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ESBL-PRODUCING ENTEROBACTERIACEAE IN ICU PATIENTS: PREVALENCE AND AMR PATTERNS IN PAKISTAN

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ABSTRACT

Background: *Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae are an escalating threat to global healthcare, particularly in Intensive Care Units (ICUs), due to their capacity for multidrug resistance and nosocomial spread.*

Objective: *To determine the prevalence and antimicrobial resistance profile of ESBL-producing Enterobacteriaceae among ICU patients in a tertiary care hospital.*

Methods: *100 clinical samples from ICU patients were processed using standard bacteriological methods. ESBL production was confirmed by phenotypic techniques including Combined Disk Test (CDT), Double Disk Synergy Test (DDST), and Three-Dimensional Test (TDT). Antimicrobial susceptibility was determined by the Kirby–Bauer method per CLSI 2024 guidelines.*

Results*: Of 100 samples, 58 isolates belonged to Enterobacteriaceae, among which 42 (72.4%) were ESBL producers. E. coli (52.3%) and Klebsiella spp. (38%) were predominant. Resistance was highest to β-lactams and third-generation cephalosporins, while carbapenems remained effective.* ***Conclusion:*** *The high prevalence of ESBL-producing Enterobacteriaceae in ICU settings highlights an urgent need for robust antibiotic stewardship and infection control policies*

Keywords: *ESBL, Enterobacteriaceae, ICU, Antibiotic resistance, Carbapenem, E. coli, Klebsiella spp*

INTRODUCTION

Antimicrobial resistance (AMR) represents one of the most significant global health crises of modern times, threatening the effectiveness of antibiotics that have revolutionized medicine. The World Health Organization (WHO) has identified antibiotic resistance as a "silent pandemic," predicting that by 2050, AMR could cause up to 10 million deaths annually if unchecked [1,2]. 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 [3–5].

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 [6,7]. In

the ICU setting, patients are often exposed to invasive procedures, broad-spectrum antibiotics, and prolonged hospitalization all of which contribute to colonization and infection by these resistant strains [8,9]. The immuno-compromised status of critically ill patients further exacerbates their vulnerability.

The biochemical basis of ESBL production involves the enzymatic hydrolysis of the β-lactam ring of penicillin and cephalosporins, rendering them ineffective. Although clavulanic acid and related inhibitors can suppress ESBLs, resistance often extends beyond β-lactams to other antibiotic classes such as aminoglycosides, fluoroquinolones, and sulfonamides due to co-localization of resistance genes on plasmids [10,11]. Molecularly, the most prevalent ESBL families include TEM, SHV, and CTX-M types, with CTX-M enzymes now dominating worldwide [12,13].

The global prevalence of ESBL producers has risen dramatically. Reports from the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) indicate that 15–30% of E. coli and Klebsiella isolates in hospitalized patients are ESBL positive [14]. In Pakistan, several multicentric studies have documented prevalence rates as high as 70– 85%, especially in tertiary care and ICU settings [15–17]. Regional variations are influenced by antibiotic misuse, hospital infection control practices, and local prescribing patterns [18,19].

The clinical implications are profound. Infections caused by ESBL producers are associated with increased mortality, longer hospital stays, higher healthcare costs, and limited treatment options. Carbapenems are considered the drugs of choice; however, overreliance on them has led to the emergence of carbapenemase-producing Enterobacteriaceae (CPE), creating a vicious cycle of resistance [20]. Consequently, understanding local resistance patterns and ESBL prevalence is essential to guiding empirical therapy and preventing therapeutic failure.

Phenotypic detection remains the cornerstone of ESBL surveillance in resource-limited settings, where molecular assays are often unavailable. Methods such as Double Disk Synergy Test (DDST), Combined Disk Test (CDT), and Three-Dimensional Test (TDT) offer reliable, cost-effective alternatives for routine laboratories [21]. The combination of these tests enhances sensitivity and ensures accurate identification of ESBL producers for infection control purposes.

This study was undertaken to determine the prevalence and antimicrobial resistance profile of ESBL-producing Enterobacteriaceae isolated from ICU patients in a tertiary care hospital. The findings help provide baseline data for local antibiotic policy formulation and reinforce the need for stringent infection prevention measures.

MATERIALS AND METHODS

This observational study was conducted in Department of Microbiology of Aziz Fatima hospital, Faisalabad.

Inclusion criteria: 1. ICU patients

Exclusion criteria:

1. OPD patients
2. Patients without indwelling medical devices
3. Refusal of consent
4. Repeat isolates of same organism from same type of sample within 72 hours
5. Unacceptable sample quality (contaminated sample).

SPECIMEN: All clinical isolates from ICU.

All the clinical samples were collected under all asceptic precautions and were transported to the bacteriology laboratory of Department of Microbiology of Aziz Fatima hospital, Faisalabad. The samples were first inoculated on MacConkey agar and Blood agar. For urine, UTI chrome agar is used. Identification of the organism was done by established departmental practices. Microscopy was performed by making wet mounts and gram staining and observations were recorded on data sheets.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Antibiotic sensitivity was performed using Kirby & Bauer's disk diffusion method on Mueller Hinton agar plates as per Clinical Laboratory standards Institute (CLSI) guideline. The antibiotic discs used for gram negative isolates were: Ceftazidime 30 mcg, Cefoxitin 30 mcg, Cefotaxime 30 mcg, Cefuroxime 30 mcg, Cefipime 30 mcg, Amikacin 30 mcg, Aztreonam 30mcg , Meropenem 10 mcg , Tobramycin 10 mcg, Gentamicin 10 mcg , Ciprofloxacin 5 mcg, Piperacillin Tazobactam 100/10 mcg, Ampicillin 10 mcg , Amoxicillin – Clavulanate 20/10 mcg , Trimethoprim-Sulfamethoxazole 1.25/23.75 mcg

, Chloremphenicol 30 mcg, Tetracycline 30 mcg. In addition Nitrofurantoin 300 mcg, Norfloxacin 10 mcg, were tested in urinary isolates. E.Coli ATCC 25922 and Pseudomonas ATCC 27853 were used as controls for the disc diffusion test. The zone of inhibition of bacterial growth around the disc is measured in mm by using measuring scale, which represents susceptibility of the organism to that particular drug.

SCREENING FOR ESBL PRODUCERS:

- If any of the isolates had zone of inhibition for 3rd generation Cephalosporins i.e., Cefpodoxime (10µg)≤ 17mm, Ceftazidime (30 µg) ≤ 22mm, Aztreonam (30µg) ≤ 27mm, Cefotaxime (30µg) ≤27mm and Ceftriaxone (30µg) ≤ 25mm (ESBL breaking point) were taken as screen positive for ESBL.
- All strains found to be ESBL screen test positive were subjected to further confirmation by CLSI recommended phenotypic confirmatory tests.

DOUBLE DISK SYNERGY TEST (DOUBLE DISK DIFFUSION TEST):

A 0.5 McFarland bacterial suspension was inoculated on Mueller hinton agar plate. Augmentin (20 µg amoxicillin and 10 µg clavulanic acid) disc was placed in the center of the inoculated plate. Three 3rd generation cephalosporin (ceftazidime 30µg, ceftriaxone 30µg, cefotaxime 30µg) and one monobactam (aztreonam 30µg) discs were placed at 20 mm distance from augmentin disc. The plate was incubated overnight at 37^{0}C.

INTERPRETATION:

A positive result (ESBL production) was indicated by an increase in zone diameter by > 5mm around the disks with clavulanic acid over the disks with cephalosporins alone.

PHENOTYPIC CONFIRMATORY TEST (COMBINED DISC TEST):

Both DDST positive and negative strains were analysed by CLSI confirmatory test. A standardized 0.5 McFarland suspension of the test isolate was swabbed on the Mueller Hinton agar. Disks of Ceftazidime (CAZ 30 mcg) and Ceftazidime with Clavulanic acid (CAC-30/10mcg) and Cefotaxime (CTX 30mcg) and Cefotaxime with Clavulanic acid (CEC 30/10mcg) were dispensed at a minimum distance of 24 mm on an MHA agar plate and incubated aerobically at 37c for 16-18 hours.

INTERPRETATION:

A positive result was indicated by Extension of the edge of the inhibition zone of ceftazidime, ceftriaxone, cefotaxime and aztreonam disc on the side exposed to the augmentin disc.This extension of edge of inhibition is due to synergy of disc of Augmentin with the four discs used, three of third generation cephalosporin and one of aztreonam disk.

Quality Control: E. coli ATCC 25922 and K. pneumoniae ATCC 700603 were used as control strains.

Statistical Analysis: Data was analyzed using Microsoft Excel. Descriptive statistics were expressed as percentages and proportions

RESULTS

A total of 100 clinical samples were processed. The majority of patients were aged 21–40 years (32%), followed by 41–60 years (26%). Males constituted 65% of the study population.

TABLE 1: SHOWING AGE DISTRIBUTION OF PATIENTS ADMITTED IN ICU

Age (in years)	Total number of patients N (%)
< 1	4 (4%)
1-20	22 (22%)
21-40	32 (32%)
41-60	26 (26%)
61-80	16 (16%)

Maximum patients belonged to the age group between 21 and 40 years (32%) followed by the age group between 41 and 60 years which constituted 26%. Least affected was the age less than 1 year.

FIGURE 1: BAR GRAPH SHOWING AGE DISTRIBUTION OF PATIENTS ADMITTED IN ICU

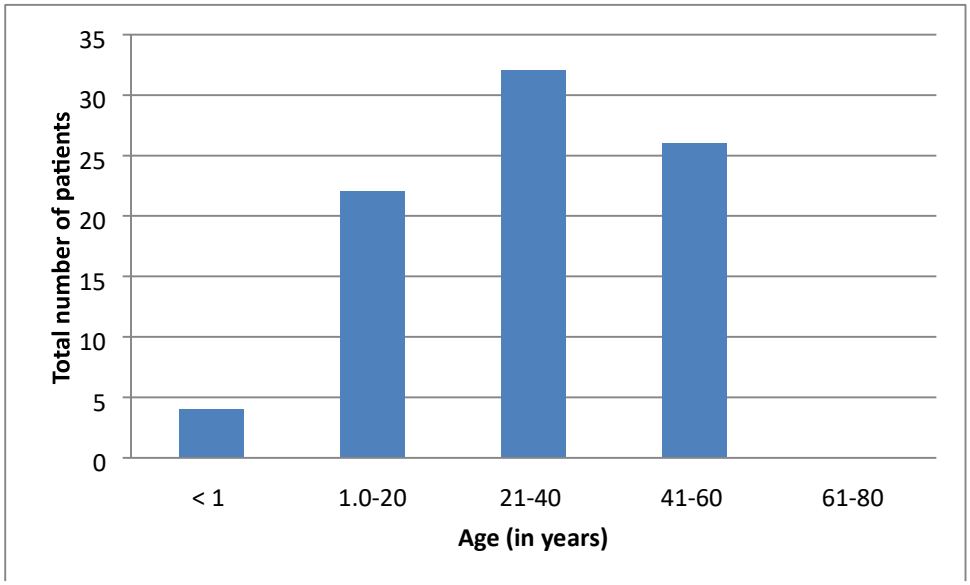
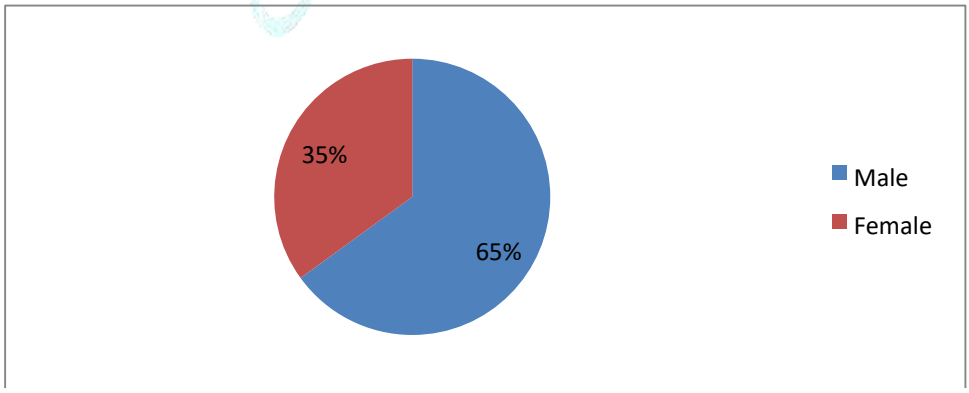


TABLE 2: SHOWING GENDER DISTRIBUTION OF PATIENTS ADMITTED IN ICU

Gender	Number N (%)
Male	65 (65%)
Female	35 (35%)

Among 100 patients admitted in ICU, maximum patients were males constituting 65% whereas females constituted only (35%).

FIGURE 2: PIE CHART SHOWING GENDER DISTRIBUTION OF PATIENTS ADMITTED IN ICU



Urine and blood were the most frequent sample types (25% each), followed by endotracheal tube aspirates (18%), pus (10%), and others. Gram-negative bacilli predominated (74%) among isolates, while gram-positive cocci and yeasts accounted for 22% and 4%, respectively.

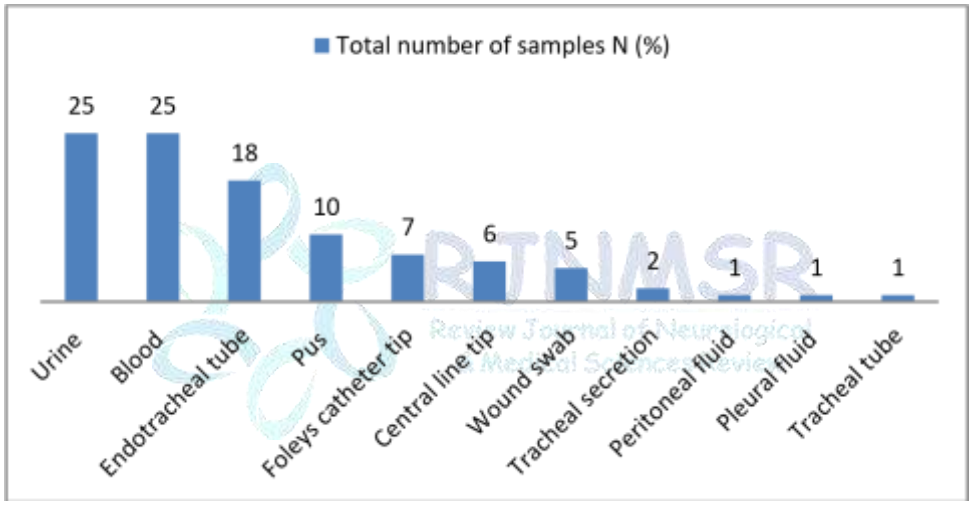
TABLE 3: DISTRIBUTION OF DIFFERENT SAMPLES COLLECTED FROM ICU

Sample	Total number of samples N (%)
Urine	25 (25%)
Blood	25 (25%)
Endotracheal tube	18 (18%)

Pus	10 (10%)
Foleys catheter tip	7 (7%)
Central line tip	6 (6%)
Wound swab	5 (5%)
Tracheal secretion	2 (2%)
Peritoneal fluid	1 (1%)
Pleural fluid	1 (1%)
Tracheal tube	1 (1%)

In our study Urine and Blood was taken as sample from 25% of patients respectively, followed by Endotracheal tube from 18% of patients, Pus from 10% of patients, Foleys catheter tip from 7% of patients , Central line tip from 6% of patients, Wound swab from 5% of patients and others (Tracheal secretion, Peritoneal fluid, Pleural fluid and Tracheal tube) from 5% of patients.

FIGURE 3: BAR GRAPH SHOWING DISTRIBUTION OF SAMPLES COLLECTED FROM ICU



Within the Gram-negative bacilli group, Enterobacteriaceae accounted for 58% of isolates. Among them, E. coli (30%) was most frequent, followed by Klebsiella spp. (20%), Proteus mirabilis (4%), Enterobacter spp. (2%), and Citrobacter spp. (2%).
Out of 58 Enterobacteriaceae isolates, 42 (72.4%) were ESBL producers. E. coli represented 52.3%, Klebsiella spp. 38%, and Proteus mirabilis 9.5% of the ESBL-producing isolates. Combined Disk Test detected the highest number of ESBLs (83%), followed by TDT (47.6%) and DDST (26%).

Table 5: Showing distribution of gram positive cocci and gram negative bacilli.

Organism	Number N(%)
Gram negative bacilli	74 (74%)
Gram positive cocci	22 (22%)
Budding yeast	4(4%)

In our study, gram negative bacilli dominated with 74%, whereas gram positive cocci constituted only 22% and budding yeast constituted 4%.

Figure 4: Pie chart showing distribution of gram positive cocci and gram negative bacilli.

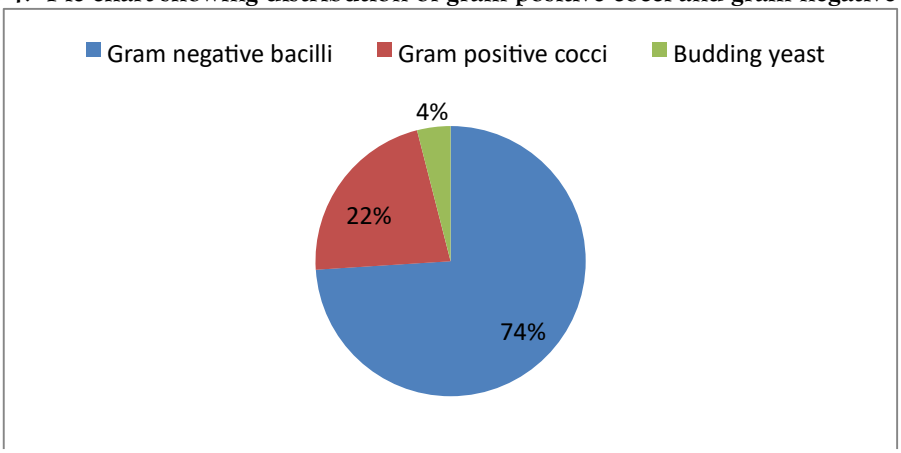


TABLE 6: TYPE AND TOTAL NUMBER OF ORGANISMS ISOLATED

Organism	Number of organism N (%)
Escherichia coli	30 (30%)
Klebsiella sp.	20 (20%)
Staphylococcus aureus	18 (18%)
Acinetobacter baumanni	8 (8%)
Pseudomonas aeruginosa	8 (8%)
Enterococcus sp	4 (4%)
Budding yeast	4 (4%)
Proteus mirabilis	4 (4%)
Citobacter sp	2 (2%)
Enterobacter sp	2 (2%)

Among gram negative bacilli, E.coli was frequently isolated (30%), followed by Klebsiella spp (20%), Acinetobacter baumanii (8%), Pseudomonas aeruginosa (8%),Proteus mirabilis (4%), Citrobacter spp (2%) and Enterobacter spp (2%).
Among gram positive , Staphylococcus aureus (18%) was frequently isolated followed by Enterococcus spp (4%).

Figure 5: Bar graph showing different types and number of organisms isolated

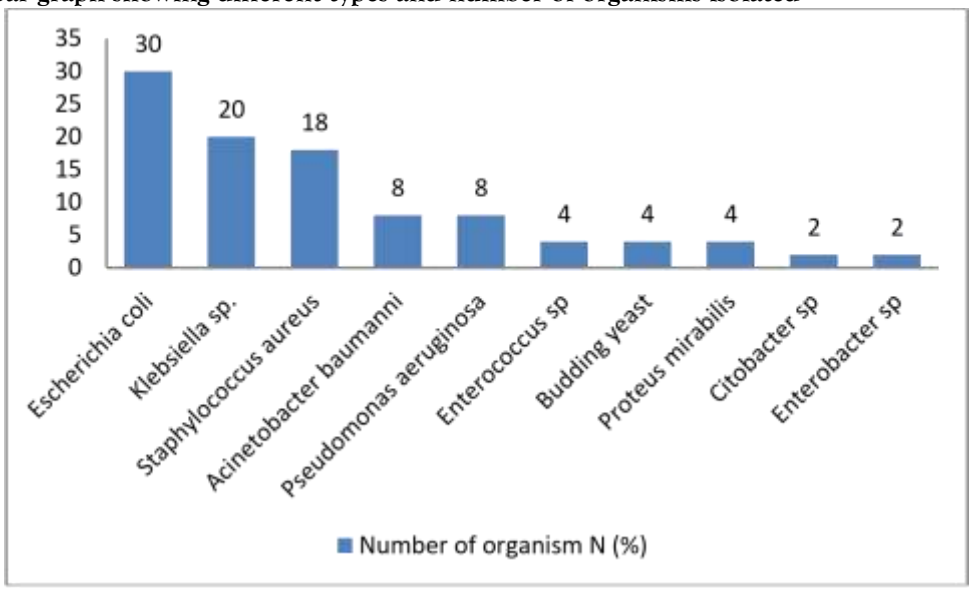


FIGURE 6. DOUBLE DISK SYNERGY TEST

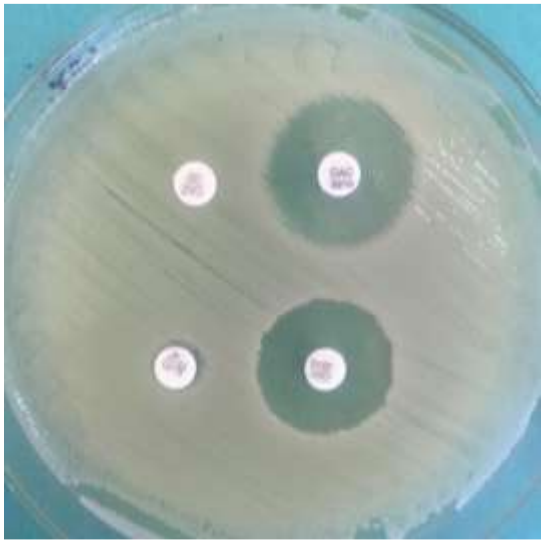


Figure 7. Phenotypic confirmatory test

TABLE 7: TABLE SHOWING RESISTANCE PATTERN OF ESBL PRODUCING E.COLI

Antibiotic	No. tested	ESBL +ve
		Resistance N (%)
Amikacin	30	7 (23%)
Ampicillin	30	30(100%)
Amoxyclavulanic acid	30	28(93%)
Aztreonam	30	24 (80%)
Cefepime	30	28 (93%)
Cefotaxime	30	29 (96.6%)
Cefpodoxime	30	28 (93%)
Ceftazidime	30	30(100%)
Ceftriaxone	30	30(100%)
Ciprofloxacin	30	22 (73%)
Ertapenem	30	3 (10%)
Gentamycin	30	7 (23%)
Meropenem	30	4 (13%)
Pipercillin/tazobactam	30	8 (26.6%)
Cotrimoxazole	30	16(53%)
Doxycyclin	30	3 (10%)
Cefuroxime	20	8 (40%)
Nitofurantoin	20	1(5%)

Figure 8: Bar graph showing resistance pattern of ESBL producing E.coli

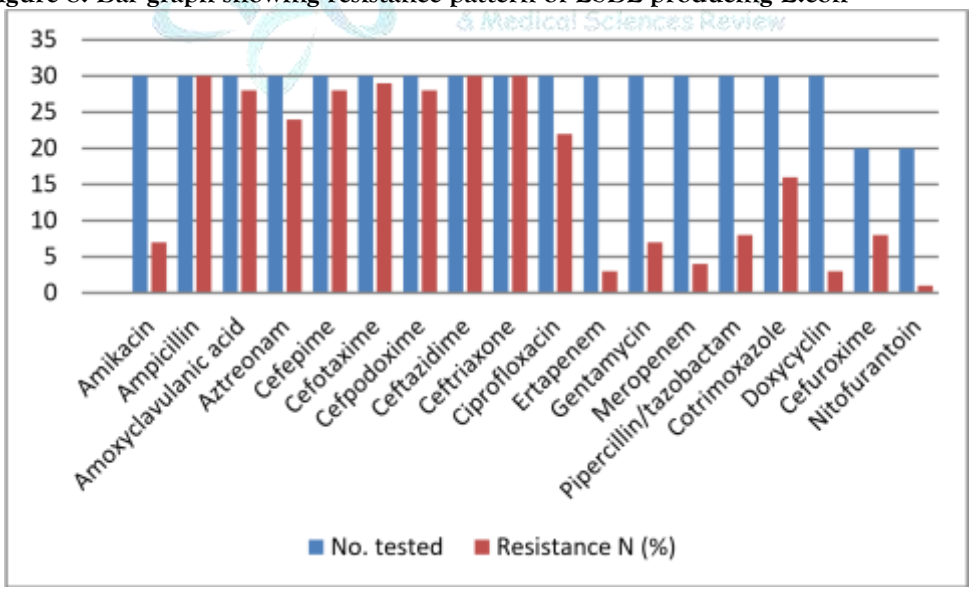


Table 8:Table showing resistance pattern of ESBL producing Klebsiella spp.

Antibiotic	No. tested	ESBL +ve
		Resistance (N%)
Amikacin	20	9 (45%)
Ampicillin	20	20 (100%)
Amoxyclavulanic acid	20	19 (95%)

Aztreonam	20	20 (100%)
Cefepime	20	16(80%)
Cefotaxime	20	20(100%)
Cefpodoxime	20	20 (100%)
Ceftazidime	20	20(100%)
Ceftriaxone	20	20(100%)
Ciprofloxacin	20	18(90%)
Doxycyclin	20	8(40%)
Ertapenem	20	3(15%)
Gentamycin	20	6(30%)
Meropenem	20	4(20%)
Pipercillin/tazobactam	20	14(70%)
Cotrimoxazole	20	15(75%)
Cefuroxime	10	7(70%)
Nitofurantoin	10	2(20%)

Figure 9: Bar graph showing resistance pattern of ESBL producing Klebsiella spp.

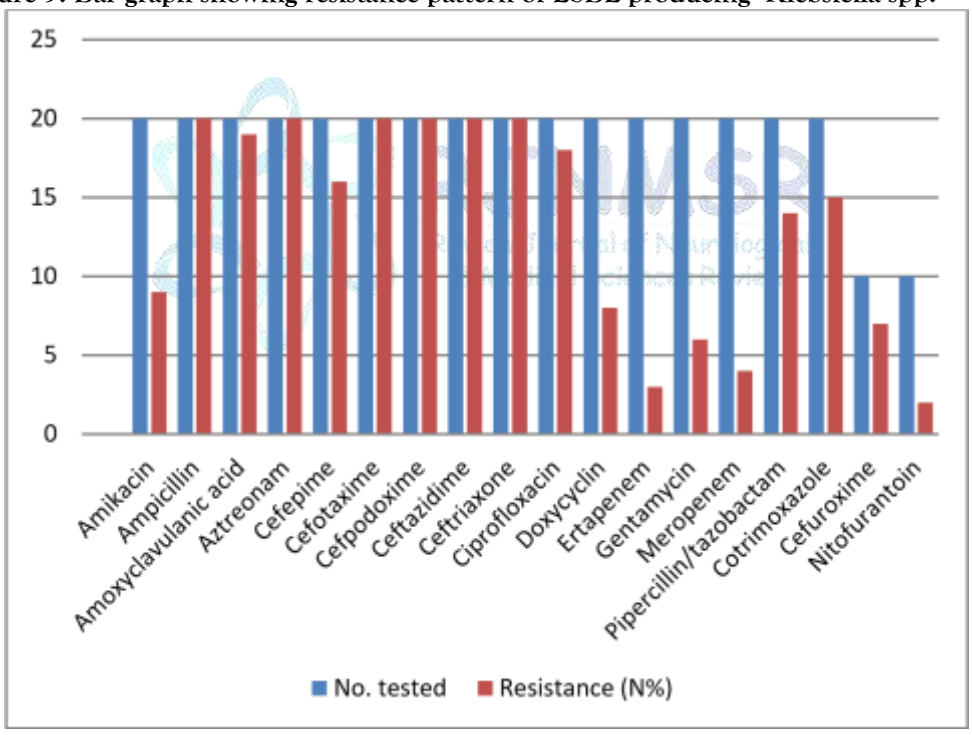
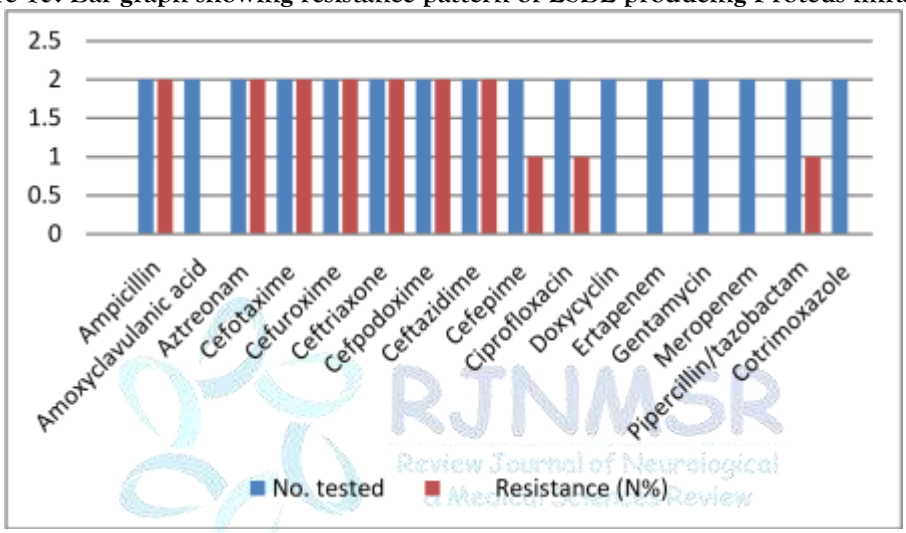


Table 9: Table showing resistance pattern of ESBL producing Proteus mirabilis.

Antibiotic	No. tested	ESBL +ve
		Resistance (N%)
Ampicillin	2	2(100%)
Amoxyclavulanic acid	2	0%
Aztreonam	2	2(100%)
Cefotaxime	2	2(100%)
Cefuroxime	2	2(100%)
Ceftriaxone	2	2(100%)

Cefpodoxime	2	2(100%)
Ceftazidime	2	2(100%)
Cefepime	2	1(50%)
Ciprofloxacin	2	1(50%)
Doxycyclin	2	0%
Ertapenem	2	0%
Gentamycin	2	0%
Meropenem	2	0%
Pipercillin/tazobactam	2	1(50%)
Cotrimoxazole	2	0%

Figure 10: Bar graph showing resistance pattern of ESBL producing Proteus mirabilis.



Antimicrobial resistance was alarmingly high to β-lactams and cephalosporins—ampicillin (100%), ceftazidime (100%), ceftriaxone (100%), cefotaxime (96.6%), and amoxyclav (93%). Resistance to aminoglycosides and fluoroquinolones was moderate. Carbapenems showed the lowest resistance:
ertapenem (10%) and meropenem (13%), indicating retained efficacy.

DISCUSSION

The present study revealed a high prevalence (72.4%) of ESBL-producing Enterobacteriaceae among ICU isolates, indicating a serious antimicrobial resistance burden in the tertiary care setting. This finding is comparable to studies by Kaur et al. [10] and Datta et al. [13], who reported ESBL rates of 70–75% among Gram-negative ICU isolates. The predominance of E. coli and Klebsiella spp. mirrors the epidemiological trends observed globally, reaffirming their central role in healthcare-associated infections [20,25].

The proportion of E. coli (52.3%) among ESBL producers in this study aligns with the findings of Sharma et al. [22] and Bhattacharya [12], who documented E. coli as the most frequent ESBL producing pathogen in both community and hospital settings. In contrast, some studies, such as Behera et al. [14], found Klebsiella pneumoniae as the leading ESBL producer, possibly due to differences in sample type and patient demographics.

Antibiotic susceptibility patterns showed near-universal resistance to ampicillin and third-generation cephalosporins. This pattern is consistent with multiple studies worldwide, reflecting extensive use and consequent selection pressure of these drugs [7,26]. The persistence of susceptibility to carbapenems (meropenem and ertapenem) offers some therapeutic relief; however, isolated carbapenem resistance (10–13%) signals the emergence of carbapenemase-

producing strains, which have been increasingly reported in Asia and the Middle East [24,27].

Our results also resonate with reports by Iqbal et al. [19], who noted that over 70% of Enterobacteriaceae in ICU patients were ESBL positive, with similar resistance trends to β-lactams and aminoglycosides. A multicentric study by Gandra et al. [28] reported 64% ESBL production among E. coli isolates, highlighting the nationwide spread of resistance.

The differences in ESBL detection rates by phenotypic methods observed in our study (CDT 83%, TDT 47.6%, DDST 26%) emphasize the variable sensitivity of each technique. Similar observations were reported by Thomson and Sanders [26], who found the Combined Disk Test to be more reliable for routine laboratory detection. CLSI (2024) guidelines also recommend confirmatory testing using combination methods for increased accuracy [27].

From a clinical standpoint, infections by ESBL producers are associated with increased mortality, prolonged hospital stay, and higher healthcare costs. Schwaber and Carmeli [7] reported a 57% higher mortality risk in patients infected with ESBL-producing Klebsiella pneumoniae compared to non-ESBL strains. In ICUs, empirical therapy with broad-spectrum cephalosporins often fails, necessitating timely detection of resistance to prevent therapeutic delays [8,20].

Comparatively, studies from Europe and the US show lower ESBL prevalence (15–25%) due to stringent antimicrobial stewardship programs and infection control surveillance [14,29]. The significantly higher rates in Pakistan and other low- and middle-income countries reflect challenges such as over-the-counter antibiotic sales, inadequate infection control infrastructure, and limited laboratory resources [17,28].

The global spread of ESBL genes, particularly CTX-M-15, underscores the importance of molecular surveillance [13,21]. Although this study relied on phenotypic detection, it provides crucial epidemiological insights into the magnitude of resistance within ICUs—a high-risk zone for multidrug-resistant organisms.

CONCLUSION

This study confirms the high prevalence of ESBL-producing Enterobacteriaceae among ICU patients, with E. coli and Klebsiella spp. being the leading isolates. High resistance to β-lactams and cephalosporins, coupled with emerging carbapenem resistance, demands urgent attention. Implementation of antibiotic stewardship programs, strict infection control practices, and continuous surveillance are vital to control the spread of these resistant pathogens.

LIMITATIONS

1. The study used only phenotypic methods without molecular confirmation (TEM, SHV, CTX-M).
2. The sample size was modest and limited to one institution.
3. Temporal trends and patient outcomes were not analyzed longitudinally.

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