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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF EXTENDED-SPECTRUM β-LACTAMASE–PRODUCING ENTEROBACTERIACEAE ISOLATED FROM URINARY SAMPLES IN A TERTIARY CARE HOSPITAL

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ABSTRACT Urinary tract infections (UTIs) are among the most common bacterial infections and are increasingly complicated by multidrug resistance and extended-spectrum β-lactamase (ESBL) production. This prospective observational study was conducted over one year (January–December 2025) in a tertiary care hospital in central India to determine the prevalence and antimicrobial susceptibility patterns of ESBL-producing uropathogens. A total of 418 urine samples from clinically suspected UTI patients were processed using standard microbiological methods. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method and interpreted according to CLSI 2024 guidelines. Significant bacterial growth was observed in 128 (32.0%) samples, with a female predominance (57.8%). Escherichia coli was the most common isolate (79.7%), followed by Klebsiella pneumoniae (11.7%). Overall, 72 (56.3%) isolates were multidrug resistant. ESBL screening was positive in 52 isolates, of which 22 (42.3%) were phenotypically confirmed as ESBL producers, predominantly E. coli (81.8%). High resistance was observed to amoxicillin, third-generation cephalosporins, and fluoroquinolones, whereas imipenem, gentamicin, and nitrofurantoin retained good activity. These findings highlight the importance of continuous local surveillance and rational antibiotic stewardship to guide empirical therapy.

KEYWORDS : Urinary tract infection, Antimicrobial resistance, ESBL-producing Enterobacterales

INTRODUCTION

Urinary tract infections (UTIs) represent a substantial clinical burden in both community and healthcare settings, accounting for nearly 3 million healthcare visits annually.[1] Severe UTIs may result in complications such as renal failure and sepsis.[2,3] Although antibiotics remain the mainstay of treatment, they do not completely prevent recurrence and are associated with the emergence of multidrug resistance.[4] Globally, an estimated 0.26 million deaths (95% uncertainty interval: 0.18–0.36) were attributed to bacterial antimicrobial resistance related to UTIs in 2019,[5] resulting in an annual societal cost of approximately USD 3.5 billion.[6] In India, UTIs continue to pose a significant health concern, with prevalence estimates ranging from 21.8% to 31.3% across various clinical settings.[7]

Members of the order *Enterobacterales* dominate the etiology of UTIs, with *Escherichia coli* accounting for 70–90% of cases, followed by *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter* species.[8] Extended-spectrum β-lactamase (ESBL)-producing *Enterobacterales* pose a critical therapeutic challenge due to their ability to hydrolyze third-generation cephalosporins such as ceftriaxone and cefotaxime through plasmid-mediated CTX-M, TEM, and SHV enzymes. ESBL prevalence among Indian urinary isolates has been reported to exceed 50–70%, driven by indiscriminate antibiotic use, inadequate infection control practices, and clonal dissemination. These organisms frequently exhibit co-resistance to fluoroquinolones, aminoglycosides, and cotrimoxazole, further limiting treatment options.[9]

ESBL-associated UTIs often result in empirical treatment failure with cephalosporins, necessitating reliance on carbapenems, fosfomycin, or nitrofurantoin. The emergence of carbapenemase co-production, particularly New Delhi metallo-β-lactamase (NDM), threatens the effectiveness of last-line agents and contributes to prolonged hospital stays and increased healthcare costs.[10] Given the marked regional variability in resistance patterns, continuous local surveillance is essential to guide empirical therapy and antimicrobial stewardship initiatives.[11] The present study aimed to determine the prevalence and antimicrobial susceptibility patterns of ESBL-producing *Enterobacterales* isolated from urine samples in a tertiary care hospital.

MATERIALS AND METHODS

Study Site

The study was conducted in the Department of Microbiology, K.M.S.K. Government Medical College, Chandrapur, a tertiary care center in Maharashtra. Ethical approval was obtained from the Institutional Ethics Committee (IEC No: 97/2024), and written informed consent was obtained from all participants prior to sample collection.

Study Design And Duration

This was a prospective observational study conducted from January 2025 to December 2025.

Study Population

All patients of any age with clinical suspicion of urinary tract infection, from whom urine samples were received for culture during the study period, were included. Samples were obtained from both outpatient and inpatient departments.

Inclusion Criteria

Clinically suspected UTI Midstream clean-catch or catheterized urine samples

Specimen Collection And Processing

Urine samples were collected under strict aseptic conditions and transported immediately to the microbiology laboratory. A calibrated 1 µL loop was used to inoculate urine onto MacConkey agar and blood agar plates (HiMedia Laboratories Pvt. Ltd., India), followed by aerobic incubation at 37 °C for 18–48 hours. Growth of ≥10^5 colony-forming units (CFU)/mL, corresponding to ≥100 colonies using the calibrated loop, was considered significant bacteriuria. Isolates were identified based on Gram staining, colony morphology, and standard biochemical tests as described in the *Manual of Clinical Microbiology*.[12]

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method, and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines.[13]

ESBL Detection

Isolates showing reduced susceptibility to third-generation cephalosporins (ceftriaxone ≤25 mm, ceftazidime ≤22 mm, cefotaxime ≤27 mm, or cefpodoxime ≤17 mm) were screened for ESBL production. Phenotypic confirmation was carried out using the combined disk method, comparing ceftazidime (30 µg) and cefotaxime (30 µg) discs alone and in combination with clavulanic

acid (10 µg). An increase in zone diameter of ≥5 mm indicated ESBL production. *Klebsiella pneumoniae* ATCC 700603 was used as the quality control strain.[13]

Statistical Analysis

Data were entered into Microsoft Excel and analyzed descriptively. Results were expressed as frequencies and percentages.

RESULTS

Study Population And Sample Distribution

A total of 418 urine samples were collected from patients with suspected urinary tract infections. Of these, 128 (32.0%) samples showed significant bacterial growth. Among the study population, females constituted the majority of cases (94, 57.8%) compared to males (54, 42.2%). Most patients were outpatients, with females (70, 54.7%) and males (20, 31.3%) outnumbering inpatients (females: 24, 37.5%; males: 14, 21.9%).

Distribution Of Bacterial Isolates

Of the 128 isolates, *Escherichia coli* was the most predominant organism, accounting for 102 (79.7%) isolates, followed by *Klebsiella pneumoniae* 15 (11.7%), *Proteus mirabilis* 3 (2.3%), and others 8 (6.3%). Among the 128 isolates, 72 (56.3%) were multidrug-resistant (MDR), with *E. coli* showing the highest MDR proportion (55/102; 53.9%) (Table 1).

Note: Although the primary focus of the study was ESBL-producing *Enterobacterales*, all uropathogens isolated from urine cultures were included to provide a comprehensive overview of UTI etiology.

Table 1. Distribution Of Bacterial Isolates And ESBL Status Among Organisms

Organism	Total isolates n (%)	MDR strains n (%)	ESBL screening positive n	ESBL confirmed n (%)
Escherichia coli	102 (79.7)	55 (53.9)	46	18 (81.8)
Klebsiella pneumoniae	15 (11.7)	8 (53.3)	6	4 (18.2)
Klebsiella oxytoca	2 (1.6)	1 (50.0)	0	0 (0)
Proteus mirabilis	3 (2.3)	2 (66.7)	NT	NT
Proteus vulgaris	2 (1.6)	1 (50.0)	NT	NT
Pseudomonas spp.	2 (1.6)	2 (100)	NT	NT
Acinetobacter spp.	1 (0.8)	0 (0)	NT	NT
Citrobacter spp.	1 (0.8)	0 (0)	NT	NT
Enterobacter spp.	1 (0.8)	0 (0)	NT	NT
Enterococcus spp.	1 (0.8)	1 (100)	NT	NT
Staphylococcus spp.	1 (0.8)	1 (100)	NT	NT
Total	128 (100)	72 (56.3)	52	22 (100)

NT – Not tested (non-Enterobacterales / Gram-positive organisms).

ESBL Testing Was Performed Only For Enterobacterales.

ESBL Detection

ESBL screening was positive in 52/128 (40.6%) isolates, of which 22 (42.3%) were phenotypically confirmed as ESBL producers. *Escherichia coli* constituted the majority of ESBL-producing isolates (18/22; 81.8%), followed by *Klebsiella pneumoniae* (4/22; 18.2%).

Antibiotic Resistance Pattern

Escherichia coli showed the highest resistance to amoxicillin (75/102; 73.5%) and the lowest resistance to imipenem (2/102; 2.0%). *Klebsiella pneumoniae* demonstrated 86.7% resistance to amoxicillin, while complete susceptibility was observed to gentamicin and imipenem.

Sex-wise Distribution Of ESBL Producers

Among ESBL-producing *E. coli*, 13 (72.2%) isolates were from female patients and 5 (27.8%) from male patients. Similarly, among ESBL-producing *Klebsiella pneumoniae*, 3 (75.0%) isolates were from females and 1 (25.0%) from males (Table 2).

Age-wise Distribution of ESBL Producers

The highest proportion of ESBL-producing *E. coli* was observed in the 21–40 years age group (9/18; 50.0%), followed by 41–60 years (6/18; 33.3%). ESBL-producing *Klebsiella pneumoniae* isolates were predominantly observed in patients aged more than 60 years (2/4; 50.0%) (Table 3).

Table 2. Sex-wise Distribution Of ESBL Producers

Sex	E. coli isolates n (%)	MDR n (%)	ESBL screening positive n (%)	ESBL confirmed n (%)	Klebsiella pneumoniae isolates n (%)	MDR n (%)	ESBL screening positive n (%)	ESBL confirmed n (%)
Male	43 (42.2)	23 (53.5)	20 (43.5)	5 (27.8)	4 (26.7)	2 (50.0)	2 (33.3)	1 (25.0)
Female	59 (57.8)	32 (54.2)	26 (44.1)	13 (72.2)	11 (73.3)	6 (54.5)	4 (66.7)	3 (75.0)
Total	102 (100)	55 (100)	46 (100)	18 (100)	15 (100)	8 (100)	6 (100)	4 (100)

Table 3. Age-wise Distribution Of ESBL-producing Isolates

Age group (years)	Total isolates n (%)	MDR n (%)	ESBL screening positive n (%)	ESBL confirmed n (%)
Escherichia coli (n = 102)				
≤10	10 (9.8)	5 (9.1)	4 (7.7)	1 (5.6)
11–20	6 (5.9)	3 (5.5)	2 (3.8)	1 (5.6)
21–30	30 (29.4)	16 (29.1)	13 (25.0)	5 (27.8)
31–40	24 (23.5)	13 (23.6)	11 (21.2)	4 (22.2)
41–50	15 (14.7)	8 (14.5)	7 (13.5)	2 (11.1)
51–60	11 (10.8)	7 (12.7)	5 (9.6)	2 (11.1)
>60	6 (5.9)	3 (5.5)	6 (11.5)	3 (16.6)
Total	102 (100)	55 (100)	52 (100)	18 (100)
Klebsiella pneumoniae (n = 15)				
≤10	1 (6.7)	0 (0.0)	1 (16.7)	0 (0.0)
11–20	1 (6.7)	1 (12.5)	1 (16.7)	1 (25.0)
21–30	2 (13.3)	1 (12.5)	1 (16.7)	0 (0.0)
31–40	2 (13.3)	1 (12.5)	1 (16.7)	1 (25.0)
41–50	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
51–60	1 (6.7)	1 (12.5)	0 (0.0)	0 (0.0)
>60	8 (53.3)	4 (50.0)	2 (33.3)	2 (50.0)
Total	15 (100)	8 (100)	6 (100)	4 (100)

Antibiotic Resistance Pattern Of The Isolates

Escherichia coli were highly resistant to amoxicillin 75 (73.5%) and least resistant to imipenem 2 (2.0%). Likewise, most *Klebsiella pneumoniae* were highly resistant to amoxicillin 13 (86.7%) and least resistant to gentamicin 0 (0.0%) and imipenem 0 (0.0%) (Table 4).

Table 4. Antibiotic Resistance Pattern Of The Isolates

Antibiotic	Escherichia coli (n = 102) Resistant n (%)	Klebsiella pneumoniae (n = 15) Resistant n (%)
Amoxicillin	75 (73.5)	13 (86.7)
Cefixime	68 (66.7)	10 (66.7)
Cefotaxime	65 (63.7)	9 (60.0)
Ceftazidime	61 (59.8)	8 (53.3)
Ceftriaxone	64 (62.7)	9 (60.0)
Ciprofloxacin	56 (54.9)	7 (46.7)
Cotrimoxazole	49 (48.0)	6 (40.0)
Gentamicin	12 (11.8)	0 (0.0)
Nitrofurantoin	18 (17.6)	4 (26.7)
Imipenem	2 (2.0)	0 (0.0)

DISCUSSION

The culture positivity rate of 32.0% and female predominance observed in the present study are consistent with previous Indian studies conducted in community and tertiary care settings. Behera et al.[14] and Mohapatra et al.[10] similarly reported higher UTI prevalence among females and identified *Escherichia coli* as the predominant uropathogen. The predominance of *E. coli* (79.7%) in this study closely parallels findings reported by Rath et al.[15] and Ahmad et al.[16] The overall MDR rate of 56.3% is comparable to reports by Singh et al.[17] and reflects sustained antimicrobial pressure in healthcare settings.

The prevalence of phenotypically confirmed ESBL producers (17.2% of total isolates) falls within the range reported in recent Indian literature. High resistance to amoxicillin, fluoroquinolones, and third-generation cephalosporins limits their utility for empirical therapy. Preserved susceptibility to imipenem, gentamicin, and nitrofurantoin supports their continued use, although carbapenem use must remain judicious to prevent further resistance.

CONCLUSION

The study demonstrates a high burden of multidrug-resistant and ESBL-producing *Enterobacterales* among urinary isolates in a tertiary care hospital. *Escherichia coli* remains the predominant uropathogen, with substantial resistance to commonly prescribed oral antibiotics. The presence of ESBL producers among both outpatient and inpatient populations indicates expanding community dissemination. Continuous antimicrobial resistance surveillance, rational antibiotic prescribing, and robust antimicrobial stewardship programs are essential to optimize treatment outcomes and curb the spread of resistant uropathogens.

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