Isolation and Identification of Bacterial Pathogens from Blood Cultures in a Tertiary Care Hospital and Their Clinical Correlation

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Clinical and Laboratory Standards Institute (CLSI), bloodstream infections (BSIs), BACTEC.

ABSTRACT
Objective: This study aimed to isolate and identify bacterial pathogens from blood cultures of patients with suspected bloodstream infections (BSIs) in a tertiary care hospital, analyze their antimicrobial susceptibility patterns, and establish clinical correlations. Methods: A cross-sectional study was conducted in the Microbiology Department of a tertiary care hospital. Blood samples from 60 patients were collected aseptically, processed using BACTEC, and cultured on blood and MacConkey agar. Bacterial isolates were identified based on colonial morphology, Gram staining, and biochemical tests. Antibiotic susceptibility testing was performed using the modified Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Results: The overall culture positivity rate was 26%, with higher positivity among female patients (31.57%) and neonates or infants under six months (26%). Klebsiella pneumoniae was the most frequently isolated pathogen (53.33%), followed by Staphylococcus aureus (20.00%), Escherichia coli (13.33%), and Citrobacter species (13.33%). Klebsiella pneumoniae showed high resistance to ceftazidime and piperacillin, while remaining sensitive to cefoperazone and tobramycin. Staphylococcus aureus isolates were resistant to penicillin and erythromycin but sensitive to vancomycin and cefazolin. The prevalence of multidrug resistance among isolates underscores the need for targeted empirical therapy. Conclusion: Klebsiella pneumoniae emerged as the primary pathogen in BSIs, with significant resistance to commonly prescribed antibiotics. These findings highlight the necessity for enhanced infection control measures, especially in NICUs, and the implementation of local antibiograms to guide effective antibiotic therapy and mitigate resistance trends. Further research across multiple centers is recommended to validate these findings and inform broader clinical guidelines.

1. Introduction

Bloodstream infections (BSIs) are a critical cause of morbidity and mortality globally, particularly in healthcare settings where timely diagnosis and treatment are essential to patient outcomes (1,2). BSIs occur when pathogenic microorganisms enter the bloodstream, leading to systemic infection and, potentially, to life-threatening conditions such as sepsis and septic shock (3). The global burden of BSIs has increased with the rise of multidrug-resistant organisms (MDROs), which complicates treatment and leads to poorer patient outcomes, longer hospital stays, and higher healthcare costs (4,5).

The detection and characterization of organisms responsible for BSIs remain primary functions of clinical microbiology laboratories. Blood culture is the gold standard for diagnosing BSIs, as it allows for the isolation and identification of causative pathogens as well as determination of their antimicrobial susceptibility (6). However, various factors influence culture positivity, including the method of blood collection, volume, and the number of blood samples, along with the patient's prior exposure to antibiotics (7). Blood culture positivity rates vary widely across regions, with studies reporting rates from 9.2% to 44% in developing countries, where the burden of resistant pathogens is notably higher (8). The lack of surveillance data in certain regions, such as Nigeria, poses challenges in creating effective treatment guidelines for BSIs, highlighting the importance of local and regional studies (9).

In India, BSIs are commonly caused by both Gram-positive and Gram-negative bacteria, with Gram-negative organisms such as Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa being especially prevalent in hospital settings (10). The rise of multidrug resistance among these organisms, particularly within the Enterobacteriaceae family, presents a significant challenge in managing BSIs. These organisms often

produce extended-spectrum beta-lactamases (ESBLs), conferring resistance to many first-line antibiotics, which limits treatment options and complicates infection control (11). Gram-positive organisms, including methicillin-resistant Staphylococcus aureus (MRSA) and Streptococcus species, are also important BSI pathogens that exhibit varying resistance profiles and add complexity to patient management (12).

Despite the established role of blood cultures in diagnosing BSIs, isolation rates can be low, often due to previous antimicrobial exposure or suboptimal sample collection methods (13). The present study aims to address these challenges by isolating and identifying bacterial pathogens from blood cultures in a tertiary care hospital in India, examining their antimicrobial susceptibility patterns, and analyzing clinical correlations. These findings aim to inform empirical treatment strategies and support local antimicrobial stewardship efforts in managing BSIs effectively.

2. Methodology

Study Design and Setting

This cross-sectional study was conducted in the Microbiology Department of Late Baliram Kashyap Memorial Government Medical College, Jagdalpur, India, over a two-month period from February to March 2024. The study population included patients from various hospital units suspected of having bloodstream infections (BSIs) based on clinical criteria.

Study Population and Sampling

A total of 60 patients with clinically suspected BSIs were recruited. Patients were selected using a simple random sampling method, ensuring representation across different hospital units, including the neonatal intensive care unit (NICU), surgical intensive care unit (SICU), and general wards. Inclusion criteria encompassed patients without prior antibiotic exposure within 72 hours before blood sample collection, to minimize potential alterations in culture positivity. Exclusion criteria included any patient with a documented history of antibiotic administration before blood sampling.

Sample Collection and Blood Culture Processing

For each patient, two venous blood samples were collected from different anatomical sites with an interval of 30 minutes between collections to reduce contamination risk. The blood collection followed strict aseptic techniques. Sample volumes varied based on patient age and weight:

- Adults: 8-10 mL of venous blood
- Neonates: 2 mL of venous blood
- Pediatric Patients (2 months - 5 years): 2-5 mL of venous blood

Each sample was inoculated into BACTEC culture bottles and incubated for a preliminary period of 48-72 hours. If growth was indicated, subculturing onto blood agar and MacConkey agar plates was performed on days 5 and 7 to ensure sufficient growth and recovery of both Gram-positive and Gram-negative bacteria.

Pathogen Identification

Once growth was detected in the culture bottles, colonies were further analyzed through a combination of:

1. Colonial Morphology: Examination of colony characteristics on blood and MacConkey agars.

2. Gram Staining: Initial differentiation of bacterial isolates into Gram-positive and Gram-negative categories.

3. Biochemical Tests: Conventional biochemical assays were conducted, including catalase, oxidase, and urease tests, as per standard laboratory protocols. These tests aided in the precise identification of pathogens, particularly among Gram-negative isolates such as Klebsiella pneumoniae and Escherichia coli.

Antibiotic Susceptibility Testing (AST)

Antibiotic susceptibility testing was performed on all identified bacterial isolates using the modified Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA). This standardized method was conducted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (1), and antibiotic susceptibility results were interpreted as “sensitive,” “intermediate,” or “resistant.”

The following antibiotics were tested:

- Beta-lactams: Cefoperazone, ceftazidime, piperacillin, and ampicillin
- Carbapenems: Doripenem
- Beta-lactamase inhibitors: Clavulanic acid
- Other classes: Levofloxacin (fluoroquinolone), tobramycin (aminoglycoside), and vancomycin (for Gram-positive isolates)

Quality control was maintained by including control strains of known sensitivity patterns in each AST batch to ensure accuracy.

Data Collection and Analysis

Patient demographic data (age, sex), clinical background, and culture results were recorded systematically. Clinical correlations were established based on patient records. Data entry and statistical analysis were performed using SPSS (Statistical Package for the Social Sciences) software version 24. Descriptive statistics were used to analyze the prevalence of bacterial pathogens and their antibiotic susceptibility patterns. Results were expressed as percentages or frequencies, providing insights into pathogen distribution and resistance trends among patient groups.

3. Results

Patient Demographics and Blood Culture Positivity

Among the 60 patients with suspected bloodstream infections (BSIs), 41 were male (68.34%) and 19 female (31.66%) (Figure 1). Blood culture positivity was observed in 17.07% of male patients and 31.57% of female patients, indicating a higher culture positivity rate among females.

Table 1: Gender Distribution and Blood Culture Positivity

Gender	Total Patients	Culture Positive	Culture Negative	Culture Positivity Rate (%)
Male	41	7	34	17.07
Female	19	6	13	31.57

Table 1 demonstrates the gender distribution and corresponding culture positivity rates among the study population.

Age-wise Distribution of Culture Positivity

The highest culture positivity rate was observed in neonates and infants under 6 months, accounting for 26% of positive cases. Culture positivity rates declined with increasing age, as shown in Figure 1.

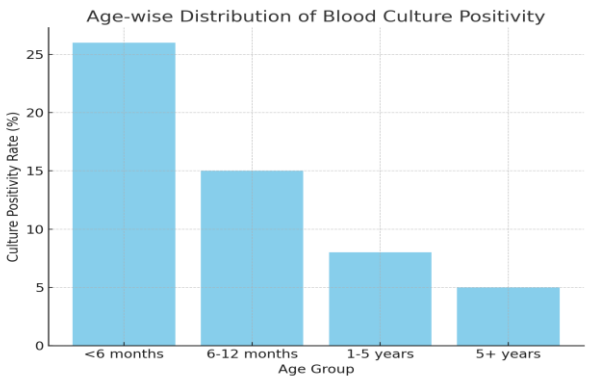

Figure 1: Age-wise Distribution of Blood Culture Positivity

Figure 1: This bar chart illustrates age distribution across culture-positive cases, highlighting higher rates in neonates and infants.

Bacterial Pathogens Isolated

A total of 15 isolates were identified from culture-positive samples. The most common pathogens included Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, and Citrobacter species. Gram-negative bacteria accounted for 73% of isolates, with Gram-positive bacteria representing the remaining 27%, as shown in Table 2.

Table 2: Distribution of Bacterial Pathogens in Blood Cultures

Pathogen	Number of Isolates	Percentage (%)
Klebsiella pneumoniae	8	53.33
Staphylococcus aureus	3	20.00
Escherichia coli	2	13.33
Citrobacter species	2	13.33
Total	15	100

Table 2 lists the bacterial pathogens isolated, with Klebsiella pneumoniae as the most prevalent.

Antibiotic Susceptibility Patterns

Antibiotic susceptibility testing revealed significant resistance among the isolates, particularly to commonly used antibiotics. Klebsiella pneumoniae showed notable resistance to ceftazidime and piperacillin but was generally sensitive to cefoperazone and tobramycin. Staphylococcus aureus exhibited resistance to penicillin and erythromycin while remaining sensitive to vancomycin and cefazolin (Figure 2, Table 3).

Table 3: Antibiotic Susceptibility Patterns of Major Pathogens

Pathogen	Antibiotic	Sensitive (S)	Intermediate (I)	Resistant (R)
Klebsiella pneumoniae	Cefoperazone	S	-	-
	Tobramycin	S	-	-
	Ceftazidime	-	-	R
	Piperacillin	-	-	R
Staphylococcus aureus	Vancomycin	S	-	-
	Cefazolin	S	-	-
	Penicillin	-	-	R
	Erythromycin	-	-	R

Table 3 provides the antibiotic susceptibility patterns for the primary pathogens isolated in this study.

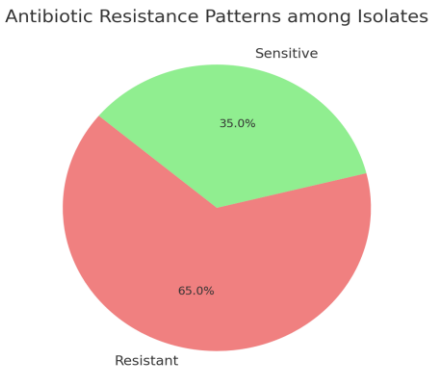

Figure 2: Antibiotic Resistance Patterns among Isolates

Figure 2: This pie chart highlights the resistance distribution among bacterial isolates, with a substantial proportion demonstrating resistance to first-line antibiotics.

Summary of Findings

- Culture Positivity: The overall blood culture positivity rate was 26%.
- Demographics: Higher positivity rates were observed in female patients and neonates/infants under six months.

- Pathogen Prevalence: Klebsiella pneumoniae was the most common isolate, predominantly resistant to ceftazidime and piperacillin.
- Antibiotic Resistance: Resistance to multiple antibiotics, including beta-lactams, was prevalent, underscoring the need for targeted therapy and antimicrobial stewardship programs.

4. Discussion

The results of this study underscore the prevalence of Gram-negative bacteria, particularly Klebsiella pneumoniae, as primary pathogens in bloodstream infections (BSIs) within a tertiary care setting. This aligns with findings from similar studies in resource-limited healthcare facilities where Gram-negative organisms are often predominant (14). The high incidence of K. pneumoniae infections may be partly attributable to environmental contamination in hospitals, inadequate infection control practices, and the organism's propensity to develop antimicrobial resistance (15). The substantial antibiotic resistance exhibited by K. pneumoniae isolates, particularly to ceftazidime and piperacillin, reflects global trends in antimicrobial resistance among Enterobacteriaceae, where extended-spectrum beta-lactamase (ESBL)-producing strains are increasingly observed (16).

Notably, the culture positivity rate was higher among female patients and infants under six months, which is consistent with other studies that highlight a heightened vulnerability to BSIs in neonates and young children due to immature immune defenses (17). BSIs in neonates often arise from perinatal factors, such as preterm birth or complications during delivery, and the colonization of hospital surfaces with multidrug-resistant organisms further compounds the risk (18). This demographic distribution emphasizes the need for robust infection prevention protocols, particularly in neonatal intensive care units (NICUs), where infants are exposed to invasive procedures and prolonged hospitalization (19).

The antibiotic susceptibility patterns observed in this study reveal critical insights for managing BSIs effectively. Resistance rates were notably high for commonly prescribed antibiotics, such as ceftazidime, suggesting a potential over-reliance on these agents in empirical therapy. High resistance to piperacillin also underscores the need for periodic reviews of empirical antibiotic policies, as overuse and misuse contribute to resistance in hospital-acquired infections (20). The observed sensitivity of K. pneumoniae to cefoperazone and tobramycin provides an alternative treatment option; however, rotating antibiotic classes in empirical treatment protocols should be considered to prevent the emergence of resistance (21). For Gram-positive isolates, such as Staphylococcus aureus, the high resistance to penicillin and erythromycin emphasizes the importance of using beta-lactamase-resistant options like vancomycin, which remains effective against methicillin-resistant S. aureus (MRSA) (22).

Additionally, the role of adequate blood volume in culture positivity was highlighted by this study. While blood culture remains the gold standard for diagnosing BSIs, its diagnostic yield is heavily influenced by the volume of blood sampled, the timing of sample collection, and prior antibiotic exposure (23). In settings where previous antibiotic use is prevalent before hospitalization, culture results may underestimate the true incidence of BSIs. Studies suggest that at least 20 mL of blood should be collected for adults and adjusted for pediatric patients to optimize detection rates (24). Moreover, the timing of blood sample collection relative to fever onset and patient symptoms can also impact the likelihood of pathogen isolation (25).

These findings carry significant implications for antibiotic stewardship. The widespread resistance to first-line agents reinforces the importance of tailoring antibiotic therapy based on local susceptibility data. Implementing routine surveillance of pathogen profiles and resistance patterns is essential to guide empirical therapy and prevent further escalation of resistance (26). Developing hospital-specific antibiograms can also assist clinicians in selecting the most effective antibiotics, reducing the misuse of broad-spectrum agents, and promoting the judicious use of last-resort drugs like carbapenems and vancomycin (27).

The study has certain limitations. The sample size was relatively small, and factors such as prior antibiotic exposure in community settings may have influenced culture results. Additionally, the study was conducted in a single center, which may limit the generalizability of the findings. Nonetheless, this study highlights the pressing need for ongoing monitoring of BSI pathogens and resistance patterns in hospitals, especially in regions with limited healthcare resources (28).

The findings underscore the importance of localized antimicrobial policies, particularly in high-risk units such as NICUs. Enhanced infection control measures and judicious use of antibiotics are essential to curtail the spread

of multidrug-resistant organisms. Future studies should focus on larger, multicenter datasets to validate these findings and inform national BSI management guidelines.

5. Conclusion

This study identifies Klebsiella pneumoniae as the predominant pathogen in bloodstream infections (BSIs) within a tertiary care hospital, with significant resistance to common first-line antibiotics such as ceftazidime and piperacillin. Neonates and infants under six months were notably vulnerable, emphasizing the need for enhanced infection control in NICUs. The observed antimicrobial resistance underscores the urgent requirement for tailored empirical treatment guided by local susceptibility data and robust antimicrobial stewardship programs. Effective infection prevention practices, combined with the judicious use of antibiotics, are essential to limit the spread of multidrug-resistant organisms. Regular surveillance and development of hospital-specific antibiograms are recommended to guide therapeutic strategies, improve patient outcomes, and reduce the burden of antimicrobial resistance. Further multicenter studies with larger samples are essential to validate these findings and aid in formulating comprehensive, evidence-based BSI management guidelines.

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