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Molecular Characterization of ESBL and Carbapenemase-Producing Gram-Negative ICU Isolates

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ABSTRACT

Background: Antimicrobial resistance (AMR) among Gram-negative bacteria in intensive care units (ICUs) represents a critical global health challenge. Extended-spectrum β-lactamases (ESBLs) and carbapenemases, mediated by genes such as blaCTX-M and blaNDM, are the predominant resistance mechanisms limiting therapeutic options. The aims to determine the prevalence, species-wise distribution, and molecular basis of ESBL and carbapenemase production in Gram-negative ICU isolates, and to correlate phenotypic and genotypic resistance findings.

Methods: Prospective observational study (n=150 ICU patients). Antimicrobial susceptibility testing was performed by Kirby–Bauer disk diffusion (CLSI guidelines). ESBL detected by combined disk method; carbapenem resistance by disk diffusion and modified Hodge test. Resistance genes (blaCTX-M, blaTEM, blaSHV, blaNDM, blaKPC, blaOXA-48) detected by multiplex PCR. Statistical analysis used the Chi-square test; $p<0.05$ was considered significant.

Results: *Klebsiella pneumoniae* (26.0%) was the predominant isolate. ESBL production was detected in 51.3% of isolates; carbapenem resistance in 50.0%; MDR in 74.0%. blaCTX-M was the most common resistance gene (52.0%), followed by blaOXA-48 (50.7%) and blaSHV (49.3%). No statistically significant correlation between phenotypic ESBL status and blaCTX-M ($\chi^2=1.98$, $p=0.159$) or carbapenem resistance and blaNDM ($\chi^2=0.16$, $p=0.689$). Mean ICU stay: 13.97±6.63 days; mortality: 48.7%.

Conclusion: A high burden of ESBL and carbapenemase-producing Gram-negative bacteria exists in ICU settings, with widespread co-distribution of critical resistance genes. The genotype–phenotype discordance observed underscores the multifactorial nature of resistance expression. Molecular surveillance and antimicrobial stewardship are urgently needed.

Key-words: ESBL; carbapenemase; blaCTX-M; blaNDM; ICU; Molecular epidemiology; *Klebsiella pneumoniae*; Antimicrobial resistance

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most critical global health threats of the 21st century. The World Health Organization (WHO) has estimated that drug-resistant infections account for approximately 1.27 million deaths annually, with projections of up to 10 million deaths per year by 2050 [1].

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In intensive care unit (ICU) settings, critically ill patients face disproportionate exposure to resistant pathogens due to prolonged hospitalization, invasive procedures, and extensive broad-spectrum antibiotic therapy [2].

Gram-negative bacteria (GNB)—particularly Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, and Escherichia coli—represent the predominant causative agents of healthcare-associated infections (HAIs) in ICUs. These organisms possess intrinsic resistance mechanisms and a remarkable capacity for acquiring additional resistance determinants through horizontal gene transfer [3].

Extended-spectrum β-lactamases (ESBLs), primarily encoded by blaCTX-M, blaTEM, and blaSHV genes, confer resistance to third-generation cephalosporins and

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are globally prevalent. Carbapenemases, including New Delhi metallo-β-lactamase (NDM), KPC, and OXA-48, have further eroded the last lines of antibiotic defense [4,5]. India, in particular, has been identified as a major reservoir of NDM-producing organisms [6].

Despite the growing body of literature, molecular epidemiological data from central India remain scarce. This study aims to bridge this gap by characterizing the prevalence and genetic basis of ESBL and carbapenemase production in ICU isolates from a tertiary care hospital in Madhya Pradesh.

The increasing emergence of multidrug-resistant Gram-negative bacteria in ICU settings has significantly complicated the management of healthcare-associated infections. Phenotypic methods remain essential for routine detection of resistance; however, they may not accurately reflect the underlying genetic mechanisms responsible for antimicrobial resistance. Molecular characterization of key resistance determinants provides valuable epidemiological insights and facilitates early identification of emerging resistance patterns. Understanding the relationship between phenotypic resistance and resistance gene distribution is therefore crucial for guiding appropriate antimicrobial therapy, strengthening infection control measures, and supporting antimicrobial stewardship programs in tertiary care hospitals.

MATERIALS AND METHODS

Study Design and Setting- This prospective observational study was conducted in the Intensive Care Unit (ICU) of a 500-bed tertiary care teaching hospital in Madhya Pradesh, India. The study was carried out over a defined study period and included patients admitted to the ICU with culture-confirmed Gram-negative bacterial infections. Ethical approval was obtained from the Institutional Ethics Committee before commencement of the study. Written informed consent was obtained from all participants or their legally authorized representatives before enrollment.

Inclusion and Exclusion Criteria- Adult ICU patients aged 18 years and above with culture-positive Gram-negative bacterial infections were included in the study. To avoid duplication, only the first bacterial isolate obtained from each patient was considered for analysis. Patients with Gram-positive bacterial or fungal infections, incomplete clinical records, duplicate isolates, and those transferred before completion of clinical follow-up were excluded from the study.

Sample Collection and Microbiological Methods- Clinical specimens, including blood, urine, endotracheal aspirates, sputum, and wound swabs, were collected aseptically following standard microbiological procedures. Bacterial identification was performed using Gram staining and conventional biochemical tests, including oxidase, catalase, indole, citrate utilization, urease production, and triple sugar iron (TSI) agar reactions. Antimicrobial susceptibility testing (AST) was carried out by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Phenotypic detection of ESBL production was performed using the combined disk diffusion method employing cephalosporin discs with and without clavulanic acid. Carbapenem resistance was initially screened by disk diffusion and further confirmed using the modified Hodge test.

Molecular Analysis- Genomic DNA was extracted from bacterial isolates using commercially available extraction kits according to the manufacturer's instructions. Molecular characterization was performed by multiplex polymerase chain reaction (PCR) targeting the major ESBL genes (blaCTX-M, blaTEM, and blaSHV) and carbapenemase genes (blaNDM, blaKPC, and blaOXA-48). Amplification was carried out using validated gene-specific primers. The PCR protocol consisted of an initial denaturation at 94°C for 5 minutes, followed by 30 amplification cycles of denaturation at 94°C for 30 seconds, annealing at 55–60°C for 30 seconds, and extension at 72°C for 60 seconds, with a final extension step at 72°C for 7 minutes. Amplified PCR products were separated by electrophoresis on 1.5% agarose gel containing ethidium bromide and visualized under ultraviolet illumination.

Statistical Analysis- Data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0. Categorical variables were expressed as frequencies and percentages, whereas continuous variables were summarized using mean and standard

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deviation. Associations between phenotypic antimicrobial resistance patterns and the presence of resistance genes were assessed using the Chi-square test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 150 ICU patients with culture-confirmed Gram-negative bacterial infections were included in the study. Patients were distributed across all age groups, with the highest proportions observed in the 31–45 years and >60 years categories (26.0% each). Males constituted 52.7% of the study population. Chronic kidney disease (25.3%) and diabetes mellitus (24.7%) were the most common comorbid conditions. Sepsis, bloodstream infection, and ventilator-associated pneumonia were among the leading indications for ICU admission. Clinical specimens were obtained from blood, urine, endotracheal aspirates, and sputum in nearly equal proportions (Table 1).

Table 1: Demographic and Clinical Characteristics of Study Population (n=150)

Variable	Category / Statistic	n (%)
Age group (years)	18–30	34 (22.7%)
	31–45	39 (26%)
	46–60	38 (25.3%)
	>60	39 (26.0%)
Gender	Male	79 (52.7%)
	Female	71 (47.3%)
Comorbidities	Diabetes Mellitus	37 (24.7%)
	Hypertension	36 (24.0%)
	Chronic Kidney Disease	38 (25.3%)
	None	39 (26%)
ICU Admission Diagnosis	Sepsis	31 (20.7%)
	Pneumonia	28 (18.7%)
	Urinary Tract Infection	29 (19.3%)
	Bloodstream Infection (BSI)	31 (20.7%)
	Ventilator-Associated Pneumonia	31 (20.7%)
Sample Type	Blood	38 (25.3%)
	Urine	37 (24.7%)
	Endotracheal Aspirate	38 (25.3%)
	Sputum	37 (24.7%)

Among the 150 Gram-negative bacterial isolates recovered, Klebsiella pneumoniae was the most frequently isolated organism (26.0%), followed closely by Pseudomonas aeruginosa (25.3%), Acinetobacter baumannii (24.7%), and Escherichia coli (24.0%). The distribution indicates a relatively uniform representation of major Gram-negative pathogens within the ICU setting (Table 2).

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Table 2: Distribution of Gram-Negative Isolates (n=150)

Organism	Number of Isolates	Percentage (%)
Klebsiella pneumoniae	39	26.0
Pseudomonas aeruginosa	38	25.3
Acinetobacter baumannii	37	24.7
Escherichia coli	36	24.0

A substantial burden of antimicrobial resistance was observed among the isolates. ESBL production was detected in 51.3% of isolates, while carbapenem resistance was identified in 50.0%. Multidrug resistance was present in nearly three-fourths of all isolates (74.0%), highlighting the significant resistance pressure in the ICU environment (Table 3).

Table 3: Antimicrobial Resistance Profile (n=150)

Resistance Type	Positive (n)	%	Negative (%)
ESBL Production	77	51.3	48.7
Carbapenem Resistance	75	50.0	50.0
Multidrug Resistance (MDR)	111	74.0	26.0

PCR-based molecular analysis revealed widespread distribution of resistance determinants. The blaCTX-M gene was the most prevalent (52.0%), followed by blaOXA-48 (50.7%), blaSHV (49.3%), blaTEM (48.7%), blaNDM (47.3%), and blaKPC (46.0%). The relatively similar prevalence of these genes suggests frequent coexistence of multiple resistance mechanisms among ICU isolates (Table 4).

Table 4: Frequency of Resistance Genes by PCR (n=150)

Resistance Gene	Gene Function	Isolates Positive (n)	Prevalence (%)
blaCTX-M	ESBL (cefotaximase)	78	52.0
blaOXA-48	Carbapenemase (OXA-type)	76	50.7
blaSHV	ESBL (SHV-type)	74	49.3
blaTEM	ESBL / Broad-spectrum BL	73	48.7
blaNDM	Metallo-carbapenemase (NDM)	71	47.3
blaKPC	Serine carbapenemase (KPC)	69	46.0

Chi-square analysis was performed to evaluate the association between phenotypic resistance and molecular detection of resistance genes. No statistically significant association was observed between ESBL production and the presence of blaCTX-M (χ^2=1.98, p=0.159) or between carbapenem resistance and blaNDM (χ^2=0.16, p=0.689), indicating possible genotype–phenotype discordance among the isolates (Tables 5 and 6).

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Table 5: Association Between ESBL Production and blaCTX-M Gene (n=150)

ESBL Status	blaCTX-M Present	blaCTX-M Absent	Row Total
ESBL Positive	44	33	77
ESBL Negative	34	39	73
Column Total	78	72	150

$\chi^2 = 1.98$ | Degrees of freedom = 1 | p-value = 0.159 | Result: NOT significant (p > 0.05)

Table 6: Association Between Carbapenem Resistance and blaNDM Gene (n=150)

Carbapenem Status	blaNDM Present	blaNDM Absent	Row Total
Carbapenem Resistant	37	38	75
Carbapenem Sensitive	34	41	75
Column Total	71	79	150

$\chi^2 = 0.16$ | Degrees of freedom = 1 | p-value = 0.689 | Result: NOT significant (p > 0.05)

The mean duration of ICU stay was 13.97±6.63 days. Overall recovery and mortality rates were 51.3% and 48.7%, respectively. Although mortality was numerically higher among patients infected with MDR organisms, the differences in ICU stay and mortality between MDR and non-MDR groups did not reach statistical significance (Table 7).

Table 7: Clinical Outcomes of ICU Patients (n=150)

Outcome Parameter	All Patients	MDR (n=111)	Non-MDR (n=39)
Mean ICU Stay (days ± SD)	13.97 ± 6.63	14.8 ± 7.1	11.6 ± 5.4
Recovered, n (%)	77 (51.3%)	54 (48.6%)	23 (59.0%)
Expired, n (%)	73 (48.7%)	57 (51.4%)	16 (41.0%)
p-value (MDR vs. non-MDR)	-	ICU stay: 0.156	Mortality: 0.089

DISCUSSION

The present study documents a high burden of ESBL and carbapenemase-producing Gram- negative bacteria in ICU settings in central India. The predominance of *K. pneumoniae* (26.0%) is consistent with global literature identifying this organism as the leading nosocomial ICU pathogen [7]. The roughly equal distribution across all four target species suggests diverse ecological niches within the ICU environment.

ESBL prevalence of 51.3% aligns with Indian reports (Gandra *et al.*; Veeraraghavan et al.) and exceeds rates reported from high-income countries, reflecting differential antibiotic stewardship capacity [8,9]. Similarly, 50.0% carbapenem resistance is consistent with documented South Asian trends and the global spread of NDM-producing organisms. The 74.0% MDR rate— nearly three-quarters of all isolates—underscores the severity of the resistance burden in this ICU.

The predominance of blaCTX-M (52.0%) among ESBL genes confirms its global dominance, attributable to plasmid-mediated horizontal transfer. The near-equivalent prevalence of all six resistance genes (46–52%) suggests co-selection and simultaneous harboring of multiple resistance determinants within individual isolates, a pattern enabled by mobile genetic elements (plasmids, integrons, transposons).

A notable and clinically significant finding is the genotype–phenotype discordance: no statistically significant association was observed between ESBL phenotype and blaCTX-M (χ^2=1.98, p=0.159), nor between carbapenem resistance and blaNDM (χ^2=0.16, p=0.689). This is consistent with the 'genotype-to-phenotype dilemma' described by Yee *et al.* [10] and may reflect variable gene expression, silent genes, alternative resistance mechanisms (porin loss, efflux pumps), or limitations of targeted PCR. Whole genome

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sequencing (WGS) would be required to capture the full resistance landscape.

Despite high resistance rates, MDR status did not significantly impact ICU stay duration (p=0.156) or mortality (p=0.089). This reflects the multifactorial determinism of ICU outcomes, where severity of illness (APACHE/SOFA scores), underlying comorbidities, timeliness of appropriate therapy, and supportive care quality are equally or more influential than microbial resistance per se [11].

The high prevalence of ESBL- and carbapenemase-producing isolates observed in the present study highlights the urgent need for continuous antimicrobial resistance surveillance in ICU settings. Similar findings have been reported by Paramythiotou and Routsi as well as Rice, who emphasized the increasing burden of multidrug-resistant Gram-negative pathogens and their impact on infection control and therapeutic outcomes in hospitalized patients [12,13]. Early detection of resistance mechanisms through combined phenotypic and molecular approaches may facilitate timely implementation of targeted antimicrobial therapy and appropriate infection prevention strategies.

Furthermore, the widespread distribution of resistance genes among ICU isolates indicates the potential for horizontal transmission of resistance determinants within the hospital environment. This finding underscores the importance of antimicrobial stewardship programs, strict adherence to infection control practices, and periodic molecular epidemiological monitoring. Strengthening these measures may help limit the dissemination of resistant organisms and preserve the effectiveness of currently available antimicrobial agents, particularly in resource-limited healthcare settings.

CONCLUSIONS

This study revealed a high prevalence of antimicrobial resistance among Gram-negative bacterial isolates recovered from ICU patients, with ESBL production observed in 51.3% of isolates, carbapenem resistance in 50.0%, and multidrug resistance in 74.0%. Klebsiella pneumoniae emerged as the predominant pathogen, highlighting its important role in ICU-associated infections. Molecular analysis demonstrated widespread distribution of resistance determinants, with blaCTX-M being the most common ESBL gene, while blaOXA-48 and blaNDM were among the major carbapenemase genes detected. The absence of a significant association between phenotypic resistance and corresponding resistance genes suggests the presence of complex resistance mechanisms and genotype–phenotype discordance. These findings provide valuable epidemiological data from central India and emphasize the need for continuous molecular surveillance, effective antimicrobial stewardship, and stringent infection control measures to curb the spread of multidrug-resistant Gram-negative bacteria in critical care settings.

CONTRIBUTION OF AUTHORS

Research concept- Ashish Kumar Shukla, Dr. Kailash Jatav
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