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Original Research Article

Antimicrobial Resistance Profiles of Bacterial Isolates from Bloodstream Infections in a Tertiary Care Hospital in Western India

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Abstract:

Background: Bloodstream infections (BSIs) are a major cause of morbidity and mortality in hospitalized patients. Timely identification of causative organisms and their antimicrobial susceptibility are essential for effective management and infection control. This study aimed to evaluate the prevalence of bacterial pathogens that cause BSIs and their resistance patterns in a tertiary care hospital.

Methods: This prospective observational study was conducted from October 2023 to October 2024 at the NAMO Medical Education and Research Institute, Silvassa. A total of 2,290 blood culture samples from patients with suspected BSIs were processed using both manual and automated methods. Bacterial isolates were identified using conventional biochemical tests and the VITEK-2 compact system. Antimicrobial susceptibility testing was performed using disk diffusion and automated methods in accordance with the CLSI 2023 guidelines.

Results: Of the 2,290 samples, 199 (8.7%) were culture-positive and 116 isolates were confirmed to be pathogenic. Escherichia coli (27.6%) and Klebsiella pneumoniae (26.7%) were the most common Gram-negative isolates, while Staphylococcus aureus and Enterococcus faecalis predominated among Gram-positive organisms. High levels of resistance were observed against third-generation cephalosporins and fluoroquinolones, especially against Klebsiella and Acinetobacter. Colistin and linezolid retained 100% efficacy against all the isolates.

This study highlights the growing challenge of multidrug-resistant pathogens in BSIs, underscoring the need for routine susceptibility testing, local antibiogram-guided therapy, and robust antimicrobial stewardship. Empirical treatments should be regularly updated based on regional resistance trends to improve patient outcomes and curb the spread of resistant organisms.

Introduction

Bloodstream infections (BSIs) are severe infections characterized by the presence of viable microorganisms in the blood, a condition known as bacteremia. The detection of bacteria in the bloodstream, typically through blood culture, is considered clinically significant and warrants immediate attention because the bloodstream is normally a sterile environment (Viscoli 2016; Akter et al. 2021). It is important to distinguish BSIs from sepsis, which refers to a host's dysregulated immune response to infection, potentially leading to life-threatening organ dysfunction (Fan et al. 2016; Singer et al. 2016).

Bacteria can enter the bloodstream through various routes, including complications of localized infections, such as pneumonia or meningitis, during surgical procedures involving mucosal surfaces, or via invasive medical devices, such as intravenous catheters. Intravenous drug use and dental procedures may also lead to transient or persistent bacteremia (Sligl et al. 2006, Perez-Chaparro et al. 2011). Once in the bloodstream, pathogens may

disseminate to distant sites, potentially resulting in metastatic infections, such as endocarditis, a phenomenon known as hematogenous spread.

BSIs can present in various clinical forms, from asymptomatic bacteremia to severe septicemia, depending on host factors and pathogen virulence. Asymptomatic bacteremia, although usually transient and self-limiting, may have serious consequences in immunocompromised individuals. In contrast, septicemia arises when the immune system fails to contain the infection, often resulting in a systemic inflammatory response and multi-organ failure. Notably, the spectrum of causative pathogens varies with geographical region, healthcare exposure, and patient demographics (Coronado, 2024).

BSIs are commonly classified by the origin of infection as community-acquired or healthcare-associated. The increasing prevalence of gram-positive bacteria, such as Staphylococcus aureus, Streptococcus spp., and Enterococcus spp., in both community and hospital settings has been well documented (Ryan 2018; Drew and Perfect 2022).S. aureus remains a dominant cause in the Americas, particularly in association with skin ulcers, respiratory infections, and intravenous drug use (Lowy 1998). Streptococcal species, including Group A, Group B, Streptococcus bovis, and Viridans streptococci, are significant contributors to BSIs, particularly in newborns, patients with cancer, and individuals undergoing dental procedures (Cockerham 2017; Kasper et al. 2015). Enterococcal bacteremia is often linked to prolonged hospital stays, antibiotic exposure, and the use of invasive devices, with resistant strains posing significant treatment challenges (Cockerham 2017).

Gram-negative organisms are responsible for a substantial proportion of BSIs, accounting for approximately 45% of community-acquired cases and 24% of healthcare-associated cases (Weinstein et al. 2005; Diekema et al. 2003). They frequently enter the bloodstream via the gastrointestinal, hepatobiliary, or respiratory tracts. Escherichia coli is the most common gram-negative isolate in community-acquired BSIs and is responsible for up to 75% of all cases (Deen et al. 2012). Other notable pathogens included Klebsiella pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis. In sub-Saharan Africa, Salmonella species, particularly HIV-positive individuals and children lacking protective antibodies, contribute significantly to the BSI burden (Kurtz et al. 2017).

Intensive care units (ICUs) represent high-risk environments for bacteremia, primarily due to invasive procedures and devices such as urinary and vascular catheters (Harris et al. 201; Graff et al. 2002). Risk factors for BSI include HIV infection, diabetes mellitus, long-term hemodialysis, solid organ or stem cell transplantation, glucocorticoid therapy, and hepatic failure (Brigden, 2001). Diagnosis involves culturing blood samples in nutrient-rich media such as brain-heart infusion broth to detect bacterial growth (Coburn et al. 2012). Strict aseptic techniques are essential to avoid contamination, although certain organisms, such as E. coli, S. pneumoniae, and S. aureus, rarely represent contaminants. Because a single positive culture may not confirm true bacteremia, multiple blood cultures from different sites are typically recommended for diagnosis (Hall and Lyman 2006).

Given the increasing prevalence of multidrug-resistant organisms and the variability of regional antimicrobial susceptibility patterns, regular surveillance of BSIs and their resistance profiles is critical. This study aimed to evaluate the spectrum of bacterial pathogens isolated from blood cultures and assess their antimicrobial susceptibility patterns in a tertiary-care hospital setting.

Materials and Methods

Study Design and Setting: This prospective observational study was conducted at the Department of Microbiology, NAMO Medical Education and Research Institute, Silvassa, over a period of 12 months from October 2023 to October 2024.The objective of this study was to isolate and identify bacterial pathogens from the blood cultures of patients with suspected bloodstream infections (BSIs) and to determine their antimicrobial susceptibility profiles.

Sample Size and Inclusion Criteria: A total of 2,290 blood culture samples were collected from patients presenting with clinical suspicion of bacteremia or sepsis. Only samples collected under aseptic conditions and with adequate volumes were included. Among the total samples processed, 199 showed positive bacterial growth, whereas 2,091 were reported to have no growth after the incubation period.

Sample Collection and Culture Methods: Venous blood samples were collected aseptically using sterile syringes and inoculated into blood culture bottles containing brain-heart infusion (BHI) broth. Each inoculated bottle was incubated at 37°C using both the manual and automated methods. In the manual method, bottles were monitored for signs of turbidity or gas production, whereas in the automated method, the BACTEC™ blood culture system (Becton Dickinson) was used to detect microbial growth through fluorescence-based carbon dioxide detection.

When positive growth was indicated, either by turbidity in manual cultures or by a positive signal in the BACTEC system, subcultures were performed on nutrient, MacConkey, and blood agar plates.

These plates were incubated aerobically at 37°C for 18–24 h to allow visible colony formation.

Identification of Bacterial Isolates: Bacterial isolates were identified using conventional biochemical methods and the VITEK-2 Compact automated system (bioMérieux).

In the manual method, bacterial identification is based on standard biochemical reactions. The tests performed included the indole test for tryptophan metabolism, Simmon's citrate utilization test for carbon source usage, and the Triple Sugar Iron (TSI) test for carbohydrate fermentation and hydrogen sulfide production. These tests helped distinguish bacterial species based on their enzymatic profiles and metabolic activity.

The VITEK-2 Compact System was used to identify gram-negative bacilli, gram-positive cocci, and yeast-like organisms. Bacterial suspensions were adjusted to a McFarland turbidity of 0.50–0.63 for GN and GP cards, and 1.80–2.20 for YST cards. These suspensions were then inoculated into the corresponding reagent cards to detect metabolic reactions using colorimetric changes. The system automatically analyzed the data and provided the bacterial identification results.

Antimicrobial Susceptibility Testing (AST): Antibiotic susceptibility testing was performed for each isolate using both the Kirby-Bauer disk diffusion method and automated VITEK-2 system. The selection of antibiotics included a broad range of antimicrobial classes commonly used in clinical practice. These included aminoglycosides (amikacin, gentamicin, and netilmicin), cephalosporins (cefuroxime, cefixime, cefotaxime, ceftriaxone, ceftazidime, and cefepime), carbapenems (meropenem, imipenem, and ertapenem), fluoroquinolones (ciprofloxacin, levofloxacin, and ofloxacin), tetracyclines (doxycycline, minocycline, and tetracycline), macrolides (azithromycin and erythromycin), sulfonamides (co-trimoxazole), glycopeptides (vancomycin, teicoplanin), oxazolidinones (linezolid), polymyxins (colistin), and other agents such as piperacillin/tazobactam, fosfomycin, clindamycin, ampicillin, and amoxicillin.

Susceptibility was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines. Each isolate was categorized as sensitive, intermediate, or resistant to the antibiotics tested based on zone diameter breakpoints or minimum inhibitory concentration (MIC) values.

Data Analysis: All data were manually compiled and analyzed. The percentage of resistant, sensitive, and intermediate isolates was calculated for each antibiotic and organism. The results are summarized in tables and represented graphically using bar charts to illustrate the resistance trends and antibiotic effectiveness against the different bacterial pathogens isolated during the study period.

Results and Discussion

Blood Culture Positivity and Bacterial Distribution: A total of 2,290 blood culture samples were processed during the one-year study period from patients with clinical suspicion of bloodstream infections. Of these, 199 (8.7%) tested positive for microbial growth. After excluding probable contaminants, 116 isolates were confirmed as pathogenic. As shown in Table 1: Gender-wise Distribution of Blood Culture Samples, 58.3% of samples were obtained from male patients and 41.7% from female patients, indicating a slightly higher prevalence in males, consistent with previous epidemiological trends.

Table 1: Gender wise distribution of patients

Gender of patients	No. of positive blood cultures (n=2290)	% of positive blood culture (n=2290)
Male	1335	58.30 %
Female	955	41.70 %
Total	2290	100%

The predominant isolates were Escherichia coli (32 isolates), Klebsiella pneumoniae (31), Pseudomonas aeruginosa (16), and Acinetobacter spp. (15), Enterococcus faecalis (14), and Staphylococcus aureus (8), as summarized in Table 2: Frequency and Percentage of Pathogenic Bacterial Isolates. These organisms represent the most common etiological agents of bloodstream infections in both community and hospital settings.

Table 2: Frequency of Pathogenic Bacterial Isolates

Organism	No. of Isolates (n=116)	% of Total Isolates
Escherichia coli	32	27.6%
Klebsiella pneumoniae	31	26.7%
Pseudomonas aeruginosa	16	13.8%
Acinetobacter spp.	15	12.9%
Enterococcus faecalis	14	12.1%
Staphylococcus aureus	8	6.9%

Antibiotic Susceptibility Patterns of Gram-negative Isolates: Among Gram-negative pathogens, E. coli and Klebsiella pneumoniae were the most frequently recovered. In the comparative analysis shown in Figure 1,E. coli demonstrated higher sensitivity to amikacin (80%) and piperacillin/tazobactam (54%) than Klebsiella, which showed lower susceptibility (50% and 34%, respectively).Both organisms exhibited complete sensitivity (100%) to colistin, confirming its role as a last-resort antibiotic. However, high resistance was noted for ciprofloxacin and cefuroxime in both species, particularly Klebsiella, where the resistance exceeded 60%.

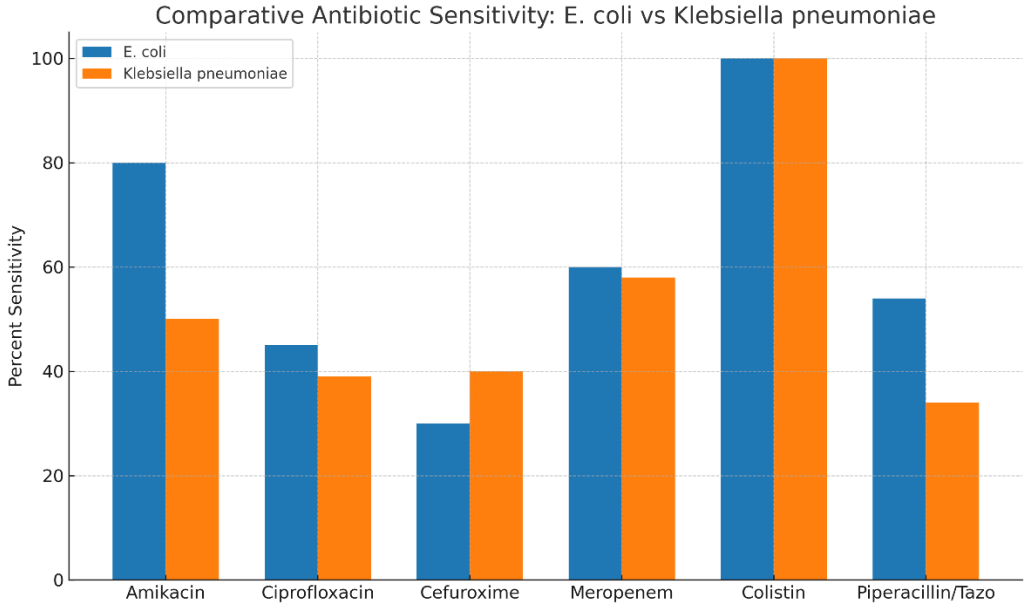

Figure 1: Comparative antibiotic sensitivity patterns of E. coli and Klebsiella pneumoniae.

These patterns are consistent with the widespread occurrence of extended-spectrum beta-lactamase (ESBL)-producing strains, which limits the effectiveness of cephalosporins and fluoroquinolones. Regional studies from South Asia support similar findings, highlighting the need for empirical therapy guided by local antibiograms rather than generalized treatment protocols.

The organism-wise susceptibility patterns for E. coli and Klebsiella pneumoniae are shown in Figure 2 and 3, respectively.

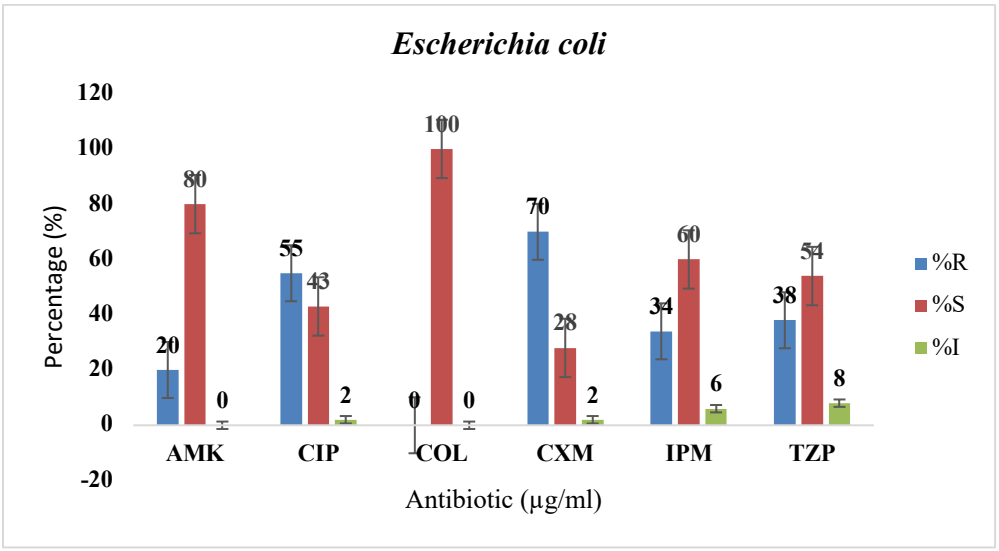

Figure 2: Antibiotic susceptibility profiles of Escherichia coli isolates.

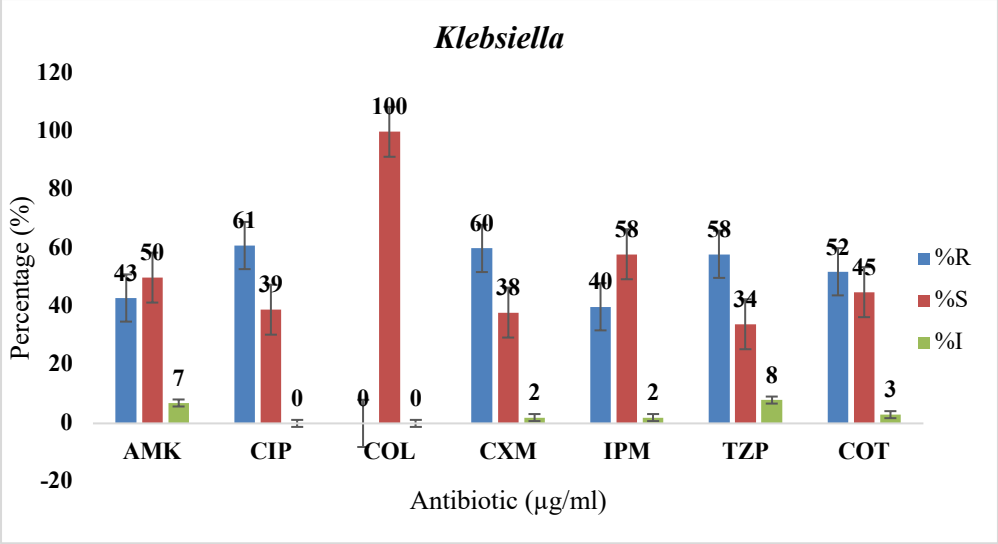

Figure 3: Antibiotic susceptibility profile of Klebsiella pneumoniae.

Pseudomonas and Acinetobacter Resistance Profiles: Pseudomonas aeruginosa showed favorable susceptibility to gentamicin (93%), meropenem (76%), and colistin (100%) (Figure 4). The resistance to ceftazidime and aztreonam was moderate. These findings indicate that aminoglycosides and carbapenems are viable treatment options for P. aeruginosa, although caution must be exercised owing to the potential for rapid resistance development.

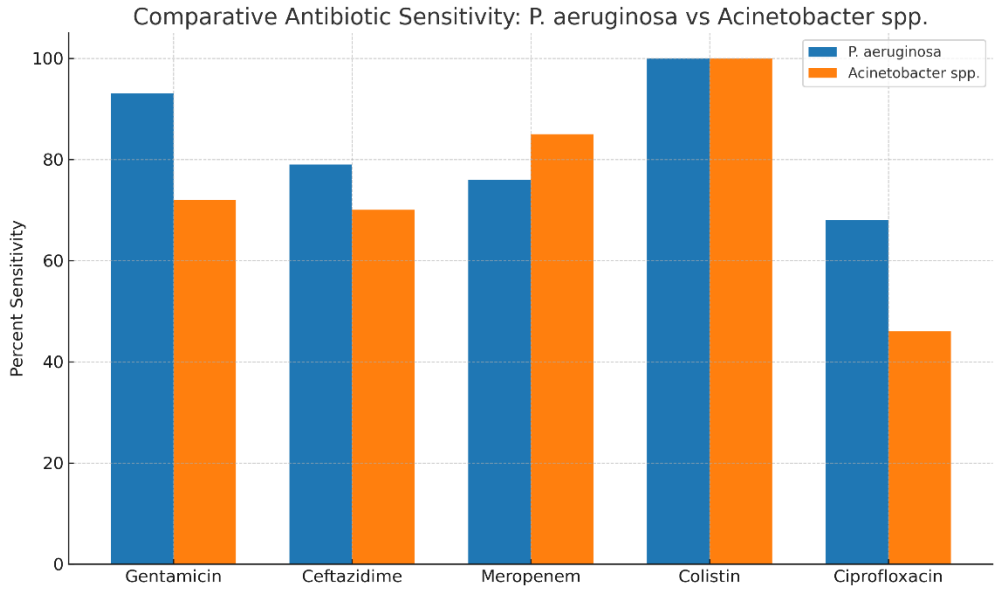

Figure 4: Comparative antibiotic sensitivities of Pseudomonas aeruginosa and Acinetobacter spp.

In contrast, Acinetobacter spp. demonstrated alarming resistance trends, with only 46% sensitivity to ciprofloxacin and 70% sensitivity to ceftazidime. Colistin (100%) and meropenem (85%) were the most effective antibiotics. As shown in Figure 4, the resistance gap between Pseudomonas and Acinetobacter is significant, and emphasizes the threat posed by multidrug-resistant Acinetobacter, particularly in ICU settings.

The organism-specific susceptibility patterns are detailed in Figure 5 and 6.

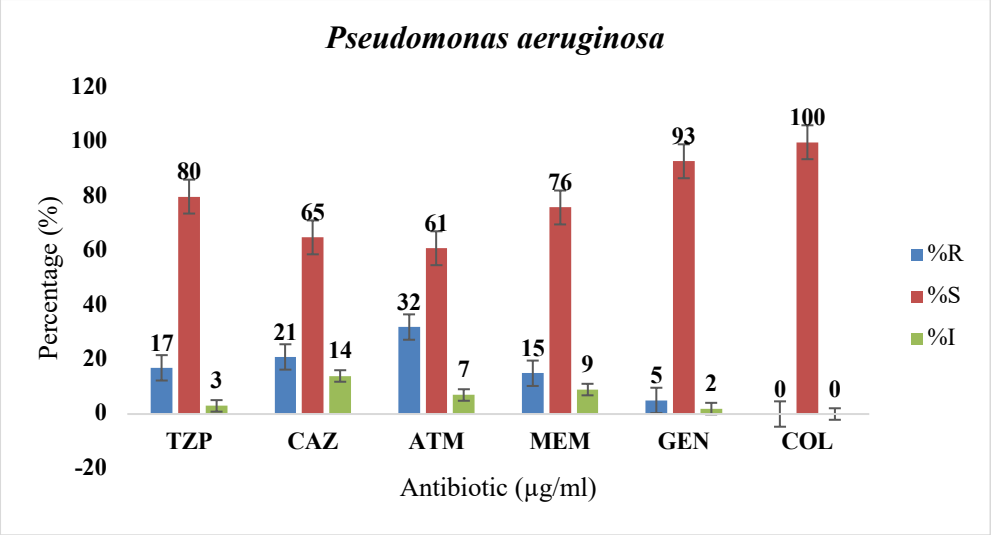

Figure 5: Antibiotic susceptibility profile of Pseudomonas aeruginosa.

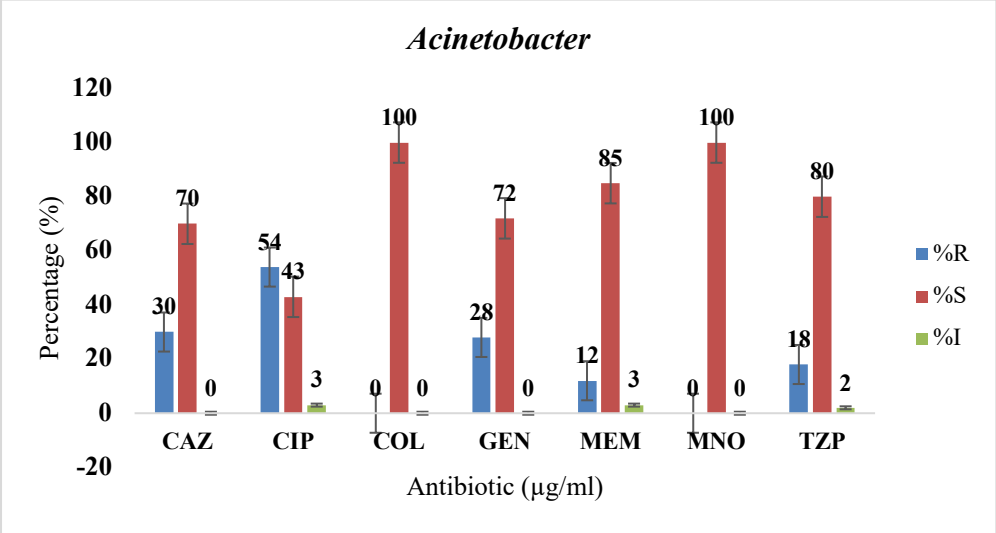

Figure 6: Antibiotic susceptibility profiles of Acinetobacter spp.

Resistance Trends in Gram-positive Pathogens: Among Gram-positive isolates, Staphylococcus aureus showed moderate resistance to erythromycin (41%) and clindamycin (23%), while maintaining full sensitivity to linezolid and trimethoprim-sulfamethoxazole (100%). Only 21% resistance to cefoxitin was observed, indicating a relatively low methicillin-resistant S. aureus (MRSA) burden in this cohort. These results are comparable to national reports, where MRSA prevalence varies between 20% and 50%, depending on the region and hospital practices.

Enterococcus faecalis displayed excellent susceptibility to vancomycin and linezolid (100% each), with moderate resistance to doxycycline (28%) and ciprofloxacin (30%). The comparison in Figure 7 shows that, while both organisms responded well to glycopeptides and oxazolidinones, Enterococcus had slightly reduced sensitivity to other agents.

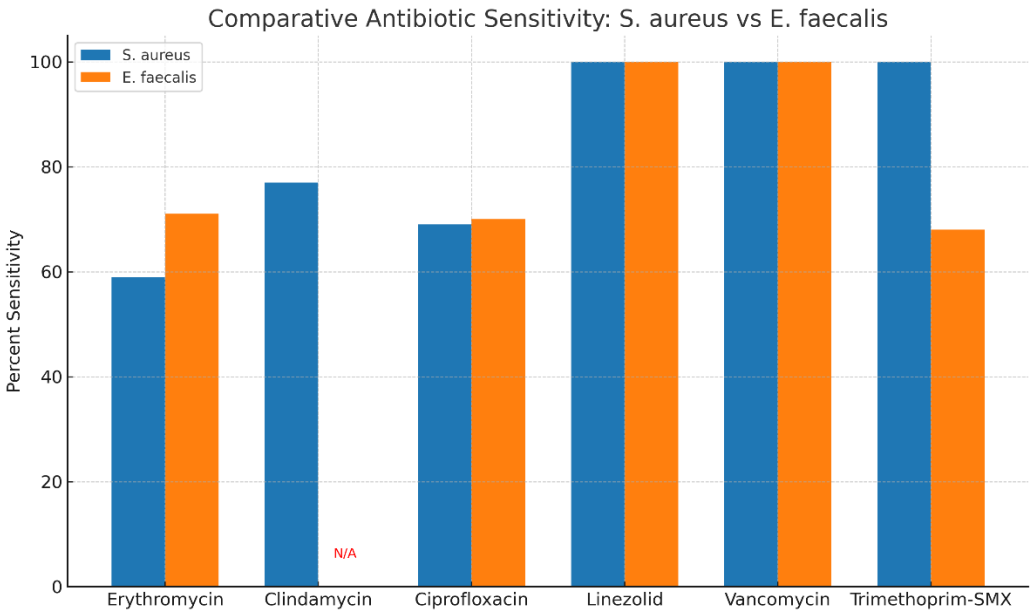

Figure 7: Comparative antibiotic sensitivity patterns of Staphylococcus aureus and Enterococcus faecalis. Both strains retained full sensitivity to vancomycin and linezolid.

The detailed antibiotic response of each gram-positive organism is shown in Figure 8 and 9.

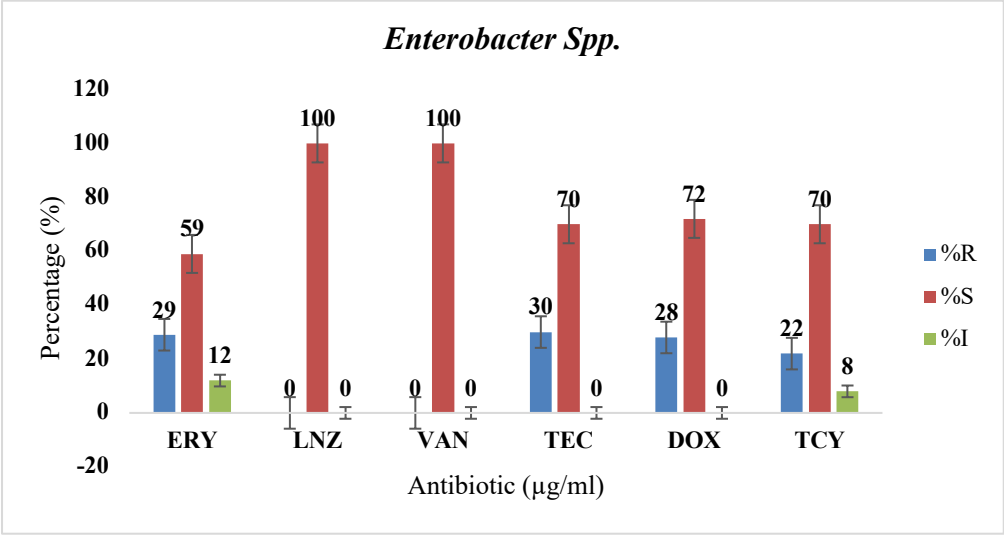

Figure 8: Antibiotic susceptibility profile of Enterococcus faecalis.

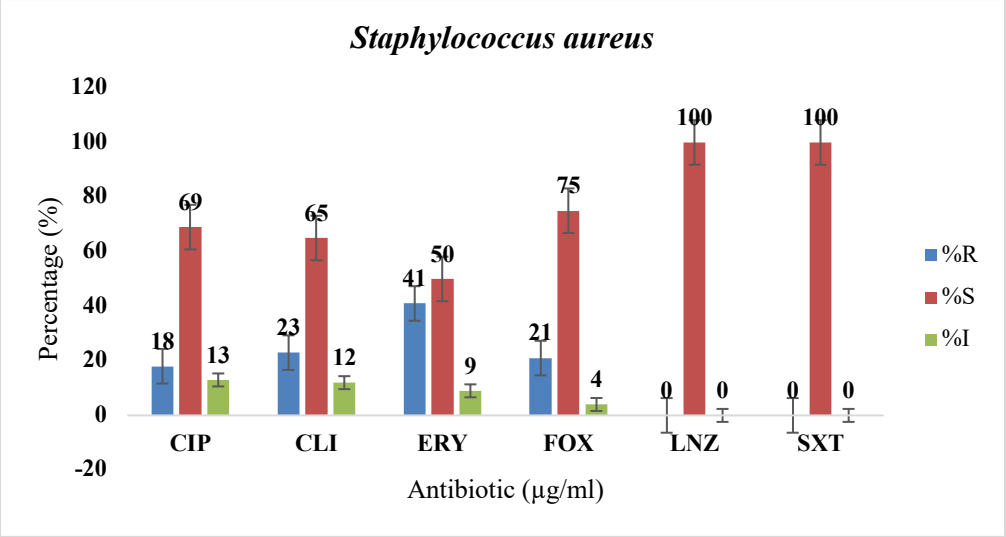

Figure 9: Antibiotic susceptibility profile of Staphylococcus aureus.

Broader Implications and Stewardship Considerations: The findings of this study provide critical insights into the antimicrobial resistance landscape of bloodstream infections. The predominance of gram-negative organisms and their substantial resistance to fluoroquinolones and third-generation cephalosporins call for prudent empirical antibiotic use. Although colistin and linezolid remain universally effective, their status as last-resort agents necessitates strict regulation to prevent further development of resistance.

These results reinforce the importance of antimicrobial stewardship programs, particularly the use of local antibiograms, for guiding therapy. Rapid diagnostics, automated systems, such as VITEK-2, and timely susceptibility testing can significantly improve patient outcomes by enabling targeted treatment. Furthermore, investing in research on bacteriophage therapy, antimicrobial peptides, and resistance-breaking adjuvants may be key strategies in the future.

Regular surveillance studies, continuous training of clinicians, and integration of hospital-specific resistance data into treatment protocols are crucial steps toward managing the growing threat of antimicrobial resistance in bloodstream infections.

Conclusion

In conclusion, this study revealed a pattern of antimicrobial resistance among key bacterial pathogens isolated from bloodstream infections, particularly Klebsiella pneumoniae, Acinetobacter spp., and Escherichia coli, which exhibited significant resistance to commonly used antibiotics, such as cephalosporins and fluoroquinolones. While last-resort agents, such as colistin and linezolid, remain effective, their exclusive efficacy emphasizes the urgency of implementing targeted antimicrobial stewardship and routine susceptibility testing. These findings highlight the importance of local resistance surveillance to guide empirical therapy and the need for strengthened infection control measures to mitigate the spread of multidrug-resistant organisms in clinical settings.

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