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Original Research Article

Burden and Genotypic Characterization of Extended-Spectrum Beta-Lactamase (ESBL) Producing Enterobacteriaceae in ICU patients: A tertiary care study

Article History:

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Abstract: ***Background:*** Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae represent a significant cause of healthcare-associated infections in intensive care units (ICUs), contributing to increased antimicrobial resistance and limited treatment options. This study aimed to determine the burden and genotypic characteristics of ESBL-producing Enterobacteriaceae in ICU patients. ***Material and Methods***: A prospective observational study was conducted over 12 months in a tertiary care ICU. A total of 164 non-duplicate Enterobacteriaceae isolates from clinical specimens were included. Identification and antimicrobial susceptibility testing were performed using standard microbiological techniques. ESBL production was confirmed phenotypically, and detection of blaCTX-M, blaTEM, and blaSHV genes was carried out using polymerase chain reaction. Clinical and demographic data were analyzed to identify associated risk factors. ***Results:*** Out of 164 isolates, 74 (45.1%) were ESBL producers. Klebsiella pneumoniae (35.4%) and Escherichia coli (30.5%) were the predominant organisms, with ESBL production rates of 51.7% and 48.0%, respectively. Endotracheal aspirates (26.8%) and urine (24.4%) were the most common specimen sources. ESBL-producing isolates showed complete resistance to cefotaxime (100%) and high resistance to ceftazidime (94.6%), cefepime (81.1%), ciprofloxacin (75.7%), and gentamicin (64.9%), while carbapenem resistance remained low (≤13.5%). Molecular analysis revealed blaCTX-M in 67.6%, blaTEM in 37.8%, and blaSHV in 32.4% of isolates, with 45.9% harboring multiple genes. Prolonged ICU stay, prior antibiotic exposure, mechanical ventilation, and central venous catheterization were significantly associated with ESBL production. ***Conclusion:*** A high prevalence of ESBL-producing Enterobacteriaceae with predominant blaCTX-M genes and multidrug resistance was observed, highlighting the need for strict infection control and antimicrobial stewardship in ICUs.

Keywords: ESBL, Enterobacteriaceae, antimicrobial resistance, ICU, blaCTX-M, molecular characterization.

INTRODUCTION

Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae have emerged as a major global public health concern due to their ability to inactivate a wide range of β-lactam antibiotics, including third-generation cephalosporins. These organisms are increasingly implicated in both community-acquired and healthcare-associated infections, significantly

complicating therapeutic management and clinical outcomes [1]. The rapid dissemination of ESBL-producing strains is largely driven by plasmid-mediated resistance mechanisms, which facilitate horizontal gene transfer across bacterial species, thereby accelerating the spread of antimicrobial resistance [2].

The burden of ESBL-producing Enterobacteriaceae is particularly pronounced in intensive care units (ICUs), where critically ill patients are highly susceptible to infections due to prolonged hospitalization, invasive procedures, and extensive exposure to broad-spectrum antibiotics. These factors collectively contribute to increased colonization and infection rates with multidrug-resistant organisms, leading to higher morbidity, mortality, and healthcare costs [3]. Recent epidemiological data suggest that the prevalence of ESBL-producing Enterobacteriaceae in hospital settings may exceed 50% in certain regions, underscoring the magnitude of the problem [4].

Among Enterobacteriaceae, Escherichia coli and Klebsiella pneumoniae are the most frequently isolated pathogens associated with ESBL production. These organisms possess a remarkable ability to acquire and disseminate resistance determinants, making them key contributors to the global antimicrobial resistance crisis [5,6].

At the molecular level, ESBL production is predominantly mediated by genes such as blaCTX-M, blaTEM, and blaSHV, which encode enzymes capable of hydrolyzing extended-spectrum cephalosporins. Among these, the CTX-M family has become the most prevalent globally, often surpassing TEM and SHV variants in clinical isolates [4,7]. The co-existence of multiple ESBL genes within a single isolate further enhances resistance potential and limits therapeutic options [1].

Furthermore, ESBL-producing organisms frequently exhibit co-resistance to other antimicrobial classes, including fluoroquinolones and aminoglycosides, thereby narrowing treatment choices and often necessitating the use of carbapenems. However, increasing reliance on carbapenems has contributed to the emergence of carbapenem-resistant strains, posing an additional challenge to infection control and antimicrobial stewardship efforts [2].

Given the rising prevalence, evolving molecular epidemiology, and significant clinical implications of ESBL-producing Enterobacteriaceae, particularly in ICU settings, continuous surveillance and molecular characterization are essential. Therefore, the present study was undertaken to assess the burden and genotypic profile of ESBL-producing Enterobacteriaceae among ICU patients in a tertiary care setting.

MATERIAL AND METHODS

Study Design and Setting: A hospital-based prospective observational study was carried out over in the ICU of a tertiary care teaching hospital. The study was designed to evaluate the burden and genotypic characteristics of ESBL-producing Enterobacteriaceae isolated from critically ill patients.

Study Population: All patients admitted to the ICU during the study period who developed clinical features suggestive of infection after at least 48 hours of hospitalization were screened. Only those patients from whom Enterobacteriaceae were isolated in significant numbers from appropriate clinical specimens were included in the study.

Inclusion Criteria:

• Patients aged ≥18 years admitted to ICU for ≥48 hours
• Clinical suspicion of infection with microbiologically confirmed Enterobacteriaceae isolates
• Specimens including blood, urine, respiratory samples (endotracheal aspirate/bronchoalveolar lavage), pus, and sterile body fluids

Exclusion Criteria:

• Duplicate isolates from the same patient during a single infectious episode
• Patients with polymicrobial infections where Enterobacteriaceae were not the predominant isolate
• Patients receiving antibiotic therapy prior to ICU admission for the same infection episode

Sample Size Determination: The sample size was estimated using the single proportion formula: $n = Z^2 \times p \times q / d^2$
Assuming an anticipated prevalence of ESBL-producing Enterobacteriaceae of 45% based on recent ICU-based studies, with a 95% confidence level (Z = 1.96) and an absolute precision of 8%, the minimum required sample size was calculated to be approximately 149 isolates. Accounting for potential exclusions and incomplete data, a total of 160–170 non-duplicate isolates were targeted for inclusion to ensure adequate statistical power and reliability of findings.

Sample Collection and Processing: Clinical specimens were collected under strict aseptic precautions following standard infection control practices. Samples were transported promptly to the microbiology laboratory and processed without delay.

• Blood cultures were processed using an automated continuous monitoring system
• Urine samples were cultured using a calibrated loop

method
• Respiratory samples and other specimens were inoculated on appropriate culture media
All samples were cultured on standard media such as MacConkey agar and blood agar and incubated aerobically at 35–37°C for 18–24 hours.

Identification of Isolates: Bacterial isolates were identified as members of Enterobacteriaceae based on colony characteristics, Gram staining, and a battery of standard biochemical tests. Confirmation at the species level was performed using automated identification systems wherever available.

Antimicrobial Susceptibility Testing: Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. The results were interpreted according to the latest Clinical and Laboratory Standards Institute (CLSI) guidelines. A panel of antibiotics representing different classes, including β-lactams, aminoglycosides, fluoroquinolones, and carbapenems, was tested.

Phenotypic Detection of ESBL Production: Initial screening for ESBL production was performed using third-generation cephalosporins such as cefotaxime and ceftazidime. Isolates exhibiting reduced susceptibility were further subjected to confirmatory tests:
• Combination disk method using cephalosporin with and without clavulanic acid
• Double disk synergy test (DDST)
An increase in inhibition zone diameter of ≥5 mm in the presence of clavulanic acid compared to cephalosporin alone was considered indicative of ESBL production.

Genotypic Characterization of ESBL Genes: Genomic DNA was extracted from phenotypically confirmed ESBL-producing isolates using a standardized extraction protocol. Polymerase chain reaction (PCR) was performed to detect the presence of common ESBL genes including blaCTX-M, blaTEM, and blaSHV using specific primers.
Amplified products were subjected to agarose gel electrophoresis and visualized under ultraviolet illumination. A subset of representative amplicons was sequenced to validate gene identity and ensure accuracy of amplification.

Data Collection: Relevant clinical and demographic information, including age, gender, duration of ICU stay, comorbid conditions, use of invasive devices (mechanical ventilation, urinary catheterization, central venous catheter), and prior antibiotic exposure, was collected using a structured data collection form.

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using statistical software such as SPSS version 25.0. Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as mean ± standard deviation. Associations between categorical variables were assessed using the Chi-square test. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 164 non-duplicate Enterobacteriaceae isolates obtained from ICU patients were included in the present study. The majority of patients belonged to the age group of 51–70 years (36.6%), followed by 31–50 years (31.7%). Patients aged more than 70 years constituted 20.7% of the study population, while younger adults (18–30 years) accounted for 11.0%. Male patients predominated, comprising 59.8% of cases. Regarding ICU stay, 42.6% of patients had a prolonged stay of more than 10 days, while 35.4% stayed for 6–10 days. Among invasive interventions, urinary catheterization was the most common (72.0%), followed by mechanical ventilation (53.7%) and central venous catheterization (45.1%) (Table 1).

With respect to specimen distribution, endotracheal aspirates were the most frequent source of isolates, accounting for 26.8%, followed by urine (24.4%) and blood (19.5%). Pus or wound swabs and body fluids contributed 15.9% and 13.4% of isolates, respectively (Table 2).

Among the bacterial isolates, Klebsiella pneumoniae was the predominant organism (35.4%), followed by Escherichia coli (30.5%). Other isolates included Enterobacter spp. (13.4%), Citrobacter spp. (11.0%), and Proteus spp. (9.8%) (Table 3).

Out of the total isolates, 74 (45.1%) were confirmed as ESBL producers, while 90 (54.9%) were non-ESBL producers (Table 4). The highest proportion of ESBL production was observed in K. pneumoniae (51.7%), followed by E. coli (48.0%). Moderate ESBL rates were noted in Enterobacter spp. (45.5%), whereas comparatively lower rates were observed in Citrobacter spp. (33.3%) and Proteus spp. (25.0%) (Table 5).

Antimicrobial susceptibility testing of ESBL-producing isolates demonstrated universal resistance to cefotaxime

(100%) and a very high level of resistance to ceftazidime (94.6%). Resistance to cefepime was also substantial (81.1%). Among non-β-lactam antibiotics, high resistance rates were observed for ciprofloxacin (75.7%) and gentamicin (64.9%). Moderate resistance was seen with piperacillin–tazobactam (43.2%) and amikacin (37.8%). Carbapenem resistance remained relatively low, with 13.5% resistance to imipenem and 10.8% to meropenem (Table 6).

Molecular analysis revealed that blaCTX-M was the most frequently detected gene, present in 67.6% of ESBL-producing isolates. This was followed by blaTEM (37.8%) and blaSHV (32.4%). Notably, co-existence of multiple ESBL genes was identified in 45.9% of isolates, indicating the presence of combined resistance mechanisms (Table 7).

Analysis of associated risk factors demonstrated that prolonged ICU stay (>10 days) and prior antibiotic exposure were strongly associated with ESBL production ($p<0.001$). Mechanical ventilation and central venous catheterization were also found to have statistically significant associations ($p=0.02$ and $p=0.03$, respectively). However, urinary catheterization did not show a statistically significant association with ESBL production ($p=0.09$) (Table 8).

Table 1. Demographic and Clinical Characteristics of ICU Patients (n = 164)

Variable	Number (n)	Percentage (%)
Age Group (years)		
18–30	18	11.0
31–50	52	31.7
51–70	60	36.6
>70	34	20.7
Gender		
Male	98	59.8
Female	66	40.2
ICU Stay Duration		
≤5 days	36	22.0
6–10 days	58	35.4
>10 days	70	42.6
Invasive Devices		
Urinary catheter	118	72.0
Mechanical ventilation	88	53.7
Central venous catheter	74	45.1

Table 2. Distribution of Clinical Specimens (n = 164)

Specimen Type	Number (n)	Percentage (%)
Endotracheal aspirate	44	26.8
Urine	40	24.4
Blood	32	19.5
Pus/Wound swab	26	15.9
Body fluids	22	13.4

Table 3. Distribution of Enterobacteriaceae Isolates (n = 164)

Organism	Number (n)	Percentage (%)
Klebsiella pneumoniae	58	35.4
Escherichia coli	50	30.5
Enterobacter spp.	22	13.4
Citrobacter spp.	18	11.0
Proteus spp.	16	9.8

Table 4. Prevalence of ESBL-Producing Isolates (n = 164)

Category	Number (n)	Percentage (%)
ESBL Producers	74	45.1
Non-ESBL Producers	90	54.9

Table 5. ESBL Production among Different Organisms

Organism	Total Isolates	ESBL Positive (n)	ESBL (%)
K. pneumoniae	58	30	51.7
E. coli	50	24	48.0
Enterobacter spp.	22	10	45.5
Citrobacter spp.	18	6	33.3
Proteus spp.	16	4	25.0

Table 6. Antibiotic Resistance Pattern of ESBL Producers (n = 74)

Antibiotic	Resistant (n)	Resistance (%)
Cefotaxime	74	100
Ceftazidime	70	94.6
Cefepime	60	81.1
Ciprofloxacin	56	75.7
Gentamicin	48	64.9
Amikacin	28	37.8
Piperacillin-tazobactam	32	43.2
Imipenem	10	13.5
Meropenem	8	10.8

Table 7. Distribution of ESBL Genes among Isolates (n = 74)

Gene Detected	Number (n)	Percentage (%)
blaCTX-M	50	67.6
blaTEM	28	37.8
blaSHV	24	32.4
Multiple genes	34	45.9

Table 8. Association of Risk Factors with ESBL Production

Risk Factor	ESBL Positive (n=74)	ESBL Negative (n=90)	p-value
ICU stay >10 days	46	24	<0.001
Prior antibiotic use	60	42	<0.001
Mechanical ventilation	44	44	0.02
Central venous catheter	38	36	0.03
Urinary catheter	54	64	0.09

DISCUSSION

The present study demonstrated an ESBL prevalence of 45.1% among Enterobacteriaceae isolates obtained from ICU patients, indicating a substantial burden of antimicrobial resistance in critical care settings. Comparable prevalence rates have been reported in recent hospital-based and ICU-focused studies, where ESBL occurrence ranges widely depending on geographic location and healthcare practices. A recent meta-analysis reported persistent high prevalence trends of ESBL-producing Enterobacteriaceae across hospital settings up to 2024, reflecting the ongoing global challenge of antimicrobial resistance [8].

In the current study, Klebsiella pneumoniae and Escherichia coli were the predominant isolates, accounting for the majority of ESBL producers. Similar findings have been consistently observed in recent ICU-based studies, where these organisms constitute over 90% of ESBL-producing Enterobacterales isolates [9]. Their predominance is attributed to their enhanced ability to acquire plasmid-mediated resistance genes and persist in hospital environments.

The higher proportion of ESBL production observed in K. pneumoniae compared to E. coli in this study is in agreement with several recent reports highlighting the increasing role of K. pneumoniae as a major nosocomial pathogen, particularly in ICU settings [10]. Additionally, the predominance of respiratory samples, especially endotracheal aspirates, as a source of isolates reflects the increased vulnerability of mechanically ventilated patients to ventilator-associated infections.

The antimicrobial susceptibility pattern observed in the present study revealed universal resistance to cefotaxime and high resistance to other cephalosporins and fluoroquinolones. Similar resistance profiles have been documented in recent

studies, emphasizing the multidrug-resistant nature of ESBL-producing organisms and their association with co-resistance to non-β-lactam antibiotics [11]. The relatively low resistance to carbapenems observed in this study is consistent with findings from recent literature; however, the gradual emergence of carbapenem resistance remains a serious concern due to increased reliance on these agents [12].

Molecular analysis in the present study identified blaCTX-M as the predominant ESBL gene, followed by blaTEM and blaSHV. This observation aligns with recent global data demonstrating the dominance of CTX-M-type enzymes, with pooled prevalence estimates exceeding 70% in ESBL-producing isolates [8]. Furthermore, studies have shown that blaCTX-M genes are widely distributed among E. coli and K. pneumoniae isolates in ICU settings, reinforcing their epidemiological significance [9]. The detection of multiple ESBL genes in a considerable proportion of isolates in this study supports the role of plasmid-mediated gene transfer in enhancing resistance complexity [13].

Risk factor analysis revealed that prolonged ICU stay and prior antibiotic exposure were strongly associated with ESBL production. These findings are consistent with recent studies that identify prolonged hospitalization and antibiotic pressure as key drivers of ESBL colonization and infection in critically ill patients [12]. Additionally, invasive interventions such as mechanical ventilation and central venous catheterization were significantly associated with ESBL production, highlighting the importance of device-associated infections in ICU settings [14].

CONCLUSION

The present study highlights a substantial burden of ESBL-producing Enterobacteriaceae among ICU patients, with Klebsiella pneumoniae and Escherichia coli emerging as the leading pathogens. The high level of resistance to third- and fourth-generation cephalosporins, along with considerable co-resistance to other antimicrobial classes, underscores the limited therapeutic options in critical care settings. The predominance of the blaCTX-M gene and frequent coexistence of multiple ESBL genes indicate active dissemination of plasmid-mediated resistance mechanisms. Significant associations with prolonged ICU stay, prior antibiotic exposure, and invasive interventions further emphasize the role of hospital-related factors in the propagation of these multidrug-resistant organisms. These findings reinforce the need for rigorous infection control strategies, judicious antibiotic use, and ongoing molecular surveillance to curb the spread of ESBL-producing pathogens in intensive care units.

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