



PREVALENCE, ANTIBIOTIC SUSCEPTIBILITY PATTERNS, AND CLINICAL CORRELATES OF ESBL-PRODUCING ENTEROBACTERIACEAE AMONG ICU PATIENTS IN A TERTIARY CARE HOSPITAL.

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Abstract

Background: Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae are a major cause of multidrug-resistant infections in Intensive Care Units (ICUs), posing significant treatment challenges. This study aimed to determine the prevalence, antibiotic susceptibility patterns, and clinical correlates of ESBL-producing Enterobacteriaceae among ICU patients in a tertiary care hospital.

Methods: A cross-sectional observational study was conducted from December 2024 to November 2025 in the Department of microbiology Sarojini Naidu Medical College Agra, with the collaboration Department of Microbiology, Mahamaya Rajkiya Allopathic Medical College, Ambedkar nagar. ICU patients aged 18–75 years with culture-positive Enterobacteriaceae isolates and an ICU stay ≥ 48 hours were included. Clinical specimens were processed for culture, identification, and antibiotic susceptibility testing according to standard protocols.

Results: Among 129 ICU patients, 101 clinical specimens yielded 100 isolates, of which 42 (41.6%) were ESBL-positive. Escherichia coli (28%) and Klebsiella spp. (18%) were the most common ESBL-producing organisms. Urine and endotracheal tube samples showed the highest ESBL positivity. Most isolates exhibited high resistance to β -lactams and fluoroquinolones, whereas carbapenems, nitrofurantoin, and aminoglycosides retained good activity.

Conclusion: ESBL-producing Enterobacteriaceae are prevalent in ICU patients and exhibit multidrug resistance, limiting therapeutic options. Carbapenems and select non- β -lactams remain effective,

highlighting the need for robust antimicrobial stewardship, strict infection control, and regular surveillance in ICUs to curb the spread of resistant organisms.

Keywords: ESBL, Enterobacteriaceae, ICU, antimicrobial resistance, carbapenems, multidrug resistance

Introduction:

Antimicrobial resistance (AMR) has emerged as a major global health threat, undermining the life-saving power of antibiotics that transformed modern medicine. The World Health Organization describes antibiotic resistance as a “silent pandemic,” warning that if current trends continue, AMR could lead to as many as 10 million deaths each year by 2050.¹

Among the various resistant pathogens, Enterobacteriaceae producing Extended-Spectrum Beta Lactamases (ESBLs) have emerged as critical priority organisms because of their ability to inactivate broad-spectrum β -lactam antibiotics, including third-generation cephalosporins and monobactams.² ESBL-producing Enterobacteriaceae, particularly *Escherichia coli* and *Klebsiella pneumoniae*, are frequently implicated in hospital-acquired infections such as urinary tract infections, bloodstream infections, ventilator-associated pneumonia, and surgical site infections.³

These treatments include the creation of new antibiotics, complementary and alternative medicine, and worldwide policy campaigns that are centered on monitoring, education, and stewardship. When it comes to the development of medications that can endure the test of time, having a thorough understanding of the complexities of antibiotic resistance is absolutely necessary.⁴

The proliferation and spread of germs that are resistant to antibiotics has been made possible as a result of this. Microorganisms can become resistant to antibiotics by developing mechanisms that allow them to withstand the effects of medications that were in formerly effective against them. This phenomenon is referred to as antibiotic resistance. Overprescribing antibiotics and patients not following prescribe treatments are two examples of human causes that contribute to the acceleration of the progression of this natural phenomenon.⁵

The major resistance issues overall, are those which are related to the methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE), extended-spectrum β -lactamase producing Enterobacteriaceae, and the multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.⁶

The extended- spectrum β -lactamases (ESBLs) are mutant, plasmid mediated β -lactamases which are derived from the older, broad spectrum β -lactamases and they confer resistance to all the extended spectrum cephalosporins and aztreonam, except to the cephamycin and the carbapenems.⁷

Study place and duration: A study was conducted in the Department of microbiology Sarojini Naidu Medical College Agra, with the collaboration Department of Microbiology, Mahamaya Rajkiya Allopathic Medical College, Ambedkar nagar, from December 2024- November 2025.

Study design: Cross-sectional observational study

Inclusion criteria:

1. ICU patients aged 18-70 years.
2. Patients who had clinical specimens collected for culture during the study period.
3. Culture-positive Enterobacteriaceae isolates.
4. ICU stay of ≥ 48 hours.

Exclusion criteria:

1. Patients aged < 18 years.
2. Contaminated or inadequate specimens.
3. Mixed growth where Enterobacteriaceae cannot be clearly identified.

4. Known ESBL colonization prior to ICU admission

Sample Collection

Clinical specimens-including urine, blood, endotracheal tube aspirates, pus, catheter tips, wound swabs, and sterile body fluids-were collected using standard aseptic techniques by trained healthcare personnel. Each specimen was transported promptly to the microbiology laboratory for processing.

Material and Methods: A cross-sectional observational study was conducted ICU patients aged 18–75 years with culture-positive Enterobacteriaceae isolates and an ICU stay ≥ 48 hours were included. Clinical specimens were processed for culture, identification, and antibiotic susceptibility testing according to standard protocols and according CLSI guideline

Laboratory Processing

1. Culture and Identification

All specimens were inoculated onto appropriate culture media including Blood agar, MacConkey agar, and CLED agar (for urine samples), and incubated at 37°C for 18–24 hours.

Bacterial growth was identified based on:

- Colony morphology
- Gram staining
- Biochemical tests (e.g., indole, citrate, urease, TSI, oxidase)
- Additional confirmatory tests when required

Enterobacteriaceae isolates were included for further testing.

2. Antibiotic Susceptibility Testing

Antimicrobial susceptibility was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines.

Detection of ESBL Production

ESBL screening and confirmation were carried out as per CLSI recommendations.

1. Screening Test

Isolates showing reduced susceptibility to any of the following antibiotics were considered potential ESBL producers.

2. Phenotypic Confirmatory Test

Potential ESBL producers were subjected to Combined Disk Test (CDT) using:

- Ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/10 µg)
- Cefotaxime (30 µg) and cefotaxime-clavulanic acid (30/10 µg)

An increase in zone diameter of ≥ 5 mm in the presence of clavulanic acid confirmed ESBL production.

Result:

Table 1: Gender distribution of the patients.

Gender	Number of Patients	Percentage (%)
Male	76	59
Female	53	41
Total	129	100%

Study population consisted of 129 patients in total. Among them, 76 were male, representing 59% of the sample, while 53 were female, accounting for the remaining 41%. This indicates that male patients formed the majority of the study group, with a noticeably higher proportion compared to females.

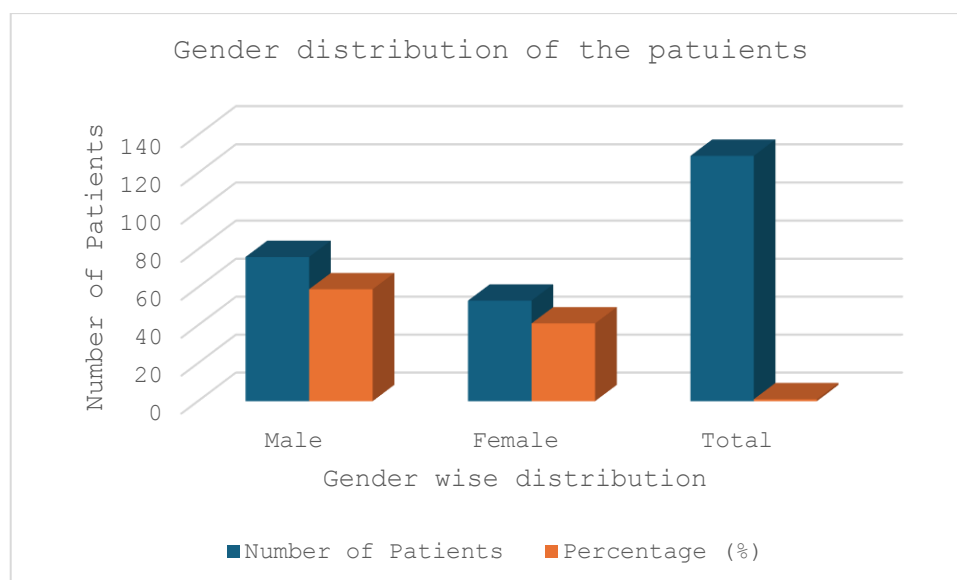


Figure 1: Graphical represents gender distribution of the patients.

Table 2: Age distribution of the patients.

Age Group (in years)	Number of Patients	Percentage (%)	p value
18–29	22	17	0.78
30–39	28	22	
40–49	26	20	
50–59	27	21	
60–70	26	20	
Total	129	100	

Study included 129 patients, distributed across five age groups. The largest proportions were observed in the 30–39 years (22%), 50–59 years (21%), and 40–49 / 60–70 years groups (20% each), while the 18–29 years group accounted for 17% of the sample. The p-value of 0.78 indicates no statistically significant difference in the distribution of patients across age groups. In other words, the ages of participants were evenly distributed, and there is no meaningful variation suggesting age-related clustering within the study population.

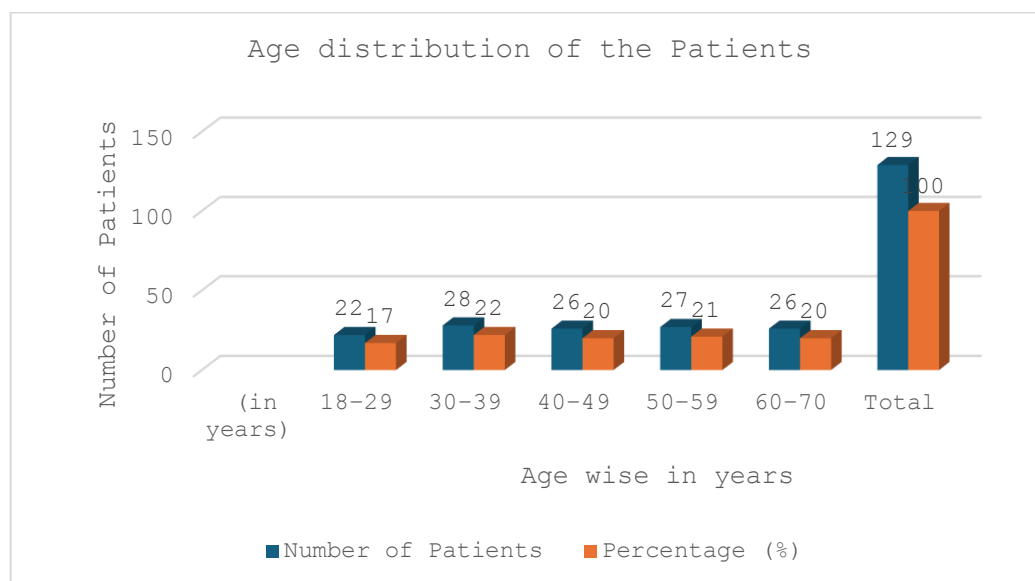


Figure 2: Graphical represents age wise distribution of the patients.

Table 3: Distribution of ESBL-Positive Isolates Among Different Clinical Specimens.

Specimen Type	Total Samples (N)	ESBL-Positive (N)
Urine	25	12
Blood	25	8
Endotracheal tube	18	9
Pus	10	5
Foley's catheter tip	7	3
Central line tip	6	2
Wound swab	5	2
Tracheal secretion	2	1
Peritoneal fluid	1	0
Pleural fluid	1	0
Tracheal tube	1	0
Total	101	42

Out of 101 specimens, 42 (41.6%) were ESBL-positive. Urine and blood were the most common specimen types, with urine showing the highest ESBL positivity (12/25). Endotracheal tube samples also showed a high positivity rate (9/18). No ESBL-positive isolates were found in peritoneal fluid, pleural fluid, or tracheal tube specimens.

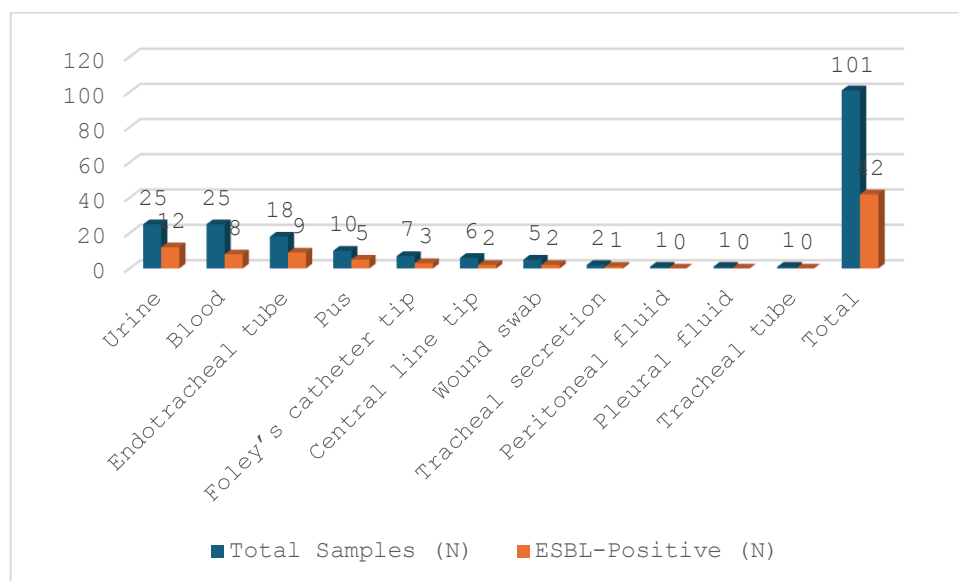


Figure 3: Graphical represents of ESBL-Positive Isolates Among Different Clinical Specimens.

Table 4: Total Number of Organisms Isolated.

Organism Type	Gram Reaction	Number (N)
Gram-negative bacilli (GNB)	GNB	71
Gram-positive cocci (GPC)	GPC	19
Budding yeast	Fungal	10
Total	-	100

Among the 100 isolates, Gram-negative bacilli (71%) were the most common, followed by Gram-positive cocci (19%) and budding yeasts (10%). This indicates a predominance of Gram-negative organisms in the sample set.

Table 5: Total Number of Organisms Isolated.

Organism	Number (N)	Percentage (%)
Escherichia coli	28	28%
Klebsiella spp.	18	18%
Staphylococcus aureus	18	18%
Acinetobacter spp.	8	8%
Pseudomonas aeruginosa	8	8%
Enterococcus spp.	6	6%
Budding yeast	4	4%
Proteus mirabilis	5	5%
Citrobacter spp.	2	2%
Enterobacter spp.	3	3%
Total	100	

Among the 100 isolates, Escherichia coli (28%) was the most common organism. This was followed by Klebsiella spp. (18%) and Staphylococcus aureus (18%). Other organisms were present in smaller proportions, including Acinetobacter spp. and Pseudomonas aeruginosa (8% each), Enterococcus spp. (6%), Proteus mirabilis (5%), Enterobacter spp. (3%), Citrobacter spp. (2%), and budding yeast (4%). Overall, the distribution shows a predominance of Enterobacterales and common hospital-associated pathogens.

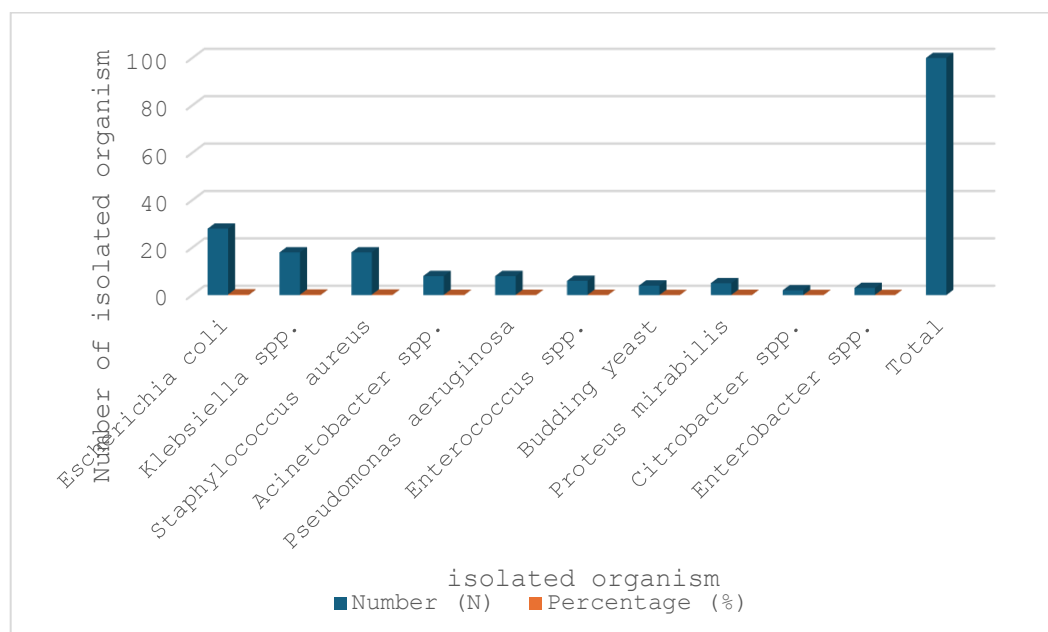


Figure 4: Graphical represents total number of organisms isolated.

Table 6: Antibiotic Resistance Pattern of ESBL-Producing E. coli Isolates from ICU Patients.

Antibiotic	No. Tested	Resistant (N)	Percentage (%)
Amikacin	30	7	23
Ampicillin	30	30	100
Amoxycavulanic acid	30	28	93
Aztreonam	30	24	80
Cefepime	30	28	93
Cefotaxime	30	29	97
Cefpodoxime	30	28	93
Ceftazidime	30	30	100
Ceftriaxone	30	30	100
Ciprofloxacin	30	22	73
Ertapenem	30	3	10
Gentamicin	30	7	23
Meropenem	30	4	13
Piperacillin–Tazobactam	30	8	27
Cotrimoxazole	30	16	53
Doxycycline	30	3	10
Cefuroxime	20	8	40
Nitrofurantoin	20	1	5

High levels of resistance were observed to several antibiotics, including ampicillin, ceftazidime, and ceftriaxone (100%), as well as cefotaxime (97%) and multiple other cephalosporins (>90%). Moderate resistance was seen to ciprofloxacin (73%) and cotrimoxazole (53%). In contrast, resistance to carbapenems was low, with ertapenem (10%) and meropenem (13%) showing good activity. Nitrofurantoin (5%), doxycycline (10%), amikacin (23%), gentamicin (23%), and piperacillin–tazobactam (27%) also showed relatively low resistance rates, indicating better effectiveness. Overall, the isolates demonstrated high resistance to β -lactams but better susceptibility to carbapenems and nitrofurantoin.

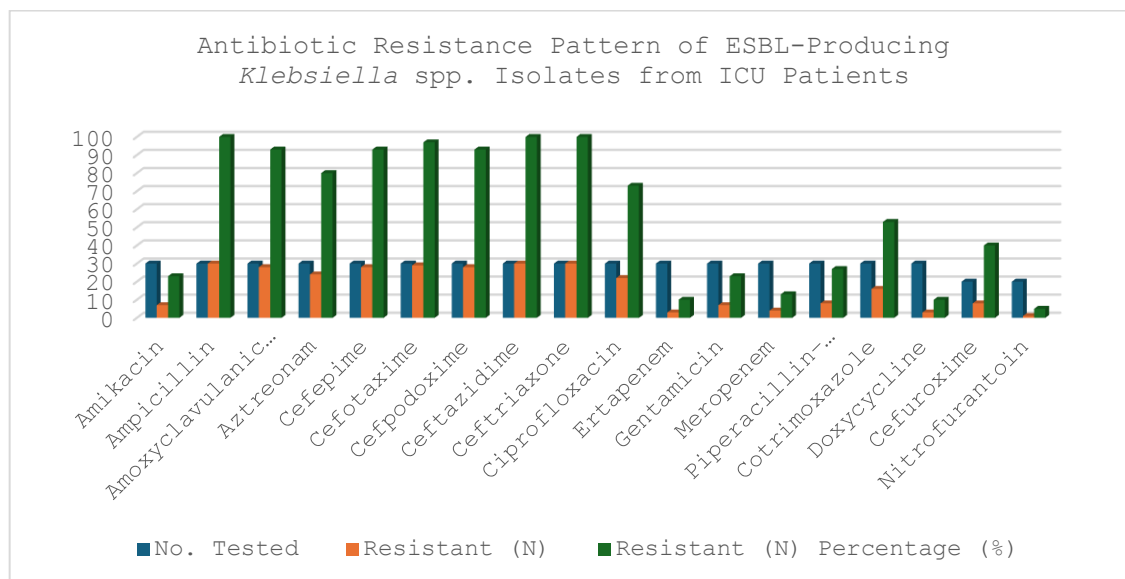


Figure 5: Graphical represents antibiotic Resistance Pattern of ESBL-Producing *Klebsiella* spp. Isolates from ICU Patients.

Table: 7: table represents distribution of Antibiotic Resistance and Sensitivity Among Tested Isolates.

Antibiotic	No. Tested	Resistant N (%)	Sensitive N (%)
Amikacin	20	9 (45%)	11 (55%)
Ampicillin	20	20 (100%)	0 (0%)
Amoxycylavulanic acid	20	19 (95%)	1 (5%)
Aztreonam	20	20 (100%)	0 (0%)
Cefepime	20	16 (80%)	4 (20%)
Cefotaxime	20	20 (100%)	0 (0%)
Cefpodoxime	20	20 (100%)	0 (0%)
Ceftazidime	20	20 (100%)	0 (0%)
Ceftriaxone	20	20 (100%)	0 (0%)
Ciprofloxacin	20	18 (90%)	2 (10%)
Doxycycline	20	8 (40%)	12 (60%)
Ertapenem	20	3 (15%)	17 (85%)
Gentamicin	20	6 (30%)	14 (70%)
Meropenem	20	4 (20%)	16 (80%)
Piperacillin/Tazobactam	20	14 (70%)	6 (30%)
Cotrimoxazole	20	15 (75%)	5 (25%)
Cefuroxime	10	7 (70%)	3 (30%)
Nitrofurantoin	10	2 (20%)	8 (80%)

There was high resistance to most β -lactams and fluoroquinolones, with 100% resistance to several cephalosporins and ampicillin. Moderate resistance was seen to piperacillin–tazobactam, cotrimoxazole, and amikacin. Carbapenems (ertapenem, meropenem) and nitrofurantoin showed the highest sensitivity, indicating they remain the most effective options.

Table 8: showing resistance pattern of ESBL producing *Proteus mirabilis*.

Antibiotic	No. Tested	Resistant N (%)	Sensitive N (%)
Ampicillin	2	2 (100%)	0 (0%)
Amoxycyclavulanic acid	2	0 (0%)	2 (100%)
Aztreonam	2	2 (100%)	0 (0%)
Cefotaxime	2	2 (100%)	0 (0%)
Cefuroxime	2	2 (100%)	0 (0%)
Ceftriaxone	2	2 (100%)	0 (0%)
Cefpodoxime	2	2 (100%)	0 (0%)
Ceftazidime	2	2 (100%)	0 (0%)
Cefepime	2	1 (50%)	1 (50%)
Ciprofloxacin	2	1 (50%)	1 (50%)
Doxycycline	2	0 (0%)	2 (100%)
Ertapenem	2	0 (0%)	2 (100%)
Gentamicin	2	0 (0%)	2 (100%)
Meropenem	2	0 (0%)	2 (100%)
Piperacillin/Tazobactam	2	1 (50%)	1 (50%)
Cotrimoxazole	2	0 (0%)	2 (100%)

Among the 2 isolates tested per antibiotic, there was complete resistance (100%) to most cephalosporins and ampicillin. Amoxycyclavulanic acid, doxycycline, carbapenems (ertapenem, meropenem), gentamicin, and cotrimoxazole were fully sensitive (100%). Ciprofloxacin, cefepime, and piperacillin–tazobactam showed intermediate resistance (50%). Overall, carbapenems and a few non- β -lactams remain highly effective.

Clinical correlation:

- High ESBL prevalence (41.6%) in ICU patients is likely linked to urinary catheterization and mechanical ventilation, common in critical care settings.⁷
- Previous antibiotic exposure, especially β -lactams, is a known risk factor for ESBL colonization and infection.
- Colonization surveillance (e.g., rectal swabs) may help guide empirical therapy and reduce overuse of carbapenems.
- These findings support the need for strict infection control, antimicrobial stewardship, and targeted empirical therapy in ICU patients.

Discussion: The study found a 41.6% prevalence of ESBL-producing Enterobacteriaceae among ICU patients, with *Escherichia coli* (28%) and *Klebsiella* spp. (18%) being the most common. Urine and endotracheal tube specimens showed the highest positivity, highlighting urinary and respiratory infections as key reservoirs.⁸ Most isolates were highly resistant to β -lactams and fluoroquinolones, while carbapenems, nitrofurantoin, and aminoglycosides remained largely effective. These findings underscore the need for antimicrobial stewardship, infection control, and routine surveillance in ICUs.⁹

Conclusion: ESBL-producing Enterobacteriaceae are common in ICU patients and show multidrug resistance, limiting treatment options. Carbapenems and select non- β -lactams remain effective, emphasizing the importance of judicious antibiotic use and strict infection control to prevent the spread of resistant organisms.

Limitations:

- Single-center design limits generalizability.
- No molecular characterization of ESBL genes.
- Prior antibiotic exposure not fully accounted for.
- Clinical outcomes (e.g., mortality, ICU stay) not measured.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this study.

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