ORIGINAL RESEARCH PAPER
Volume - 15 | Issue - 02 | February - 2026 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

UROPATHOGENIC *ESCHERICHIA COLI*: A CROSS-SECTIONAL EVALUATION OF VIRULENCE FACTORS AND ANTIMICROBIAL RESISTANCE

Microbiology

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ABSTRACT

Introduction: The increasing emergence of multidrug-resistant (MDR) *Escherichia coli* with opportunistic uropathogenic potential poses a significant challenge in the management of urinary tract infections (UTIs), leading to increased healthcare burden and mortality. Uropathogenic *E. coli* (UPEC) remains the predominant causative agent of UTIs, including cystitis and pyelonephritis, and may result in severe complications such as acute renal failure in both immunocompetent individuals and renal transplant recipients. **Aim:** To assess the prevalence of *E. coli* and UPEC among UTI cases and to comparatively evaluate their antimicrobial resistance patterns and associated determinants in order to guide effective management strategies. **Materials And Methods:** A cross-sectional study was conducted from March 2023 to February 2024 in the Microbiology Laboratory of Adichunchanagiri Hospital and Research Centre. A total of 450 clinical specimens, including 150 urine samples, were analysed to determine the burden of *E. coli* and the proportion of UPEC. Antimicrobial susceptibility testing was performed using the disc diffusion method. Resistance mechanisms were evaluated and statistically analysed using Chi-square test, two-tailed p-values, odds ratios (ORs), and confidence intervals with Microsoft Excel 2022. **Results:** *Escherichia coli* was the most prevalent uropathogens, accounting for 45% of UTI isolates. Among these, 62.9% were Extended Spectrum Beta-Lactamase (ESBL) producers, exhibiting complete resistance to penicillins and cephalosporins. ESBL-producing UPEC showed high resistance to carbapenems and fluoroquinolones, while retaining good susceptibility to Nitrofurantoin (100%) and Fosfomycin (75%). Carbapenem resistance was observed in 33.3% of *E. coli* isolates, with 14.8% producing Metallo-Beta-Lactamase (MBL). A history of hospitalisation within the previous year was identified as a significant risk factor ($p<0.05$). **Conclusion:** Nitrofurantoin and Fosfomycin remain effective options for empirical therapy of UTIs. However, optimal management of antimicrobial resistance requires definitive, culture-guided therapy and rational use of synergistic antimicrobial combinations. The rising incidence of carbapenem resistance, limited treatment options for ESBL-producing isolates, and constrained phenotypic detection of carbapenemases highlight the need for molecular assays to accurately identify resistance mechanisms and guide targeted clinical management.

KEYWORDS

INTRODUCTION

Escherichia coli is a Gram-negative bacillus belonging to the family *Enterobacteriaceae*. In humans, it exists as a predominant commensal of the gastrointestinal tract and plays a beneficial role when present in balance; however, through the acquisition of virulence-associated genes, it can transform into a versatile pathogen capable of causing both intestinal and extra-intestinal infections [1]. Among its extra-intestinal pathotypes, uropathogenic *E. coli* (UPEC).

UPEC typically originates from the rectal flora and ascends the urinary tract, accounting for the majority of community- and hospital-acquired UTIs. Globally, UTIs affect nearly 150 million individuals annually. These infections pose a significant health burden, as approximately 50% of untreated or inadequately managed cases of cystitis may progress to pyelonephritis, leading to serious complications such as renal failure, sepsis, and increased morbidity and mortality [2–4].

The growing burden of antimicrobial resistance (AMR), recognised as one of the top ten global public health threats, has further complicated the management of UPEC-associated UTIs. *E. coli* is designated as a critical priority pathogen in the Indian Priority Pathogens List (IPPL 2021) [5]. The increasing emergence of Extended Spectrum Beta-Lactamase (ESBL)-producing and carbapenemase-producing *E. coli*, including UPEC strains, combined with the high recurrence rate of UTIs, presents substantial therapeutic limitations and life-threatening clinical challenges. [6,7]

Key drivers of AMR include inappropriate and excessive antimicrobial use, poor regulatory oversight, inadequate infection prevention and control practices within healthcare settings, and environmental contamination. Addressing AMR requires robust infection control strategies, including continuous environmental surveillance, periodic revision of antimicrobial policies, and routine monitoring of clinical isolates with their corresponding antibiogram [8,9]. In addition, recurrent UTIs compounded by evolving resistance patterns and the lack of region-specific antibiogram data significantly hinder effective disease management. In this context, the present study was designed to evaluate the prevalence of UPEC among UTI cases and to analyse the antimicrobial resistance patterns of regional *E. coli* isolates. By assessing the resistance spectrum and associated determinants, this study aims to provide clinically relevant data to support antibiotic stewardship initiatives, inform empirical treatment strategies, and contribute to measures aimed at controlling the spread of AMR and reducing infection-related morbidity and mortality.

MATERIALS AND METHODS

A cross-sectional study was conducted from March 2022 to February 2024 in the Microbiology Laboratory of Adichunchanagiri Hospital and Research Centre. (AH&RC), India. Ethical approval for the study was obtained from the Institutional Ethics Committee. The sample size was calculated using the standard formula for cross-sectional studies based on power analysis.

Clinical specimens were collected from symptomatic patients presenting with suspected infections. A total of different clinical samples were included for the comparative evaluation of uropathogenic *Escherichia coli* (UPEC)

Inclusion Criteria: Different clinical specimens obtained from symptomatic patients were included for the comparative study of uropathogenic E. coli (UPEC) and non UPEC isolates. **Exclusion Criteria:** Isolates from asymptomatic patients were excluded from the study

Specimen Collection, Transportation And Processing

A total of 450 clinical specimens were collected aseptically from symptomatic patients under the supervision of trained medical personnel and transported promptly to the microbiology laboratory for further processing. Of these, 150 specimens were urine samples. Standard operating procedures and established guidelines were followed for specimen collection, transportation, and laboratory processing [10,11]. Significant bacteriuria was defined by a colony count of $\geq 10^5$ CFU/mL, while insignificant growth was interpreted in conjunction with clinical findings [10]. Preliminary identification of isolates was based on colony morphology, Gram staining, and motility assessment using the hanging drop method. Biochemical characterisation was performed using standard tests.

Escherichia coli isolates were identified as motile, Gram-negative bacilli producing lactose-fermenting colonies on MacConkey agar. The isolates demonstrated fermentation of 1% sugars with acid and gas production, an acidic slant and butt with gas formation on TSI agar, and positive reactions for Indole and MR tests. Isolates recovered from urine samples were categorised as uropathogenic *E. coli* (UPEC), whereas isolates obtained from non-urinary clinical specimens were designated as non-UPEC strains. ATCC 25922 was used as a quality control strain throughout the study.

Antibiotic Susceptible

Antibiotic Susceptibility Testing (AST) of UPEC and non-UPEC isolates was performed using the Kirby–Bauer disc diffusion method. The zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. [12]

Detection of Extended Spectrum Beta-Lactamase (ESBL) production was carried out using the Double Disc Synergy Test (DDST). Lawn cultures of the test organism were prepared on MHA plates, and Ceftazidime (CAZ) and Ceftazidime–Clavulanic Acid (CAC) discs were placed at the recommended distance and incubated at 37°C overnight. An increase in zone diameter of ≥5 mm for the antimicrobial agent tested in combination with clavulanic acid was considered confirmatory for ESBL production [12].

Carbapenemases production was detected using the Modified Carbapenem Inactivation Method (mCIM), and Metallo-Beta-Lactamase (MBL) production was identified using the EDTA-modified Carbapenem Inactivation Method (eCIM). For mCIM, the test organism was emulsified in peptone water along with a Meropenem (MRP) disc and incubated at 35°C for four hours. The treated MRP disc was then placed onto an MHA plate lawn-cultured with *E. coli* ATCC 25922 and incubated overnight at 37°C. A zone of inhibition ≤15 mm was interpreted as positive for carbapenemases production [12]. For eCIM, the test organism was inoculated in peptone water containing 20 µL of 0.5 M EDTA along with an MRP disc and processed similarly to mCIM. An increase in zone diameter of ≥5 mm compared to the MRP disc without EDTA indicated MBL production, whereas a zone diameter difference of ≤4 mm was interpreted as serine carbapenemases production [12].

Statistical Analysis

Clinical data were entered into Microsoft Excel 2022 (Microsoft Corporation, USA) and analysed using descriptive statistics expressed as relative frequencies. Categorical variables were assessed for associations with potential risk factors using the uncorrected Chi-square test. Statistical significance was determined using a two-tailed p-value of <0.05. Odds ratios (ORs) with corresponding confidence intervals were calculated to measure the strength of associations.

RESULTS

Out of the 450 clinical specimens processed for culture and sensitivity testing, 150 were urine samples obtained from symptomatic patients. Among these, 58 (37.91%) urine samples yielded significant growth and were included for further analysis. A higher proportion of symptomatic UTI cases was observed among inpatients, with 89 cases (58.17%) from the Inpatient Department (IPD) compared to 64 cases (41.83%) from the Outpatient Department (OPD). Overall, UTIs were more frequently observed in males, accounting for 31 cases (53.45%), whereas females constituted 27 cases (46.55%).

Age-wise distribution revealed that UTIs were more common among individuals aged 40–80 years. Male patients predominantly belonged to the 40–59-year age group, while female patients showed a higher incidence in the 60–79-year age group. Risk factor analysis was performed among 82 IPD patients diagnosed with UTIs. Among the associated co-morbid conditions evaluated, only a history of hospitalisation within the previous year demonstrated a statistically significant association with UTIs, evidenced by a Chi-square value exceeding 3.84 and a p-value <0.05, as detailed in [Table 1]

Table1: Risk Factors Associated With UTIs

Risk factors	UTI Positive	UTI Negative	p-value	Odd Ratio (CI)	λ2 value
Hypertension	8	11	0.26	1.81	1.23
Diabetes mellitus	9	14	0.36	1.58	0.81
Renal calculus	6	6	0.14	2.5	2.17
Steroid use	1	1	0.57	2.2	0.31
Catheterization	16	25	0.15	1.98	2.02
Previous hospitalisation in the last 1 year	6	3	0.016	5.3	5.70
Duration of hospitalisation >48 hours	23	52	0.50	0.5	0.43

Of the 150 urine specimens analysed, 58 were culture-positive. *Escherichia coli* was the predominant uropathogens, accounting for 23 cases (39.66%). This was followed by *Candida* spp. in 11 cases (18.97%), *Pseudomonas* spp. in 8 cases (13.79%), and Coagulase-negative *Staphylococci* (CoNS) in 3 cases (5.17%). (Fig 1)

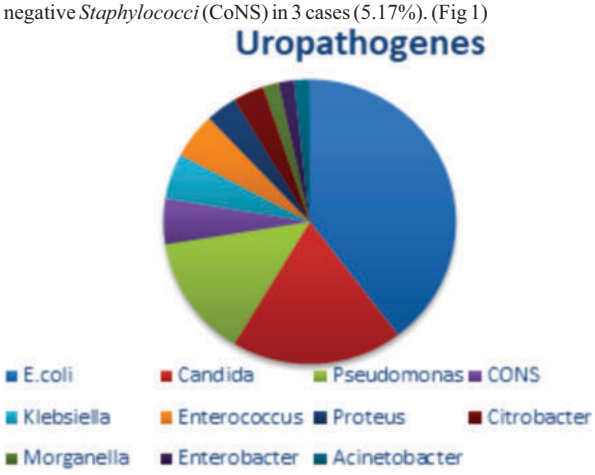

Fig-1: Rate Of Uropathogens Isolated From Urine Specimens.

A total of 51 *E. coli* isolates were recovered from various clinical specimens. Of these, 23 isolates (45.09%) were obtained from urine samples and categorized as UPEC, indicating their association with UTIs. The remaining 28 isolates (54.91%) were obtained from non-urinary specimens and classified as non-UPEC strains. Among the non-urinary isolates, stool samples constituted the majority, accounting for 15 isolates (29.41%), as shown in [Fig-2].

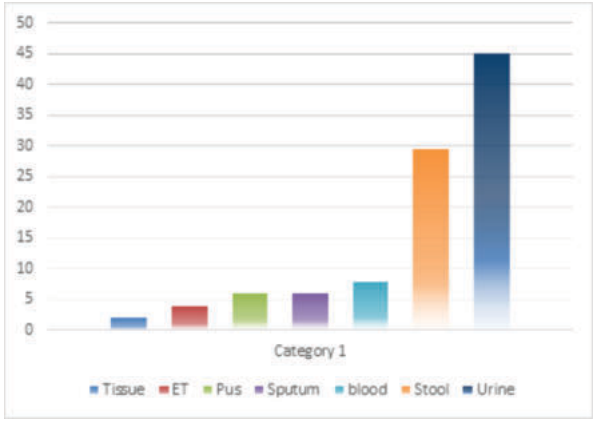

Fig-2: Distribution Of E. coli In Various Clinical Specimens.

Extended Spectrum Beta-Lactamase (ESBL) and carbapenemases activity were evaluated among 27 *E. coli* isolates. The overall prevalence of ESBL production was 62.96%, with a higher proportion observed among non-UPEC isolates (68.42%). ESBL-producing isolates from both UPEC and non-UPEC groups exhibited 100% resistance to ampicillin and all generations of cephalosporin. ESBL-producing UPEC isolates demonstrated a higher degree of resistance to carbapenems and fluoroquinolones compared to non-UPEC isolates, as shown in [Fig-3]. Despite this resistance, all ESBL-producing isolates showed good susceptibility to aminoglycosides, chloramphenicol, Fosfomycin and nitrofurantoin. Notably, ESBL-producing UPEC isolates exhibited 100% sensitivity to nitrofurantoin and 75% sensitivity to Fosfomycin.

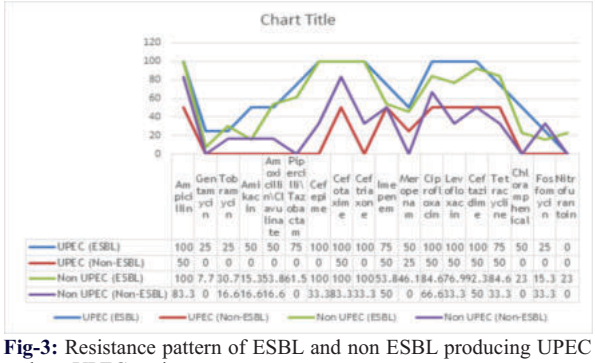

Fig-3: Resistance pattern of ESBL and non ESBL producing UPEC and non UPEC strains.

Meropenem resistance was observed in 33.33% of the total *E. coli* isolates, comprising 37.50% of UPEC and 31.57% of non-UPEC isolates. Carbapenem-resistant isolates were further evaluated for carbapenemase production using mCIM and eCIM assays. Carbapenemase activity was detected in 14.81% of isolates by mCIM, and all carbapenemase-positive isolates were confirmed as Metallo-Beta-Lactamase (MBL) producers by eCIM.

DISCUSSION

Uropathogenic *Escherichia coli* (UPEC) remains the most important aetiological agent of urinary tract infections (UTIs) and poses a significant clinical challenge due to its potential to cause recurrent infections and severe complications, including renal impairment and sepsis [13].

Uncomplicated UTIs are traditionally more common in females owing to anatomical predisposition and hormonal variations across different life stages, with recurrence frequently observed among sexually active women [14,15]. In contrast, the present study demonstrated a higher overall incidence of UTIs among males, similar to observations reported by Karishetti MS and Shaik HB [16]. The majority of affected patients belonged to the 40–79-year age group. Increased susceptibility in this population may be attributed to comorbid conditions such as diabetes mellitus, hypertension, prior catheterisation, surgical interventions, and hospitalisation, as previously reviewed by Medina M and Castillo-Pino E [14]. Notably, a history of hospitalisation within the preceding year was identified as a statistically significant risk factor in the present study. Despite the higher prevalence of UTIs among males, UPEC isolation was more frequent in females, likely reflecting a greater propensity for ascending infection from rectal flora [17].

E. coli accounted for 39.66% of culture-positive UTIs, followed by *Candida* spp. (18.97%), *Pseudomonas* spp. (13.79%), and lower proportions of Coagulase-negative *Staphylococci*, *Klebsiella* spp., and *Enterococcus* spp. Less frequently isolated pathogens included *Proteus* spp. and *Citrobacter* spp. (3.45%), while *Acinetobacter*, *Morganella*, and *Enterobacter* spp. were identified in smaller numbers (1.72%). These findings highlight the polymicrobial nature of UTIs, particularly in hospitalised patients.

Several studies have documented a rising prevalence of Extended Spectrum Beta-Lactamase (ESBL)-producing *E. coli* over recent decades. Ghazvini H et al. observed a 32% ESBL rate among UPEC isolates [19]. Kateregga JN et al. reported ESBL production in 62% of *E. coli* isolates from diverse clinical specimens, which closely aligns with the findings of the present study [18]. In this study, the overall ESBL prevalence among *E. coli* isolates was 62.96%, with a higher proportion observed among non-UPEC strains (68.42%).

Carbapenems remain the drugs of choice for severe infections caused by ESBL-producing organisms. However, the present study demonstrated an overall carbapenem resistance rate of 33.33% among *E. coli* isolates, detected using meropenem disc diffusion, with comparable rates observed in UPEC and non-UPEC strains.

Phenotypic detection using mCIM and eCIM revealed carbapenemase production in 14.8% of *E. coli* isolates, all of which were confirmed as Metallo-Beta-Lactamase (MBL) producers. Among the nine meropenem-resistant isolates, four were MBL-positive, while five were negative by both mCIM and eCIM.

Therapeutic options for ESBL-producing UPEC are increasingly limited. The ICMR 2019 guidelines recommend nitrofurantoin and Fosfomycin as first-line empirical agents for uncomplicated cystitis, with cotrimoxazole, fluoroquinolones, and carbapenems as alternative options based on susceptibility patterns [19]. In the present study, ESBL-producing UPEC isolates demonstrated complete resistance to ampicillin, all generations of cephalosporin, fluoroquinolones, and trimethoprim. In contrast, high susceptibility was retained to nitrofurantoin (100%) and fosfomycin (75%), supporting their continued use in empirical therapy.

LIMITATION

Data from Outpatient Department (OPD) patients could not be comprehensively included due to incomplete clinical information; therefore, risk factor analysis was restricted to Inpatient Department (IPD) cases. Additionally, the analysis of ESBL- and non-ESBL-producing *Escherichia coli* was limited to a subset of 27 isolates, including both UPEC and non-UPEC strains, owing to the unavailability of additional strains for resistance mechanism evaluation.

CONCLUSION

The antimicrobial resistance (AMR) landscape is highly dynamic and varies across regions due to factors such as host susceptibility, healthcare exposure, and local antibiotic prescribing practices. In the present study, multidrug-resistant uropathogenic *Escherichia coli* (MDR-UPEC) isolates demonstrated good susceptibility to nitrofurantoin and fosfomycin, which are also recommended by national guidelines. These agents should therefore continue to be considered for the empirical management of urinary tract infections.Definitive therapy should be guided by culture and antimicrobial susceptibility testing of isolated pathogens. The use of synergistic antimicrobial combinations may further aid in limiting resistance development. Regular implementation of culture and sensitivity testing is strongly advocated to facilitate timely transition from empirical to targeted therapy and to enhance understanding of local AMR trends, thereby supporting effective antimicrobial stewardship strategies.

Furthermore, the increasing prevalence of Metallo-Beta-Lactamase (MBL) production has compromised the efficacy of carbapenems, which are considered last-resort antibiotics. This highlights the urgent need for continued surveillance and the development of effective MBL inhibitors, along with the integration of molecular diagnostic approaches, to improve clinical management and preserve the utility of existing antimicrobial agents.

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