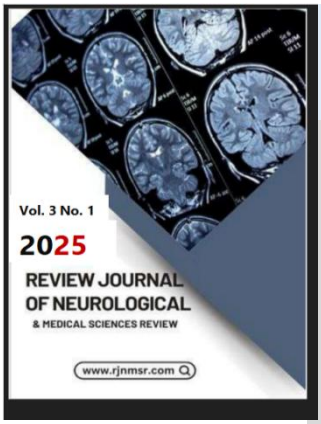

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Bacteriological Profile and Antibiotic Susceptibility Patterns of Blood Stream Infections

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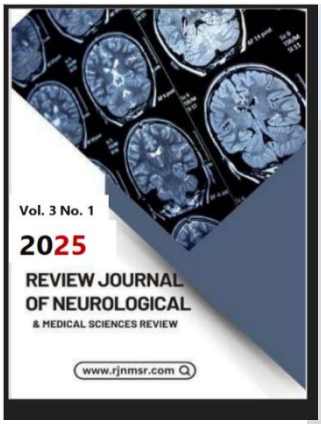
Abstract

Bloodstream infections (BSIs) significantly contribute to global morbidity and mortality, exacerbated by rising antimicrobial resistance. A thorough understanding of the bacterial profiles and their antibiotic susceptibility is crucial for effective treatment. This study aims to identify the pathogens associated with BSIs, assess their prevalence, and evaluate their antibiotic resistance patterns, considering demographic factors and comorbidities. A cross-sectional study was conducted at the Microbiology Lab of Amjad Naeem Clinic Rawalpindi, from January to June 2024. We analyzed 135 blood culture samples using aseptic techniques. Blood samples were cultured in Brain Heart Infusion (BHI) broth under aerobic and anaerobic conditions for up to 7 days. Isolates were classified using Gram staining and biochemical tests. Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method. Staphylococcus aureus was the most prevalent pathogen, accounting for 41.2%, followed by Escherichia coli at 21.8%. Other notable isolates included Acinetobacter baumannii (10.3%), Pseudomonas aeruginosa (7.4%), and Klebsiella pneumoniae (8.1%). Antibiotic susceptibility revealed that Staphylococcus aureus and Enterococcus faecalis were highly sensitive to linezolid (92.3%) but resistant to erythromycin (72.7%). Escherichia coli showed significant resistance to cephalexin (61.5%) but high sensitivity to meropenem (91.7%). Klebsiella pneumoniae was



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similarly sensitive to meropenem (83.3%) and resistant to cephalexin (88.9%). Ciprofloxacin was effective against Acinetobacter baumannii (84.6%) and Pseudomonas aeruginosa (66.7%), while Salmonella typhi was sensitive to sulfamethoxazole (71.9%). Our findings indicate that Staphylococcus aureus is the predominant pathogen in BSIs, with Escherichia coli as the leading gram-negative organism. The observed antibiotic susceptibility patterns underscore the need for awareness programs to mitigate antibiotic resistance.

Keywords: Bloodstream infection, Antimicrobial susceptibility, CLSI 2023, Rawalpindi

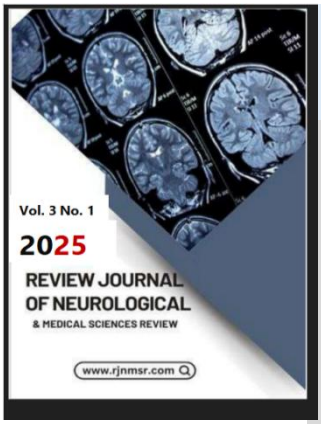
Introduction

Bloodstream infections (BSIs) are serious medical conditions characterized by the presence of viable bacteria within the bloodstream, leading to inflammatory responses that affect hemodynamic and clinical parameters. These infections can arise from various sources within the body and pose significant health risks, particularly in healthcare settings (Klevens et al., 2017; Rangel-Frausto et al., 2019).

Globally, BSIs represent a significant category of healthcare-associated infections, accounting for approximately 9% to 11% of hospital-acquired infections in developed countries and up to 19% in low- and middle-income nations (Klevens et al., 2007). In the U.S., the prevalence of BSIs has decreased by 16% from 2011 to 2015, but they still contribute to around 250,000 deaths annually across North America and Europe (CDC, 2019). In Pakistan, prevalence rates of BSIs have been documented to range from 8.4% to 13.1% (Khan et al., 2020).

Mortality rates associated with BSIs are concerning, with estimates of 21 to 32 deaths per 100,000 individuals and case fatality rates of 10% to 28% depending on the infection's origin (Vincent et al., 2014). Risk factors for BSIs include poor hand hygiene, inadequate isolation of high-risk patients, and improper antibiotic use (CDC, 2019). BSIs can be categorized into central line-associated, community-acquired, and hospital-acquired infections, each with distinct origins and associated pathogens. Gram-positive bacteria like Staphylococcus aureus and Gram-negative bacteria such as Escherichia coli are common culprits, with rising antibiotic resistance posing a significant public health challenge (WHO, 2014; Tzeng et al., 2015).

Recent studies highlight the increasing prevalence of multidrug-resistant organisms contributing to BSIs, complicating treatment options and emphasizing the need for effective infection control measures. For instance, the emergence of carbapenem-resistant Enterobacteriaceae (CRE) has raised alarms due to its association with high mortality rates and limited therapeutic options (Brolund et al., 2021). Furthermore, the COVID-19 pandemic has exacerbated the situation, with reports indicating a rise in secondary infections, including BSIs, among hospitalized patients (Paltiel et al., 2021). This underscores the urgent need for ongoing surveillance and research to adapt treatment strategies and mitigate the impact of BSIs.Understanding the patterns of antibiotic susceptibility among these pathogens is essential for effective treatment and infection control strategies. Continuous monitoring of the microbiological landscape and susceptibility profiles is crucial for guiding empirical antibiotic therapy and preventing the spread of resistant strains (Murray et al., 2021).



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Methodology

This study employed an experimental, cross-sectional design conducted at the Microbiology Lab of Amjad Naeem Clinic Rawalpindi, from January to June 2024. The study spanned four months following approval from the research board. Data collection occurred three times a week throughout this period.

Participants included patients with confirmed bloodstream infections who had not received antibiotic treatment for at least two weeks prior to enrollment. Sufficient blood samples were required for bacterial profiling and antimicrobial susceptibility testing. Individuals of any age were eligible if they met these criteria.

Patients who had received antibiotics before sample collection, those with infections unrelated to bloodstream infections, individuals with insufficient blood samples, and pregnant women were excluded from the study.

A total of 135 patient specimens were analyzed over the four-month study period, specifically focusing on blood culture specimens. Patient records and history charts from the past two years, along with current records of individuals presenting with bloodstream infections, were reviewed in the microbiology lab. Purposive random sampling was utilized to select research participants.

Blood samples were collected following strict aseptic techniques to prevent contamination. The procedure included wearing gloves and cleansing the venipuncture site with 70% ethanol, followed by 2% tincture of iodine. Using a sterile syringe and needle, approximately 10-15 mL of blood was withdrawn from adults or around 2 mL from young children for both aerobic and anaerobic cultures. Multiple blood cultures (at least two) were collected from different venipuncture sites to enhance pathogen detection. The blood was inoculated into diphasic culture medium (Brain Heart Infusion broth) and incubated promptly. Blood was inoculated into 50 mL of Brain Heart Infusion (BHI) broth, with a blood-to-broth ratio of approximately 1:10. The samples were incubated at 37°C for up to 5-7 days, with daily monitoring for signs of bacterial growth.

Upon detecting bacterial growth, subculturing was performed onto solid media such as Blood agar, MacConkey agar, or Chocolate agar. Gram staining was conducted to categorize bacteria as either Gram-positive or Gram-negative, aiding in preliminary identification.

Biochemical tests, including the catalase, coagulase, oxidase, indole, urease, and Triple Sugar Iron tests, were employed to confirm bacterial identification. Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method, determining the sensitivity, intermediate, or resistance profiles of the isolates.

Results

The data of 135 patients with Bloodstream infections were collected, the parameters that were taken for this study included their age (years), gender, comorbidities, length of hospital stay(days), symptoms (fever, vomiting, diarrhea, respiratory distress, body pain) and organisms causing the bloodstream infection and their respective antibiotic susceptibility profiles

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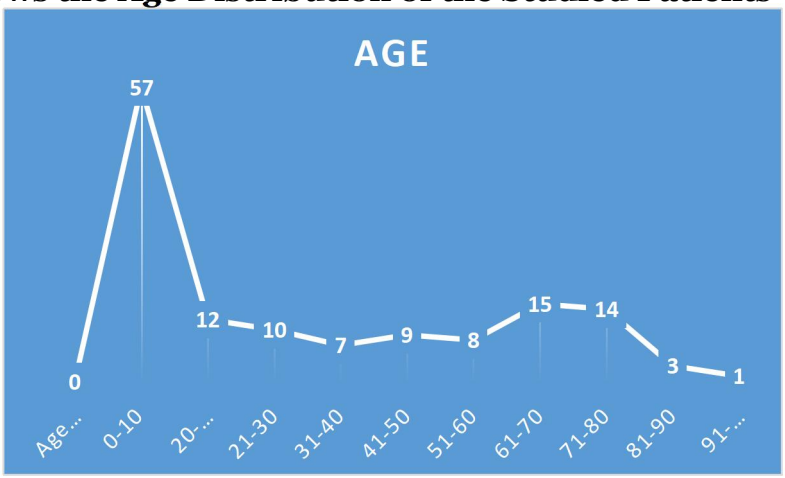
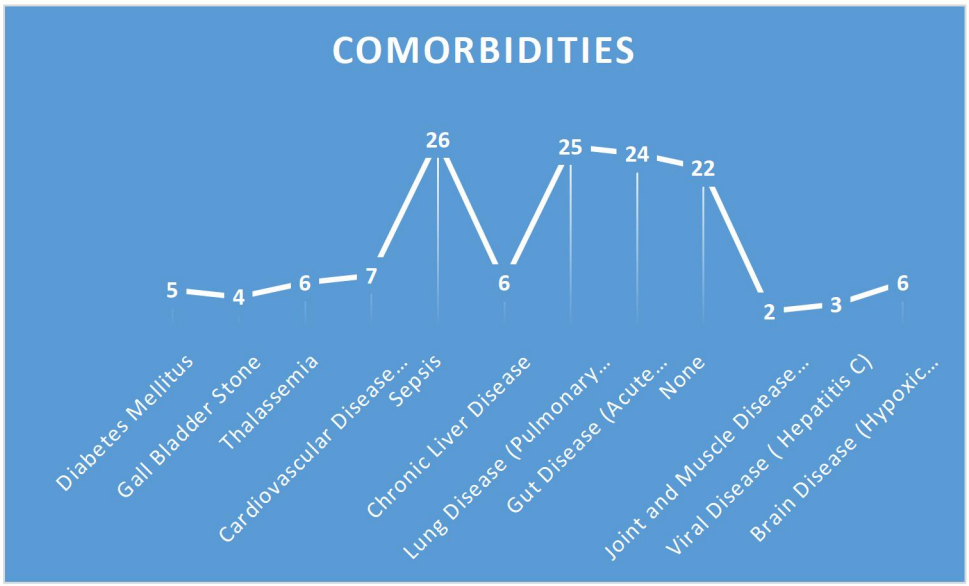
Figure 1: Shows the Age Distribution of the Studied Patients



Out of our total collected samples N=135 the patients between the age of 0-10 years have N=57 (41.9%) have the highest percentage of BSIs infection, 11-20 years N=12 (8.8%) have second highest percentage, 21-30 years N=10 (7.4%) of patients, then 31-40 years N=7 (5.1%) patients , 41-50 years N=9 (6.6%) patients, 51-60 years N=8 (5.9%) having the BSIs infection, 61-70 years N=15 (11.0%) patients, 71-80 years N=14 (10.3%) patients, 81- 90 years N=3 (2.2%) patients, and then 91-100 years N=1(0.7%). having least number of patients having the bloodstream infection.

Figure 2: Shows the Comorbidities of the Studied Patients



Out of our total samples N=135 it was noticed that about N=26 (19.1%) of BSIs patients having sepsis as a comorbidities, N= 25 (18.4%) lung disease (Pulmonary Fibrosis) , N=24 (17.6) patients having Gut disease (Acute Gastroenteritis) ,then N=22 (16.2%) patients do not have any type of Comorbidities , cardiovascular disease

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(Angina) N=7 (5.1%) , brain disease (Hypoxic Ischemic Encephalopathy), N=6 (4.4%), thalassemia N=6 (4.4%), chronic liver disease N=6 (4.4%),then Diabetes Mellitus N=5 (3.7%), gall bladder Stone N=4(2.9%), viral disease (hepatitis C) N=3 (2.2%) ,then least number of patients have the joint and muscle disease (Arthritis) N=2 (1.5%).

Figure 3: Shows The Symptoms Of The Participants

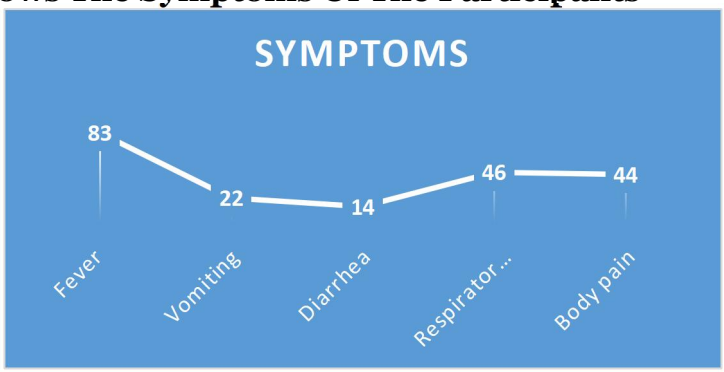
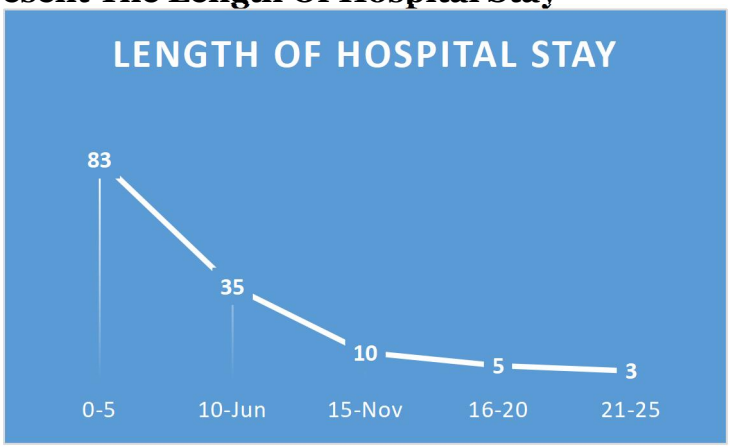


Figure 4: Represent The Length Of Hospital Stay



Out of total twelve organisms, there are four gram positive organisms Staphylococcus aureus N= 56 (41.2 %), Enterococcus faecalis N= 6 (4.4%), Streptococcus sp. N=3 (2.2%) then least bacteria is Staphylococcus epidermis N=2(1.5%), which comprises (49.2%) N= 67 of the total samples.

Out of total twelve organisms, there are four gram positive organisms Staphylococcus aureus N= 56 (41.2 %), Enterococcus faecalis N= 6 (4.4%), Streptococcus sp. N=3 (2.2%) then least bacteria is Staphylococcus epidermis N=2(1.5%), which comprises (49.2%) N= 67 of the total samples.

The gram negative organisms were eight and more prevalent organisms which included Escherichia coli N=16 (11.8%), Acinetobacter baumannii N=14 (10.3%), Pseudomonas aeruginosa N=11 (8.1%), Klebsiella pneumoniae N= 10 (7.4%), ,Salmonella typhi N=8 (5.8%), Pseudomonas stutzeri N=6 (4.4%), Enterobacter cloacae N=3 (2.2%),then least noumber of bacteria was Stenotrophomonas sp.N=1(0.7%), and they comprise N=69 (50.8%) of the total samples.

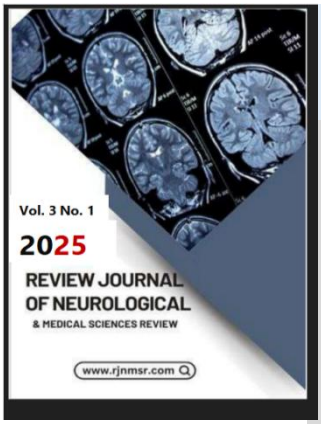


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Figure 6: Prevalence Of Gram Negative Isolates Among Pregnant Women

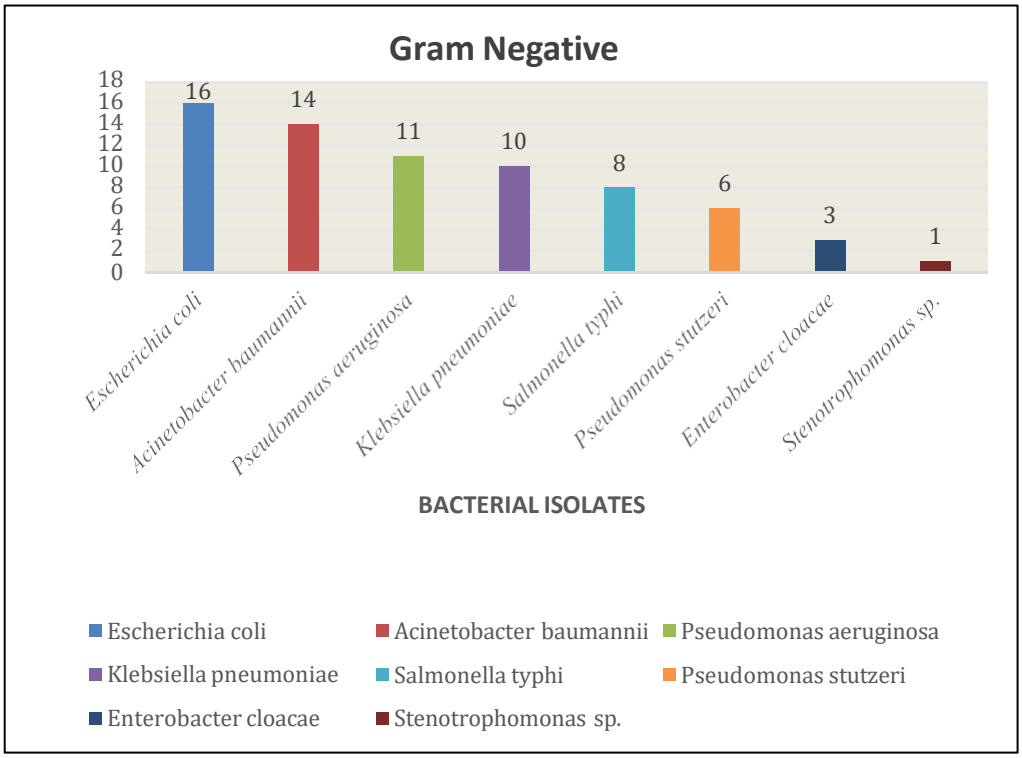
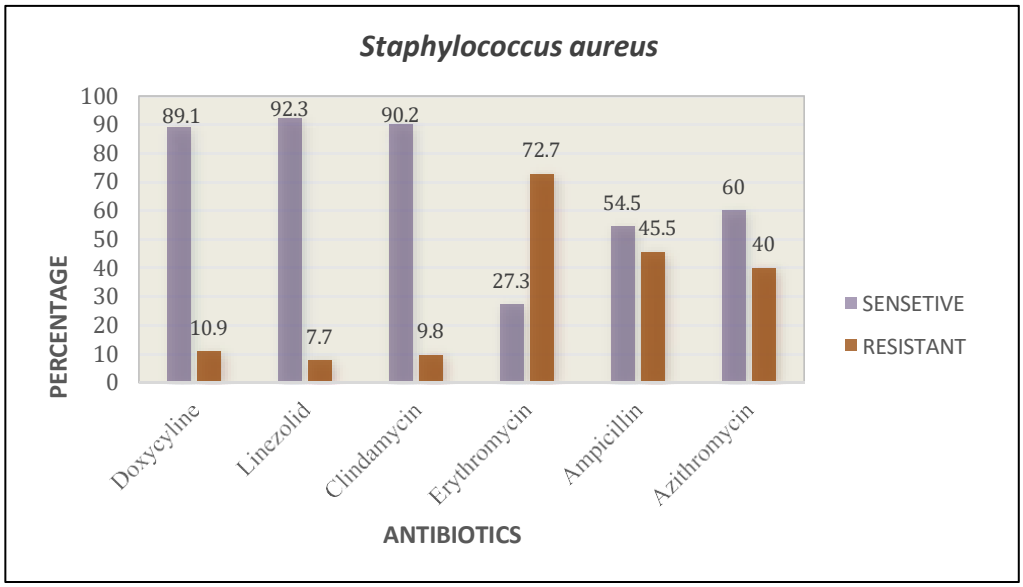


Figure 7: Antibiotic Susceptibility Profiles Of Gram Positive Isolates



Staphylococcus aureus showed high resistance rates against azithromycin 72.7% and 45.5% from ampicilin but linezolid showed high effectiveness 92.3%, and

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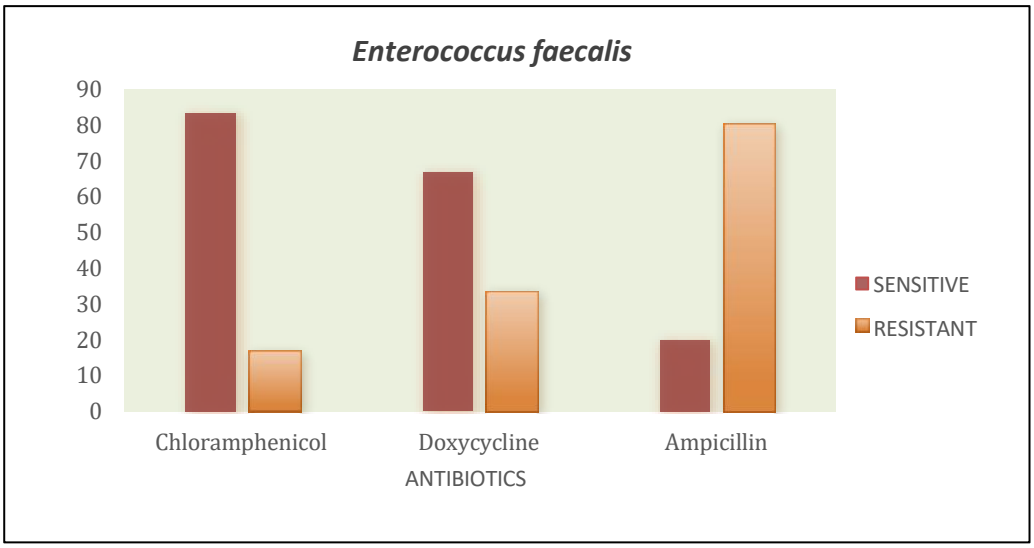
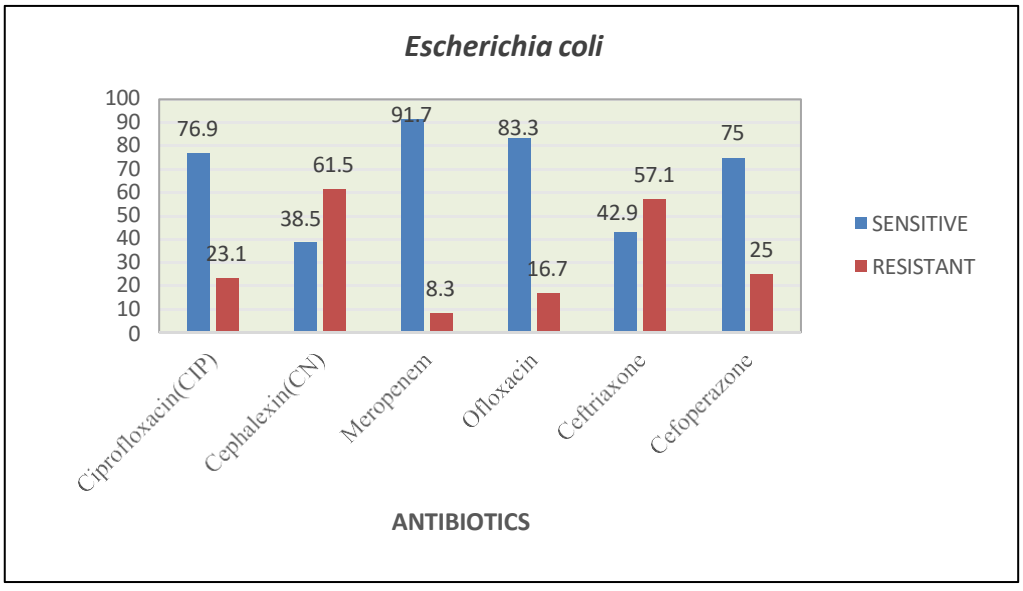
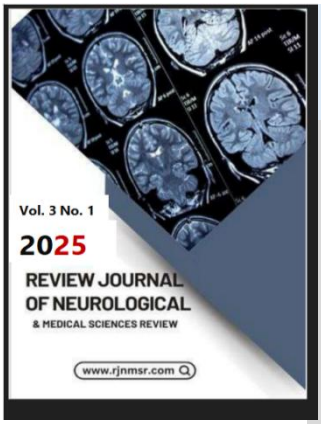
clindamycin have effectivesness 90.2% against Staphylococcus aureus , having the low resistant rate linezolid 7.7% and clindamycin 9.8%. doxycycline having the effective against bacteria is 89.1% with little resistant rate of 10.9%, azithromycin have high sensitivity of 60% then resistance 40% against the Staphylococcus aureus infection.





This chart shows the antibiotic susceptibility profile of Escherichia coli isolates. Meropenem appears to be the most sensitive occurring up to 91.7%, with the lowest resistance rate of 8.3%. This suggests that Meropenem could be a potential treatment option for E. coli infections, especially in cases of multidrug resistance. Among the tested antibiotics, Cephalexin (CN) stands out with the highest resistance rate, with 76.9% of isolates being resistant. This indicates a significant challenge in treating E.



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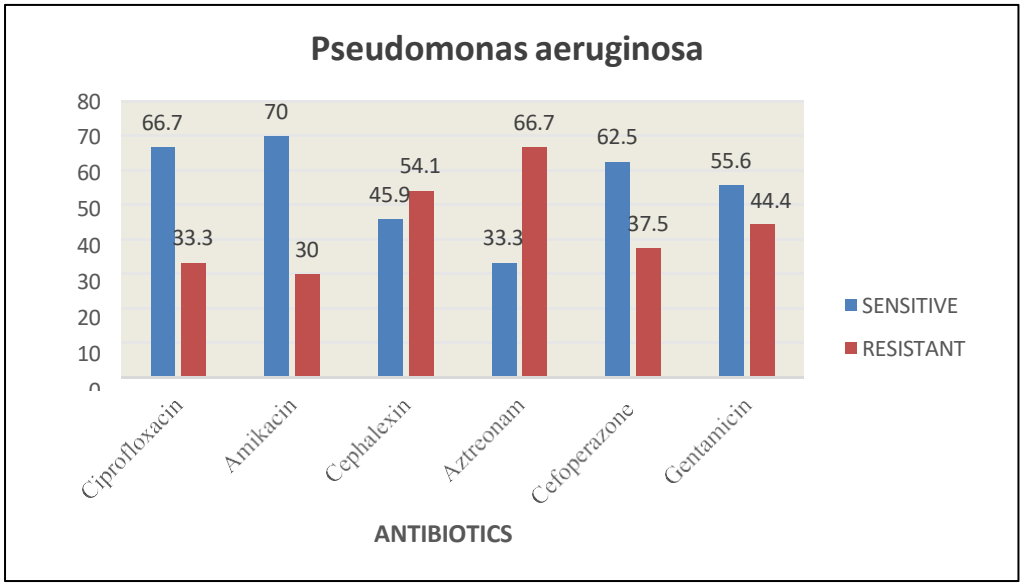
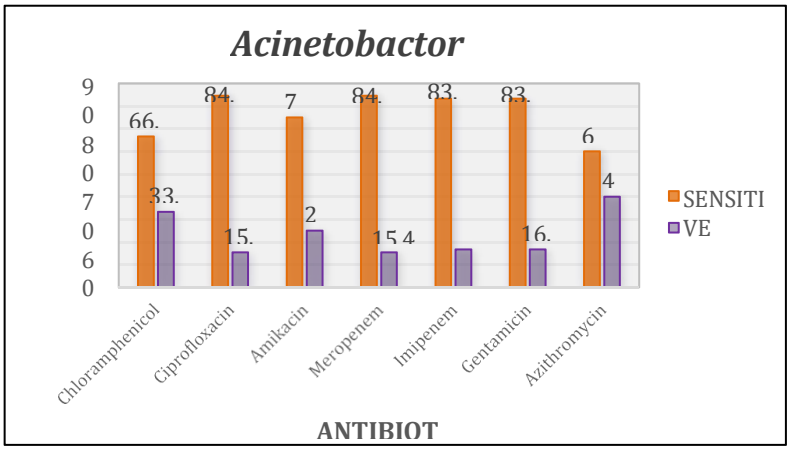
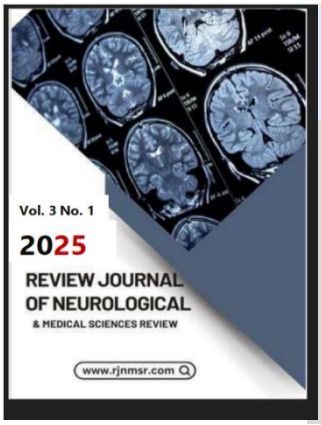
coli infections with this antibiotic. Ciprofloxacin (CIP) also exhibits substantial resistance (61.5%). While Meropenem, Ofloxacin, Ceftriaxone, and Cefoperazone demonstrate lower resistance rates, the presence of any resistance emphasizes the importance of careful antibiotic selection.



Amikacin emerged as the most effective antibiotic, with a sensitivity rate of 70% and only 30% resistance, indicating its continued efficacy in combating infections caused by Pseudomonas aeruginosa. Ciprofloxacin and Aztreonam also demonstrated promising sensitivity levels, both at 66.7%, while resistance was observed at 33.3%. Cefozoparone exhibited a sensitivity of 62.5% and resistance of 37.5%, suggesting moderate effectiveness. Gentamicin showed slightly lower sensitivity at 55.6% and a resistance rate of 44.4%, which could limit its reliability in certain cases. Cephalexin, however, displayed an equal sensitivity and resistance rate of 50%, making it a less dependable choice for treating Pseudomonas aeruginosa bloodstream infection.





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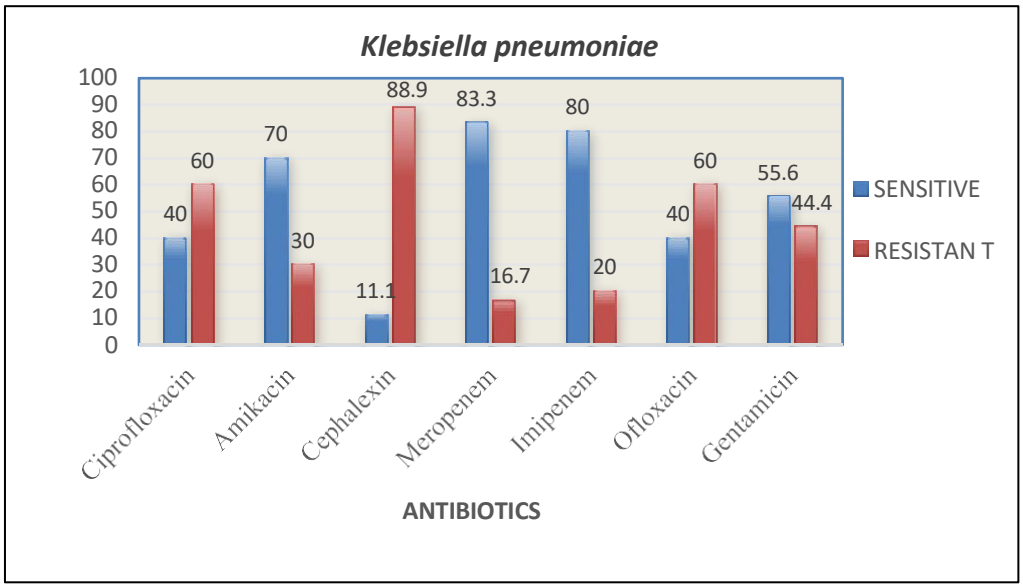
In this graph the antibiotic susceptibility profile of Acinetobactor baumannii isolates. Ciprofloxacin and Meraopenem appears to be the most sensitive occurring up to 84.6.%, with the lowest resistance rate of 15.4%. Gentamicin and Imipenem also have great sensitivity towards Acinetobactor baumannii the infection. This suggests that Ciprofloxacin and Meropenem could be a potential treatment option for Acinetobactor baumannii infections, especially in cases of multidrug resistance. Among the tested antibiotics, Azithromycin stands out with the highest resistance rate, with 40% of isolates being resistant. This indicates a challenge in treating Acinetobactor baumannii infections with this antibiotic. Chloramphenicol also exhibits substantial resistance of 33,3. Gentamicin and Imipenem has the resistant rate about 16.7% While Meropenem, Ciprofloxacin has the lowest resistant rate about 15.4% the presence of any resistance emphasizes the importance of careful antibiotic selection.



Among the antibiotics, Meropenem exhibited the highest sensitivity rate at 83.3%, followed closely by Imipenem at 80%, suggesting their efficacy as carbapenem agents in combating Klebsiella pneumoniae bloodstream infections. However, resistance to Meropenem and Imipenem, though comparatively low at 16.7% and 20%, indicates emerging concerns about resistance even in these potent antibiotics. Amikacin also showed a high sensitivity rate of 70%, with resistance at 30%, making it a strong candidate for treatment. Conversely, Cephalexin exhibited the lowest sensitivity (11.1%) and the highest resistance (88.9%), underscoring its limited utility against this pathogen.

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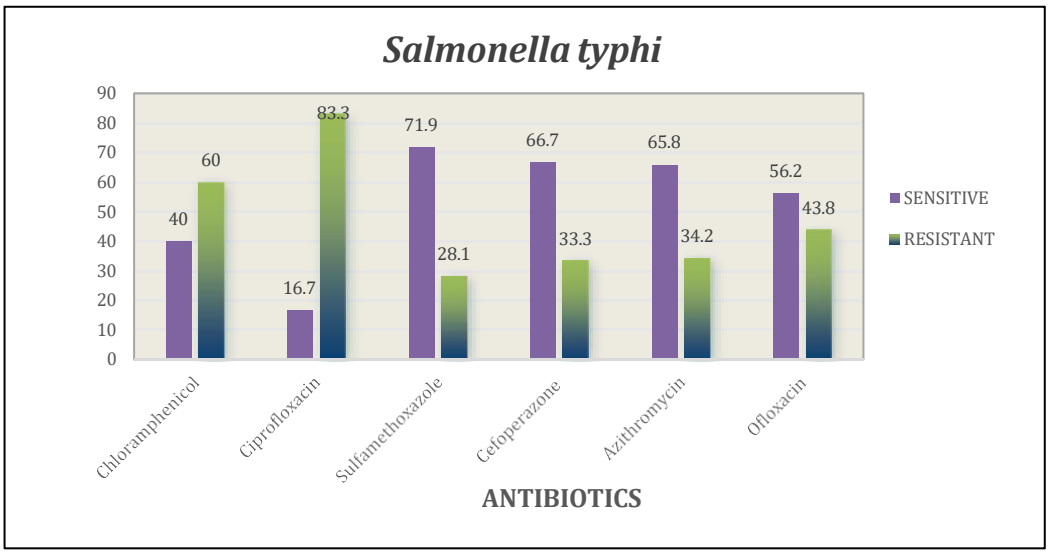


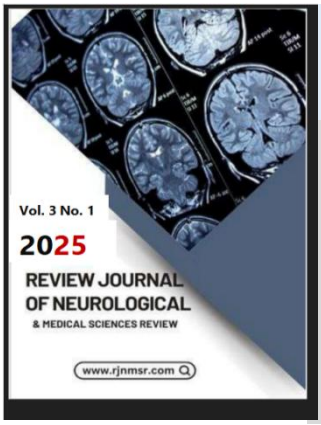
Salmonella Typhi showed the greatest resistance to ciprofloxacin 83.3% while it exhibited the highest sensitivity to Sulfamethoxazole 71.9%, cefoperazone 66.7%, azithromycin, showed 65.8% and Ofloxacin shows 56.2% sensitiviy from Salmonella Typhi infection resistance and 50% sensitivity. Therefore, cefoperazone proved to be the most effective antibiotic against Salmonella Typhi, with the resistant rate of Sulfamethoxazole 29.1%, cefoperazone 33.3%, azithromycin, showed 34.2% and Ofloxacin shows a bit high resistant to infection about 43.8%.

Discussion

Bloodstream infections (BSIs) are significant infectious diseases marked by the presence of bacteria in the bloodstream, leading to considerable morbidity and mortality worldwide. Despite advancements in treatment and prevention, BSIs remain a leading cause of death and disability, particularly in developing countries. The increasing trend of bacteremia in certain regions necessitates urgent and invasive management with antimicrobial therapies.

In our study, we observed a higher prevalence of BSIs among females (55.1%) compared to males (44.9%). This gender disparity may stem from biological, clinical, and social factors. Biologically, hormonal differences can influence immune responses; for instance, estrogen is linked to enhanced immunity in females, potentially leading to more robust inflammatory responses. Clinically, women often undergo more invasive procedures, such as catheterizations and gynecological surgeries, which are known risk factors for BSIs. Socially, disparities in healthcare access may result in women seeking medical attention later, exacerbating infection severity. These findings align with global studies, such as Muleta D. (2022) in Ethiopia, which reported similar trends, while Cohen et al. (2018) noted a marginally higher incidence among males in the U.S., primarily due to lifestyle-related risk factors.

The microbiological profile of BSIs also varies by gender. Women are more frequently infected with Gram-negative organisms like Escherichia coli, often linked



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to urinary tract infections (UTIs), whereas men are more susceptible to multidrug-resistant organisms such as Methicillin-Resistant Staphylococcus aureus (MRSA). Our findings highlight the need for gender-specific strategies in prevention and management, including tailored empirical antibiotic treatments and enhanced catheter care protocols.

Our study revealed that the highest incidence of BSIs occurred in patients aged 0-5 years (36.8%), followed by the 11-15 and 71-75 age groups (6.6% each). In contrast, a study in Nepal found the highest prevalence in the 16-30 age group. This discrepancy may be due to differences in demographic composition and healthcare-seeking behaviors. The significant burden of BSIs in the pediatric population could be attributed to their developing immune systems and higher exposure to pathogens.

Most patients in our study were discharged within 1-5 days, indicating effective early intervention protocols. Recent studies have shown that patients with multidrug-resistant bacteria tend to have longer hospital stays due to the complexities of managing such infections. In our case, the majority of patients had short hospital stays, suggesting the effectiveness of rapid diagnostics and targeted therapy. Timely and appropriate antimicrobial treatment is critical in reducing hospital stays and associated costs.

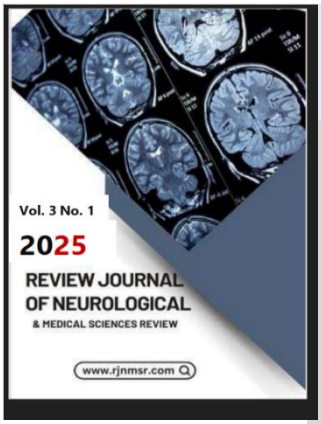
In terms of comorbidities, sepsis was the most common condition observed in our study (19%), contrasting with Marwan Jabr's study, which identified hypertension as the leading comorbidity (56.5%). Symptoms associated with BSIs included fever (61%), respiratory distress (34%), and less commonly, vomiting and diarrhea. These findings are consistent with previous studies highlighting fever as a primary symptom.

Our study identified Staphylococcus aureus as the dominant microorganism, with a prevalence of 41.2%. This aligns with findings from Ayoyi et al., who reported a 29.7% prevalence among pregnant women. However, other studies indicated a higher prevalence of Gram-negative organisms, particularly Escherichia coli, in similar populations. The variability in microbial profiles may reflect differences in study populations and healthcare settings.

Antibiotic resistance patterns revealed that Staphylococcus aureus exhibited high resistance rates against ampicillin (88%) and azithromycin (87%), consistent with other studies. Linezolid demonstrated high effectiveness (98%) against Staphylococcus aureus, aligning with previous research. Enterococcus faecalis showed 80% sensitivity to linezolid but high resistance to azithromycin, contrasting with findings from India.

Among Gram-negative organisms, Escherichia coli showed a high resistance rate against ampicillin (97%) but significant sensitivity to amikacin (93%) and fosfomycin (83%). Klebsiella pneumoniae displayed high sensitivity to gentamicin (84%) and cefotaxime (80%), while Acinetobacter baumannii and Pseudomonas aeruginosa showed 90% and 88% sensitivity to gentamicin, respectively.

In conclusion, our study underscores the critical need for gender-specific approaches in managing BSIs, emphasizing the importance of timely diagnosis and tailored antibiotic therapies. The findings highlight the evolving landscape of microbial



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resistance and the necessity for ongoing surveillance to optimize treatment strategies and improve patient outcomes.

Conclusion

In conclusion, our study highlights the significant impact of gender and age on the prevalence of bloodstream infections, emphasizing the need for tailored prevention and management strategies. The findings underscore the importance of early diagnosis and targeted antibiotic therapies to combat evolving antimicrobial resistance, ultimately improving patient outcomes and reducing hospital stays. Continuous surveillance and adaptation of treatment protocols are essential to address the complexities of BSIs effectively.

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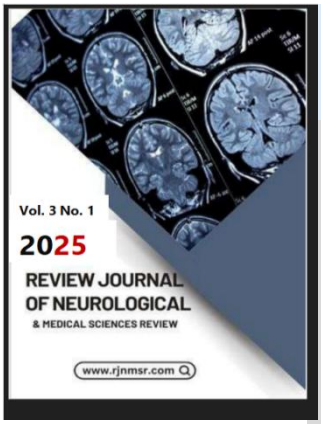

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