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CHARACTERIZATION OF MOLECULAR DRUG RESISTANCE IN BACTERIAL ISOLATES FROM CLABSI CASES IN A TERTIARY CARE HOSPITAL JAIPUR, RAJASTHAN

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ABSTRACT

Objective: Central Line-Associated Bloodstream Infection (CLABSI) is a major healthcare-associated infection associated with increased morbidity, mortality, and rising antimicrobial resistance. This study aimed to determine the microbial profile of CLABSI and characterize molecular mechanisms of antimicrobial resistance among bacterial isolates recovered from patients admitted to a tertiary care hospital in Jaipur, Rajasthan.

Methods: A prospective observational study was conducted from May 2024 to Aug 2025 at Jaipur National University Institute for Medical Sciences & Research Centre, Jaipur. Blood culture samples from clinically suspected CLABSI patients were processed using standard microbiological techniques. Isolates were identified by conventional biochemical methods, and antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to CLSI guidelines. Multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug resistant (PDR) phenotypes were determined. Resistance genes including mecA, blaKPC, blaNDM, blaOXA-48, blaVIM, and blaIMP were detected using "Multiplex real-time PCR (qPCR)".

Results: A total of 125 microbiologically confirmed CLABSI cases were analyzed. Males accounted for 57.6% of cases, and the most affected age group was 41–60 years (48.8%). MRSA (24.0%) and Acinetobacter species (20.0%) were the predominant pathogens. Enterobacteriaceae demonstrated the highest MDR (74.0%) and XDR (26.0%) rates. Molecular analysis identified blaNDM as the most prevalent resistance gene (26.7%), followed by mecA and blaIMP (20.0% each). Chills (34.8%) and bradycardia (25.5%) were the commonest clinical manifestations.

Conclusion: The study revealed a substantial burden of multidrug-resistant and carbapenemase-producing pathogens among CLABSI cases. Integration of phenotypic susceptibility testing with molecular resistance profiling facilitates early detection of resistant pathogens and supports effective antimicrobial stewardship and infection control practices.

KEYWORDS: CLABSI; antimicrobial resistance; MDR; carbapenemase; blaNDM; MRSA; molecular characterization.

INTRODUCTION

Central Line-Associated Bloodstream Infections (CLABSIs) remain a critical concern in healthcare, especially within intensive care units (ICUs) where patients are at heightened risk due to the frequent use of invasive devices. These infections significantly contribute to patient morbidity and mortality worldwide and present considerable challenges for healthcare providers. The situation is exacerbated by the rising prevalence of antimicrobial resistance (AMR) among CLABSI pathogens, which severely limits the effectiveness of conventional antimicrobial therapies and complicates infection control efforts.1

In India, Central Line-Associated Bloodstream Infections (CLABSIs) remain a major healthcare challenge. The pooled incidence rate sits at roughly 8.8 to 9 cases per 1,000 central line days. Neonatal Intensive Care Units (NICUs) face the highest burden at nearly 14 cases per 1,000 days. The crisis is heavily driven by multidrug-resistant (MDR) pathogens like Klebsiella pneumoniae and Acinetobacter baumannii.2The most frequently isolated pathogens were Klebsiella pneumoniae and Acinetobacter baumannii, both showing alarmingly high resistance to carbapenems—87.1% and 77.7%, respectively (Parveen et al., 2025). Similarly, a tertiary care ICU study identified Klebsiella species and Acinetobacter baumannii as common CLABSI pathogens, with a significant correlation between comorbidities and infection risk, alongside high antibiotic resistance among isolates.3

Recent extensive surveillance data highlight the alarming dominance of World Health Organization (WHO) priority pathogens in CLABSI cases, particularly in ICU settings. A landmark study evaluating over 5,000 patients at risk for CLABSI revealed that these priority pathogens accounted for approximately 76% of CLABSI isolates, with a predominance of critical priority organisms such as carbapenem-resistant Enterobacterales (CRE) and carbapenem-resistant Acinetobacter baumannii (CRAB). The multidrug resistance observed in these isolates rendered most standard treatments ineffective, leaving colistin as one of the last-resort options despite its known toxicity concerns. This study

also underscored the devastating impact of CRAB infections, which were associated with mortality rates exceeding 90% among affected patients in critical care.4

Antimicrobial monitoring in different healthcare settings reveals shifting resistance patterns. For example, oncology units have displayed a distinct pathogen distribution, with fluoroquinolone-resistant Escherichia coli and vancomycin-resistant Enterococcus faecium emerging as significant contributors to CLABSI cases. Notably, fluoroquinolone resistance increased over time, highlighting the pressing need to reassess antimicrobial prophylaxis and empiric therapies in these vulnerable populations.5

The COVID-19 pandemic has also impacted CLABSI dynamics, with increased infection rates reported in ICUs during this period, alongside greater use of broad-spectrum antimicrobials. Importantly, despite increased antimicrobial consumption, the susceptibility profiles of the causative microorganisms did not notably change, suggesting that intensified stewardship and infection control measures remain critical.6

Taken together, these findings illustrate the urgent need for integrated surveillance that combines molecular identification of resistance genes with phenotypic antimicrobial susceptibility testing to fully characterize the resistance mechanisms of CLABSI pathogens. Such a comprehensive approach is essential to tailor targeted therapeutic strategies and inform effective containment measures in regions like Jaipur, Rajasthan, where local epidemiology may influence clinical outcomes. Understanding the genetic basis of resistance can help optimize antimicrobial selection, improve stewardship, and enhance infection prevention protocols in critical care environments, ultimately reducing the burden imposed by these challenging infections.

The Aim of the study is to identify and characterize molecular drug resistance mechanisms in pathogens causing Central Line-Associated Bloodstream Infections in Jaipur, Rajasthan.

MATERIALS & METHODS

Sample Collection and Pathogen Isolation

Blood samples were aseptically collected from patients clinically diagnosed with CLABSI at Jaipur National University Institute for Medical Sciences & Research Centre, Jaipur, following strict infection control protocols, Patient demographic and clinical data, including central line details, were recorded between May 2024 to Aug, 2025. Pathogens were isolated using aerobic and anaerobic culture techniques on selective media. Identification was confirmed by biochemical assays and automated systems.

Phenotypic Antimicrobial Susceptibility Testing

Isolates underwent AST using Kirby-Bauer disk diffusion with interpretation based on CLSI/EUCAST guidelines. The antibiotic panel included beta-lactams, carbapenems, glycopeptides, aminoglycosides, and fluoroquinolones. Multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug resistant (PDR) phenotypes were documented.7

Molecular Detection of Resistance Genes

Genomic DNA was extracted from pure cultures using validated protocols. Targeted RT-PCR assays detected resistance genes including mecA, mecC, blaCTX-M, blaTEM, blaSHV, vanA, vanB, blaKPC, blaNDM, blaOXA-48, blaVIM, and blaIMP. “Multiplex real-time PCR (qPCR)” was employed to enhance detection efficiency. Positive and negative controls were included in all PCR runs.7

Data Analysis

Phenotypic and molecular data were integrated to assess concordance and investigate alternative resistance mechanisms. Statistical analyses examined associations between resistance patterns and clinical variables including catheterization duration and prior antibiotic use. Resistance trends were analyzed temporally and spatially within hospital units.7

RESULTS

Out of 125 microbiologically confirmed Central Line-Associated Bloodstream Infection (CLABSI) cases, males accounted for 72 (57.6%) cases while females constituted 53 (42.4%) cases. The age group most frequently affected was 41–60 years, comprising 62 (48.78%) patients, followed by 0–40 years [32 (26.02%)] and 61–90 years [31 (25.20%)] patients. The age distribution of patients with CLABSI is shown in Table 6 and Figure 5.

The predominant pathogens isolated from CLABSI cases were Methicillin-Resistant Staphylococcus aureus (MRSA) [30, 24.0%], Acinetobacter species [25, 20.0%], Escherichia coli [19, 15.2%], Klebsiella pneumoniae [12, 9.6%], Enterococcus species [10, 8.0%], and Methicillin-Sensitive Staphylococcus aureus (MSSA) [10, 8.0%]. The microbial etiology of CLABSI cases is summarized in Table 1 and Figure 1.

The gender distribution analysis demonstrated that males were more frequently affected than females, accounting for 57.6% and 42.4% of CLABSI cases, respectively. The gender-wise distribution of CLABSI patients is presented in Table 2 and Figure 2.

Antimicrobial resistance analysis demonstrated a high prevalence of multidrug resistance among isolates. Enterobacteriaceae exhibited the highest MDR rate (74.0%) and XDR rate (26.0%), with 4.1% showing PDR characteristics. S. aureus isolates showed MDR and XDR rates of 63.3% and 13.3%, respectively, while Enterococcus spp. demonstrated MDR and XDR rates of 50.0% and 10.0%, respectively. The antimicrobial resistance patterns among major pathogens are summarized in Table 3 and Figure 3.

Molecular characterization revealed the presence of clinically significant resistance genes. The most frequently detected gene was blaNDM [4, 26.7%], followed by mecA [3, 20.0%], blaIMP [3, 20.0%], blaKPC [2, 13.3%], blaVIM [2, 13.3%], and blaOXA-48 [1, 6.7%]. These findings indicate the circulation of carbapenemase-producing and methicillin-resistant

pathogens among CLABSI cases. The distribution of resistance genes detected by multiplex RT-PCR is illustrated in Table 4 and Figure 4.
Among clinical manifestations, chills were the most common symptom [49, 34.8%], followed by bradycardia [37, 25.5%], fever [26, 17.7%], and hypotension [16, 11.3%]. The distribution of clinical manifestations among patients with CLABSI is described in Table 5.

TABLE 1: Microbial etiology of patients with central line-associated blood stream infection (CLABSI)

SR	Micro-organism	Total cases	Percentage %
1	S. aureus (MRSA)	30	24.0%
2	Acinetobacter species	25	20.0%
3	E. coli	19	15.2%
4	S. aureus (MSSA)	10	8.0%
5	K. pneumoniae	12	9.6%
6	Enterococcus species	10	8.0%
7	E. aerogenes	5	4.0%
8	Burkholderia spp.	5	4.0%
9	CONS	5	4.0%
10	P. aeruginosa	2	1.6%
11	P. mirabilis	2	1.6%
	Total	125	100%

The microbial etiology of CLABSI patients is shown in the table. MRSA and Acinetobacter species were the most often found organisms associated with CLABSI, followed by E. Coli and K. pneumoniae.

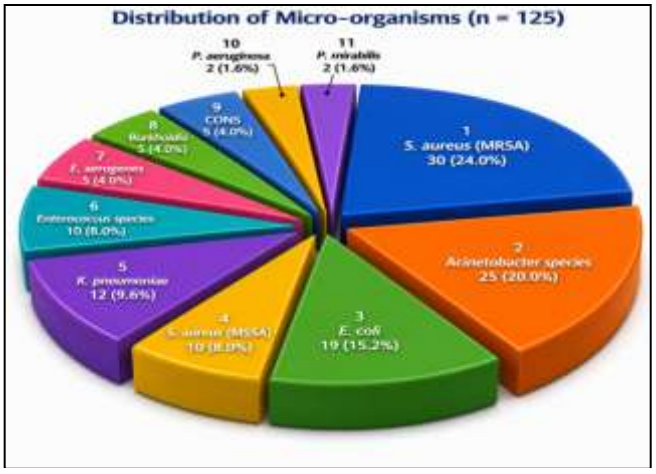

Figure 1. Distribution of microorganisms isolated from CLABSI cases (n = 125).

TABLE 2: Description of central line-associated blood stream infection rates by patient gender

Gender of patients with CLABSI	N (%) Total = 125
Male	72 (57.6%)
Female	53 (42.4%)

The gender distribution of patients with CLABSI is shown in the table. Males had a higher rate of CLABSI (57.6%) than females (42.4%).

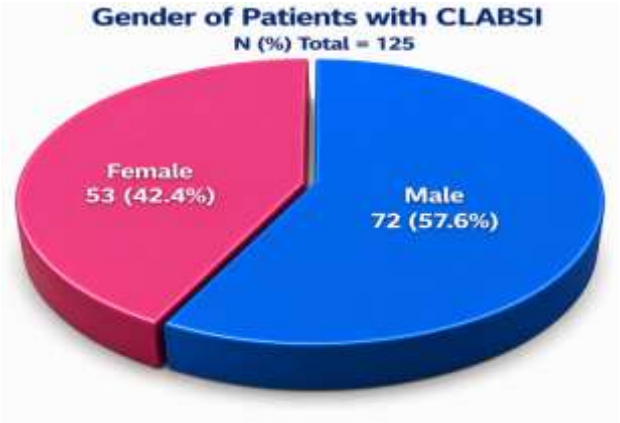

Figure 2. Gender distribution of patients with CLABSI (n = 125).

Table 3: MDR, XDR and PDR Analysis Graph

Pathogen	Number of Isolates	MDR (%)	XDR (%)	PDR (%)
S. aureus	30	63.3	13.3	0.0
CONS	12	58.3	8.3	0.0
Enterobacteriaceae	73	74.0	26.0	4.1
Enterococcus spp.	10	50.0	10.0	0.0

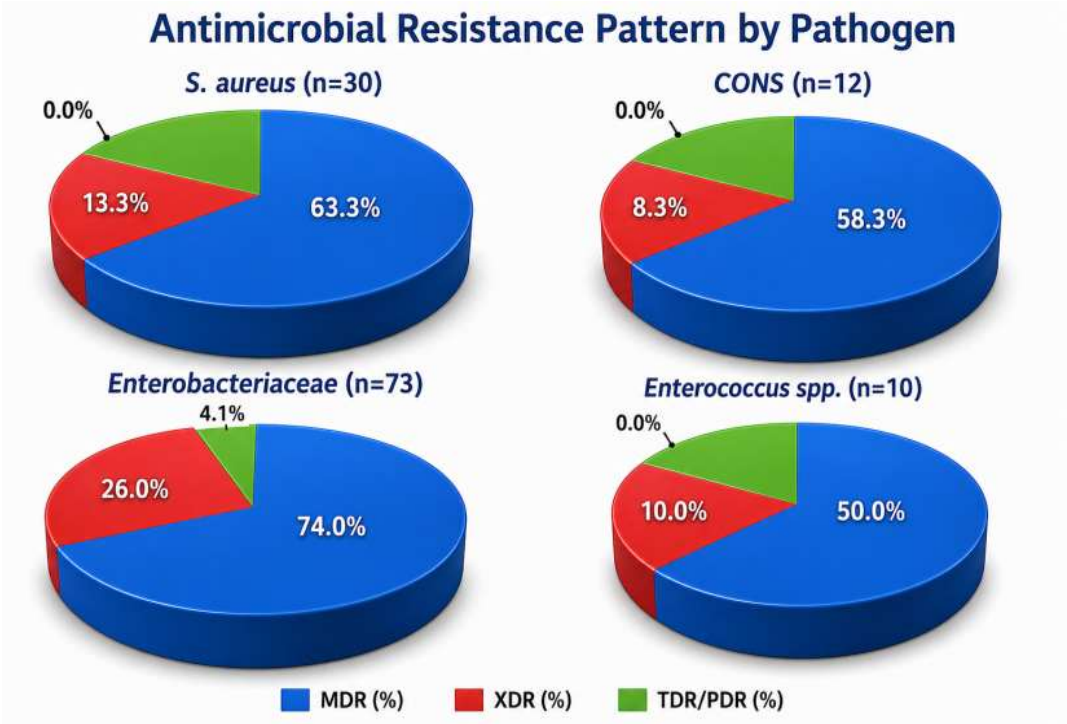

Figure 3. Antimicrobial resistance pattern among major bacterial pathogens causing CLABSI.

Table 4: Resistance Gene Distribution Table and Graph

Resistance Gene	Number Positive	Percentage (%)	Associated Phenotypic Resistance
mecA	3	20.0	Methicillin resistance in Staphylococci
Bla KPC	2	13.3	Carbapenemase production
blaNDM	4	26.7	Carbapenemase production
blaOXA-48	1	6.7	Carbapenemase production
blaVIM	2	13.3	Carbapenemase production
blaIMP	3	20.0	Carbapenemase production

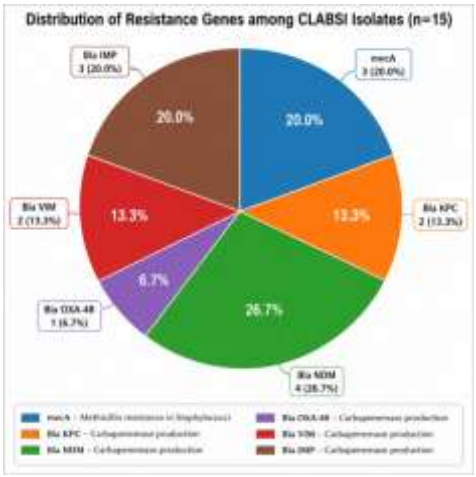

Figure 4. Distribution of resistance genes detected by multiplex RT-PCR.

Table 5: Distribution of Clinical Manifestations among Patients with Central Line-Associated Bloodstream Infection (CLABSI)

SR	Symptoms	Count	Percentage (%)
1	CHILLS	49	34.8
2	BRADYCARDIA	37	25.5
3	FEVER	26	17.7
4	HYPOTENSION	16	11.3

Demonstrates that chills constituted the most common presenting symptom (34.8%), followed by bradycardia (25.5%), fever (17.7%), and hypotension (11.3%). The symptom profile observed in this study highlights the varied clinical manifestations of CLABSI among ICU patients and underscores the importance of vigilant monitoring for early identification of bloodstream infections.

TABLE 6: Frequency of patients with central line-associated blood stream infection (CLABSI) in different age groups

Age group of patients (years)	CLABSI n (%) Total=125
0-40	32 (26.02%)
41-60	62 (48.78%)
61-90	31(25.20%)

The age group that is most frequently associated with CLABSI is shown in the table. With a mean age was 48.78 ± 20.1 years, the age group that was most frequently linked to CLABSI was 41–60 years.

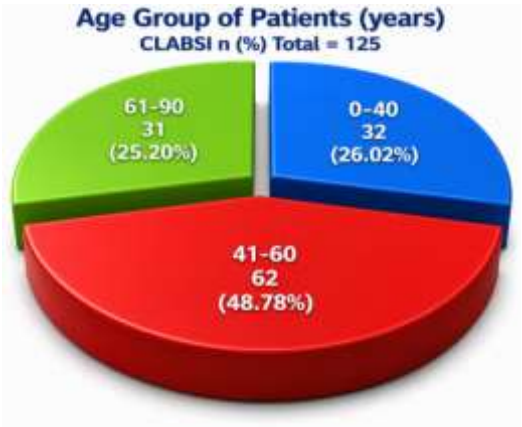

Figure 5. Age-wise distribution of patients with CLABSI (n = 125).

DISCUSSION

Central Line-Associated Bloodstream Infection (CLABSI) continues to represent a major challenge in intensive care units because of its association with prolonged hospitalization, increased healthcare expenditure, and poor clinical outcomes. The present study provides valuable insight into the demographic characteristics, microbial profile, antimicrobial resistance patterns, and molecular mechanisms of resistance among bacterial isolates causing CLABSI in a tertiary care hospital in Jaipur, Rajasthan.

In the present study, males constituted 57.6% of microbiologically confirmed CLABSI cases, while females accounted for 42.4%. Similar findings have been reported by Rosenthal et al., who observed a predominance of male patients among ICU-acquired bloodstream infections, likely due to higher ICU admission rates and increased exposure to invasive procedures among males.8 Likewise, Parameswaran et al. reported a higher incidence of catheter-related bloodstream infections among male patients in Indian healthcare settings.9 The higher proportion of male patients in our study may also reflect the greater prevalence of comorbid conditions and healthcare utilization among this population.

Age-wise analysis demonstrated that patients aged 41–60 years represented the largest proportion of CLABSI cases (48.78%), followed by patients aged 0–40 years (26.02%) and those aged 61–90 years (25.20%). Similar observations have been documented in studies by Vincent et al. and Mermel, where middle-aged and elderly individuals were found to be at increased risk of CLABSI because of prolonged hospitalization, underlying chronic illnesses, immunosuppression, and frequent central venous catheter use.10,11 These findings emphasize the importance of strict catheter care protocols, particularly among vulnerable age groups.

The microbiological profile of CLABSI in our study revealed that MRSA (24.0%) was the most frequently isolated pathogen, followed by Acinetobacter species (20.0%), Escherichia coli (15.2%), and Klebsiella pneumoniae (9.6%). Similar studies from India have reported Staphylococcus aureus and Acinetobacter baumannii as predominant etiological agents of ICU-associated bloodstream infections.9 The predominance of MRSA highlights the persistent burden of methicillin-resistant staphylococcal infections despite ongoing infection prevention efforts. Moreover, the high prevalence of Acinetobacter species is of significant concern because of their ability to survive in hospital environments, form biofilms on medical devices, and acquire multiple resistance determinants. Lee et al. described Acinetobacter baumannii as an emerging nosocomial pathogen characterized by remarkable genomic plasticity and extensive antimicrobial resistance.12

The antimicrobial susceptibility profile observed in this study demonstrated alarmingly high rates of multidrug resistance among the isolates. Enterobacteriaceae exhibited the highest MDR rate (74.0%) and XDR rate (26.0%), with 4.1% of isolates displaying PDR characteristics. Similarly, S. aureus isolates demonstrated MDR and XDR rates of 63.3% and 13.3%, respectively. These findings are comparable to reports from Indian surveillance studies that have documented increasing resistance to third-generation cephalosporins, fluoroquinolones, aminoglycosides, and carbapenems among Gram-negative bacilli isolated from ICUs.13 The emergence of PDR isolates in our study is particularly concerning, as these organisms severely limit therapeutic options and may contribute to increased mortality.

Molecular characterization of resistant isolates revealed that blaNDM was the most frequently detected resistance determinant (26.7%), followed by mecA (20.0%) and blaIMP (20.0%). The detection of blaNDM is particularly significant because New Delhi Metallo-β-lactamase-producing organisms confer resistance to nearly all β-lactam antibiotics, including carbapenems. Yong et al. first described blaNDM-1 as a transferable resistance mechanism with considerable global dissemination potential.14 The presence of blaKPC, blaVIM, and blaOXA-48 genes in the present study further confirms the coexistence of multiple carbapenemase mechanisms among CLABSI pathogens. Nordmann et al. reported that the widespread dissemination of carbapenemase genes represents one of the greatest threats to modern antimicrobial therapy.15 The identification of mecA among staphylococcal isolates corroborated the phenotypic diagnosis of MRSA and emphasized the value of molecular diagnostics in confirming resistance mechanisms.

The clinical presentation of CLABSI observed in our study was characterized predominantly by chills (34.8%) and bradycardia (25.5%), followed by fever (17.7%) and hypotension (11.3%). Although fever is often considered the hallmark manifestation of bloodstream infections, several studies have shown that critically ill patients may exhibit atypical clinical features because of altered immune responses and concurrent medical conditions.11 Consequently, reliance solely on clinical manifestations may delay diagnosis and treatment, reinforcing the need for early microbiological investigations in suspected CLABSI cases.

The integration of phenotypic antimicrobial susceptibility testing with molecular detection techniques significantly enhanced the understanding of resistance mechanisms in the study isolates. While conventional susceptibility testing remains indispensable for routine clinical management, molecular assays facilitate the rapid identification of specific resistance genes, thereby enabling early optimization of antimicrobial therapy and implementation of targeted infection control measures. Recent recommendations by the Infectious Diseases Society of America (IDSA) have emphasized the importance of incorporating molecular diagnostic tools into antimicrobial stewardship programs to improve patient outcomes and reduce the spread of resistant pathogens.16

The present study has several limitations. First, it was conducted at a single tertiary care center, which may limit the generalizability of the findings to other healthcare settings. Second, the number of isolates subjected to molecular characterization was relatively small. Finally, advanced techniques such as whole genome sequencing and clonal relatedness analysis were not performed, which could have provided deeper insight into transmission dynamics and resistance evolution. Nevertheless, this study provides valuable regional data regarding the molecular epidemiology of antimicrobial resistance among CLABSI pathogens in Rajasthan.

Overall, the findings of the present study highlight the urgent need for continuous surveillance of antimicrobial resistance, strict adherence to central line insertion and maintenance bundles, implementation of antimicrobial stewardship interventions, and incorporation of molecular diagnostic techniques into routine microbiological practice. Such integrated strategies are essential to reduce the burden of CLABSI and improve clinical outcomes among critically ill patients.

CONCLUSION

Central Line-Associated Bloodstream Infection (CLABSI) remains one of the most important healthcare-associated infections in critically ill patients, contributing significantly to prolonged hospital stay, increased healthcare costs, and mortality. The emergence of multidrug-resistant (MDR) and carbapenem-resistant pathogens has further complicated the

management of these infections. In the present study, molecular and phenotypic characterization of bacterial isolates from CLABSI patients revealed a high burden of antimicrobial resistance among pathogens isolated from a tertiary care hospital in Jaipur, Rajasthan.17, 18

Among the 125 confirmed CLABSI cases, male patients constituted the majority (57.6%) compared to females (42.4%). Similar findings have been reported in previous studies where male predominance was attributed to higher ICU admissions, increased exposure to invasive procedures, and a greater prevalence of underlying comorbid conditions. The most affected age group in our study was 41–60 years (48.78%), indicating that middle-aged and elderly patients are particularly vulnerable to CLABSI because of prolonged hospitalization, compromised immunity, and frequent use of central venous catheters.19,20

The microbiological profile of CLABSI in the present study demonstrated that MRSA (24.0%) and Acinetobacter species (20.0%) were the predominant pathogens, followed by Escherichia coli (15.2%) and Klebsiella pneumoniae (9.6%). These findings are consistent with several Indian and international studies reporting Staphylococcus aureus and Acinetobacter baumannii as major causative agents of bloodstream infections in ICU settings. The high prevalence of Acinetobacter species is particularly concerning because of its remarkable ability to survive in hospital environments and acquire multiple resistance determinants. Likewise, MRSA continues to be a major nosocomial pathogen associated with catheter-related bloodstream infections.21,22

Antimicrobial resistance analysis revealed alarming levels of multidrug resistance among the isolates. Enterobacteriaceae demonstrated the highest MDR rate (74.0%) and XDR rate (26.0%), while S. aureus exhibited MDR and XDR rates of 63.3% and 13.3%, respectively. These findings indicate extensive antibiotic selection pressure within intensive care units and emphasize the challenges faced during empirical antimicrobial therapy. The detection of PDR characteristics among 4.1% of Enterobacteriaceae isolates further highlights the growing threat of untreatable bacterial infections. Similar trends have been observed in surveillance studies across India, where increasing resistance to third-generation cephalosporins, fluoroquinolones, and carbapenems has been reported.23,24

Molecular characterization provided important insights into the genetic basis of antimicrobial resistance. The most frequently detected resistance gene was blaNDM (26.7%), followed by mecA (20.0%) and blaIMP (20.0%). The presence of blaNDM indicates the circulation of New Delhi Metallo-β-Lactamase-producing organisms, which confer resistance to most β-lactam antibiotics including carbapenems. The detection of blaKPC, blaVIM, and blaOXA-48 genes further confirms the coexistence of multiple carbapenemase mechanisms among CLABSI pathogens. These findings are clinically significant because carbapenemase-producing organisms are associated with limited therapeutic options and poor clinical outcomes. The detection of the mecA gene among Staphylococcal isolates confirms the molecular basis of methicillin resistance and supports the phenotypic identification of MRSA isolates.25,26,27

The symptom profile observed in this study showed that chills (34.8%) and bradycardia (25.5%) were the most common clinical manifestations, followed by fever (17.7%) and hypotension (11.3%). These findings indicate that clinical presentation alone may not be sufficient for early diagnosis of CLABSI, highlighting the importance of timely microbiological investigations and molecular diagnostics.28

The combined use of phenotypic antimicrobial susceptibility testing and molecular resistance gene detection significantly enhanced the understanding of resistance mechanisms in the study isolates. While phenotypic methods remain essential for routine clinical decision-making, molecular techniques provide rapid and accurate identification of resistance determinants, facilitating early implementation of targeted antimicrobial therapy and infection control measures. Such integrated approaches are increasingly recommended for surveillance and management of healthcare-associated infections.29

The present study has certain limitations. It was conducted at a single tertiary care center and included a relatively limited number of molecularly characterized resistant isolates. Additionally, whole genome sequencing and clonal analysis were not performed. Nevertheless, the study provides valuable regional data regarding the molecular epidemiology of antimicrobial resistance among CLABSI pathogens in Rajasthan and contributes to the growing body of evidence supporting molecular surveillance in healthcare settings.30

Overall, the findings of this study underscore the urgent need for continuous antimicrobial resistance monitoring, implementation of strict central line care bundles, antimicrobial stewardship programs, and routine molecular surveillance to reduce the burden of CLABSI and improve patient outcomes in intensive care units.17,30,31

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