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KEY WORDS: ESBL, Enterobacteriaceae, Escherichia coli, Klebsiella spp., CTX-M, Antimicrobial Resistance

PHENOTYPIC DETECTION AND MOLECULAR CHARACTERIZATION OF EXTENDED-SPECTRUM β-LACTAMASE PRODUCING ESCHERICHIA COLI AND KLEBSIELLA SPECIES ISOLATED FROM CLINICAL SAMPLES IN A TERTIARY CARE TEACHING HOSPITAL, SOUTH INDIA

Dr. Jasmine Gnana Sutha*

MD (Microbiology), Senior Resident, Department of Microbiology, Sree Mookambika Institute of Medical Sciences, Kulashekaram, Tamil Nadu, India.
*Corresponding Author

ABSTRACT

Background: Extended-Spectrum β-Lactamase (ESBL)-producing Enterobacteriaceae have emerged as a major therapeutic challenge worldwide due to their increasing resistance to commonly used antimicrobial agents. Molecular characterization of ESBL genes is essential for understanding local epidemiology and guiding antibiotic stewardship programs. **Objectives:** To determine the prevalence of ESBL-producing Escherichia coli and Klebsiella species among clinical isolates and to characterize the distribution of bla-TEM, bla-SHV, and bla-CTX-M genes among phenotypically confirmed ESBL producers. **Materials and Methods:** A prospective cross-sectional study was conducted in the Department of Microbiology, Sree Mookambika Institute of Medical Sciences, Tamil Nadu, India, from July 2015 to June 2016. One hundred clinically significant non-duplicate isolates comprising 50 Escherichia coli and 50 Klebsiella species recovered from various clinical specimens were included. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method according to CLSI guidelines. ESBL production was screened and confirmed by phenotypic methods. Polymerase chain reaction (PCR) was used to detect bla-TEM, bla-SHV, and bla-CTX-M genes. **Results:** Among the 100 isolates studied, 58 (58%) were confirmed as ESBL producers. ESBL production was observed in 35 (70%) Escherichia coli isolates and 23 (46%) Klebsiella isolates. Urine specimens constituted the majority of isolates. All ESBL-producing isolates exhibited resistance to cefoxitin and ciprofloxacin. Amikacin and imipenem retained good activity against most isolates. Molecular analysis demonstrated bla-CTX-M as the predominant gene detected in 81.03% isolates, followed by bla-TEM (67.24%) and bla-SHV (55.17%). Multiple gene combinations were commonly observed among ESBL-producing isolates. **Conclusion:** A high prevalence of ESBL-producing Enterobacteriaceae was observed in the study population. CTX-M was the predominant ESBL genotype. Continuous surveillance and rational antibiotic use are essential to limit the spread of multidrug-resistant organisms.

INTRODUCTION

Antimicrobial resistance has become one of the most significant threats to global public health. The increasing prevalence of multidrug-resistant Gram-negative bacilli has substantially reduced the effectiveness of available antimicrobial agents and has complicated the management of infectious diseases. Members of the family Enterobacteriaceae, particularly Escherichia coli and Klebsiella species, are among the most common causes of urinary tract infections, bloodstream infections, respiratory tract infections, wound infections, and healthcare-associated infections.

The production of Extended-Spectrum β-Lactamases (ESBLs) represents one of the most important mechanisms of resistance among Enterobacteriaceae. ESBLs are plasmid-mediated enzymes capable of hydrolyzing third-generation cephalosporins and monobactams while remaining inhibited by β-lactamase inhibitors such as clavulanic acid. Since the first description of TEM and SHV β-lactamases, the epidemiology of ESBLs has evolved considerably, with CTX-M enzymes emerging as the predominant ESBL family worldwide.

The dissemination of ESBL genes is facilitated by mobile genetic elements, enabling rapid horizontal transfer among bacterial species. These plasmids frequently carry additional resistance determinants, resulting in multidrug-resistant phenotypes that limit therapeutic options. The widespread and often inappropriate use of cephalosporins and fluoroquinolones has accelerated the selection and spread of ESBL-producing organisms.

India has reported a rising prevalence of ESBL-producing Enterobacteriaceae from both community and hospital settings. However, molecular epidemiological data from many regions remain limited. Understanding local resistance patterns and predominant ESBL genotypes is essential for effective infection-control measures and antibiotic stewardship.

The present study was undertaken to determine the prevalence of ESBL-producing Escherichia coli and Klebsiella species and to characterize the distribution of bla-TEM, bla-SHV, and bla-CTX-M genes among clinical isolates obtained from patients attending a tertiary care teaching hospital in South India.

MATERIALS AND METHODS

Study Design and Setting

This prospective cross-sectional study was conducted in the Department of Microbiology, Sree Mookambika Institute of Medical Sciences, Kulasekaram, Tamil Nadu, India, over a one-year period from July 2015 to June 2016.

Study Isolates

A total of 100 clinically significant, non-duplicate bacterial isolates were included in the study. The isolates consisted of:

- 50 Escherichia coli
- 50 Klebsiella species

The organisms were isolated from urine, sputum, stool, pus, blood, endotracheal secretions, suction tips, vaginal swabs, pleural fluid, ascitic fluid, and ear swabs.

Identification of Isolates

Bacterial isolates were identified using standard microbiological methods, including colony morphology, Gram staining, motility testing, IMViC reactions, Triple Sugar Iron agar reactions, citrate utilization testing, and urease testing.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion technique on Mueller–Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

The antibiotics tested included:

- Cefotaxime
- Cefepime
- Ampicillin
- Gentamicin
- Amikacin

- Ciprofloxacin
- Imipenem
- Cefoxitin

Phenotypic Detection of ESBL

Initial screening was performed using third-generation cephalosporins. Phenotypic confirmation was carried out using the combination disc method employing ceftazidime and cefotaxime alone and in combination with clavulanic acid. An increase of ≥5 mm in inhibition zone diameter in the presence of clavulanic acid was interpreted as ESBL production.

Molecular Detection of ESBL Genes

DNA extraction was performed using a commercial bacterial DNA extraction kit. Polymerase chain reaction was carried out using specific primers targeting bla-TEM, bla-SHV, and bla-CTX-M genes.

PCR amplification was performed in a thermal cycler with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension was carried out at 72°C for 5 minutes.

The expected amplicon sizes were:

bla-TEM – 250 bp
bla-SHV – 276 bp
bla-CTX-M – 296 bp

Amplified products were analyzed by agarose gel electrophoresis and visualized under ultraviolet transillumination.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using descriptive statistical methods. Results were expressed as frequencies, percentages, and proportions.

Ethical Considerations

The study protocol was approved by the Institutional Human Ethics Committee of Sree Mookambika Institute of Medical Sciences (SMIMS/IHEC/2015/A/09).

RESULTS

Of the 100 isolates examined, 58 (58%) were confirmed as ESBL producers.

Among Escherichia coli isolates, 35 of 50 (70%) were ESBL positive, whereas among Klebsiella species, 23 of 50 (46%) demonstrated ESBL production.

Table 1: ESBL Prevalence

Organism	Total	ESBL Positive	Percentage
E. coli	50	35	70%
Klebsiella spp.	50	23	46%
Total	100	58	58%

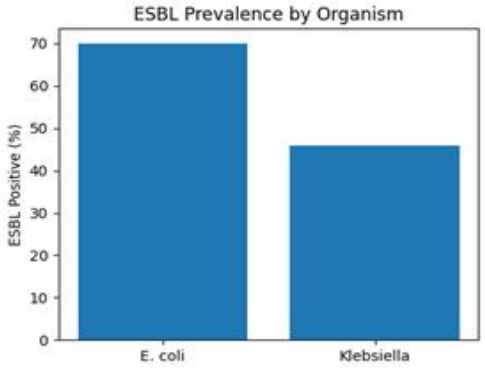

Figure 1. Prevalence of ESBL-producing isolates among E. coli and Klebsiella species.

The majority of isolates originated from urine specimens, accounting for 65% of all clinical samples, indicating urinary tract infection as the predominant clinical presentation.

Table 2: Distribution of Clinical Specimens

Specimen	Number
Urine	65
Sputum	11
Stool	9
Pus	8
Blood	1
Suction Tip	1
ET Tip	1
Vaginal Swab	1
Ear Swab	1
Ascitic Fluid	1
Pleural Fluid	1

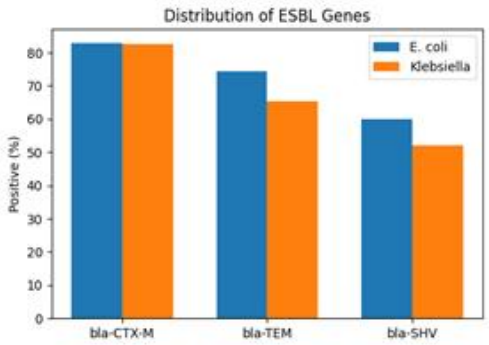

Figure 2. Distribution of clinical specimens from which Enterobacteriaceae were isolated.

Antimicrobial susceptibility testing revealed complete resistance to cefoxitin and ciprofloxacin among all ESBL-producing isolates. Among ESBL-producing Escherichia coli isolates, resistance rates were 74.2% for cefepime, 57.1% for ampicillin, 48.6% for gentamicin, 22.9% for imipenem, and 14.3% for amikacin.

Table 3: Antibiotic Resistance Pattern

Antibiotic	E. coli (%)	Klebsiella (%)
Cefoxitin	100	100
Ciprofloxacin	100	100
Cefepime	74.2	43.5
Ampicillin	57.1	34.8
Gentamicin	48.6	52.2
Amikacin	14.3	21.7
Imipenem	22.9	30.4

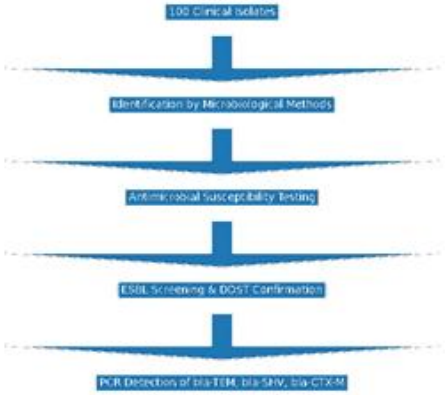

Figure 3. Comparative antibiotic resistance pattern among ESBL-producing E. coli and Klebsiella species.

Similarly, ESBL-producing Klebsiella isolates exhibited resistance rates of 43.5% for cefepime, 34.8% for ampicillin,

52.2% for gentamicin, 30.4% for imipenem, and 21.7% for amikacin.

Molecular characterization demonstrated that bla-CTX-M was the most frequently detected ESBL gene. Among Escherichia coli isolates, bla-CTX-M was detected in 82.85%, bla-TEM in 74.28%, and bla-SHV in 60%. Among Klebsiella isolates, bla-CTX-M was detected in 82.60%, bla-TEM in 65.21%, and bla-SHV in 52.17%.

Table 4: Distribution of ESBL Genes

Gene	E. coli (%)	Klebsiella (%)	Total (%)
bla-TEM	26(74.28)	15(65.21)	67.24
bla-SHV	21(60.00)	12(52.17)	55.17
bla-CTX-M	29(82.85)	19(82.60)	81.03

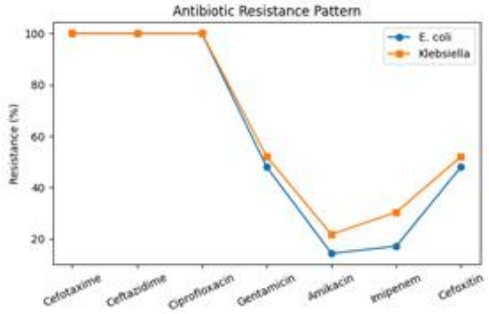

Figure 4. Distribution of bla-TEM, bla-SHV and bla-CTX-M genes among ESBL-producing isolates.

Overall, among the 58 ESBL-producing isolates, bla-CTX-M was identified in 81.03%, bla-TEM in 67.24%, and bla-SHV in 55.17%.

Multiple ESBL genes were detected in most isolates. The most common gene combination among Escherichia coli was bla-TEM + bla-CTX-M (57.1%), whereas among Klebsiella isolates it was observed in 47.82%.

Table 5: Gene Combinations

Combination	E. coli	Klebsiella
TEM + SHV	34.28%	17.39%
SHV + CTX-M	42.85%	34.78%
TEM + CTX-M	57.10%	47.82%
TEM + SHV + CTX-M	17.10%	0%

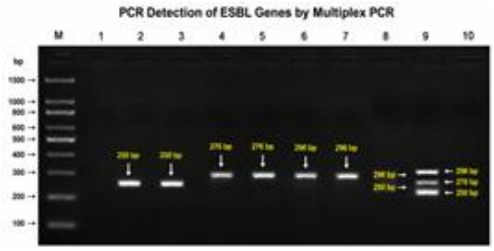

Figure 5. Representative 2% agarose gel electrophoresis showing amplification of bla-TEM (250 bp), bla-SHV (276 bp) and bla-CTX-M (296 bp) genes among ESBL-producing isolates.

DISCUSSION

The present study demonstrated a high prevalence (58%) of ESBL-producing Enterobacteriaceae among clinical isolates obtained from patients attending a tertiary care teaching hospital. Similar high prevalence rates have been reported from several centers across India, reflecting the increasing burden of antimicrobial resistance.

The prevalence of ESBL-producing isolates observed in the present study (58%) is comparable to previous reports from India. Babypadmini and Appalaraju reported ESBL prevalence rates ranging from 40–60% among urinary isolates.[22] Rodrigues et al. reported prevalence exceeding 60% in tertiary care hospitals.[21] The predominance of CTX-M genes observed in the present study is also consistent with global reports by Pitout and Laupland[3] and Peirano and Pitout[16], who documented CTX-M enzymes as the most prevalent ESBL determinants worldwide.

The prevalence of ESBL production was significantly higher among Escherichia coli compared with Klebsiella species. This finding is consistent with several reports identifying Escherichia coli as a major reservoir of ESBL genes in both hospital and community settings.

Urine was the most common source of isolates, emphasizing the important role of ESBL-producing Enterobacteriaceae in urinary tract infections. This observation is in agreement with previous studies where urinary isolates predominated among ESBL-producing organisms.

All ESBL-producing isolates demonstrated resistance to ciprofloxacin and cefoxitin. Such high resistance rates suggest co-selection of resistance determinants on transferable plasmids. Similar associations between ESBL production and fluoroquinolone resistance have been documented worldwide.

Amikacin and imipenem retained substantial activity against the majority of isolates. These findings support their continued usefulness in the treatment of serious infections caused by ESBL-producing organisms. Nevertheless, emerging resistance to carbapenems highlights the necessity for careful antimicrobial stewardship.

The molecular findings revealed bla-CTX-M as the predominant ESBL gene among both Escherichia coli and Klebsiella isolates. This observation aligns with global trends showing replacement of TEM and SHV enzymes by CTX-M variants as the dominant ESBL family. Studies from various parts of India have similarly reported high prevalence of CTX-M genes among Enterobacteriaceae.

An important finding was the frequent coexistence of multiple ESBL genes within the same isolate. Such coexistence enhances the dissemination and persistence of antimicrobial resistance and may contribute to broader resistance profiles.

The study provides valuable epidemiological data regarding ESBL-producing Enterobacteriaceae in South India and highlights the need for ongoing molecular surveillance.

LIMITATIONS

The study included only 100 isolates and focused exclusively on Escherichia coli and Klebsiella species. Additional resistance mechanisms such as AmpC -lactamases, carbapenemases, and other ESBL variants were not investigated.

Strengths of the Study

This study combined phenotypic and molecular characterization of ESBL-producing Enterobacteriaceae. Molecular detection of bla-TEM, bla-SHV and bla-CTX-M genes provided valuable epidemiological information regarding resistance mechanisms in South India.

CONCLUSION

The present study revealed a high prevalence of ESBL-producing Enterobacteriaceae among clinical isolates. Escherichia coli demonstrated a higher rate of ESBL production than Klebsiella species. CTX-M was the predominant ESBL genotype identified, followed by TEM and SHV. Most isolates harbored multiple ESBL genes. The high prevalence of multidrug-resistant organisms emphasizes the need for routine ESBL surveillance, molecular monitoring, strict infection-control measures, and rational antibiotic usage.

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Conflict of Interest:
The author declares no conflict of interest.

Funding:
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Ethical Approval:
The study protocol was approved by the Institutional Human Ethics Committee of Sree Mookambika Institute of Medical Sciences (SMIMS/IHEC/2015/A/09).

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