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Identification of ESBL and Carbapenemase Genes and their Correlation with Antibiotic Resistance Profiles in *Escherichia coli* Strains Isolated from Patients with Urinary Tract Infections in a Tertiary Care Hospital

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Abstract

The worldwide rise of antibiotic-resistant *Escherichia coli*, a major cause of urinary tract infections (UTIs), has intensified concerns over patient outcomes, illness severity, and healthcare costs. This study investigated the antimicrobial resistance patterns of uropathogenic *E. coli* (UPEC) isolates, emphasizing the prevalence of extended-spectrum β-lactamase (ESBL) and carbapenemase producers and their associated resistance trends. A prospective study was conducted at BGS Global Institute of Medical Sciences, Bengaluru, from June 2021 to May 2022, involving 226 UPEC isolates. Antimicrobial susceptibility testing revealed high resistance to ciprofloxacin (50.9%), ampicillin (59.6%), and aztreonam (61.5%), while notable susceptibility was observed to fosfomycin (97.5%), amikacin (93.7%), and imipenem (93.4%). ESBL production was confirmed in 36.7% of isolates, and 3.1% were identified as carbapenemase producers. Molecular analysis showed that bla_{TEM} and bla_{CTX} genes were associated with ESBL production, while bla_{VIM} was associated with carbapenemase production. There was no significant correlation between ESBL and carbapenemase expression. The findings highlight the alarming prevalence of multidrug-resistant UPEC and underline the necessity for continuous surveillance of antimicrobial resistance patterns. The preserved activity of fosfomycin, amikacin, and imipenem suggests their continued utility in managing resistant UTIs. Rational antibiotic use and stringent infection control measures remain imperative to curb the further spread of resistant strains.

Keywords: Antibiotic Resistance, Carbapenemase Genes, ESBL (Extended-Spectrum Beta-Lactamase) Genes, Uropathogenic *Escherichia coli* (*E. coli*), Urinary Tract Infections (UTIs)

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INTRODUCTION

Urinary Tract Infections (UTIs) - A possible pandemic menace looming large

Among the infections of bacterial origin, Urinary tract infections (UTIs) are most prevalent and annually approximately 150 million people around the globe are affected by it.[1] These infections pose a significant burden on healthcare systems, leading to high morbidity, hospitalization rates, and increased treatment costs. UTIs can affect individuals of all ages, but certain populations, such as women, diabetics, and the elderly, are more susceptible due to anatomical and immunological factors.

Pathogenic role of *Escherichia coli* (*E. coli*) in UTIs

In the reported cases of UTIs, it has been observed that a major fraction (70%-80%) is caused by uropathogenic *Escherichia coli* (UPEC)**,[2,3] which establishes its colonies in the urinary tract epithelium and evades the host immune system using through the action of virulence factors such as: Fimbriae and adhesins (e.g., type 1 pili, *P. fimbriae*) - allowing bacterial attachment to urothelial cells. Toxins (e.g., hemolysin, cytotoxic necrotizing factor) - damaging host tissues and promoting bacterial survival.

UTIs can be classified into two broad categories based on epidemiology

Community-acquired UTIs - primarily caused by non-catheter-associated infections.

Nosocomial (hospital-acquired) UTIs - often associated with catheterization, prolonged hospital stays, and multidrug-resistant (MDR) pathogens.[4] Understanding these classifications is essential, as the type of infection often determines the causative organisms, resistance patterns, and treatment strategies. The following section explores these aspects in greater detail, highlighting the predominant pathogens and their antimicrobial resistance profiles. Nosocomial UTIs pose a significant threat due to their association with MDR bacteria, often leading to treatment failures and prolonged hospitalizations. Reports indicate that these infections comprise about 40% of all the hospital-acquired infections and 50% of the infections of bacterial origin.[5]

Rising antibiotic resistance - A public health threat

The increasing resistance to antibiotics like ampicillin and ciprofloxacin among uropathogenic *E. coli* (UPEC) has complicated treatment strategies and led to the failure of antibiotics. The primary mechanisms of antibiotic resistance in UPEC include:

Efflux pumps

These are active transport systems that expel antibiotics and toxic compounds from bacterial cells, thereby reducing the intracellular concentration of drugs to sub-lethal levels. Efflux pumps can be constitutively expressed or induced upon exposure to antibiotics or environmental stress. Their activation often involves global regulatory genes (such as *marA*, *soxS*, and *rob* in *E. coli*) that upregulate efflux pump operons. The overexpression of these systems contributes to multidrug-resistance (MDR) by enabling bacteria to simultaneously eject multiple classes of antibiotics, including fluoroquinolones, tetracyclines, and β-lactams.

Heteroresistance

Subpopulations of bacteria display varying levels of antibiotic resistance, allowing a fraction of the population to survive antibiotic treatment and potentially develop full resistance upon further exposure.

Alteration in outer membrane permeability

Modifications in porin proteins or loss of specific channels reduce antibiotic entry into bacterial cells, thereby decreasing susceptibility to β-lactams and fluoroquinolones.

Production of β-lactamase enzymes

These enzymes hydrolyze the β-lactam ring of antibiotics such as penicillins and cephalosporins, rendering them inactive.[6] The emergence of extended-spectrum β-lactamases (ESBLs) and carbapenemases has further intensified resistance in uropathogens.

Among β-lactamase enzymes, extended-spectrum β-lactamases (ESBLs) have become a major concern due to their ability to break down third-generation cephalosporins (e.g., ceftriaxone, ceftazidime, cefotaxime). Mobile genetic elements

like plasmids often bear the genes that encode for ESBLs which bring about rapid horizontal gene transfer (HGT) among the bacterial populations.[7]

Carbapenem-Resistant Pathogens - The Emerging Crisis:

The last line of defense against ESBL producing bacteria is carbapenem antibiotics, such as imipenem, meropenem and ertapenem.[8] Unfortunately, the emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) has challenged the effectiveness of these drugs as the enzyme carbapenemase hydrolyzes carbapenems resulting in extreme drug resistance (XDR) infections. Notable carbapenemase enzymes include:

- *Klebsiella pneumoniae* carbapenemase (KPC)
- Verona integron-encoded metallo-β-lactamase (VIM)
- New Delhi metallo-β-lactamase (NDM)
- Oxacillinase-48 (OXA-48)

Infections caused by carbapenemase-producing bacteria have been linked to high mortality rates (up to 50%), prolonged hospital stays, and increased healthcare costs.[9]

Molecular Diagnostics - A Tool for Rapid Detection

Molecular diagnostic techniques, such as polymerase chain reaction (PCR), whole-genome sequencing (WGS), and multiplex PCR, have become essential tools in detecting ESBL- and carbapenemase-producing bacteria. These methods allow for:

- Early identification of resistant bacterial strains.
- Rapid differentiation of resistance genes.
- Guiding targeted antibiotic therapy to improve patient outcomes.

However, due to their high cost and requirement for advanced laboratory facilities, molecular techniques are not always accessible in resource-limited settings. Consequently, phenotypic methods like Kirby-Bauer disc diffusion test, double-disc synergy test and modified Hodge test are widely used for detection of ESBL and carbapenemase.

MATERIALS AND METHODS

Given the clinical and epidemiological significance of antibiotic-resistant UPEC, this study was conducted to:

1. Isolate and identify uropathogenic *E. coli* and study its antimicrobial resistance.
2. Using phenotypic and genotypic methods to detect ESBL and carbapenemase production among UPEC isolates.
3. To examine the nexus between antibiotic resistance and the production of ESBL and carbapenemase among the UPEC isolates.

This study aims to provide critical insights into antimicrobial resistance trends, which can help shape effective antibiotic stewardship programs and infection control policies to combat drug-resistant UTIs.

Patients and Methods
Study design

Upon procuring the approval from the institutional ethics committee, a prospective study was carried out from June 2021 to May 2022 at BGS Global Institute of Medical Sciences, Bengaluru. From every participating patient, a detailed informed consent was obtained and were instructed to provide mid-stream, clean-catch urine samples after proper genital toileting.

Inclusion criteria

Patients who were 18 years of age or older, exhibiting UTI symptoms, and providing their informed consent were included.

Exclusion criteria

Patients under 18 years of age, those without symptoms of UTI, and those without informed consent were excluded from the study.

Isolation and identification of UPEC isolates

Eligible patients had their urine samples taken under stringent aseptic precautions, and the samples were processed within an hour of being taken. Wet mount preparations were performed for observing pus cells, erythrocytes, bacteria, and urinary casts.

More than 5 leukocytes per high-power field were defined as significant pyuria. The presence and morphology of bacteria were examined using Gram staining method.[10,11] Samples were cultured and subjected to sensitivity testing using standard protocols; and semi-quantitative urine culture analyses were performed using

the calibrated loop technique, with inoculation on MacConkey agar and blood agar plates. An incubation time of 24 hours at 37 °C in aerobic conditions was considered significant when colony counts exceeded 10^5 CFU/mL.

Phenotypic detection
ESBLs detection
Screening method

Kirby Bauer disc diffusion test (zone of inhibition ≤17 mm) was performed using the isolates of *Escherichia coli* to screen for resistance to 2nd and 3rd generation cephalosporins: ceftazidime (30 μg) and cefotaxime (30 μg).[12] UPEC isolates that exhibited resistance to either one or both third-generation cephalosporins, were counted as positive for ESBL production in screening test.

Confirmatory method

UPEC isolates that tested positive for ESBL production in the initial screening were further confirmed using the combination disc method. Discs containing either ceftazidime (30 μg) or ceftazidime-clavulanic acid (30 μg/10 μg), or cefotaxime (30 μg) and cefotaxime-clavulanic acid (30 μg/10 μg) were used. An increase of ≥5 mm in the zone diameter around the cephalosporin-clavulanic acid disc compared to the cephalosporin disc alone was interpreted as positive for ESBL production, according to CLSI guidelines. *Escherichia coli* ATCC 35218 (non-ESBL producer) and *Escherichia coli* ATCC 700603 (ESBL producer) were used as negative and positive controls, respectively.[13]

Carbapenemase detection
Screening method

UPEC isolates were screened for resistance to imipenem (10 μg) disc by Kirby-Bauer disc diffusion test.[12] Isolates that displayed resistance to imipenem disc (<19 mm zone of inhibition) were considered positive for carbapenemase production in screening test.

Confirmatory method

UPEC isolates demonstrating presumptive carbapenemase production on initial screening were subjected to confirmation by the combined disc test. Imipenem (10 μg) and imipenem-EDTA (10 μg/0.5 μg) discs were placed on Mueller-Hinton agar plates inoculated with the test isolates. An increase of ≥7 mm in the inhibition zone diameter around the imipenem-EDTA disc, compared to the imipenem disc alone, was interpreted as indicative of carbapenemase production, as per standard guidelines.[14-16]

Anti-microbial testing

Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antibiotic discs (HiMedia Laboratories, Mumbai, India) were employed: gentamicin (10 μg), nitrofurantoin (300 μg), cotrimoxazole (1.25/23.75 μg), norfloxacin (10 μg), aztreonam (30 μg), ceftazidime (30 μg), cefotaxime (30 μg), amikacin (30 μg), amoxicillin-clavulanic acid (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), imipenem (10 μg), meropenem (10 μg), fosfomycin (200 μg) and piperacillin-tazobactam (100/10 μg). Zone diameters were interpreted according to CLSI breakpoints. *Escherichia coli* ATCC 25922 served as the quality control strain. Only isolates of *E. coli* exhibiting colony counts ≥10^5 CFU/mL were included for antimicrobial susceptibility testing and further evaluated for ESBL and carbapenemase production.[13]

Molecular genotyping for ESBL and Carbapenemase genes

Molecular genotyping of *Escherichia coli* isolates was conducted using a combination of bioinformatics tools, multiplex PCR, and colony PCR. To guide the selection of relevant resistance genes, four representative *E. coli* isolates were selected for Whole Genome Sequencing (WGS) using high throughput sequencing platforms. The WGS data were analyzed through bioinformatics pipelines to identify prevalent ESBL and carbapenemase genes circulating in southern India. Based on the genetic profiles derived from WGS, a targeted panel of resistance genes bla_{TEM}, bla_{CTX-M}, bla_{PER}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC}, and $bla_{OXA-48-like}$ was selected for screening in the remaining UPEC isolates.[14,16] This WGS-guided approach enabled focused molecular detection of clinically and regionally significant antimicrobial resistance determinants.

Table 1. Primer Sequences for Carbapenemase and ESBL Genes

No.	Primer	Sequence	Length	Temp. (°C)	GC%
1	bla_{TEM}	GGGAACCGGAGCTGAATGAA	20	60.04	55
		CAGTGCTGCAATGATACCGC	20	59.97	55
2	bla_{CTX-M}	TTACCCAGCGTCAGATTCCG	20	59.83	55
		TGCGGAAAAGCACGTCAATG	20	60.04	50
3	bla_{PER}	GCAACTGCTGCAATACTCGG	20	60.03	55
		CATCTGCGCTTCATTTG	20	59.96	55
4	bla_{IMP}	GCTACCGCAGCAGAGTCTTT	20	60.39	55
		AAAGCCCCACCCGTTAACTT	20	59.81	50
5	bla_{VIM}	GCTACCGCAGCAGAGTCTTT	20	60.39	55
		AAAGCCCCACCCGTTAACTT	20	59.81	50
6	bla_{NDM}	CCTGGACCAATGACCAGACC	20	60.04	60
		AATACCTTGAGCGGGCCAAA	20	59.96	50
7	bla_{KPC}	TCCGTTACGGCAAAAATGCG	20	60.11	50
		CGGCATAGTCATTTGCCGTG	20	59.97	55
8	$bla_{OXA-48-like}$	AATTGGCGAGGCACGTATGA	20	60.11	50
		TTCGAATACCACCGTCGAGC	20	60.18	55

Primer design for antibiotic resistance genes

Primer design for the targeted genes involved in ESBL and carbapenemase production was conducted using bioinformatics tools and established guidelines. The following steps outline the methodology:

Sequence retrieval

The nucleotide sequences of the target genes (bla_{TEM}, bla_{CTX-M}, bla_{PER}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC}, and $bla_{OXA-48-like}$) were retrieved from databases such as GenBank or relevant literature.

Sequence Alignment and Analysis

The retrieved sequences were aligned using multiple sequence alignment tools to identify conserved regions among different variants of the target genes. Conserved regions were selected for primer design to ensure specificity and efficiency.

Primer Design Software

Bioinformatics tools such as Primer3, NCBI Primer-BLAST, or similar software were utilized for primer design. Primers were designed to flank the conserved regions identified in the sequence alignment while ensuring optimal length, GC content, melting temperature (Tm), and minimizing secondary structures.

Primer Specificity Check

The designed primers were validated *in silico* against the target gene sequences and other related sequences to avoid non-specific amplification. Tools such as BLAST were used to assess primer specificity.

By following these steps, specific and efficient primer pairs were designed for the detection and characterization of mechanisms of antibiotic resistance in *E. coli* isolates.

Bacterial DNA extraction and PCR amplification

Genomic DNA was extracted from bacterial isolates using the Genei DNA extraction kit (Genei, India), following the manufacturer's protocol. PCR was performed in a total reaction volume of 25 µL containing 1× JumpStart REDTaq ReadyMix (Sigma-Aldrich, USA) and gene-specific primers. The thermal cycling conditions included an initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of (i) Denaturation at 95 °C for 30 seconds, (ii) Annealing and extension at 62.5 °C for 1 hour 30 minutes, and (iii) A final extension at 72 °C for 5 minutes.

Negative controls lacking template DNA were included in each run to check for contamination. PCR products (5 µL) were electrophoresed on 1.5% (w/v) agarose gels prepared in 0.5 × TBE buffer and run at 100 V for 30 minutes. The gels were stained with ethidium bromide and visualized under UV transillumination. Amplicon sizes were determined

by comparison with a molecular size marker.[17]

Bacterial colonies were cultured on LB agar for 18 hours at 37 °C. A small amount of pure colony was collected using a sterile toothpick and suspended in the PCR reaction mixture. Colony PCR reactions and gel electrophoresis were conducted using the same procedure described above.

Primers for ESBL and carbapenemase genes were designed and purchased from GENEI, and JumpStart REDTaq ReadyMix was procured from Sigma-Aldrich. Their sequences are listed in Table 1.

Data analysis

Data entry was done on Microsoft Excel data sheet. Data was analyzed using SPSS version [IBM SPSS Statistics, SOMERS, NY USA]. The association of ESBL and MBL with antimicrobial resistance was studied, using Chi-square test. A P-value <0.001 was considered as statistically significant.[17]

RESULTS

Characteristics of UPEC patients: A total of 226 patients were examined, of which 56.6% were females (n = 128), and 43.4% were males (n = 98) (Table 2). Most of the patients were between the ages of 18 and 25, followed by 56-65 years of age, 26-35 years of age, 36-45 years of age, 66-75 years of age, 46-55 years of age, and 76-85 years of age (Table 2). Patients with no comorbid conditions constituted the majority of the patient population (n = 116) (Table 2). A total of 105 patient isolates were found to have type-II diabetes mellitus,

Table 2. Demographic Distribution and Comorbidities of Patients with Urinary Tract Infection

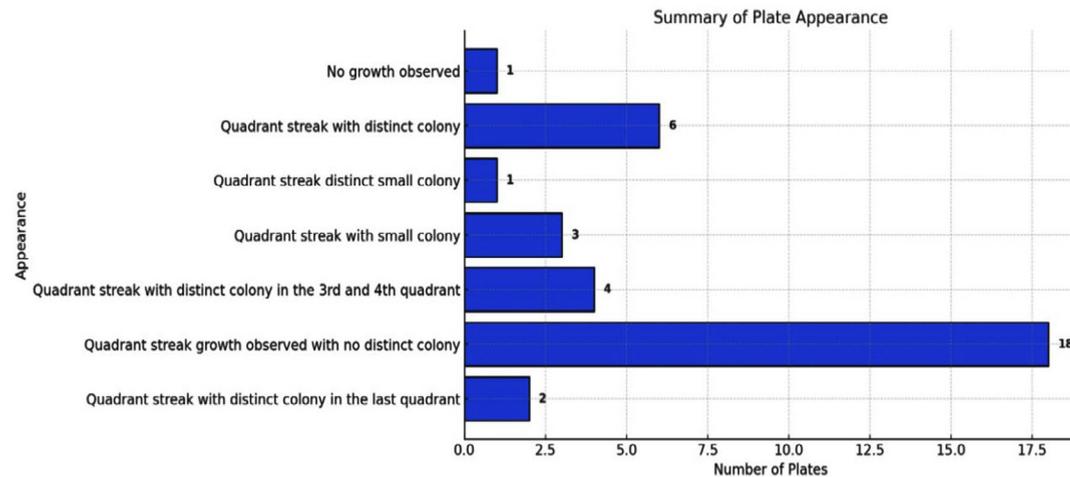| Age Group (Years)	Number of patients n (%)	Sex n (%)		Comorbidities
		Male	Female	
18-25	60 (26.5%)	98 (43.4%)	128 (56.6%)	Diabetes (105)
26-35	28 (12.4%)			CKD (10)
36-45	28 (12.4%)			Prostate carcinoma (01)
46-55	21 (9.30%)			None (116)
56-65	50 (22.1%)			
66-75	28 (12.4%)			
76-85	11 (4.90%)			

Figure 1. Bacterial appearance in culture plates

followed by chronic kidney disease (n = 10), and prostate carcinoma (1 patient isolate) (Table 2).

Bacterial Uropathogens

During the study period, a total of 4031 urine samples were processed, of which 873 (21.65%) yielded significant bacterial growth. Among these culture-positive samples, 226 non-

repetitive isolates of uropathogenic *Escherichia coli* (UPEC) that met the inclusion criteria were selected for further analysis. The colony morphology of representative isolates is depicted in Figure 1. The highest proportion of UPEC isolates was recovered from patients aged 18-25 years (26.5%), followed by those aged 56-65 years (22.1%). The overall distribution of bacterial pathogens isolated from
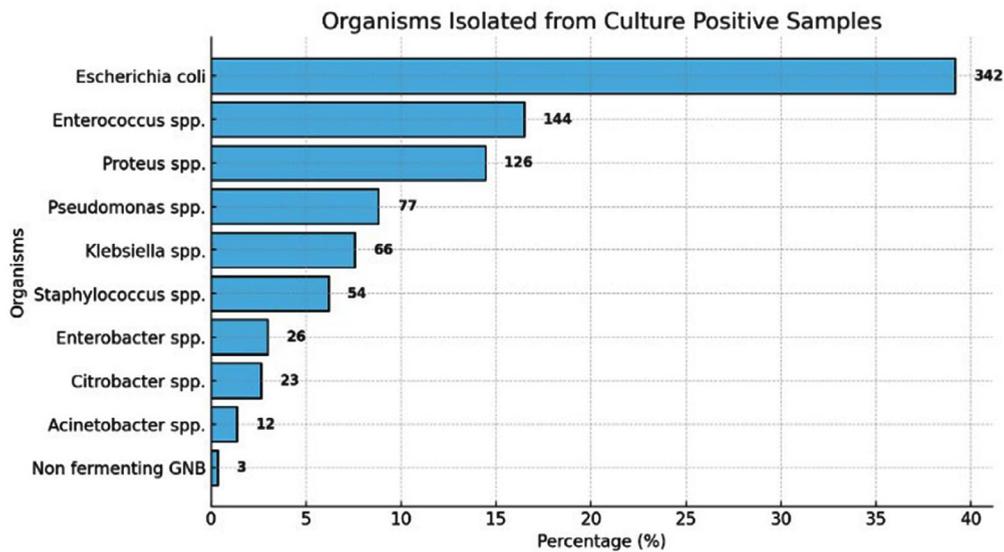

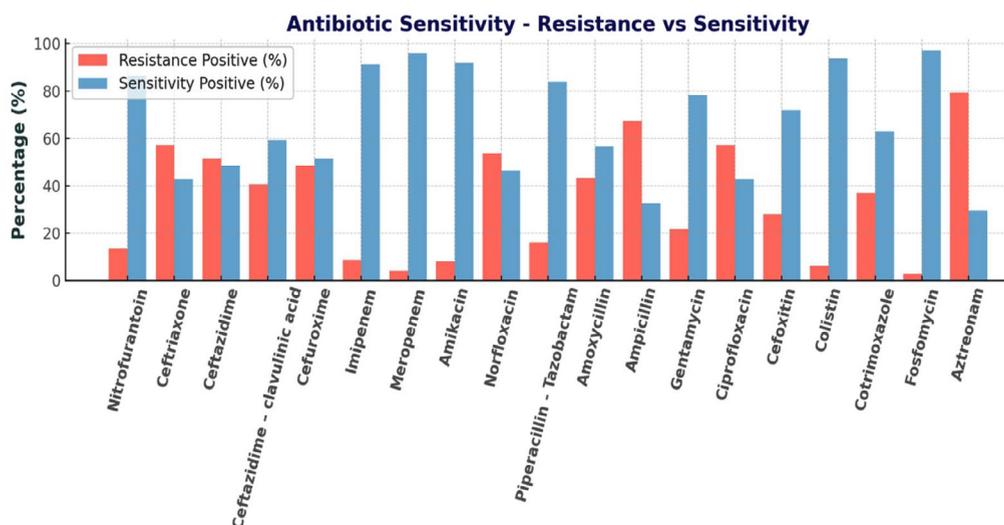**Figure 2.** Organisms isolated from the culture positive samples

Figure 3. Antibiotic susceptibility pattern of UPEC isolates

urine cultures was as follows: *E. coli* (39.17%), *Enterococcus* spp. (16.49%), *Proteus* spp. (14.43%), *Pseudomonas* spp. (8.82%), *Klebsiella* spp. (7.56%), *Staphylococcus* spp. (6.18%), *Enterobacter* spp. (2.97%), *Citrobacter* spp. (2.63%), *Acinetobacter* spp. (1.37%), and non-fermenting Gram-negative bacilli (0.34%) (Figure 2).

Antibiotic susceptibility pattern of *E. coli*

In our study, the sensitivity pattern of *Escherichia coli* showed good sensitivity to fosfomycin (97.5%), imipenem (93.4%), amikacin (93.7%), nitrofurantoin (88.5%), piperacillin-tazobactam (86.6%), gentamicin (82.3%), and cefoxitin (75.9%) (Figure 3). Moderate sensitivity was observed for cotrimoxazole (65.9%), ceftazidime-clavulinic acid (65.3%), ceftazidime (56.7%), ceftriaxone (52.2%), cefotaxime (54.4%), amoxicillin (63.6%), ciprofloxacin (49.1%),

ampicillin (40.4%) and aztreonam (38.5%) (Figure 3).

Detection of ESBL production

Of the 226 *Escherichia coli* isolates, 83 (36.7%) were identified as potential ESBL producers based on the initial screening test (Figure 4A). All isolates were subsequently subjected to phenotypic confirmation using the combined disc diffusion method. The confirmatory test also detected ESBL production in 83 (36.7%) isolates (Figure 4B). There was complete agreement between the screening and confirmatory tests, as all isolates positive by screening were confirmed as ESBL producers.

Detection of carbapenemase

In the screening method, out of 226 isolates of UPEC, 19 (8.40%) isolates were

Table 3. Carbapenemase detection by screening and confirmatory methods

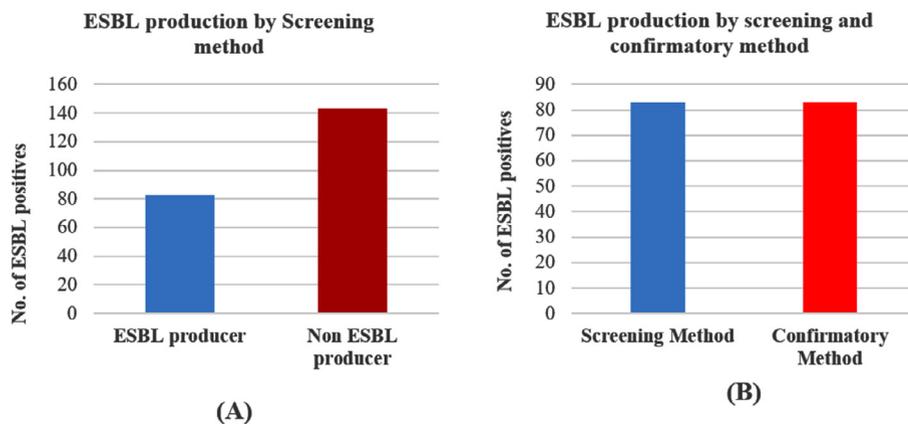| Carbapenemase detection by screening method	Number	Percentage (%)
UPEC isolates producing carbapenemase	19	8.40
UPEC isolates not producing carbapenemase	207	91.59
Carbapenemase detection by confirmatory method	**Number**	**Percentage (%)**
UPEC isolates producing carbapenemase	7	3.1
UPEC isolates not producing carbapenemase	219	96.9

Figure 4. (A) Screening test for ESBL production among UPEC isolates; (B) ESBL production by screening method and confirmatory method

Table 4. Correlation of antibiotic resistance with ESBL production

Antibiotic Sensitivity	Resistance Positive (Number)	Resistance Positive (%)	Resistance Negative (Number)	Resistance Negative (%)	Sensitivity Positive (Number)	Sensitivity Positive (%)	Sensitivity Negative (Number)	Sensitivity Negative (%)	P-value
Nitrofurantoin	24	13.7	2	3.9	151	86.3	49	96.1	0.008
Ceftriaxone	100	57.1	8	15.7	75	42.9	4	84.3	0.001
Ceftazidime	90	51.4	7	13.7	85	48.6	44	86.3	0.001
Ceftazidime-clavulinic acid	71	40.6	4	7.8	104	59.4	47	92.2	0.001
Cefuroxime	85	48.6	5	9.8	90	51.4	46	90.2	0.001
Imipenem	15	8.6	0	0	160	91.4	51	100	0.09
Meropenem	7	4	0	0	168	96	51	100	0.162
Amikacin	14	8	0	0	161	92	51	100	0.005
Norfloxacin	94	53.7	11	21.6	81	46.3	40	78.4	0.001
Piperacillin-Tazobactam	28	16	2	3.9	147	84	49	96.1	0.006
Amoxycillin	76	43.4	7	13.7	99	56.6	44	86.3	0.001
Ampicillin	118	67.4	16	31.4	57	32.6	35	68.6	0.001
Gentamycin	38	21.7	2	3.9	137	78.3	49	96.1	0.001
Ciprofloxacin	100	57.1	14	27.5	75	42.9	37	72.5	0.001
Cefoxitin	49	28	3	5.9	126	72	48	94.1	0.001
Colistin	11	6.3	0	0	164	93.7	51	100	0.056
Cotrimoxazole	65	37.1	8	15.7	110	62.9	43	84.3	0.001
Fosfomycin	5	2.9	0	0	170	97.1	51	100	0.275
Aztreonam	139	79.43	0	0	36	29.57	51	100	0.001

identified as carbapenemase producing UPEC isolates (Table 3). In the confirmatory method, out of 226 isolates, 7 (3.1%) isolates were identified as carbapenemase producing isolates (Table 3).

Correlation of antibiotic resistance with ESBL

Significant correlation (P < 0.005), between antibiotic resistance and ESBL production, was observed in this study for antibiotics like ceftazidime (P < 0.001), ceftazidime-clavulinic acid (P < 0.001), ceftriaxone (P < 0.001), cefoxitin (P < 0.001), norfloxacin (P < 0.001) and ciprofloxacin (P < 0.001) (Table 4).

Table 5. Distribution of ESBL and carbapenemase genes among UPEC isolates

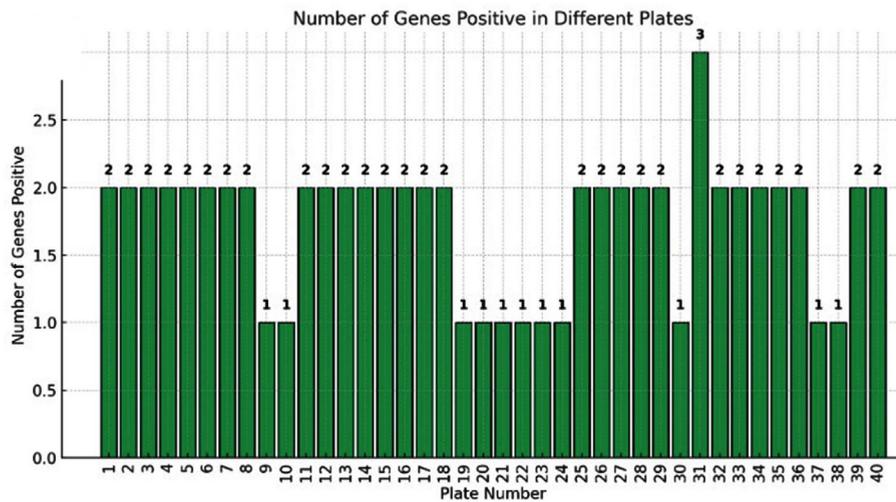| ESBL/Carbapenemase genes	Frequency	%
bla_{TEM}	34	85
bla_{CTX-M}	2	5
bla_{PER}	6	15
bla_{IMP}	0	0
bla_{VIM}	30	75
bla_{NDM}	2	5
bla_{KPC}	4	10
$bla_{OXA-48-like}$	0	0

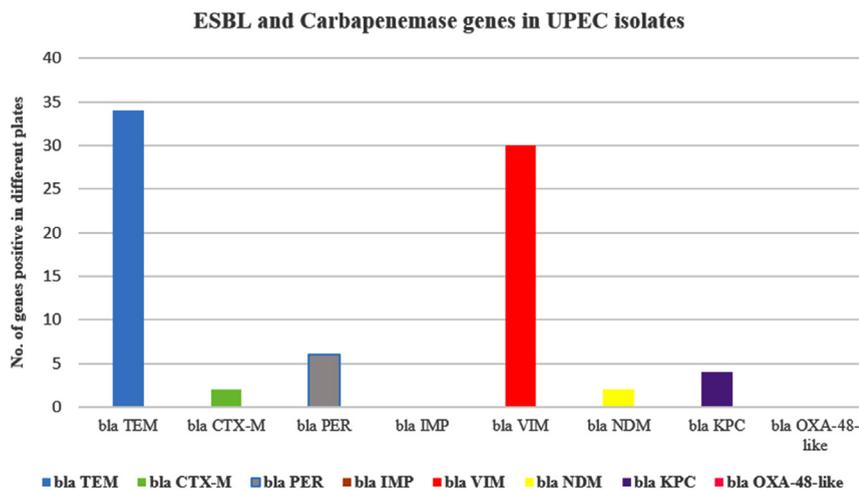**Figure 5.** Number of genes positive in different plates

Figure 6. Average number of genes expressed in *E. coli* isolates linked to ESBL and Carbapenemase production

Genotypic expression among UPEC isolates

Approximately 40 genes were identified in *Escherichia coli* (*E. coli*) isolates (Figures 5 and 6). Carbapenemase genes were detected in 39 *E. coli* isolates. The most prevalent gene was bla_{TEM}, present in all plates except one. Other commonly detected genes included bla_{VIM}, found in all plates, and bla_{CTX}, which was present in all but two plates (Figure 7).

Genes bla_{NDM}, bla_{KPC}, bla_{OXA}, and bla_{PER} were not detected in any of the bacterial isolates (Figure 5 and Table 5).

DISCUSSION

This study provides a detailed analysis of 226 non-repetitive uropathogenic *Escherichia coli* (UPEC) isolates, combining phenotypic and genotypic characterization of antimicrobial resistance due to ESBL and carbapenemase production. Uropathogenic *E. coli* (UPEC) constitutes the principal etiological agent of UTI. It has an arsenal of virulence determinants and can acquire and disseminate antibiotic resistance genes.[18,19] The findings highlight the alarming emergence of multidrug-resistance and reveal the interplay between virulence and resistance determinants in urinary pathogens.

Out of 4031 urine samples, 873 (21.65%) showed significant bacterial growth, of which *E. coli* constituted 39.17%, confirming it as the predominant urinary pathogen. These results echo the classic observations of Ronald,[3] and Foxman,[20] who established *E. coli* as the leading cause of both community- and hospital-acquired UTIs. The higher infection rate in females (56.6%) compared to males (43.4%) corresponds with the findings of Dielubanza & Schaeffer[19] and Sobel & Kaye,[21] attributing this pattern to anatomical factors such as shorter urethra and hormonal predisposition.

The 18-25 year group accounted for 26.5% of the cases, followed by the 56-65 year group (22.1%), a bimodal distribution typical for sexually active females and postmenopausal women. Among comorbidities, Type-II diabetes mellitus (46%) dominated, similar to Niranjan and Kabugo et al,[22,23] who linked diabetes with impaired host defenses and recurrent UTI risk. The antibiotic sensitivity profile revealed a dual scenario - a few highly effective agents contrasted against widespread resistance to conventional antibiotics. In our cohort, *E. coli* isolates exhibited excellent sensitivity to fosfomycin (97.5%), amikacin (93.7%), imipenem (93.4%), and nitrofurantoin (88.5%). This aligns with Sabharwal & Sharma[24] and Sultan et al.,[25] who highlighted fosfomycin's renewed therapeutic role against multidrug-resistant urinary pathogens. Conversely, resistance was markedly high to ampicillin (67.4%), ceftriaxone (57.1%), ceftazidime (51.4%), ciprofloxacin (57.1%), and aztreonam (79.4%). Comparable resistance profiles were documented by Mukherjee et al.[26]
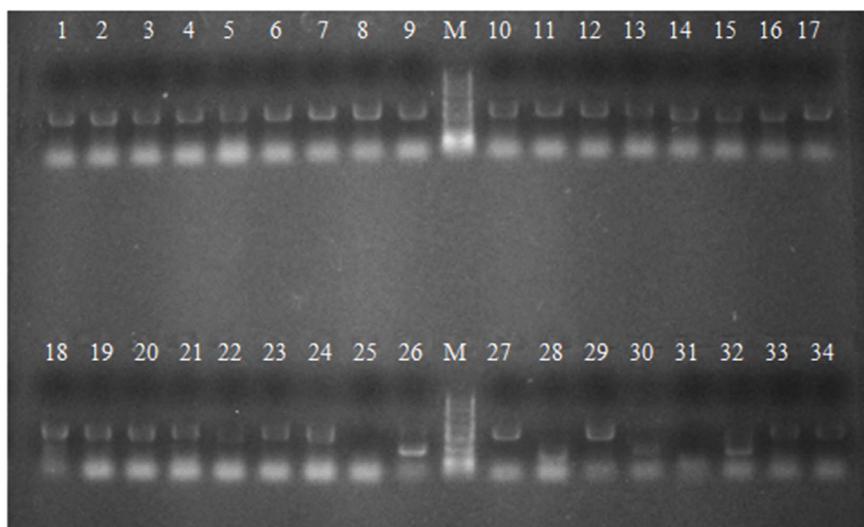

Figure 7. Expression of ESBL and Carbapenemase genes among UPEC *E. coli* isolates

in Indian tertiary centers, where empirical use of β-lactams and fluoroquinolones facilitated plasmid-borne resistance spread.

The current data further demonstrate a statistically significant (P < 0.001) correlation between ESBL production and resistance to key β-lactams and fluoroquinolones - including ceftazidime (51.4% resistant, P = 0.001), ceftriaxone (57.1% resistant, P = 0.001), cefoxitin (28%, P = 0.001), and ciprofloxacin (57.1%, P = 0.001). This co-resistance supports the assertion by Adamus-Bialek et al.[2] that resistance in *E. coli* is frequently driven by co-located resistance genes on mobile plasmids, conferring multidrug-resistance and adaptive survival advantage.

ESBL production and molecular determinants of the 226 isolates, 83 (36.7%) were confirmed as ESBL producers by both screening and confirmatory assays. This prevalence is consistent with Bajpai et al.,[17] who reported ESBL rates ranging from 30%-45% among urinary *E. coli* isolates in Indian hospitals.

Genotypically, the study detected bla_{TEM} and bla_{CTX-M} as the most dominant genes, both present in nearly all ESBL-producing isolates, while bla_{VIM} was detected in several isolates associated with carbapenemase activity. Genes such as bla_{NDM}, bla_{KPC}, and bla_{OXA} were absent.

The predominance of bla_{TEM} agrees with Bajpai et al.,[17] who identified TEM as the principal β-lactamase gene in Indian *E. coli* isolates, followed by bla_{CTX-M}.[26] The co-occurrence of bla_{TEM} and bla_{CTX-M} signifies a progressive genetic transition from classical TEM/SHV enzymes to CTX-M-type ESBLs, consistent with the molecular epidemiology described by Adamus-Bialek and Agarwal et al.[2,4]

The ESBL positivity was also significantly associated with multidrug-resistance patterns - isolates expressing ESBLs showed 2-4 times higher resistance to fluoroquinolones and aminoglycosides compared to non-ESBL strains, corroborating Ranjini et al and Sajama & Premlatha.[13,27] Such co-selection likely reflects plasmid-mediated horizontal gene transfer encompassing bla_{CTX-M}, *aac(6')-Ib-cr*, and *qnr* determinants, as noted in global genomic studies.[7,28]

Carbapenemase detection revealed 19 isolates (8.4%) positive by screening but only 7 isolates (3.1%) confirmed phenotypically. This finding indicates possible overestimation by screening assays, a discrepancy similar to that reported by Haji et al.[16] and Vamsi et al.[15] The limited occurrence of carbapenemase producers in our cohort is encouraging compared to the 6%-10% prevalence documented by Mariappan et al.[9] in Indian intensive care settings.

The genetic screening identified bla_{VIM} as the only carbapenemase gene present, consistent with Legese et al.[8] who reported dominance of VIM-type metallo-β-lactamases in African *E. coli* isolates. The absence of bla_{NDM} and bla_{KPC} contrasts with northern Indian and Middle Eastern reports[29,30] Rahdar et al., El Naggar et al., suggesting regional containment or restricted plasmid dissemination in our hospital ecosystem.

Clinically, the carbapenem susceptibility remained high (imipenem 93.4%, meropenem 96%), supporting the continued efficacy of carbapenems in our setting.[31-33] However, as emphasized by Bloemberg et al.,[14] the presence of even a few carbapenemase producers represents a sentinel warning for potential clonal outbreaks, warranting strict antibiotic stewardship and infection-control protocols.[34]

When compared with other regional and international datasets, our 36.7% ESBL and 3.1% carbapenemase rates are lower than the figures from North India (ESBL 52%, carbapenemase 7%) but closely resemble European and Latin American averages (30%-40%).[7,35] The molecular profile - bla_{TEM} and bla_{CTX-M} without bla_{NDM} - suggests that plasmid-mediated dissemination remains the primary resistance mechanism in this locale, as opposed to transposon-mediated metallo-β-lactamase spread.

Therapeutically, the study supports fosfomycin, nitrofurantoin, and amikacin as viable empirical options for uncomplicated UTIs, consistent with CLSI guidelines (2015). For complicated or catheter-associated UTIs, carbapenems remain effective but must be preserved through stringent antimicrobial stewardship.

From a public-health standpoint, the coexistence of bla_{TEM}, bla_{CTX-M}, and carbapenem tolerance presents a significant nosocomial hazard. The findings underscore the urgency of integrating molecular diagnostics into infection surveillance frameworks.

Despite its comprehensive phenotypic–genotypic design, this study was limited by its single-center scope and lack of advanced molecular typing (e.g., whole-genome sequencing or MLST). Expanding to multicentric studies incorporating plasmid profiling and virulome-resistome linkage would strengthen the understanding of UPEC pathoadaptation.

CONCLUSION

Accurate detection of extended-spectrum β-lactamase (ESBL) and carbapenemase production among *E. coli* isolates causing urinary tract infections (UTIs) is pivotal for optimizing antimicrobial therapy and minimizing treatment failures. The rising prevalence of multidrug-resistant (MDR) uropathogenic *E. coli* (UPEC) strains underscores the need for early identification of resistance mechanisms to reduce morbidity and prevent nosocomial dissemination of resistant pathogens. The high concordance observed between phenotypic and genotypic detection methods in this study reinforces the importance of an integrated diagnostic approach. While phenotypic assays such as disc diffusion and carbapenemase detection remain practical and cost-effective for routine laboratory screening, molecular techniques like PCR-based identification of bla_{TEM}, bla_{VIM}, and bla_{KPC} genes offer superior specificity and precision. The combined use of these complementary methods enhances diagnostic accuracy, thereby supporting evidence-based antimicrobial stewardship and informed therapeutic decision-making.The emergence of ESBL and carbapenemase-producing UPEC isolates in tertiary care settings presents a significant threat to public health by limiting the efficacy of β-lactam antibiotics and increasing dependence on last-line agents. These findings emphasize the urgent need for comprehensive infection control measures, periodic antimicrobial resistance surveillance, and the development of targeted antibiotic policies to preserve treatment efficacy. In the broader context of combating antimicrobial resistance, early detection, continuous monitoring, and strengthened interdisciplinary collaboration among clinicians, microbiologists, infection control personnel, and policymakers are essential for guiding empirical therapy, refining hospital antibiotic protocols, and ensuring sustainable antibiotic utilization within healthcare systems.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethical Committee, BGS Global Institute of Medical Sciences, Bengaluru vide protocol number sBGS GIMS/IEC/ dated 01.02.2021.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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