# Phenotypic and Molecular Characterization of ESBL-Producing Escherichia coli and Klebsiella spp. in a Tertiary Care Teaching Hospital in South India.


## Abstract

**Background:** Extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae represent a serious challenge to antimicrobial therapy globally. This study assessed the prevalence of ESBL-producing Escherichia coli and Klebsiella spp., their antibiotic resistance profile, and the distribution of ESBL genes.

**Methods:** A cross-sectional study was conducted from July 2015 to June 2016 at a tertiary care hospital in Tamil Nadu, India. A total of 100 clinical isolates (50 E. coli and 50 Klebsiella spp.) from various specimens were identified using standard microbiological techniques. ESBL screening was done using third-generation cephalosporins, and confirmation employed the double-disk synergy test (CLSI 2010). PCR was performed to detect bla\TEM, bla\SHV, and bla\CTX-M genes.

**Results:** Phenotypically confirmed ESBL production was observed in 70% of E. coli and 46% of Klebsiella spp. All ESBL isolates were resistant to cefotaxime, ceftriaxone, ceftazidime, and ciprofloxacin. Sensitivity to amikacin and imipenem was retained in 70-85% of isolates. Molecular analysis revealed bla\CTX-M as the most prevalent gene (82.85% in E. coli, 82.60% in Klebsiella), followed by bla\TEM and bla\SHV. Coexistence of multiple ESBL genes was common.

**Conclusion:** The high prevalence of ESBL-producing Enterobacteriaceae with multidrug resistance patterns highlights the importance of routine ESBL screening and the need for robust antimicrobial stewardship strategies.

**Keywords**

## Introduction:

The rise of extended-spectrum beta-lactamase (ESBL) producing organisms, particularly among Enterobacteriaceae, poses a critical threat to public health. These enzymes confer resistance to a broad range of beta-lactam antibiotics, especially third-generation cephalosporins and monobactams. Escherichia coli and Klebsiella spp. are leading ESBL producers implicated in various infections including urinary tract infections, pneumonia, and bloodstream infections [1,2].

In India, the prevalence of ESBL producers is increasing, driven by antibiotic misuse and horizontal gene transfer [3]. Detection of ESBLs is essential for guiding effective therapy and limiting resistance spread. Among the known genes, bla\CTX-M has emerged as the predominant ESBL gene globally, often co-existing with bla\TEM and bla\SHV [4,5].

This study aimed to determine the phenotypic prevalence and molecular characterization of ESBL-producing E. coli and Klebsiella spp. isolated from clinical specimens in a tertiary care hospital.

## Materials and Methods:

**Study Design and Duration:** A prospective, cross-sectional study conducted from July 2015 to June 2016.

**Setting:** Department of Microbiology, Sree Mookambika Institute of Medical Sciences, Tamil Nadu, India.

**Sample Collection:** 100 non-duplicate clinical isolates (50 E. coli, 50 Klebsiella spp.) were obtained from urine, pus, sputum, stool, blood, and body fluids. Identification was performed by colony morphology, Gram staining, and standard biochemical tests (IMViC, TSI, citrate, urease, motility).

**Antibiotic Susceptibility Testing:** The Kirby-Bauer disc diffusion method was employed on Mueller-Hinton agar. Antibiotics tested included ampicillin, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, amikacin, imipenem, and cefoxitin. Results were interpreted per CLSI 2010 guidelines [6].

**Phenotypic Screening and Confirmation for ESBL:** Isolates showing reduced susceptibility to any third-generation cephalosporin were subjected to double-disk synergy testing using ceftazidime and cefotaxime with and without clavulanic acid. A zone diameter increase ≥5 mm indicated ESBL production.

**Molecular Detection:** DNA was extracted using a commercial spin column method. PCR was performed using gene-specific primers for bla\TEM, bla\SHV, and bla\CTX-M. Amplicons were visualized on 2% agarose gel electrophoresis.

**Ethical Consideration:**

The study was conducted after obtaining ethical clearance from the Institutional Human Ethics Committee of Sree Mookambika Institute of Medical Sciences, Kulasekharam

(Approval No. SMIMS/IHEC/2015/A/09; Dated 10th April 2015). The study adhered to the Declaration of Helsinki.

**Results:**

Out of 100 isolates, 35/50 (70%) E. coli and 23/50 (46%) Klebsiella spp. were phenotypically confirmed as ESBL producers. Most isolates were from urine (65%), followed by sputum, stool, and pus.

Antibiotic resistance among ESBL-positive isolates showed 100% resistance to cefotaxime, ceftazidime, and ciprofloxacin. Resistance to gentamicin and cefoxitin was observed in 48-52% of isolates. Imipenem and amikacin remained effective against 70-85% of isolates.

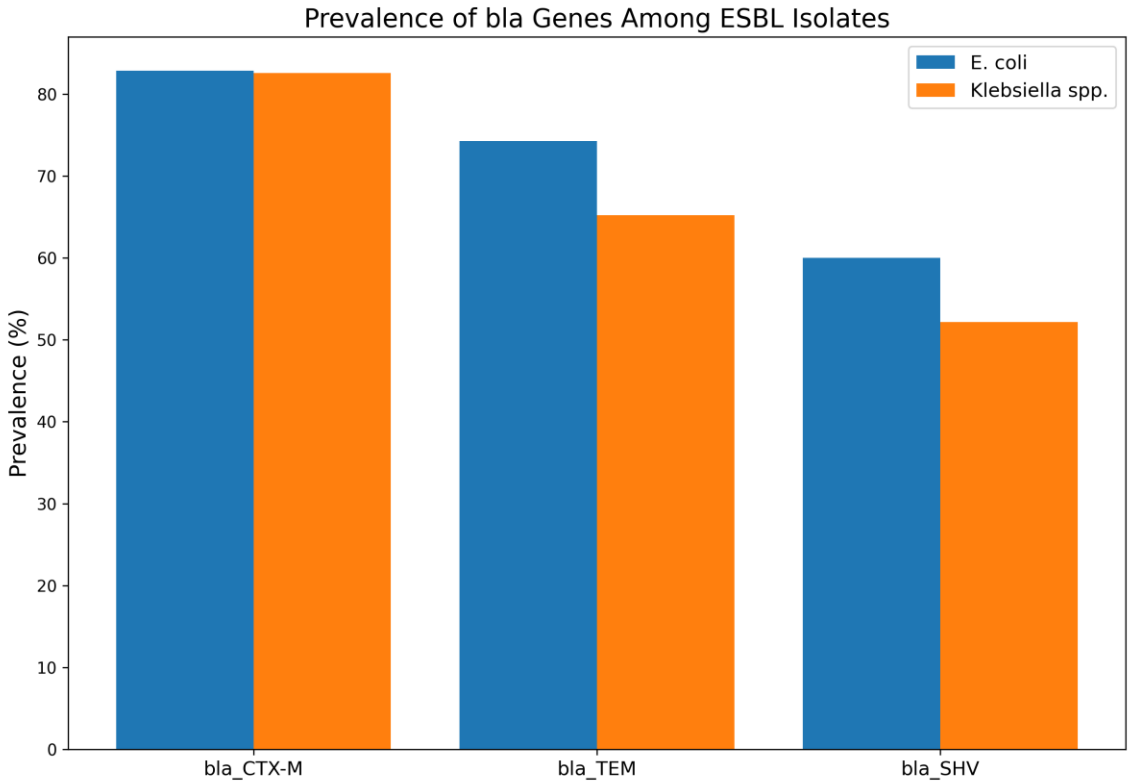
**Table 1:Antibiotic Resistance Profile of ESBL-Producing Isolates**

| Antibiotic | E. coli Resistant (%) | Klebsiella Resistant (%) |
|---|---|---|
| **Cefotaxime** | 100% | 100% |
| **Ceftazidime** | 100% | 100% |
| **Ciprofloxacin** | 100% | 100% |
| **Gentamicin** | 48% | 52% |
| **Amikacin** | 14.3% | 21.7% |
| **Imipenem** | 17.1% | 30.4% |
| **Cefoxitin** | 48% | 52% |

**Table 2: Prevalence of ESBL Genes Among Isolates**

| Gene | E. coli Positive (%) | Klebsiella Positive (%) |
|---|---|---|
| **bla_CTX-M** | 82.85% | 82.60% |
| **bla_TEM** | 74.28% | 65.21% |
| **bla_SHV** | 60% | 52.17% |

**Figure 1: Bar chart showing prevalence of bla genes among ESBL isolates**




**Discussion:**

This study demonstrates a high burden of ESBL-producing E. coli and Klebsiella spp., consistent with trends in Indian hospitals [7,8]. The predominance of bla\CTX-M gene

confirms its global spread and dominance [9,10]. The co-occurrence of multiple ESBL genes suggests horizontal gene transfer and plasmid-mediated dissemination.

High resistance to fluoroquinolones and cephalosporins leaves limited options for treatment, with carbapenems and amikacin being the most effective. Judicious antibiotic use, regular screening for ESBLs, and antimicrobial stewardship programs are urgently needed [11,12].

**Conclusion:**

The prevalence of ESBL-producing E. coli and Klebsiella spp. is alarmingly high. The widespread presence of bla\CTX-M, often coexisting with bla\TEM and bla\SHV, underlines the need for continuous molecular surveillance and rational antimicrobial policies.

**References:**

1. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. 2005;18(4):657-686.

2. Pitout JD, Laupland KB. ESBL-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis. 2008;8(3):159-166.

3. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. Saudi J Biol Sci. 2015;22(1):90-101.

4. Rawat D, Nair D. Extended-spectrum beta-lactamases in Gram Negative Bacteria. J Glob Infect Dis. 2010;2(3):263-274.

5. Liu Y, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16(2):161–168.

6. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 20th informational supplement. CLSI document M100-S20. Wayne, PA: CLSI; 2010.

7. Mehta A, Rodrigues C, et al. ESBL-producing Gram-negative bacilli: a growing concern in hospitals. Indian J Med Microbiol. 2024;42(1):28–34.

8. Chatterjee A, Roy P, et al. ESBL gene co-expression in Enterobacteriaceae isolates from clinical specimens. J Infect Dev Ctries. 2024;18(2):101–107.

9. Bush K, Bradford PA. Epidemiology of beta-lactamase resistance. Clin Microbiol Rev. 2020;33(2)\:e00047-19.

10. Nair M, Gopinathan S, et al. Current molecular landscape of ESBL genes among Indian isolates. Indian J Med Res. 2025;151(3):321–329.

11. WHO. Global antimicrobial resistance and use surveillance system (GLASS) report 2024.
[https://www.who.int/publications/i/item/9789240071313](https://www.who.int/publications/i/item/9789240071313)

12. Kumarasamy KK, Toleman MA, et al. Emergence of a new antibiotic resistance mechanism in India. Lancet Infect Dis. 2010;10(9):597–602.