International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 22s, 2025
https://theaspd.com/index.php

High Prevalence Of ESBL-Producing And Multidrug-Resistant Escherichia Coli In Urinary Tract Infections

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Abstract
Background: *Urinary tract infections (UTIs) are predominantly caused by Escherichia coli, with the rising prevalence of extended-spectrum β-lactamase (ESBL) production posing a critical challenge to effective therapy. Antimicrobial resistance (AMR) not only limits therapeutic options but also contributes to treatment failure and recurrence.*
Objectives: *This study aimed to (i) determine the prevalence of ESBL-producing E. coli isolates from urinary samples, (ii) evaluate phenotypic methods for carbapenemase detection, and (iii) assess antimicrobial susceptibility patterns, with a focus on multidrug-resistant (MDR) and extensively drug-resistant (XDR) profiles.*
Methods: *A cross-sectional study was conducted on 100 urinary E. coli isolates collected from catheterized patients with symptomatic UTIs between June 2023 and May 2024. Antimicrobial susceptibility was determined by Kirby–Bauer disc diffusion, interpreted according to CLSI (2023) guidelines. ESBL production was confirmed using the combined disc method, while carbapenemase activity was evaluated using the modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM).*
Results: *ESBL production was detected in 79% of isolates. MDR and XDR were observed in 60% and 18% of isolates, respectively, while 22% remained non-MDR. High resistance was noted against ampicillin (77.3%), cefotaxime (77%), cefixime (65%), and ciprofloxacin (60%). In contrast, fosfomycin (97.3%), gentamicin (96%), amikacin (80%), and imipenem (85%) retained good activity. Phenotypic carbapenemase assays demonstrated utility in differentiating serine-carbapenemases from metallo-β-lactamases.*
Conclusion: *The study revealed an alarmingly high prevalence of ESBL and MDR/XDR E. coli compared with regional reports. Fosfomycin, aminoglycosides, nitrofurantoin, and carbapenems remain effective agents, whereas cephalosporins and fluoroquinolones show limited utility. Continuous surveillance and antimicrobial stewardship are essential to prevent the emergence of carbapenem-resistant Enterobacteriaceae.*

Keywords: *Escherichia coli, urinary tract infections, ESBL, multidrug resistance, carbapenemase, antimicrobial susceptibility.*

1. INTRODUCTION

Urinary tract infections (UTIs) are among the most frequent bacterial infections encountered globally, affecting both community and hospital populations [1]. Escherichia coli (E. coli) is recognized as the predominant etiological agent, accounting for nearly 70–90% of cases. The widespread use and misuse of antimicrobials in clinical practice has contributed to the rapid emergence of resistant uropathogens, posing a major therapeutic challenge [2]. Of particular concern is the increasing prevalence of Extended-Spectrum β-Lactamase (ESBL)-producing E. coli, which exhibit resistance to a wide range of β-lactam antibiotics, including third-generation cephalosporins and monobactams, while retaining susceptibility to β-lactamase inhibitors [3].

The dissemination of ESBL-producing strains represents a significant global health threat due to their association with therapeutic failures, recurrent infections, and increased morbidity and mortality [4]. Detecting these organisms in routine clinical laboratories is complicated, as resistance mechanisms often overlap with those of AmpC β-lactamases and carbapenemases. While phenotypic tests such as the double-disc synergy test (DDST), combination disc method (CDM), and E-test strips are commonly employed, their performance varies depending on sensitivity, specificity, and regional prevalence rates. Lack of standardization further complicates routine diagnosis and surveillance [5].

From a clinical perspective, accurate and timely detection of ESBL producers is essential, as infections caused by these organisms are frequently associated with multidrug resistance (MDR). In many cases, therapeutic options are restricted to carbapenems, aminoglycosides, or fosfomycin, which not only increases treatment costs but also exerts additional selective pressure, potentially driving the emergence of carbapenem resistance [6]. The covert spread of ESBL-producing E. coli within healthcare and community settings underscores the need for robust diagnostic, therapeutic, and infection-control strategies.

Therefore, epidemiological studies are critical to establish the prevalence of ESBL-producing urinary isolates and to assess the reliability of different detection methods. Furthermore, evaluating the antibiotic resistance profiles of these organisms provides valuable evidence to guide empiric therapy, strengthen antimicrobial stewardship programs, and support infection-control measures [7].

The present study was undertaken to investigate the burden of antimicrobial resistance in urinary tract pathogens with a specific focus on Escherichia coli. The objectives were threefold: first, to determine the frequency of ESBL production among urinary E. coli isolates; second, to compare the performance of different phenotypic techniques commonly employed for ESBL confirmation; and third, to evaluate the antibiotic resistance patterns associated with ESBL-producing E. coli. Together, these findings aim to provide valuable insights into the detection and management of resistant urinary pathogens, thereby supporting evidence-based antimicrobial therapy and stewardship practices.

2. MATERIALS AND METHODS

2.1. Study Design and Ethical Approval

A cross-sectional study was conducted between June 2023 and May 2024 at the Department of Microbiology, St. Thomas Hospital, Chethipuzha, Kerala, affiliated to Department of Microbiology, Allied Health Sciences, Srinivas University. Ethical clearance was obtained from the Institutional Ethics Committee of Srinivas University (protocol no. 23/AHS/2023). All procedures followed institutional biosafety protocols and Clinical and Laboratory Standards Institute (CLSI) guidelines.

2.2. Sample Collection and Processing

Clinical specimens were collected from hospitalized patients with indwelling urinary catheters. A total of 100 samples were processed from patients with symptomatic UTIs were examined. Samples were directly inoculated onto culture media, while 2–3 cm segments of catheter tips were incubated in Brain Heart Infusion (BHI) broth prior to subculture to enhance microbial recovery.

2.3. Microbial Isolation and Identification

Specimens were subcultured onto MacConkey agar, blood agar and incubated at 37 °C for 18–48 hours. Isolates were identified based on colony morphology, Gram staining, and biochemical tests (IMViC, urease, oxidase, catalase, and motility) [8]. Confirmatory identification was performed using standard microbiological methods.

2.4. Biofilm Detection

Biofilm formation was assessed using the Tissue Culture Plate (TCP) method. Overnight cultures were diluted (1:100) in BHI broth with 1% glucose and incubated in 96-well plates at 37 °C for 24 hours. Wells were washed, stained with 0.1% crystal violet, and the bound dye was solubilized with ethanol. Biofilm biomass was quantified spectrophotometrically at 570 nm, and isolates were classified as strong, moderate, or weak biofilm producers [9].

2.5. Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method on Mueller–Hinton agar, interpreted according to CLSI (2023) [10] guidelines by using antibiotic discs containing ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), amikacin (30 μg), imipenem (10 μg), Ciprofloxacin (5 μg), Ampicillin (30 μg), Gentamicin (10 μg), Amikacin (30 μg), Ceftazidime (30 μg), Cefuroxime, Ceftriaxone (30 μg), Cefotaxime (30 μg), Piperacillin (10 μg), Cefoxitin (30 μg), Aztreonam (10 μg), Tetracycline (30 μg), Cefixime (5 μg),Fosfomycin (5 μg), and Norfloxacin (10 μg).

2.6. Detection of ESBL and Carbapenemase Production

Phenotypic detection of ESBL was performed using the combined disc method with ceftazidime and ceftazidime–clavulanate. A ≥5 mm increase in zone diameter in the presence of clavulanate indicated ESBL production. Carbapenemase production was assessed by the modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM).

2.7. Molecular Characterization of Resistance Genes

Genomic DNA was extracted from multidrug-resistant isolates using commercial extraction kits. Quantitative PCR (qPCR) was performed to detect blaSHV, blaTEM, and blaCTX-M genes using specific primers. Amplification curves and threshold cycle values were used to confirm gene presence. PCR runs included both positive and negative controls, with reactions performed under standard cycling conditions.

2.8. Statistical Analysis

Data were analyzed using SPSS version 22. Descriptive statistics were used to summarize isolate distribution, biofilm formation, and resistance patterns. Associations between categorical variables were evaluated using Chi-square or Fisher's exact test, with $p < 0.05$ considered statistically significant.

3. RESULTS

3.1. Frequency of ESBL-Producing E. coli

Out of 100 urinary E. coli isolates, 79 (79%) were identified as ESBL producers, whereas 21 (21%) were non-ESBL producers (Figure 1). This finding indicates a high prevalence of ESBL-mediated resistance among urinary E. coli, underscoring their clinical significance in urinary tract infections.

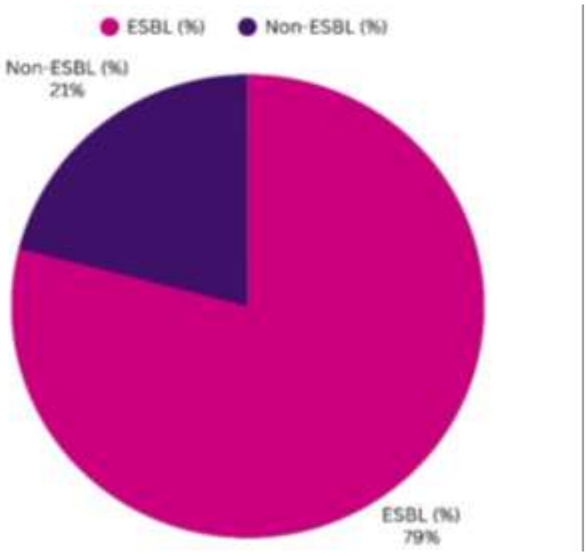

Figure 1. Frequency of ESBL production among E. coli isolates

3.2. Antimicrobial Susceptibility Patterns

The antimicrobial susceptibility testing of E. coli isolates revealed diverse resistance patterns (Table 1, Figure 2). Aminoglycosides demonstrated high efficacy, with gentamicin showing 96% sensitivity and amikacin 80% sensitivity. Carbapenems also retained strong activity, with imipenem sensitivity at 85%.

In contrast, marked resistance was observed against several β-lactams: cefotaxime (77.3%), ampicillin (77.3%), ceftazidime (62.7%), and cefixime (65.3%). Fluoroquinolone resistance was also high, with 60% of isolates resistant to ciprofloxacin. Fosfomycin emerged as the most effective agent, with 97.3% sensitivity and minimal resistance (2.7%).

Table 1: Antibiotic resistance profile of E. coli isolates (n=100)

ANTIBIOTICS	RESISTANCE %	SENSITIVITY %	INTERMEDIATE %
GENTAMYCIN	04	96	-
AMIKACIN	11	80	09
IMIPENEM	10	85	05
CEFUROXIME	52	29.33	18.66
CEFOXITIN	22.66	77.33	-
CIPROFLOXACIN	60	33.33	6.66
AZTREONAM	49.33	34.66	16
AMPICILLIN	77.33	19	3.67
TETRACYCLINE	34..66	60.66	02
CEFIXIME	65.33	22.33	07
CEFTAZIDIME	62.66	14.66	22.66
CEFOTAXIME	77.33	13.33	9.33
FOSFOMYCIN	2.66	97.33	-
COTRIMOXAZOLE	42.66	57.33	-
NORFLOXACIN	42.66	57.33	-

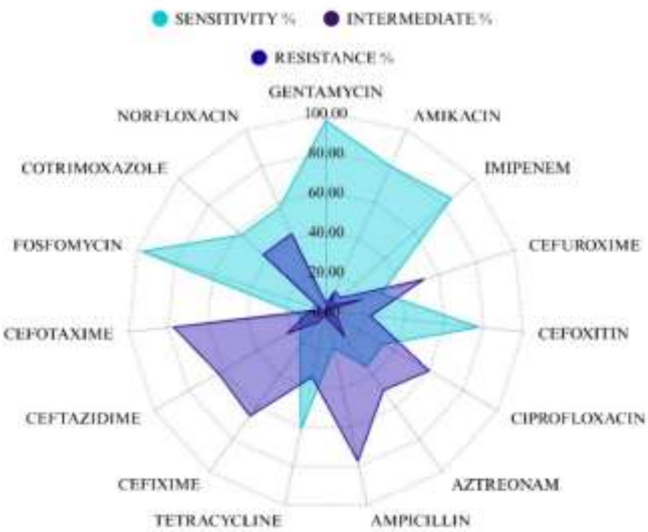

Figure 2: Illustration of Antibiotic resistance profile of E. coli isolates (n=100)

Overall, aminoglycosides, carbapenems, and fosfomycin remained the most effective therapeutic agents. In contrast, high resistance to cephalosporins, ampicillin, and fluoroquinolones highlights the limited utility of these drugs against ESBL-producing E. coli.

Multidrug Resistance Profiles

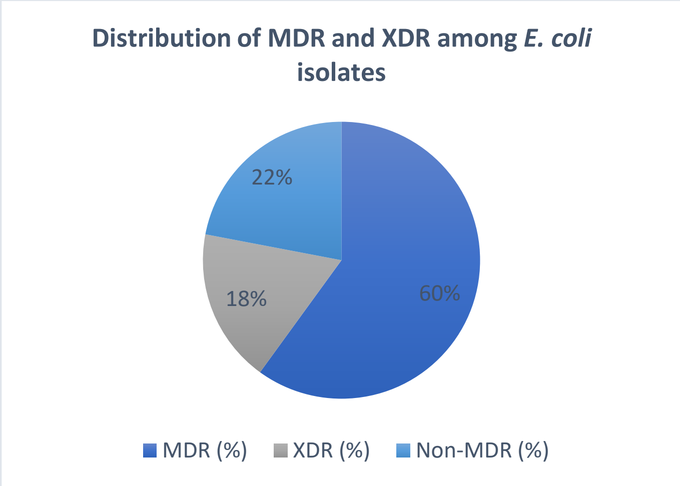

Figure 3. Distribution of MDR and XDR among E. coli isolates

Among the 100 E. coli isolates, 60% were classified as multidrug-resistant (MDR), 18% as extensively drug-resistant (XDR), and only 22% as non-MDR (Figure 3). The predominance of MDR and XDR isolates reflects the high adaptive capacity of urinary E. coli against commonly used antibiotics. This resistance trend significantly restricts treatment options, necessitating reliance on last-line antibiotics such as carbapenems and colistin.

3.3. Phenotypic Detection of Carbapenemases

Phenotypic assays, including the modified carbapenem inactivation method (mCIM) and the EDTA-modified carbapenem inactivation method (eCIM), were employed to identify carbapenemase production in E. coli isolates. Isolates that tested positive with mCIM but negative with eCIM were interpreted as serine carbapenemase producers, whereas those positive for both mCIM and eCIM indicated metallo-β-lactamase (MBL) production, as inhibition of carbapenemase activity was observed in the presence of EDTA. The combined application of mCIM and eCIM thus proved to be a practical and reliable strategy for detecting carbapenemase activity in E. coli, offering valuable guidance for therapeutic decision-making in cases involving carbapenem-resistant infections.

4. DISCUSSION

In this study, Escherichia coli isolates from urinary tract infections demonstrated a high prevalence of extended-spectrum β-lactamase (ESBL) production (79%), with multidrug resistance (MDR) observed in 60% of isolates, extensively drug-resistant (XDR) in 18%, and only 22% remaining non-MDR. These findings underscore the escalating burden of antimicrobial resistance (AMR) among uropathogens and are broadly aligned with trends reported globally, though the prevalence in our setting was markedly higher.

4.1. ESBL Prevalence

The ESBL prevalence observed in our isolates (79%) is considerably higher than reported in several comparable studies. For instance, ESBL-positive E. coli accounted for 35% of urinary isolates in Nigeria (Nwafia et al., 2019), 28% in Iran (Gharavi et al., 2021), 25.8% in Nepal (Pantha et al., 2024), and 15% in Saudi Arabia (Alghamdi et al., 2023) [6,12]. Such variation is often attributed to local prescribing practices, antibiotic availability, and infection-control measures. The markedly elevated prevalence in our cohort suggests strong local selective pressure, potentially driven by empirical and unregulated antibiotic use, a concern previously highlighted in South Asian contexts. These observations reinforce the importance of localized antibiogram surveillance to guide therapy and stewardship [11,13].

4.2. Antimicrobial Susceptibility Patterns

Our antibiogram revealed high susceptibility to fosfomycin (97.3%), gentamicin (96%), amikacin (80%), and imipenem (85%). These findings are consistent with reports from Nigeria and Iran, where >90% of ESBL-producing E. coli remained susceptible to imipenem (Nwafia et al., 2019; Gharavi et al., 2021). Similarly, Pantha et al. (2024) reported 100% imipenem susceptibility in Nepal, while Alghamdi et al. (2023) observed carbapenem sensitivity of 95–99% in Saudi Arabia. Collectively, these results confirm carbapenems, aminoglycosides, and fosfomycin as important therapeutic options against ESBL producers. Nevertheless, the 10% resistance to imipenem noted in our study is worrisome, as increased carbapenem use has been linked to the emergence of carbapenemase-producing strains (Moayednia et al., 2014) [6,11,12,13].

In contrast, resistance rates were unacceptably high against ampicillin (77.3%), cefotaxime (77%), cefixime (65%), and ciprofloxacin (60%). Similar trends of poor cephalosporin efficacy against ESBL strains have been reported across Nigeria, Iran, and Saudi Arabia (Nwafia et al., 2019; Gharavi et al., 2021; Alghamdi et al., 2023). Fluoroquinolone resistance was also concordant with earlier findings, ranging from 56–74% in Saudi Arabia (Alghamdi et al., 2023) to 74% in Iran (Gharavi et al., 2021). Taken together, these data emphasize the diminishing utility of third-generation cephalosporins and fluoroquinolones in empirical treatment of urinary infections due to E. coli [3,11,12].

4.3. MDR and XDR Profiles

The MDR (60%) and XDR (18%) rates in our study exceeded those documented in Iran (38% MDR; Gharavi et al., 2021) and Nepal (38% MDR; Pantha et al., 2024). Emerging reports from Saudi Arabia also confirm the rise of MDR and XDR phenotypes, though at relatively lower frequencies (Alghamdi et al., 2023). Such differences reflect the influence of geographic variability, stewardship strategies, and antimicrobial accessibility. Importantly, the high proportion of XDR isolates observed in our cohort represents a critical threat to therapeutic options and highlights the urgency of implementing rigorous infection-control measures [3,6,12].

4.4. Carbapenemase Detection

Phenotypic detection using the modified carbapenem inactivation method (mCIM) and EDTA-modified CIM (eCIM) enabled differentiation between serine carbapenemases and metallo-β-lactamases (MBLs), in line with international recommendations (Moayednia et al., 2014). Although widespread carbapenem resistance was not observed, the early indications of reduced susceptibility underscore the need for ongoing surveillance. Proactive stewardship is essential to curb the rise of carbapenem-resistant Enterobacteriaceae (CRE), which have already been reported in several hospitals in Iran and Turkey [12,13].

The study findings reveal a substantially higher prevalence of ESBL-producing and MDR/XDR E. coli compared with most regional and international reports [3,6,11,12]. The preserved activity of fosfomycin, aminoglycosides, nitrofurantoin, and carbapenems supports their role as cornerstone agents in managing resistant urinary isolates, while the high resistance to cephalosporins and fluoroquinolones argues against their empirical use. Routine surveillance, supported by phenotypic carbapenemase detection (mCIM/eCIM), is essential for early identification of emerging resistance and should guide evidence-based stewardship and policy interventions.

5. STRENGTHS AND LIMITATIONS

A key strength of this study is the use of standardized phenotypic assays (mCIM and eCIM) to confirm carbapenemase production, ensuring robust characterization of resistance mechanisms. Additionally, the comprehensive antibiogram provides a clear picture of local resistance patterns, which is essential for guiding empirical therapy and stewardship interventions.

However, some limitations must be acknowledged. First, this was a single-center study, and therefore, the findings may not fully represent regional resistance trends. Second, molecular confirmation of ESBL and carbapenemase genes was not performed, which could have provided deeper insights into the genetic basis of resistance. Third, the study did not assess clinical outcomes, such as treatment response or recurrence, which limits correlation between resistance profiles and patient management. Despite these limitations, the study adds valuable evidence on the high prevalence of ESBL, MDR, and XDR E. coli in our setting and underscores the need for continuous surveillance.

6. CONCLUSION

This study highlights the alarming burden of antimicrobial resistance among urinary Escherichia coli isolates, with nearly four out of five strains producing extended-spectrum β-lactamases and the majority exhibiting multidrug or extensive drug resistance. The findings underscore a troubling therapeutic landscape, where conventional first-line agents such as cephalosporins, ampicillin, and fluoroquinolones have lost much of their efficacy. In contrast, the preserved activity of fosfomycin, aminoglycosides, nitrofurantoin, and carbapenems offers valuable alternatives for empirical and definitive therapy, although emerging resistance to carbapenems warrants close monitoring. The use of phenotypic assays (mCIM/eCIM) proved effective in detecting carbapenemase activity, providing practical tools for routine laboratories to identify resistance mechanisms. However, the observed early signals of reduced carbapenem susceptibility raise concerns over the potential spread of carbapenemase-producing Enterobacteriaceae, as already reported in neighboring regions.

Overall, the study reinforces the importance of periodic local antibiogram surveillance to guide treatment decisions, rationalize antibiotic use, and inform stewardship interventions. It also emphasizes the urgent need to strengthen infection-control practices and regulate empirical prescribing to reduce selective pressure. Future studies incorporating molecular characterization and clinical outcome data would provide further insights into the evolving resistance dynamics of uropathogenic E. coli.

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