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Original Research Article

# Prevalence of Extended-Spectrum Beta-lactamase (ESBL) and AmpC Producers in Urinary Isolates: A Prospective Laboratory-Based Study in a Tertiary Care Setting in Gujarat, India

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**Abstract**

**Background:** Urinary tract infections (UTIs) are a common cause of morbidity, with Escherichia coli and Klebsiella pneumoniae being the predominant pathogens. The increasing emergence of extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase-producing organisms has complicated treatment, leading to therapeutic failures and limited antibiotic options. Data on the prevalence of these resistance mechanisms remain limited in Gujarat, making local surveillance crucial for guiding empirical therapy.

**Aim:** To determine the prevalence of ESBL and AmpC producers in urinary isolates and analyze their antimicrobial susceptibility pattern.

**Methods:** This prospective study was conducted in the Department of Microbiology, GMERS Medical College, Gandhinagar, from April 2024 to March 2025. Midstream urine samples with significant bacteriuria ($\geq 10^5$ CFU/mL) were processed. Isolates were identified by standard microbiological methods. Antimicrobial susceptibility was tested by Kirby–Bauer disc diffusion as per CLSI guidelines. ESBL detection was performed using the combined disc diffusion test with cefotaxime/ceftazidime and clavulanic acid. AmpC production was screened using cefoxitin discs and confirmed by the cefoxitin-cloxacillin double disc synergy test. Data were analyzed using SPSS version 26.0.

**Results:** Of the 1,240 urine samples processed, 312 (25.1%) yielded significant bacterial growth. E. coli (59.6%) was the most common isolate, followed by Klebsiella pneumoniae (26.3%), Proteus spp. (5.8%), Enterobacter spp. (4.5%), and other Gram-negative bacilli (3.8%). ESBL production was detected in 142 isolates (45.5%), predominantly in Klebsiella pneumoniae (57.3%) and E. coli (44.1%). AmpC production was observed in 48 isolates (15.4%), with the highest prevalence in Enterobacter spp. (35.7%). Co-production of ESBL and AmpC was identified in 22 isolates (7.0%). Antibiotic susceptibility testing revealed high resistance to third-generation cephalosporins and fluoroquinolones, while carbapenems (94%) and piperacillin-tazobactam (82%) remained effective.

**Conclusion:** The study revealed a high prevalence of ESBL-producing urinary isolates, with AmpC and co-producers also contributing significantly to multidrug resistance. Routine screening and reporting of these enzymes are essential for guiding empirical therapy. Implementation of antimicrobial stewardship and regular surveillance programs are strongly recommended to curb the growing resistance burden.

**Keywords:** ESBL, AmpC, Urinary Tract Infection, Antimicrobial Resistance, E. coli, Klebsiella pneumoniae.



## Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections encountered in both community and hospital settings, affecting individuals across all age groups. Escherichia coli and Klebsiella species are the predominant uropathogens, responsible for nearly 70–80% of cases [1]. The widespread and often irrational use of broad-spectrum antibiotics has led to the emergence of multidrug-resistant organisms, posing significant challenges in the management of UTIs [2]. Extended-spectrum β-lactamases (ESBLs) are enzymes that confer resistance to third-generation cephalosporins and monobactams by hydrolyzing the β-lactam ring. First reported in

the 1980s, ESBL-producing Enterobacteriaceae have now become a global concern, with prevalence rates ranging from 20% to as high as 70% in certain regions [3]. The problem is particularly acute in low- and middle-income countries, where over-the-counter antibiotic use and lack of antimicrobial stewardship contribute to rising resistance [4]. AmpC β-lactamases represent another clinically significant mechanism of resistance. Unlike ESBLs, AmpC enzymes confer resistance not only to penicillins and cephalosporins but also to cephamycins such as cefoxitin, and they are poorly inhibited by β-lactamase inhibitors like clavulanic acid [5]. The coexistence of ESBL and AmpC enzymes within the same isolate further complicates treatment, leading to therapeutic failures and limiting the efficacy of commonly used antibiotics such as cephalosporins, fluoroquinolones, and aminoglycosides [6].

In India, the burden of ESBL- and AmpC-producing organisms is among the highest worldwide. Studies from tertiary care hospitals have reported ESBL prevalence rates exceeding 60% among urinary isolates of Enterobacteriaceae [7], with increasing reports of AmpC producers as well [8]. This situation has serious implications for empirical therapy of UTIs, as resistance compromises the utility of first-line and even second-line agents, leaving carbapenems as one of the few remaining reliable treatment options. However, increasing carbapenem use is now driving the emergence of carbapenemase-producing organisms, escalating the antimicrobial resistance crisis [9].

In Gujarat, limited regional surveillance studies have indicated a high prevalence of ESBLs among urinary isolates, but data on AmpC producers remain sparse [10]. The lack of consistent laboratory screening and confirmatory testing further contributes to underreporting of these resistance mechanisms. Since antibiotic prescribing in UTIs is often empirical, without timely laboratory guidance, there is an urgent need to generate local prevalence data to guide therapy and strengthen antibiotic stewardship programs [11].

The present study has been undertaken at GMERS Medical College, Gandhinagar, over a one-year period from April 2024 to March 2025, with the objective of determining the prevalence of ESBL- and AmpC-producing organisms in urinary isolates. By providing updated epidemiological data, this study seeks to support rational antibiotic use, improve empirical therapy choices, and contribute to the formulation of local antimicrobial policies.

The expected outcome is to reduce treatment failures, limit the spread of resistant organisms, and strengthen the framework for antimicrobial resistance surveillance at the regional level.

## Methodology

This prospective laboratory-based study was carried out in the Department of Microbiology, GMERS Medical College, Gandhinagar, from April 2024 to March 2025. A total of non-duplicate midstream urine samples received in the microbiology laboratory during the study period were included. Samples from patients on prior antibiotic therapy, mixed growth, or contaminants were excluded. All urine specimens were cultured on Cystine Lactose Electrolyte Deficient (CLED) agar using the calibrated loop technique and incubated aerobically at 37°C for 18–24 hours. Significant bacteriuria was defined as growth of $\geq 10^5$ colony forming units (CFU)/mL.

Isolates were identified up to species level by standard microbiological methods, including colony morphology, Gram staining, and a battery of biochemical tests such as indole, citrate utilization, triple sugar iron agar reaction, and urease test. Where required, identification was confirmed using automated systems available in the laboratory.

Antimicrobial susceptibility testing was performed on all isolates by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotic panel included third-generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone), cefoxitin, aztreonam, amoxicillin-clavulanic acid, ciprofloxacin, gentamicin, piperacillin-tazobactam, and carbapenems (imipenem, meropenem).

Screening for ESBL production was carried out using ceftazidime (30 µg) and cefotaxime (30 µg) discs. Isolates showing an inhibition zone ≤22 mm for ceftazidime or ≤27 mm for cefotaxime were considered potential ESBL producers. Confirmation of ESBL production was done using the combined disc diffusion method, wherein ceftazidime and cefotaxime discs with and without clavulanic acid were placed at appropriate distances. An increase of ≥5 mm in zone diameter in the presence of clavulanic acid was interpreted as ESBL positive.

AmpC β-lactamase production was screened by reduced susceptibility to cefoxitin (30 µg) discs. Suspected isolates were confirmed using the cefoxitin-cloxacillin double disc synergy test, where a ≥4 mm increase in inhibition zone around cefoxitin in the presence of cloxacillin indicated AmpC production.

Quality control strains used in this study included Escherichia coli ATCC 25922 (negative control)

and Klebsiella pneumoniae ATCC 700603 (ESBL positive control). All data were recorded in a structured proforma and compiled in Microsoft Excel. Statistical analysis was performed using SPSS version 26.0. Descriptive statistics such as frequencies and percentages were calculated for ESBL and AmpC prevalence.

Chi-square test was applied to assess the association between resistance mechanisms and clinical variables, and a p-value of <0.05 was considered statistically significant.

**Results**

A total of 1,240 urine samples were processed during the study period, of which 312 (25.1%) showed significant bacterial growth.

Among these, Escherichia coli was the predominant isolate, accounting for 186 cases (59.6%), followed by Klebsiella pneumoniae in 82 cases (26.3%), Proteus species in 18 cases (5.8%), Enterobacter species in 14 cases (4.5%), and other Gram-negative bacilli in 12 cases (3.8%). Female patients contributed a higher proportion of isolates (62.5%) compared to males (37.5%), with the majority of cases occurring in the 21–40 years age group.

Out of the 312 urinary isolates, 142 (45.5%) were confirmed as extended-spectrum β-lactamase (ESBL) producers by the combined disc diffusion method. ESBL production was highest in Klebsiella pneumoniae (57.3%) followed by E. coli (44.1%), while lower rates were observed in Enterobacter (35.7%) and Proteus (22.2%). AmpC β-lactamase production was identified in 48 isolates (15.4%) using the cefoxitin-cloxacillin double disc synergy test. The highest prevalence was observed in Enterobacter species (35.7%), followed by Klebsiella pneumoniae (19.5%) and E. coli (13.4%). Notably, 22 isolates (7.0%) demonstrated co-production of ESBL and AmpC enzymes, which was most frequently seen in Klebsiella pneumoniae. Antimicrobial susceptibility analysis revealed high levels of resistance to third-generation cephalosporins among ESBL and AmpC producers, with susceptibility retained in 82% of isolates for piperacillin-tazobactam and 94% for carbapenems.

Resistance to fluoroquinolones was widespread, particularly in ESBL-producing E. coli (72%). Aminoglycosides such as amikacin remained effective in 78% of isolates. Overall, the prevalence of ESBL-producing urinary isolates was nearly half of all significant cultures, while AmpC producers constituted a smaller but clinically important fraction. Co-producers exhibited multidrug resistance and limited therapeutic options, underscoring the importance of routine screening and reporting of these resistance mechanisms.

**Table 1: Distribution of Urinary Isolates (n = 312)**

| Bacterial Species | No. of Isolates | Percentage (%) |
|---|---|---|
| Escherichia coli | 186 | 59.6 |
| Klebsiella pneumoniae | 82 | 26.3 |
| Proteus spp. | 18 | 5.8 |
| Enterobacter spp. | 14 | 4.5 |
| Other Gram-negative bacilli | 12 | 3.8 |
| **Total** | **312** | **100.0** |

**Table 2: Prevalence of ESBL and AmpC Producers in Urinary Isolates**

| Bacterial Species | Total Isolates | ESBL Positive n (%) | AmpC Positive n (%) | ESBL + AmpC Co-producers n (%) |
|---|---|---|---|---|
| Escherichia coli | 186 | 82 (44.1) | 25 (13.4) | 10 (5.4) |
| Klebsiella pneumoniae | 82 | 47 (57.3) | 16 (19.5) | 8 (9.8) |
| Proteus spp. | 18 | 4 (22.2) | 1 (5.6) | 0 (0.0) |
| Enterobacter spp. | 14 | 5 (35.7) | 5 (35.7) | 2 (14.3) |
| Other GNB | 12 | 4 (33.3) | 1 (8.3) | 2 (16.7) |
| **Total** | **312** | **142 (45.5)** | **48 (15.4)** | **22 (7.0)** |

**Table 3: Antibiotic Susceptibility Pattern of ESBL and AmpC Producers (n = 168)**

| Antibiotic | ESBL Producers Sensitive (%) | AmpC Producers Sensitive (%) |
|---|---|---|
| Piperacillin–Tazobactam | 82.0 | 75.0 |
| Carbapenems (Imipenem/Mero) | 94.0 | 92.0 |
| Amikacin | 78.0 | 70.0 |
| Gentamicin | 62.0 | 58.0 |
| Ciprofloxacin | 28.0 | 34.0 |
| Trimethoprim–Sulfamethoxazole | 40.0 | 37.0 |
| Cefotaxime/Ceftazidime | 0.0 | 0.0 |

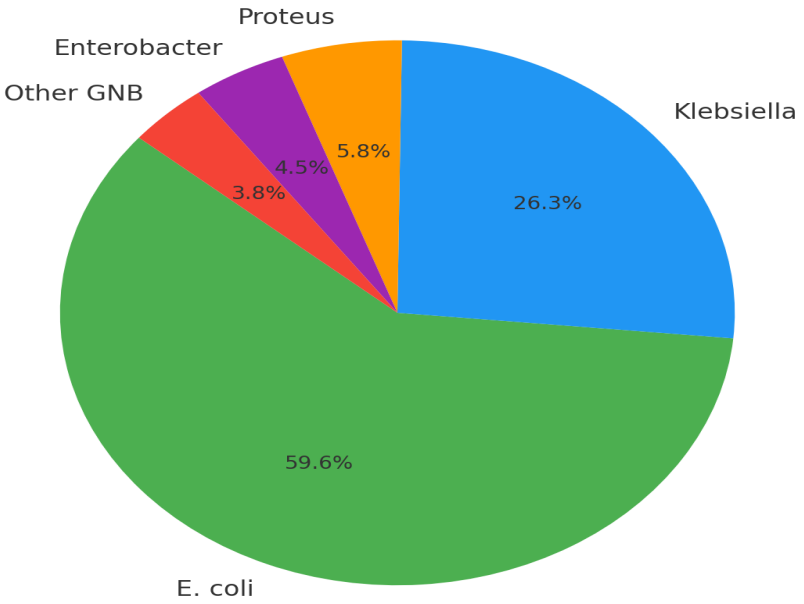


**Figure 1: Distribution of Urinary Isolates**

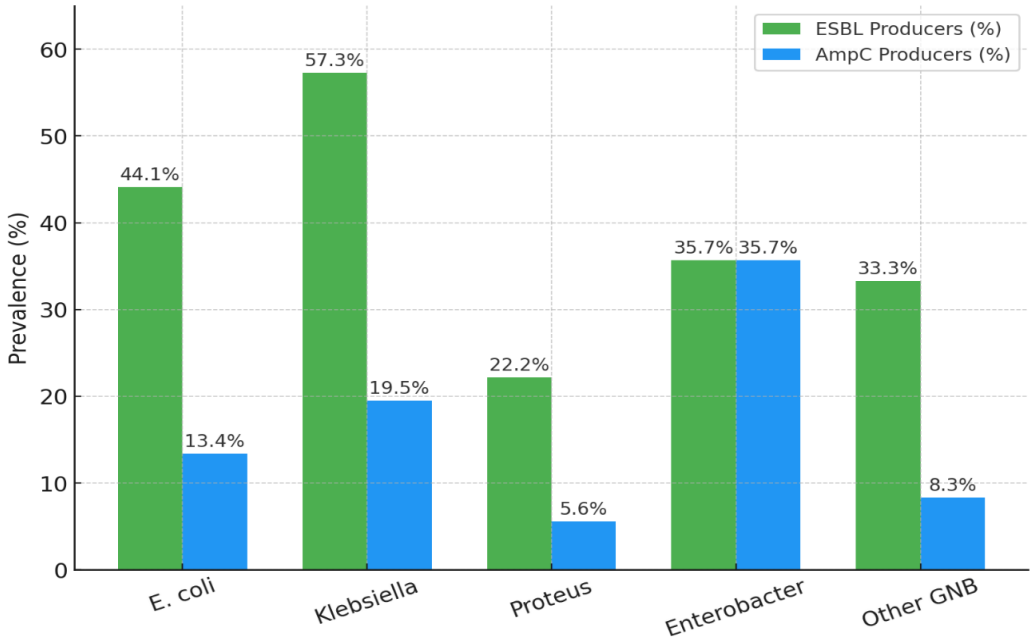


**Figure 2: prevalence of ESBL and AmpC Procedures across Species (%)**

## Discussion

In the present study, out of 312 urinary isolates, 45.5% were confirmed as extended-spectrum β-lactamase (ESBL) producers and 15.4% as AmpC producers, while 7.0% demonstrated co-production of both enzymes. These findings highlight the widespread burden of β-lactamase-mediated resistance among uropathogens in this region. The prevalence of ESBL producers in this study (45.5%) is consistent with reports from other Indian centers, though prevalence rates vary depending on study population and methodology. Tankhiwale reported ESBL prevalence of 44% among urinary isolates from Nagpur, which is nearly identical to the present findings [12].

A higher prevalence was documented by Rodrigues in Goa, where 66% of Enterobacteriaceae were found to be ESBL producers [13]. Internationally, Hawser reported ESBL rates of 25–35% in Europe and North America, which are notably lower than Indian data, reflecting regional variations in antimicrobial usage and stewardship [14].

AmpC production was noted in 15.4% of isolates in the present study, with the highest occurrence in

Enterobacter species (35.7%). This is comparable to the study by Singhal, who reported AmpC prevalence of 20% in Enterobacteriaceae [15]. Manoharan documented lower rates of AmpC production (8.5%) in southern India, suggesting regional variation in dissemination of AmpC plasmids [16]. Globally, AmpC prevalence ranges from 2–12%, as observed in studies from Europe and the US, which is much lower than Indian data [17]. Co-production of ESBL and AmpC enzymes was detected in 7% of isolates, most commonly in Klebsiella pneumoniae. This observation is important because co-producers exhibit resistance to both cephalosporins and β-lactamase inhibitor combinations, leaving carbapenems as the primary treatment option. Shah reported similar findings in Gujarat, with 6.8% of isolates showing co-production [18]. The increasing trend of co-producers is concerning, as it narrows therapeutic choices and contributes to treatment failures.

Antibiotic susceptibility testing in this study revealed that carbapenems remained the most effective agents, with >90% sensitivity, followed by piperacillin-tazobactam and amikacin. Resistance to fluoroquinolones was widespread, particularly among ESBL-producing E. coli (72%). These results are consistent with studies by Gupta and Mathur, who reported high fluoroquinolone resistance among ESBL-positive isolates, further complicating empirical therapy [19,20]. The retained activity of carbapenems highlights their role as the last line of defense, though increasing use of these agents risks the emergence of carbapenemase-producing organisms.

The present findings reinforce the urgent need for regular surveillance and reporting of ESBL and AmpC producers in urinary isolates. As highlighted by ICMR-AMR surveillance data, empirical use of third-generation cephalosporins and fluoroquinolones is no longer justified in many regions of India [21]. Instead, culture-guided therapy should be prioritized to preserve the efficacy of carbapenems and other reserve drugs. In Gujarat, limited regional data are available, and this study contributes valuable evidence supporting antimicrobial stewardship interventions tailored to local resistance trends [22].

## Conclusion

This study demonstrated a high prevalence of β-lactamase-mediated resistance among urinary isolates in this region, with 45.5% identified as extended-spectrum β-lactamase (ESBL) producers and 15.4% as AmpC producers. Co-production of both enzymes was detected in 7% of isolates, most frequently in Klebsiella pneumoniae. ESBL-producing E. coli and Klebsiella emerged as the predominant pathogens, reflecting the growing challenge of multidrug resistance in urinary tract infections. Antibiotic susceptibility analysis revealed widespread resistance to third-generation cephalosporins and fluoroquinolones, while carbapenems and piperacillin-tazobactam retained good activity. These findings underscore the urgent need for routine detection of ESBL and AmpC producers in diagnostic laboratories to guide appropriate therapy and prevent treatment failures.

## Limitations and Recommendations

The study was conducted at a single center and limited to one year, which may not capture seasonal or long-term variations in prevalence. Molecular characterization of β-lactamase genes was not performed, which could have provided deeper insights into the genetic basis of resistance. In addition, the absence of ethical committee approval restricts the study from being generalized for multicentric policy development.

Despite these limitations, the findings provide important baseline data on ESBL and AmpC prevalence in Gujarat. It is recommended that clinical microbiology laboratories routinely screen for both enzymes and report them promptly to clinicians. Empirical therapy for urinary tract infections should avoid indiscriminate use of third-generation cephalosporins and fluoroquinolones, and instead rely on culture-guided therapy. Strengthening antimicrobial stewardship programs, ensuring judicious use of carbapenems, and implementing regular regional surveillance are crucial steps to control the spread of resistance. Future studies with larger sample sizes, multicentric data, and molecular epidemiology will help establish comprehensive guidelines for managing multidrug-resistant urinary pathogens.

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