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Original Article

Microbiology Section

Comparative Evaluation of Infectious Potential, Resistance Patterns and Key Determinants of *Escherichia coli* in Urinary Tract Infection: A Cross-sectional Study

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ABSTRACT

Introduction: The emergence of Multidrug-Resistant (MDR) *Escherichia coli* (*E. coli*) and its opportunistic uropathogenicity complicates the treatment of Urinary Tract Infections (UTIs), consequently increasing healthcare costs and mortality rates. Uropathogenic *E. coli* (UPEC) is the primary cause of most UTIs, such as cystitis and pyelonephritis. These infections can lead to severe complications, including acute renal failure, affecting both healthy individuals and renal transplant patients.

Aim: To scrutinise the infectious prevalence of *E. coli* and the occurrence of UPEC, with relative proportional scrutiny of the resistance spectrum and determinants associated with UTIs to identify their management strategies.

Materials and Methods: This cross-sectional study was conducted between February 2019 and February 2023 at the Microbiology Laboratory of Charotar Hospital and Research Foundation (CHRF), Anand, Gujarat, India. A total of 461 clinical specimens, including 153 urine samples, were processed to determine the infectious magnitude of *E. coli* and the rate of UPEC. Their resistance spectrum and mechanisms were compared using disc diffusion assays and statistically analysed by Chi-square value, two-tailed p-value, odds ratios (ORs) and confidence intervals using Microsoft Excel 2021 and CDC Epi Info™ software by the US Department of Health and Human Services, Centres for Disease Control and Prevention.

Results: *E. coli* was predominant in UTIs, comprising 45% of UPECs. Of the *E. coli* samples, 62.9% were Extended Spectrum Beta-Lactamase (ESBL) producers, showing complete resistance to penicillins and cephalosporins. Furthermore, ESBL UPEC demonstrated a high level of resistance to carbapenems and fluoroquinolones, but exhibited good sensitivity to Nitrofurantoin (100%) and Fosfomycin (75%). Additionally, 33.3% of *E. coli* were carbapenem-resistant, with 14.8% found to produce Metallo-Beta-Lactamase (MBL). Hospitalisation in the past year was identified as a significant associated risk factor (p-value <0.05).

Conclusion: Nitrofurantoin and Fosfomycin are significant drugs for the empirical management of UTI cases. However, for effective management of Antimicrobial Resistance (AMR), definitive therapy must be continued in a synergistic combination after ensuring regular culture and sensitivity practices. The increasing rate of carbapenem resistance, limited therapeutic options for ESBL and the constrained results of carbapenemase phenotypes necessitate the identification of the precise causes by molecular assay for effective clinical guidance.

Keywords: Beta-lactamase, Carbapenemase, Drug resistance, Uropathogenic *Escherichia coli*

INTRODUCTION

E. coli is a gram-negative bacillus that belongs to the Enterobacteriaceae family. From the human perspective, it is a common gastrointestinal commensal flora that is essential when balanced but behaves as a complex pathogen when it thrives. By acquiring virulence genes, the intestinal flora can act as both an intestinal and extraintestinal pathotype [1]. The UPEC is a common extraintestinal pathotype that causes opportunistic UTIs. It originates from the rectal flora and enters the urinary tract, accounting for frequent UTIs that affect 150 million people worldwide. UTIs are a serious health issue, as 50% of established cystitis cases progress to pyelonephritis. The urological consequences can result in renal failure and sepsis, leading to increased mortality and morbidity [2-4]. Furthermore, within the context of AMR, which ranks among the top ten global health threats, *E. coli* is identified as a critical priority pathogen according to the Indian Priority Pathology List (IPPL-2021) [5]. The emergence of Extended ESBL and carbapenemase in *E. coli*, including UPECs, along with the frequent recurrence of UTIs, represents significant therapeutic limitations and life-threatening challenges [6,7].

The primary contributors to AMR include the misuse and overuse of antimicrobials, erratic regulation, inadequate infection prevention and control practices in healthcare facilities and environmental contamination. To combat the threat of AMR, it is essential to implement infection control measures such as environmental surveillance and regular restructuring of antimicrobial policies, along with the routine monitoring of clinical isolates and their antibiograms [8,9]. Recurrent UTIs with therapeutic limitations due to evolving resistance and inadequate regional antibiogram data complicate the disease management. Therefore, this study aimed to primarily focus on the prevalence rate of UTIs associated with UPEC and their antibiogram in regional UPEC isolates to detect their level of resistance. Understanding the AMR spectrum and resistance mechanisms will guide antibiotic stewardship interventions and underscore the necessary measures to control the spread of AMR and minimise infectious fatalities. The study aimed to scrutinise the infectious extent of *E. coli* and the occurrence of UPEC, with relative proportional scrutiny of the resistance spectrum and determinants associated with UTIs to identify their management strategies.

MATERIALS AND METHODS

This cross-sectional study was conducted from February 2019 to February 2023 at the Microbiology Laboratory of Charotar Hospital and Research Foundation (CHRF), Anand, Gujarat, India. The

study received approval from the Institutional Ethical Committee of CHARUSAT (IEC/CHARUSAT/EX/23/109). The sample size was calculated according to the standard formula (power analysis) for a cross-sectional study.

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 461 different clinical samples were collected aseptically under the supervision of medical staff and transported directly to the laboratory in an icebox for processing. Among these, 153 samples were urine. Standard protocols and guidelines were followed for specimen collection, transportation and processing [10,11]. The isolation of clinical strains was performed using the four-flame streaking method and colony counting was carried out using the direct streaking technique with a calibrated loop. Significant bacteriuria was determined by a colony count of $\geq 10^5$ CFU/mL, while insignificant growth was confirmed through clinical correlation [10]. Clinical isolates were identified by analysing their morphological features through Gram staining and motility via the hanging drop method. Their colonial characteristics were observed after cultivation on a range of media, including ordinary, differential, enriched and selective types. Additionally, biochemical assays were conducted using tests such as sugar fermentation, Indole, Triple Sugar Iron Agar (TSI), Simmons citrate, Urease, Methyl Red (MR) and Voges-Proskauer (VP).

E. coli strains were distinguished as motile, Gram-negative bacilli, displaying lactose-fermenting colonies with a greenish metallic sheen on Eosin-Methylene-Blue (EMB) agar. They exhibited fermentation of all 1% sugars with acid and gas production, an acidic slant and butt in TSI accompanied by gas formation and positive results for both Indole and MR tests. *E. coli* isolated from urine samples were classified as UPEC, while those from other clinical samples were classified as non UPEC strains. Additionally, the ATCC 25922 *E. coli* strain was used for internal quality control procedures.

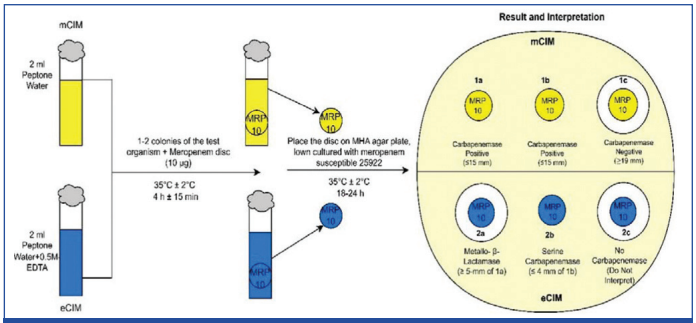
Antibiotic Susceptibility and Resistance Mechanism

The study conducted routine Antibiotic Susceptibility Testing (AST) on isolated UPEC and non UPEC strains using the Kirby-Bauer disc diffusion method. The antibiotics belonging to the Enterobacteriaceae group were placed on the inoculated Mueller-Hinton Agar (MHA) plates and incubated overnight at 37°C. The zone of inhibition was measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints [12].

ESBL detection was performed using the Double Disc Synergy Test (DDST) on MHA with the lawn-cultured test organism. Ceftazidime (CAZ) and Ceftazidime-Clavulanic Acid (CAC) combination discs were placed and incubated overnight at 37°C. An increase in the zone diameter of ≥5 mm for either antimicrobial agent tested in combination with clavulanic acid was interpreted as ESBL positive [12].

Carbapenemase detection was performed using the Modified Carbapenem Inactivation Method (mCIM), while Metallo-beta-lactamase (MBL) was detected by the EDTA-Modified Carbapenem Inactivation Method (eCIM) as indicated in [Table/Fig-1]. For mCIM, the test organism was inoculated in peptone water with a Meropenem (MRP) disc and incubated at 35°C for four hours. The treated MRP disc was then placed on an MHA plate that had been lawn cultured with a standard culture suspension of *E. coli* ATCC-25922 and incubated overnight at 37°C. The next day, a zone of inhibition (ZOI) ≤15 mm was interpreted as positive for carbapenemase [12].

For eCIM, the test organism was inoculated in peptone water mixed with 20 µL of 0.5M EDTA (ethylenediaminetetraacetic acid) and an



[Table/Fig-1]: Diagrammatic representation of carbapenemase and Metallo-Beta-Lactamase detection.

MRP disc, which was further processed similarly to mCIM. The following day, a ≥5 mm increase in zone diameter compared to the MRP disc without EDTA (the difference between 1a and 2a) was interpreted as MBL positive, while a zone diameter of ≤4 mm was interpreted as serine carbapenemase [12].

STATISTICAL ANALYSIS

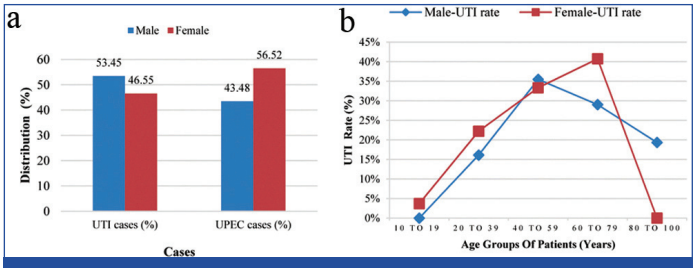
Clinical data were entered and analysed for descriptive statistics in the form of relative frequency using Microsoft Excel 2021 (Microsoft Corporation, USA). The categorical variables were examined for significant risk factor associations by analysing the uncorrected statistical chi-square value, two-tailed p-value <0.05, odds ratios (ORs) and confidence intervals as evidenced from CDC Epi Info™ software, US Department of Health and Human Services, Centres for Disease Control and Prevention.

RESULTS

UTI Occurrences and Demographic Distribution

Out of a total of 461 clinical specimens analysed for culture and sensitivity, 153 were urine samples obtained from symptomatic patients. Among these, 58 (37.91%) urine samples showed positive cultures and were subsequently categorised for further analysis. A total of 89 (58.17%) symptomatic UTI cases were observed in the Inpatient Department (IPD), compared to 64 (41.83%) in the Outpatient Department (OPD).

UTI cases were more commonly seen among males, accounting for 31 (53.45%) of the cases, compared to 27 (46.55%) in females. Conversely, when considering UTIs caused by UPEC, a higher proportion was found in females, with 13 (56.52%) compared to 10 (43.48%) in males, as shown in [Table/Fig-2a]. Regarding age distribution, a greater number of UTI cases were recorded among individuals aged between 40 and 80 years. Specifically, male UTI cases were most commonly observed in the 40 to 59-year age group, whereas in females, the incidence was higher in the 60 to 79-year age group, as illustrated in [Table/Fig-2b].



[Table/Fig-2]: Demographic distribution of UTI and UPEC cases a) Gender-wise UTI cases distribution; b) Age and gender-wise UTI rate. (Images from left to right)

UTI Co-morbid association

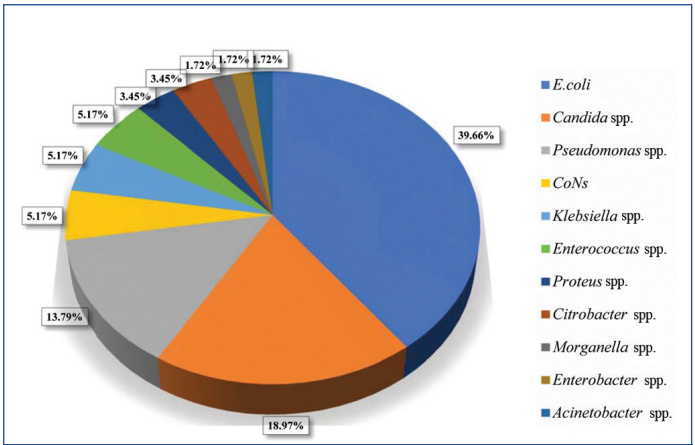
The risk association of UTI cases was ascertained in 82 IPD patients. Among concurrent conditions, only a history of previous hospitalisation in the last year showed a significant correlation with UTIs, where the p-value was below the significance threshold (<0.05) and the chi-square value exceeded 3.84, as indicated in [Table/Fig-3].

Risk factors	UTI Positive (n=26)	UTI Negative (n=56)	p-value	Odd Ratio (CI)	λ^2 value
Hypertension	8	11	0.26	1.81 (0.62-5.25)	1.23
Renal calculus	6	6	0.14	2.5 (0.72-8.68)	2.17
Diabetes mellitus	9	14	0.36	1.58 (0.57-4.35)	0.81
Steroid use	1	1	0.57	2.2 (0.13-36.61)	0.31
Catheterisation	16	25	0.15	1.98 (0.76-5.12)	2.02
Previous hospitalisation in the last 1 year	6	3	0.016*	5.3 (1.20-23.24)	**5.70**
Immunocompromised condition	14	26	0.53	1.34 (0.52-3.42)	0.391
Duration of hospitalisation > 48 hours	23	52	0.50	0.5 (0.12-2.85)	0.43
Urinary system dysfunction	18	45	0.26	0.55 (0.19-1.5)	1.23

[Table/Fig-3]: Risk factors associated with UTIs.
Indicates significance at p-value <0.05 (5%)

Occurrence of diverse Uropathogens

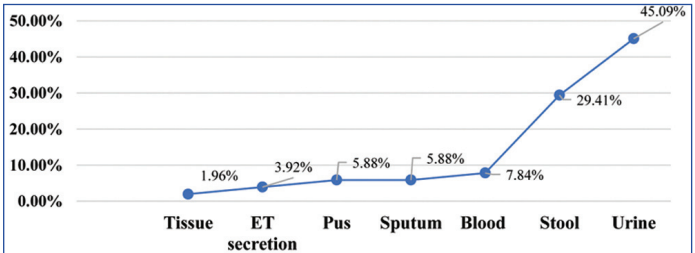
Out of 153 urine specimens, 58 were detected as culture positive, predominating with 23 (39.66%) cases of *E. coli*, followed by 11 (18.97%) cases of *Candida* spp., 8 (13.79%) cases of *Pseudomonas* spp. and 3 (5.17%) cases of Coagulase-negative Staphylococci (CoNS), with *Klebsiella* spp. and *Enterococcus* spp. each being reported, as revealed in [Table/Fig-4].



[Table/Fig-4]: Rate of uropathogens isolated from urine specimens.

Clinical Extent of *E. coli* from different clinical specimens

Overall, 51 *E. coli* strains were isolated from different clinical specimens. From a clinical perspective, 23 (45.09%) of the *E. coli* isolates were derived from urine samples and were classified as UPEC, which are typically associated with UTIs. The *E. coli* strains obtained from other clinical specimens, excluding urine, were considered non UPEC, accounting for a total of 28 (54.91%) isolates, with the majority being from stool samples, which represented 15 (29.41%), as presented in [Table/Fig-5].



[Table/Fig-5]: Distribution of *E. coli* in various clinical specimens.

Resistance spectrum of UPEC and non UPEC

The UPEC and non UPEC strains exhibit higher levels of resistance to many antibiotics, including ampicillin, cefepime, imipenem, ciprofloxacin, levofloxacin, ceftazidime, tetracycline and trimethoprim. However, the antimicrobial spectrum of these isolates demonstrates a significant difference between UPEC and non UPEC, with substantial resistance levels to cefazolin, cefuroxime and cefotaxime in non UPEC, showing p-values of 0.009, 0.004 and 0.029, respectively, as presented in [Table/Fig-6].

Furthermore, ESBL and Carbapenemase activity were identified among 27 *E. coli* strains. The overall prevalence rate of ESBL was found to be 62.96% (17/27), primarily among non UPEC, which secured 68.42% (13/19). The ESBL strains from both UPEC and non UPEC exhibited 100% resistance to ampicillin and all generations of cephalosporin agents, including cefazolin, cefotaxime, cefuroxime, ceftazidime and cefepime. ESBL UPEC demonstrated a high level of resistance to carbapenems and the fluoroquinolone group of drugs compared to non UPEC, as depicted in [Table/Fig-7].

Despite this, all ESBL strains exhibited higher sensitivity to Group A aminoglycosides, namely gentamicin, tobramycin and amikacin, as well as to the Group C antibiotic chloramphenicol and the Group U antibiotics fosfomycin and nitrofurantoin, with ESBL UPEC showing sensitivity rates of 100% and 75% to nitrofurantoin and fosfomycin, respectively.

As shown in [Table/Fig-8], meropenem resistance was observed in 33.33% (9/27) of the total *E. coli* strains, accounting for 37.50% (3/8) in UPEC and 31.57% (6/19) in non UPEC. The carbapenem-resistant strains were further screened for carbapenemase activity through the eCIM and mCIM disc diffusion assays. In the mCIM assay, 14.81% (4/27) of *E. coli* strains were found to be carbapenemase-positive and all were positive for MBL production as evaluated by eCIM.

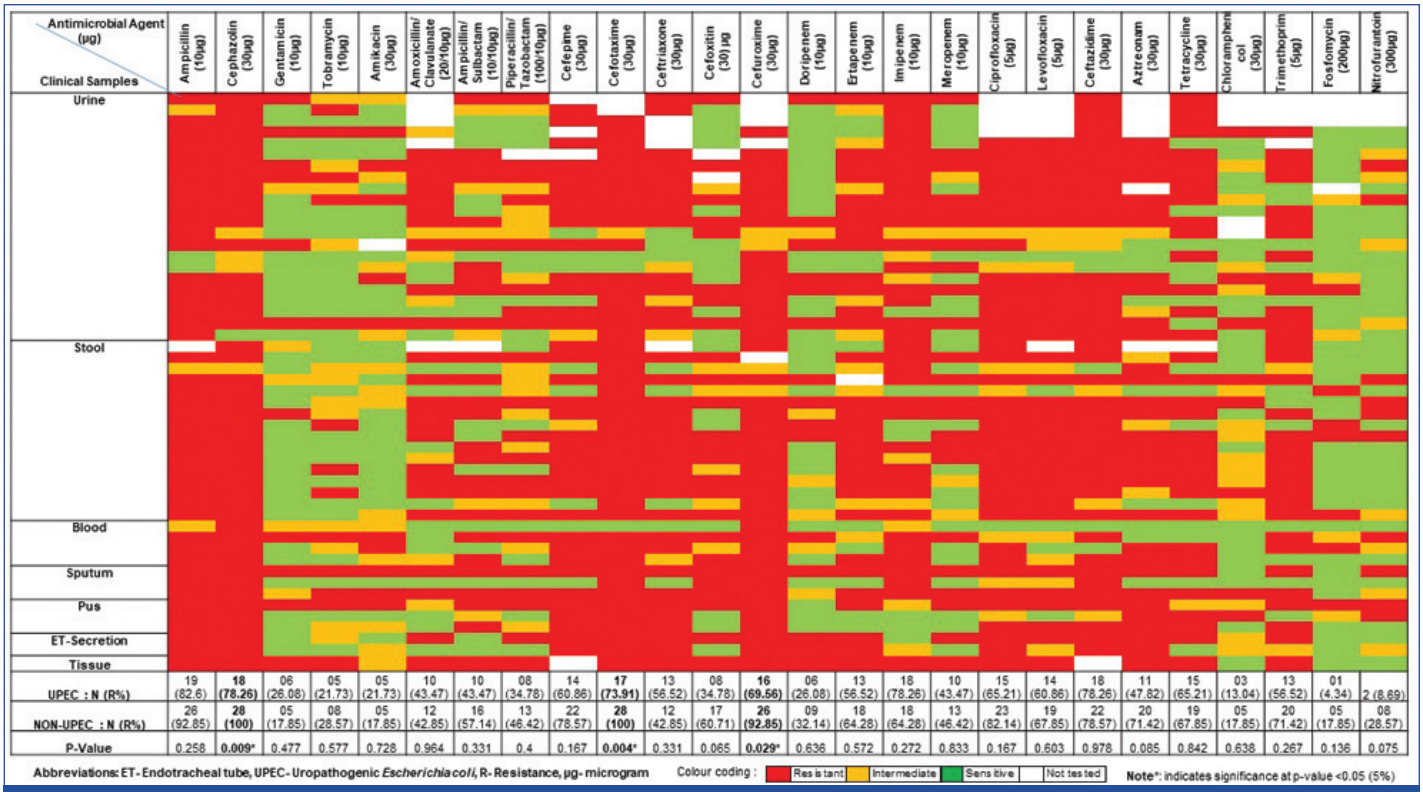
DISCUSSION

UPEC, a significant contributor to UTIs, represents a critical concern regarding ineffective treatment due to its potential progression to serious complications affecting kidney function [13]. Understanding the occurrence of diverse uropathogens and the infectious extent of *E. coli* will highlight the clinical importance of this opportunistic pathogen. The comparative exploration of their resistance spectrum and the associated risk factors is crucial for developing and implementing effective antibiotic stewardship strategies.

Uncomplicated UTIs are more commonly seen in females due to their anatomical structure and hormonal changes at various life stages. These infections are frequently recurrent in sexually active women [14,15]. However, in this study, males exhibited a higher incidence of UTIs compared to females, similar to the findings of Karishetti MS and Shaik HB [16]. The affected age group for both genders was 40-79 years. This increased susceptibility to UTIs is linked to several health conditions, including hypertension, diabetes, catheterisation, surgeries and previous hospitalisations, as reviewed by Medina M and Castillo-Pino E [14]. Previous hospitalisation, in particular, was identified as a significant risk factor in present study. Despite the higher number of UTIs in males, the detection rate of UPEC was higher in females, which could be attributed to a predisposition to proximal seeding of *E. coli*, the predominant aerobic coliform [17].

UPEC is the most common uropathogen identified, in concordance with regional, national and global studies [18-23]. Present study presents *E. coli* as the leading cause of UTIs at 23 (39.66%), followed by 11 (18.97%) instances of *Candida* spp., 8 (13.79%) instances of *Pseudomonas* spp. and 3 (5.17%) instances each of CoNS, *Klebsiella* spp. and *Enterococcus* spp. *Proteus* spp. and *Citrobacter* spp. accounted for 3.45%, while *Acinetobacter* spp., *Morganella* spp. and *Enterobacter* spp. comprised 1.72%.

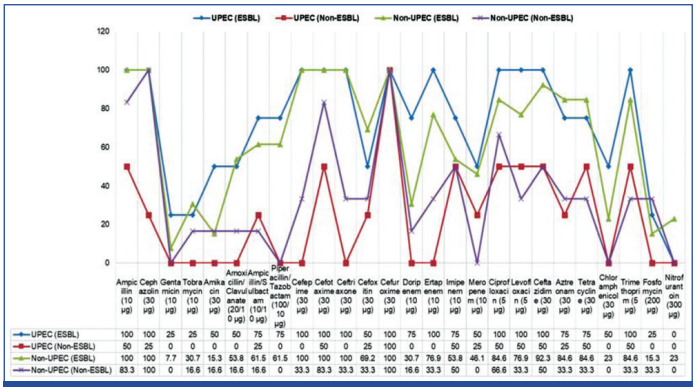
The AMR is a global concern [24,25]. Shkalim Zemer V et al., have shown a substantial increase in ESBL prevalence from 2007 to 2021, rising from 6.1% to 25.4% [22] and Ghazvini H et al., reported a 32% ESBL rate in UPEC [19]. Kateregga JN et al., have indicated that 62% of ESBL *E. coli* were isolated from different clinical specimens,



Antimicrobial Agent (µg) / Clinical Samples	Ampicillin (10µg)	Cephazolin (30µg)	Gentamicin (10µg)	Tobramycin (10µg)	Amikacin (30µg)	Amoxicillin/ Clavulanate (20/10µg)	Ampicillin/ Sulbactam (10/10µg)	Piperacillin/ Tazobactam (100/10µg)	Cefepime (30µg)	Cefotaxime (30µg)	Ceftriaxone (30µg)	Cefoxitin (30) µg	Cefuroxime (30µg)	Doripenem (10µg)	Ertapenem (10µg)	Imipenem (10µg)	Meropenem (10µg)	Ciprofloxacin (5µg)	Levofloxacin (5µg)	Ceftazidime (30µg)	Aztreonam (30µg)	Tetracycline (30µg)	Chloramphenicol (30µg)	Trimethoprim (5µg)	Fosfomycin (200µg)	Nitrofurantoin (300µg)
Urine																										
Stool																										
Blood																										
Sputum																										
Pus																										
ET-Secretion																										
Tissue																										
UPEC : N (R%)	19 (82.6)	**18 (78.26)**	06 (26.08)	05 (21.73)	05 (21.73)	10 (43.47)	10 (43.47)	08 (34.78)	14 (60.86)	**17 (73.91)**	13 (56.52)	08 (34.78)	**16 (69.56)**	06 (26.08)	13 (56.52)	18 (78.26)	10 (43.47)	15 (65.21)	14 (60.86)	18 (78.26)	11 (47.82)	15 (65.21)	03 (13.04)	13 (56.52)	01 (4.34)	2 (8.69)
NON-UPEC : N (R%)	26 (92.85)	**28 (100)**	05 (17.85)	08 (28.57)	05 (17.85)	12 (42.85)	16 (57.14)	13 (46.42)	22 (78.57)	**28 (100)**	12 (42.85)	17 (60.71)	**26 (92.85)**	09 (32.14)	18 (64.28)	18 (64.28)	13 (46.42)	23 (82.14)	19 (67.85)	22 (78.57)	20 (71.42)	19 (67.85)	05 (17.85)	20 (71.42)	05 (17.85)	08 (28.57)
P-Value	0.258	**0.009***	0.477	0.577	0.728	0.964	0.331	0.4	0.167	**0.004***	0.331	0.065	**0.029***	0.636	0.572	0.272	0.833	0.167	0.603	0.978	0.085	0.842	0.638	0.267	0.136	0.075

Abbreviations: ET- Endotracheal tube, UPEC- Uropathogenic *Escherichia coli*, R- Resistance, µg- microgram Colour coding : Resistant Intermediate Sensitive Not tested Note*: indicates significance at p-value <0.05 (5%)

[Table/Fig-6]: Resistance spectrum of UPEC and non UPEC.



	Ampicillin (10 µg)	Cephazolin (30 µg)	Gentamicin (10 µg)	Tobramycin (10 µg)	Amikacin (30 µg)	Amox/Ampicillin/Clavulanate (20/10 µg)	Ampicillin/Sulbactam (10/10 µg)	Piperacillin/Tazobactam (100/10 µg)	Cefepime (30 µg)	Cefotaxime (30 µg)	Ceftriaxone (30 µg)	Cefoxitin (30 µg)	Cefuroxime (30 µg)	Doripenem (10 µg)	Ertapenem (10 µg)	Imipenem (10 µg)	Meropenem (10 µg)	Ciprofloxacin (5 µg)	Levofloxacin (5 µg)	Ceftazidime (30 µg)	Aztreonam (30 µg)	Tetracycline (30 µg)	Chloramphenicol (30 µg)	Trimethoprim (5 µg)	Fosfomycin (200 µg)	Nitrofurantoin (300 µg)
UPEC (ESBL)	100	100	25	25	50	50	75	75	100	100	100	50	100	75	100	75	50	100	100	100	75	75	50	100	25	0
UPEC (Non-ESBL)	50	25	0	0	0	0	25	0	0	50	0	25	100	0	0	50	25	50	50	50	25	50	0	50	0	0
Non-UPEC (ESBL)	100	100	7.7	30.7	15.3	53.8	61.5	61.5	100	100	100	69.2	100	30.7	76.9	53.8	46.1	84.6	76.9	92.3	84.6	84.6	23	84.6	15.3	23
Non-UPEC (Non-ESBL)	83.3	100	0	16.6	16.6	16.6	16.6	0	33.3	83.3	33.3	33.3	100	16.6	33.3	50	0	66.6	33.3	50	33.3	33.3	0	33.3	33.3	0

[Table/Fig-7]: Resistance pattern of ESBL and non ESBL producing UPEC and non UPEC strains.

Total no. of *E. coli*	Meropenem-Resistant *E. coli*	Carbapenemase-producing *E. coli* by mCIM	MBL Producing *E. coli* by eCIM
27	9 (33.33%)	4 (14.81%)	4 (14.81%)

[Table/Fig-8]: Phenotypic detection of carbapenem resistance and carbapenemase activity.

aligning with present study [26]. The present study identified a higher prevalence rate of ESBL among overall *E. coli*, accounting for 62.96% (17/27), predominantly among non UPEC at 68.42% (13/19). The elevated rate of ESBL signifies the potential risk of harbouring resistance genes to other Gram-negative bacteria and highlights the urgent need for preventive and treatment strategies for AMR clinical isolates.

Carbapenem is the drug of choice against ESBL pathogens. The Carbapenem group of drugs, such as Ertapenem, is recommended as first-line empirical therapy for acute prostatitis. Imipenem and Meropenem are the preferred drugs for acute pyelonephritis and acute prostatitis [24]. However, in present study, an overall carbapenem resistance of 33.33% was detected using the meropenem disc diffusion assay, with 37.50% in UPEC and 31.60% in non UPEC. Alternatives to carbapenem include β-lactam/β-lactamase inhibitors, fourth-generation cephalosporins, tigecycline and several oral antibiotic agents such as fluoroquinolones, fosfomycin and nitrofurantoin [24].

As previously noted, our isolated ESBL UPEC and ESBL non UPEC strains demonstrated complete resistance to cefepime, the fourth-generation cephalosporin and high-level resistance to amoxicillin-clavulanate, ampicillin sulbactam and piperacillin tazobactam, which are β-lactam/β-lactamase inhibitors, as well as to fluoroquinolones like ciprofloxacin and levofloxacin. Carbapenem resistance may be attributed to factors such as carbapenemase production, efflux pumps and hyperproduction of ESBL and/or Ampicillinase C (AmpC) with porin loss.

Through phenotypic detection of carbapenemase via the eCIM and mCIM tests, 14.8% of MBL-producing *E. coli* were identified, where β-lactamase hydrolysis is enhanced by the presence of Zn^{2+} ions at the active site of the enzyme. The β-lactamase inhibitors that do not impede MBL and selectively constrain serine carbapenemases are also reflected in present study [27].

As shown in [Table/Fig-8], out of the nine Meropenem-resistant strains, four were identified as MBL-positive, while five strains were neither mCIM nor eCIM positive. However, upon further analysis, the four strains were found to be ESBL producers, indicating the mechanism of ESBL hyperproduction, while one strain remains unidentified. Molecular assays may play a significant role in further screening and identifying the possibilities of their resistance mechanisms and carbapenemase-resistant UPEC clones.

The National Treatment Guidelines of ICMR 2019 recommend nitrofurantoin and fosfomycin as the empirical drugs of choice for treating acute uncomplicated cystitis, while cotrimoxazole, ertapenem and amikacin are suggested as alternative options [24]. Present study presented good sensitivity to nitrofurantoin, fosfomycin and amikacin but showed a higher level of resistance to ertapenem. Amikacin, being an aminoglycoside and a protein synthesis inhibitor, is associated with common side-effects of nephrotoxicity and ototoxicity, which limits its use [28].

The Infectious Diseases Society of America (IDSA) 2023 recommends nitrofurantoin and cotrimoxazole as the drugs of choice for uncomplicated cystitis caused by ESBL-E (Enterobacteriaceae), while ciprofloxacin, levofloxacin and carbapenem drugs are considered alternative agents for uncomplicated cystitis caused by ESBL-E [25]. In present study, the isolated ESBL UPECs

demonstrated complete resistance to ampicillin and all generations of cephalosporin agents, including cefuroxime and cefepime, as well as to fluoroquinolone antimicrobial agents such as ciprofloxacin and levofloxacin, along with the sulphonamide drug trimethoprim. Additionally, Group U antibiotics, nitrofurantoin and fosfomycin, exhibited high sensitivity rates of 100% and 75%, respectively, among our ESBL-UPECs.

Nitrofurantoin operates via a multifactorial mechanism of action. Bacterial nitro reductase generates various electrophilic intermediates that target bacterial ribosomal proteins and inhibit DNA, RNA, protein synthesis and cell wall synthesis. It is a broad-spectrum bactericidal antibiotic with good pharmacokinetics in the urinary system and the development of resistance is low due to its action on multiple targets simultaneously. However, because of potential long-term side-effects, such as pulmonary toxicity, it is essential to establish specific criteria for use in cases of chronic UTI [29].

Fosfomycin is a phosphonic acid-derived antibiotic with broad-spectrum bactericidal activity that remains effective against MDR-ESBL strains, biofilms and intracellular bacterial clearance. It also exhibits favourable pharmacokinetics and pharmacodynamics in urine, inhibiting cell wall biogenesis while stimulating host immune activity [30,31]. Due to its unique structure, Fosfomycin has minimal cross-resistance with other antibiotics. Consequently, the older drugs Nitrofurantoin and Fosfomycin emerge as significant options for the empirical management of UTI cases. However, definitive therapy should be adjusted following the susceptibility patterns of isolated and identified strains. Furthermore, synergistic drug combination therapy should be considered to reduce levels of resistance.

Limitation(s)

The study encountered a significant constraint by not incorporating data from outpatient department (OPD) patients due to a lack of information. Consequently, the analysis relied solely on inpatient department (IPD) patient records to explore risk factor associations. Additionally, the examination of ESBL and non ESBL data was limited to a subset of 27 *E. coli* strains, which included both UPEC and non UPEC strains, due to the absence of other strains.

CONCLUSION(S)

The AMR landscape appears highly dynamic across regions due to multiple factors, including host susceptibility and local antibiotic usage. Our multidrug-resistant uropathogenic *E. coli* (MDR-UPEC) strains demonstrated good sensitivity to nitrofurantoin and fosfomycin, which are also recommended by the National Centre for Disease Control; thus, these should continue to be used for the empirical management of UTI cases. After determining the susceptibility patterns of the isolated and identified strains, definitive therapy must be administered. Furthermore, employing synergistic drug combinations can help reduce resistance levels.

Authors strongly advocate for the regular endorsement of culture and sensitivity practices to facilitate the transition to definitive therapy and to enhance the understanding of local AMR patterns, which will support effective antimicrobial strategies. Comparative phenotypic and molecular assays of resistant strains will yield more precise findings and can provide valuable guidance for clinicians. The increasing MBL activity within strains has limited the efficacy of the last-resort antibiotic (carbapenem), making the search for MBL inhibitors essential to enhance carbapenem effectiveness.

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