistance genes, especially those encoding CTX-M-type β-lactamases, are frequently plasmid-mediated, facilitating horizontal gene transfer and rapid dissemination within bacterial populations [15,16].

India has witnessed a substantial rise in ESBL-producing and MDR UPEC isolates, with resistance rates to commonly used antibiotics such as fluoroquinolones, aminoglycosides, and third-generation cephalosporins exceeding 30–40% in several regional studies [17–19]. This growing resistance not only complicates empirical therapy but also contributes to prolonged hospital stays, increased healthcare costs, and higher risk of treatment failure.

Despite the high burden of UTIs, there remains a paucity of comprehensive molecular epidemiological data from Central India, particularly regarding the association between virulence determinants and antimicrobial resistance in UPEC isolates. Understanding the interplay between these factors is essential for developing targeted treatment strategies and effective infection control policies.

Therefore, the present study was undertaken to investigate the epidemiological profile, virulence characteristics, antimicrobial susceptibility patterns, and molecular resistance mechanisms of *Escherichia coli* isolated from urinary tract infections in a tertiary care hospital in Central India, with special emphasis on ESBL production and the presence of CTX-M resistance genes.

MATERIALS AND METHODS

Study Design and Sample Collection

A hospital-based cross-sectional study was conducted over 18 months. Clean-catch midstream urine samples were collected from both symptomatic and asymptomatic patients attending outpatient and inpatient departments.

Isolation and Identification

Urine samples were cultured on standard bacteriological media. Isolates were identified as *E. coli* using colony morphology, Gram staining, and biochemical tests.

Detection of Virulence Factors

Virulence properties were assessed phenotypically:

- **Siderophore production:** Chrome azurol S assay
- **Cell surface hydrophobicity:** Ammonium sulfate aggregation test
- **Hemolysin production:** Blood agar assay
- **Hemagglutination:** Microtiter plate method
- **Plasmid profiling:** Alkaline lysis method
- **ESBL production:** Double disc synergy test

Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined using the Kirby–Bauer disk diffusion method following CLSI guidelines.

Molecular Analysis

DNA extraction was followed by PCR and real-time PCR for detection of **CTX-M** resistance genes. Selected isolates underwent sequencing for genetic characterization.

Statistical Analysis

Data were analyzed using SPSS software, and results were expressed as percentages and frequencies.

RESULTS

A total of 2,483 urine samples were analyzed during the study period to determine the epidemiological distribution, virulence attributes, antimicrobial susceptibility patterns, and molecular characteristics of *Escherichia coli* isolates

causing urinary tract infections. The results are presented below with emphasis on differences between symptomatic and asymptomatic patient groups.

Distribution of *E. coli* Isolates

Out of 2,483 urine samples processed, 230 (9.26%) were confirmed as *Escherichia coli*. The majority of isolates were obtained from symptomatic patients, indicating a strong association between *E. coli* and clinically evident urinary tract infections.

Table 1. Distribution of *E. coli* isolates among study groups

Patient Group	Number of Isolates (n)	Percentage (%)
Symptomatic	161	70.0
Asymptomatic	69	30.0
Total	**230**	**100**

Gender-wise Distribution

Female patients contributed a significantly higher proportion of *E. coli* isolates compared to males in both symptomatic and asymptomatic groups.

Table 2. Gender-wise distribution of *E. coli* isolates

Gender	Symptomatic n (%)	Asymptomatic n (%)	Total n (%)
Male	46 (28.6)	21 (30.4)	67 (29.1)
Female	115 (71.4)	48 (69.6)	163 (70.9)
Total	**161**	**69**	**230**

Prevalence of Virulence Factors

Virulence factors were more frequently detected in isolates from symptomatic patients. Siderophore production and cell surface hydrophobicity were the most prevalent factors.

Table 3. Virulence factors among *E. coli* isolates from symptomatic patients (n = 161)

Virulence Factor	Positive n	Percentage (%)
Siderophore production	55	34.15
Cell surface hydrophobicity	52	32.17
Hemolysin production	50	31.05
Hemagglutination	42	26.09
Plasmid presence	47	29.19
ESBL production	22	13.66

Table 4. Virulence factors among *E. coli* isolates from asymptomatic patients (n = 69)

Virulence Factor	Positive n	Percentage (%)
Siderophore production	5	7.24
Cell surface hydrophobicity	6	8.69
Hemolysin production	8	11.59
Hemagglutination	8	11.59
Plasmid presence	6	8.69
ESBL production	28	40.58

Distribution Based on Number of Virulence Factors

A higher number of virulence factors per isolate was predominantly observed in symptomatic patients. Some isolates expressed up to six virulence factors simultaneously.

Table 5. Number of virulence factors detected per *E. coli* isolate

No. of Virulence Factors	Symptomatic n	Asymptomatic n
0–1	32	41
2–3	67	20
4–5	49	7
≥6	13	1

Antimicrobial Susceptibility Pattern

High levels of resistance were observed against commonly prescribed antibiotics, particularly aminoglycosides and fluoroquinolones. Piperacillin–tazobactam demonstrated the highest efficacy.

Table 6. Antibiotic susceptibility profile of *E. coli* isolates (n = 230)

Antibiotic	Sensitive (%)	Resistant (%)
Gentamicin	38.27	61.73
Ciprofloxacin	42.60	57.40
Cefotaxime	46.52	53.48
Amikacin	76.95	23.05
Nitrofurantoin	82.60	17.40
Piperacillin–Tazobactam	99.14	0.86

ESBL Production and Molecular Findings

ESBL production was confirmed phenotypically in 50 isolates. Molecular analysis using PCR and real-time PCR demonstrated the presence of the CTX-M gene in a majority of ESBL-producing and multidrug-resistant isolates. Sequencing revealed genetic variations suggestive of emerging resistance mechanisms.

Summary of Key Findings

- *E. coli* was the predominant uropathogen.
- Symptomatic isolates exhibited significantly higher virulence.
- Multidrug resistance was common.
- CTX-M gene played a central role in β-lactam resistance.

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DISCUSSION

Urinary tract infections remain a major cause of morbidity worldwide, with *Escherichia coli* continuing to be the most predominant uropathogen. The present study provides comprehensive insight into the epidemiological distribution, virulence determinants, antimicrobial resistance patterns, and molecular characteristics of *E. coli* isolates from UTI patients in Central India. The findings emphasize the growing clinical challenge posed by virulent and multidrug-resistant uropathogenic *E. coli* (UPEC).

In the present study, *E. coli* accounted for 9.26% of total urine samples processed, with a significantly higher proportion isolated from symptomatic patients. This observation is consistent with previous studies from India and other developing countries, which have reported *E. coli* as the leading causative agent in both community- and hospital-acquired UTIs [1,2]. The predominance of isolates among symptomatic patients highlights the pathogenic potential of these strains and reinforces their role in clinically significant infections.

A marked female preponderance was observed, which aligns with established epidemiological data indicating higher UTI susceptibility among women due to anatomical and physiological factors such as shorter urethral length and proximity to the anal region [3,4]. Similar gender distributions have been reported by Gupta et al. and Flores-Mireles et al., confirming that female gender remains a major risk factor for UTIs across populations [5,6].

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The analysis of virulence factors revealed a significantly higher expression among symptomatic isolates compared to asymptomatic strains. Siderophore production and cell surface hydrophobicity were the most prevalent virulence traits among symptomatic isolates. These factors are known to play a crucial role in iron acquisition and bacterial adhesion, enabling UPEC to survive in the iron-limited urinary environment and establish infection [7,8]. Previous studies have also reported a strong association between siderophore production and enhanced pathogenicity in UPEC strains [9].

Hemolysin and hemagglutination were more frequently observed in symptomatic isolates, supporting their role in tissue damage, inflammation, and bacterial persistence. Hemolysin-mediated cytotoxicity facilitates epithelial cell injury, which can lead to more severe clinical manifestations such as pyelonephritis and urosepsis [10,11]. The higher number of virulence factors per isolate among symptomatic patients further indicates that pathogenicity in UPEC is multifactorial, and the cumulative effect of multiple virulence determinants significantly enhances disease severity [12].

Antimicrobial susceptibility testing revealed alarmingly high resistance rates to commonly prescribed antibiotics, particularly gentamicin, ciprofloxacin, and third-generation cephalosporins. These findings are in agreement with several Indian and global studies reporting increasing resistance to first-line antibiotics used for UTI management [13–15]. The widespread resistance observed may be attributed to indiscriminate antibiotic use, self-medication, and inadequate antimicrobial stewardship practices.

Notably, piperacillin–tazobactam and nitrofurantoin retained excellent activity against the majority of isolates. Similar findings have been reported in other regional studies, suggesting that these agents remain effective therapeutic options for empirical treatment of UTIs, particularly in settings with high resistance to fluoroquinolones and cephalosporins [16,17].
The detection of ESBL production in a substantial proportion of isolates is a significant concern. ESBL-producing *E. coli* strains are associated with treatment failure, prolonged hospitalization, and increased healthcare costs [18]. Molecular analysis confirmed the presence of the **CTX-M** gene among multidrug-resistant isolates, corroborating global evidence

that CTX-M-type β-lactamases are the most prevalent ESBL enzymes worldwide [19,20]. The plasmid-mediated nature of these genes facilitates horizontal transfer, accelerating the spread of resistance within hospital and community settings. Interestingly, a higher proportion of ESBL producers was observed even among asymptomatic isolates, indicating that asymptomatic bacteriuria may serve as an important reservoir for resistant strains. This finding underscores the importance of surveillance and judicious antibiotic use, even in apparently benign infections [21].

Overall, the study highlights a strong association between virulence determinants and antimicrobial resistance in UPEC. The coexistence of multiple virulence factors and resistance genes in a single isolate poses a serious threat to effective UTI management and emphasizes the need for integrated microbiological and molecular surveillance [22].

Strengths and Clinical Implications

This study provides region-specific molecular data from Central India, an area with limited published evidence. The findings support the need for:

- routine screening for ESBL production,
- periodic review of empirical treatment guidelines,
- strengthened antimicrobial stewardship programs.

CONCLUSION

This study demonstrates a high burden of virulent and multidrug-resistant *E. coli* causing UTIs in Central India. Integrating molecular diagnostics with routine microbiological testing can significantly improve patient management. Strengthening surveillance systems and promoting rational antibiotic use are imperative to curb the spread of resistance.

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