

## Detection of Esbl-Producing Uropathogens and Their Antimicrobial Susceptibility Profile in Urine Samples

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### Abstract

**Background:** Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and are increasingly associated with antimicrobial resistance. The emergence of extended-spectrum beta-lactamase (ESBL)-producing uropathogens has significantly limited therapeutic options and contributed to treatment failure.

**Objectives:** To detect ESBL-producing Enterobacteriaceae in urine samples and to evaluate their antimicrobial susceptibility patterns.

**Methods:** This prospective observational study was conducted over a period of 12 months in a tertiary care hospital. Adult patients with clinically suspected UTIs were included. Urine samples were processed using standard microbiological techniques. Identification of isolates was performed using conventional biochemical methods. Antibiotic susceptibility testing was carried out by the Kirby–Bauer disk diffusion method as per CLSI guidelines. ESBL production was screened and confirmed using the double disk synergy test (DDST) and combined disk diffusion test.

**Results:** Out of 235 Enterobacteriaceae isolates, 55.7% were identified as ESBL producers. *Escherichia coli* were the predominant organism (54.5%) and showed the highest ESBL production (64.8%). ESBL isolates exhibited high resistance to beta-lactam antibiotics and fluoroquinolones, whereas carbapenems and amikacin were found to be the most effective drugs.

**Conclusion:** The high prevalence of ESBL-producing uropathogens emphasizes the need for routine surveillance, early detection, and rational use of antibiotics to limit the spread of resistance and improve patient outcomes.

**Keywords:** Urinary tract infection, ESBL, Enterobacteriaceae, antimicrobial resistance, antibiotic susceptibility, uropathogens.

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### Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections encountered in clinical practice and represent a significant cause of morbidity worldwide. They affect individuals across all age groups and contribute substantially to the healthcare burden due to frequent recurrences, diagnostic investigations, and antimicrobial use [1, 2]. UTIs may involve any part of the urinary tract and are classified as uncomplicated or complicated depending on host factors and underlying comorbidities [2]. The majority of UTIs are caused by Gram-negative bacteria, predominantly members of the Enterobacteriaceae family.

*Escherichia coli* remains the leading uropathogen, followed by *Klebsiella pneumoniae*, *Proteus mirabilis*, and other related organisms [3]. Recently, antimicrobial resistance among uropathogens has emerged as a major global concern. Of particular importance is the increasing prevalence of extended-spectrum beta-lactamase (ESBL)-producing organisms. These enzymes hydrolyze a wide range of beta-lactam antibiotics, including third-generation cephalosporins, thereby limiting therapeutic options and contributing to treatment failure [4]. ESBL-producing Enterobacteriaceae are often associated with

multidrug resistance due to the presence of plasmid-mediated resistance genes, which can be easily transferred between bacteria [5]. The prevalence of ESBL-producing uropathogens has increased significantly, especially in developing countries such as India, where studies report rates ranging from 40% to 70% among urinary isolates [6].

### Materials and Methods

This prospective observational study was conducted in the Department of Microbiology of a tertiary care hospital over a period of one year. Adult patients aged 18 years and above presenting with symptoms suggestive of urinary tract infection were included. A total of 235 urine samples showing significant growth of Enterobacteriaceae were analyzed.

Urine samples were collected under aseptic precautions using the midstream clean-catch method and processed without delay in the microbiology laboratory. Each sample was cultured on Blood Agar and MacConkey Agar and incubated at 37°C for 18–24 hours. Growth of  $\geq 10$  colony-forming units (CFU)/mL of a single organism were considered significant.

The isolates were identified using routine microbiological techniques, including Gram staining and standard biochemical tests. Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton Agar, and results were interpreted according to CLSI 2023 guidelines. Isolates showing reduced susceptibility to third-generation

cephalosporins were screened for ESBL production. Confirmation was carried out using phenotypic methods, namely the double disk synergy test and the combined disk diffusion test.

### Inclusion Criteria

- Patients aged  $\geq 18$  years
- Patients with clinical features suggestive of UTI
- Urine samples showing significant bacteriuria due to Enterobacteriaceae

### Exclusion Criteria

- Patients below 18 years of age
- Patients who had received antibiotics within the last 72 hours
- Samples showing polymicrobial growth
- Non-Enterobacteriaceae isolates
- Patients who did not consent

**Statistical Analysis:** Data were entered into Microsoft Excel and analyzed using SPSS version 26.0. Categorical variables were expressed as frequencies and percentages. The Chi-square test was applied to assess associations and a p-value  $< 0.05$  was considered statistically significant.

### Results

A total of 235 urine samples with significant bacteriuria due to Enterobacteriaceae were included in the study.

The isolates were evaluated for distribution of uropathogens, ESBL production, and antimicrobial susceptibility patterns. The detailed findings are presented in the following tables and figures.

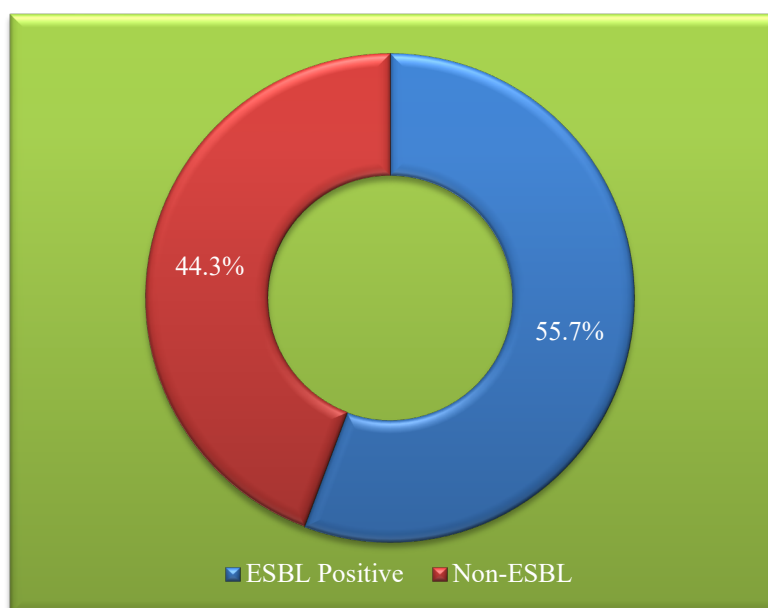


Figure 1: Overall Prevalence of ESBL Production among Urinary Enterobacteriaceae Isolates (n = 235)

**Table 1: Distribution of Enterobacteriaceae Isolates (n = 235)**

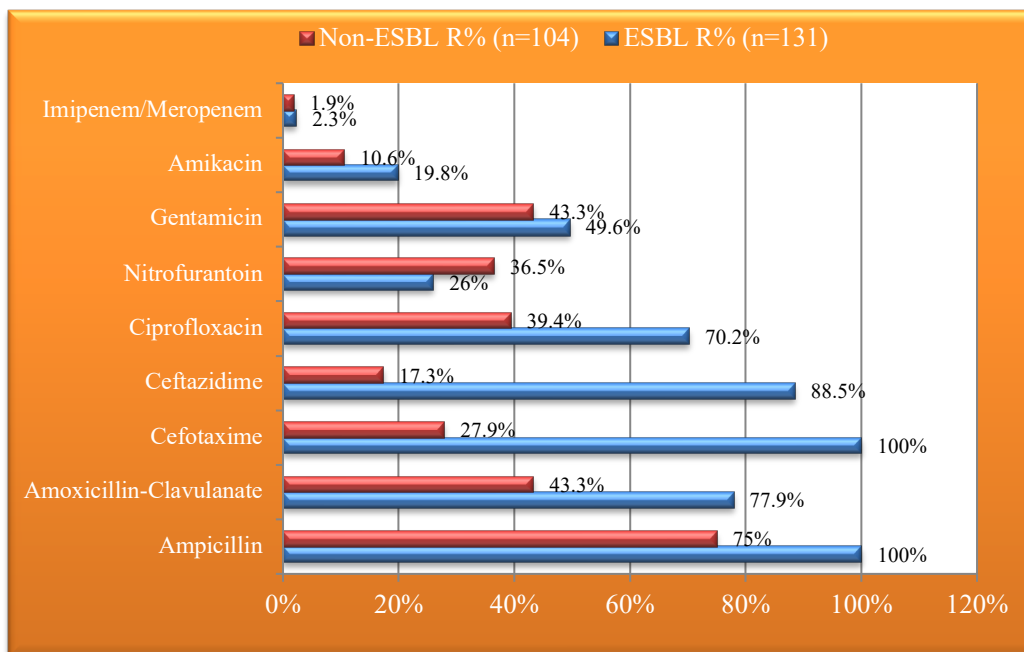
| Organism              | Number of Isolates | Percentage (%) |
|-----------------------|--------------------|----------------|
| Escherichia coli      | 128                | 54.5%          |
| Klebsiella pneumoniae | 48                 | 20.4%          |
| Proteus mirabilis     | 22                 | 9.4%           |
| Citrobacter freundii  | 14                 | 6.0%           |
| Enterobacter cloacae  | 12                 | 5.1%           |
| Proteus vulgaris      | 5                  | 2.1%           |
| Morganella morganii   | 4                  | 1.7%           |
| Klebsiella oxytoca    | 2                  | 0.9%           |

**Table 2: ESBL Production by Organism**

| Organism              | ESBL Positive | ESBL Negative | ESBL Rate (%) |
|-----------------------|---------------|---------------|---------------|
| Escherichia coli      | 83            | 45            | 64.8%         |
| Klebsiella pneumoniae | 24            | 24            | 50.0%         |
| Proteus mirabilis     | 9             | 13            | 40.9%         |
| Citrobacter freundii  | 6             | 8             | 42.9%         |
| Enterobacter cloacae  | 5             | 7             | 41.7%         |
| Proteus vulgaris      | 2             | 3             | 40.0%         |
| Morganella morganii   | 0             | 4             | 0.0%          |
| Klebsiella oxytoca    | 2             | 0             | 100.0%        |
| Total                 | 131           | 104           | 55.7%         |

**Table 3: Antibiotic Susceptibility Pattern of ESBL vs Non-ESBL Isolates**

| Antibiotic              | ESBL Isolates (n=131) |            |             | Non-ESBL Isolates (n=104) |            |            |
|-------------------------|-----------------------|------------|-------------|---------------------------|------------|------------|
|                         | S (%)                 | I (%)      | R (%)       | S (%)                     | I (%)      | R (%)      |
| Ampicillin              | 0 (0%)                | 0 (0%)     | 131 (100%)  | 23 (22.1%)                | 3 (2.9%)   | 78 (75.0%) |
| Amoxicillin-Clavulanate | 16 (12.2%)            | 13 (9.9%)  | 102 (77.9%) | 42 (40.4%)                | 17 (16.3%) | 45 (43.3%) |
| Cefotaxime              | 0 (0%)                | 0 (0%)     | 131 (100%)  | 59 (56.7%)                | 16 (15.4%) | 29 (27.9%) |
| Ceftazidime             | 4 (3.1%)              | 11 (8.4%)  | 116 (88.5%) | 78 (75%)                  | 8 (7.7%)   | 18 (17.3%) |
| Ciprofloxacin           | 29 (22.1%)            | 10 (7.6%)  | 92 (70.2%)  | 54 (51.9%)                | 9 (8.7%)   | 41 (39.4%) |
| Nitrofurantoin          | 67 (51.1%)            | 30 (22.9%) | 34 (26%)    | 56 (53.8%)                | 10 (9.6%)  | 38 (36.5%) |
| Gentamicin              | 53 (40.5%)            | 13 (9.9%)  | 65 (49.6%)  | 51 (49%)                  | 8 (7.7%)   | 45 (43.3%) |
| Amikacin                | 82 (62.6%)            | 23 (17.6%) | 26 (19.8%)  | 85 (81.7%)                | 8 (7.7%)   | 11 (10.6%) |
| Imipenem/Meropenem      | 124 (94.7%)           | 4 (3.1%)   | 3 (2.3%)    | 100 (96.2%)               | 2 (1.9%)   | 2 (1.9%)   |



**Figure 2: Comparative Antibiotic Resistance (R%) — ESBL vs Non-ESBL with Statistical Significance**

## Discussion

This study observed a high prevalence (55.7%) of ESBL-producing uropathogens among Enterobacteriaceae isolates, indicating a substantial burden of antimicrobial resistance in urinary tract infections. This finding is consistent with reports from developing countries, particularly India, where ESBL prevalence ranges between 40% and 70% [6]. The increasing trend may be attributed to the widespread and often inappropriate use of antibiotics. In the present study, *Escherichia coli* was the predominant uropathogen and demonstrated the highest ESBL production. Similar findings have been reported in recent studies identifying *Escherichia coli* as the principal ESBL-producing organism in UTIs [7,8]. *Klebsiella pneumoniae* was the second most common isolate and also showed significant ESBL production, which is in agreement with previous studies highlighting its role in hospital-associated infections [9]. The antimicrobial susceptibility pattern revealed high resistance to third-generation cephalosporins, which is expected due to ESBL enzyme activity [4]. In addition, a high level of resistance to fluoroquinolones was observed, suggesting co-resistance mediated by plasmids [5]. Nitrofurantoin showed moderate effectiveness against ESBL isolates and may be considered a useful oral option for uncomplicated UTIs [10]. Aminoglycosides, particularly amikacin, were found to be effective, consistent with earlier reports [11]. Carbapenems demonstrated the highest sensitivity and remain the drugs of choice for severe ESBL infections; however, the emergence of resistance is an increasing concern [12]. Routine detection of ESBL-producing organisms and implementation of antimicrobial stewardship programs are essential to control the spread of resistance [13].

**Conclusion:** The study demonstrates a high prevalence of ESBL-producing uropathogens, predominantly *Escherichia coli*, with significant resistance to commonly used antibiotics. Carbapenems and amikacin remain the most effective therapeutic options, while nitrofurantoin may be useful in selected cases. These findings emphasize the importance of routine ESBL detection, rational antibiotic use, and continuous surveillance to limit antimicrobial resistance and improve patient outcomes.

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