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Original Article

Microbiology Section

Bacteriological Profile and Antimicrobial Susceptibility Pattern of Blood Culture Isolates in Immunocompromised Patients: A Hospital-based Cross-sectional Study

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ABSTRACT

Introduction: Immunocompromised individuals have weaker immune systems and they are more likely to develop Bloodstream Infections and have lower ability to fight against infections. Multidrug-resistant bacterial infections require rapid pathogen identification because it enables healthcare providers to start targeted antimicrobial treatment, which leads to better patient outcomes.

Aim: To determine the bacterial profile and antimicrobial susceptibility pattern of isolates from blood culture of immunocompromised patients admitted to a tertiary care hospital.

Materials and Methods: This hospital-based cross-sectional study was carried out in the Department of Microbiology, National Institute of Medical Sciences and Research, Jaipur, Rajasthan, India, between October 2023 and April 2024. A total of 246 blood samples were collected from immunocompromised individuals under aseptic conditions and processed in the BD BACTEC FX40 automated blood culture system. The standard microbiological and biochemical techniques were used to identify the positive blood cultures. The modified Kirby-Bauer disk diffusion method was used to test for antimicrobial susceptibility, and the Clinical and Laboratory Standards Institute (CLSI) criteria for 2023 were followed in interpreting the results.

Results: A total of 246 blood samples were processed, and 75 (30.49%) were culture-positive. Of the patients with culture-positive results, 30 (40%) were female, and 45 (60%) were male. Most of the patients were below 60 years of age. There were 57 (76%) Gram-negative and 18 (24%) Gram-positive bacteria. Gram-negative bacteria commonly present were *Escherichia coli* 10 (17.54%), *Klebsiella pneumoniae* 15 (26.31%), and *Pseudomonas aeruginosa* 18 (31.57%). *Staphylococcus aureus*, constituting 7 (38.89%) of Gram-positive bacteria, all Methicillin-Resistant *Staphylococcus aureus* (MRSA). Multidrug resistance was commonly present in *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter baumannii*.

Conclusion: The current research demonstrated that people with weakened immune systems face higher risks of developing BSIs, which are caused by multidrug-resistant Gram-negative bacteria. The establishment of enhanced local BSI surveillance systems, together with prompt microbiological testing, will lead to improved antimicrobial treatment decisions, which will result in better treatment results for patients in high-risk categories.

Keywords: Antibiotic resistance, Hospital-acquired infection, Multidrug resistance, Sepsis

INTRODUCTION

The immune system has developed to defend the host against an ever-evolving cosmos of dangerous microorganisms [1,2]. The ability to differentiate between self and non-self is crucial for triggering the right immunological response [2]. Innate immunity and adaptive immunity are the immune system's two main lines of defense against infection. The imbalance between these two can lead to several disorders, including immunodeficiency, autoimmunity, hypersensitivity, and chronic inflammation [3,4]. People who have weak immune systems face a higher risk of getting infections. The underlying causes of this condition arise from multiple clinical conditions, which include cancer, Human Immunodeficiency Virus (HIV), diabetes, and genetic disorders, together with environmental factors, which include organ transplantation, malnourishment, immunosuppressive drugs, and radiation/chemotherapy treatments [5].

The BSI represents one of the most critical complications that affects these patients because it involves the existence of viable pathogens in their bloodstream [6]. BSIs, which include bacteraemia and septicaemia, represent the primary reason for hospital admissions and fatalities throughout all age groups [7]. The excessive or inappropriate use of broad-spectrum antibiotics results in more difficult bacteraemia cases because it enables bacterial pathogens to establish permanent infections, which develop into severe life-threatening conditions like septic shock, multiple organ failure, and disseminated intravascular coagulation [8,9]. According to worldwide statistics, 30 million individuals experience BSIs every year, which results in six million fatalities. The total number of deaths includes three million newborns and 1.2 million children developing sepsis annually [10]. The most common pathogen causing BSI is Gram-positive bacteria such as *Staphylococcus aureus,* coagulase-negative *Staphylococcus, Enterococcus, Streptococcus pneumoniae*, and Gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii* [11].

Constant surveillance is necessary due to the changing epidemiology and resistance tendencies of these pathogens [12]. Empirical treatment is generally based on clinical suspicion. The local bacteria present in the area, together with their specific antimicrobial resistance patterns, must be understood for successful medical treatment [13], while definitive therapy relies on isolating pathogens and sensitivity testing, traditionally dependent on culture-based systems. Today, the implementation of automated rapid culture systems for timely diagnosis is on the rise [14].

According to recent hospital-based investigations, the bacteriological profile of BSIs varies significantly, especially among

immunocompromised individuals. Gram-positive organisms used to predominate, but recent research from India and other developing nations has shown that Gram-negative pathogens, many of which are multidrug resistant, are becoming more common [15]. Local antibiotic consumption, infection control strategies, and patient demographics have an impact on the distribution of pathogenic organisms and their patterns of antimicrobial susceptibility, which vary drastically across regions and healthcare settings. This heterogeneity underscores the importance of region-specific surveillance data for guiding empirical treatment [16].

Even though BSIs are becoming more common in immunocompromised individuals, there remains a dearth of targeted data from Indian tertiary care facilities that describe the bacteriological profile and patterns of antibiotic susceptibility in this high-risk population [17]. Many research studies that are currently available either do not stratify outcomes according to immunocompromised state or include heterogeneous patient populations. Additionally, the usefulness of earlier data is limited by changing patterns of antimicrobial resistance, highlighting the necessity of updated, institution-specific data to enable rational antimicrobial prescribing and successful infection control measures [8].

The study aimed to determine the bacterial profile and antimicrobial pattern of isolates from blood culture of immunocompromised patients. The primary objective was to determine the bacteriological profile of BSIs in immunocompromised patients, and the secondary objectives were to assess the incidence of multidrug-resistant organisms in this population and to evaluate the antimicrobial susceptibility patterns of the isolated pathogens.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted from October 2023 to April 2024 in the Department of Microbiology at National Institute of Medical Sciences and Research, Jaipur, Rajasthan, India. The Institutional Ethics Committee (IEC No.: NIMSUR/IEC/2023/787) approved the research after it received ethical approval. Written informed consent was obtained from all study participants and their legal guardians before conducting sample collection. A total of 246 blood samples were collected during the duration of the study.

Sample size: The sample size was calculated using the formula:

$$n=\frac{Z^2\times p\times(1-p)}{d^2}$$

Where:

- Z=1.96 (standard normal deviate corresponding to a 95% confidence level)
- p=0.20 (The assumed prevalence was based on previous study [18].
- d=0.05 (margin of error)

The assumed prevalence was based on previous studies reporting BSI rates around 16-20% among immunocompromised patients [18].

$$n=\frac{(1.96)^2\times 0.20\times 080}{(0.05)^2}=245.86\approx 246$$

Inclusion criteria: This study included only blood culture specimens collected from immunocompromised patients irrespective of age or sex. The study population comprised individuals with diabetes mellitus, patients receiving immunosuppressive therapy following organ transplantation, cancer patients undergoing chemotherapy or radiotherapy, patients infected with HIV, and other immunocompromised conditions such as malnutrition, chronic kidney disease, and chronic liver disease. Only the first isolate obtained from each patient was included for analysis to avoid duplication of isolates.

Exclusion criteria: Immunocompetent patients and non-blood specimens such as urine, sputum, cerebrospinal fluid, ascitic fluid, pleural fluid, and tissue specimens were excluded from the study.

Coagulase-Negative Staphylococci (CoNS) were considered significant and included only when isolated from clinically suspected cases with supportive clinical findings, while isolates suspected to represent contamination were excluded.

Study Procedure

A total of 246 blood samples were collected from immunocompromised patients aseptically. Venipuncture was used to draw 5-10 mL of blood from adult patients and 1-5 mL from paediatric patients, which were then inoculated into blood culture bottles. The samples were processed using the BD BACTEC FX40 automated blood culture apparatus. When the blood culture bottle indicated positive, the broth was smeared onto a slide for a Gram stain examination.

After subculturing on blood agar and MacConkey agar from the positive blood culture bottle, then incubated at 37°C for 24-48 hours. Preliminary identification of bacteria was carried out based on colony morphology and culture characteristics observed on blood agar, MacConkey agar, and nutrient agar. Observations included colony size, shape, surface texture, pigmentation, elevation, haemolytic activity on Blood agar, and lactose fermentation on MacConkey. The colonies obtained on culture plates, blood agar, and nutrient agar was further subjected to gram staining to identify bacteria. Rapid assays such as catalase, oxidase, and coagulase, as well as a series of biochemical reactions such as the indole, citrate utilisation, urease hydrolysis, triple sugar iron, and methyl-red tests, were performed to further determine the organism.

Antimicrobial susceptibility testing of Mueller-Hinton agar was done by the modified Kirby-Bauer disk diffusion technique according to CLSI 2023 guidelines [19]. These following antimicrobial agents were tested in the present study: amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ceftazidime (30 µg), cefepime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg), meropenem (10 µg), cotrimoxazole (23 µg), tetracycline (30 µg), doxycycline (30 µg), minocycline (30 µg), chloramphenicol (30 µg), vancomycin (30 µg), and linezolid (30 µg), for Gram-negative and Gram-positive isolates.

The organisms were identified using CLSI 2023 breakpoints, which were unique to each organism, and zone diameter measurements. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 were used as reference strains for testing purposes [19].

STATISTICAL ANALYSIS

To analyse the data after importing it into a Microsoft Excel data bank, Statistical Package for Social Science (SPSS) version 23.0 was used. Descriptive statistics, which include frequency and percentage use, were used in the study.

RESULTS

A total of 246 blood samples were processed in the study, of which 75 (30.49%) were culture-positive, and 171 (69.51%) were culture-negative. Of the 75 blood culture-positive cases, 30 (40%) were female, and 45 (60%) were male. Patients under 60-year-old accounted for the majority of culture-positive cases, with the highest percentage, 20 (26.67%), in the 41-50 age range [Table/Fig-1].

Among the isolates, 57 (76%) were Gram-negative bacteria and 18 (24%) were Gram-positive organisms. *Pseudomonas aeruginosa and Klebsiella pneumoniae* were the most commonly isolated Gram-negative pathogens [Table/Fig-2].

MRSA was the most common isolate among Gram-positive cocci [Table/Fig-3].

Age (years)	n (%)
<1	3 (4)
1-10	2 (2.67)
11-20	6 (8)
21-30	8 (10.67)
31-40	13 (17.33)
41-50	20 (26.67)
51-60	13 (17.33)
>60	10 (13.33)

[Table/Fig-1]: Age-wise distribution of isolates (n=75).

Isolate	n (%)
Pseudomonas aeruginosa	18 (31.57)
Klebsiella pneumoniae	15 (26.31)
Escherichia coli	10 (17.54)
Acinetobacter baumannii	8 (14.03)
Burkholderia spp.	3 (5.26)
Enterobacter spp.	2 (3.50)
Proteus mirabilis	1 (1.75)

[Table/Fig-2]: Distribution of Gram-negative bacterial isolates (n=57).

Isolate	n (%)
Methicillin-Resistant *Staphylococcus aureus* (MRSA)	7 (38.89)
Enterococcus spp.	6 (33.33)
Methicillin-Sensitive *Staphylococcus aureus* (MSSA)	4 (22.22)
Streptococcus spp.	1 (5.56)

[Table/Fig-3]: Distribution of Gram-positive bacterial isolates (n=18).

Immunocompromised patients, especially those with immunosuppression 11 (14.66%) and chronic kidney illness 26 (34.66%), had a greater percentage of positive cultures. Amikacin 17 (94.44%) and imipenem/meropenem 15 (83.33%) were highly susceptible to *Pseudomonas aeruginosa* among Gram-negative isolates. *Acinetobacter* species demonstrated complete susceptibility to minocycline 8 (100%) and high susceptibility to doxycycline and gentamicin 7 (87.5% each). Among the Enterobacteriaceae family, *Escherichia coli* 10 (17.54%) showed susceptibility to minocycline in 8 (80%), doxycycline in 6 (60%), and chloramphenicol in 9 (90%) isolates. Similarly, *Klebsiella pneumoniae* 15 (26.31%) demonstrated susceptibility to minocycline in 13 (86.67%) and doxycycline in 11 (73.33%) isolates. The gram-positive isolates exhibited complete susceptibility to linezolid and vancomycin, while they showed increased susceptibility to doxycycline at 83.33% and chloramphenicol at 94.44% [Table/Fig-4].

Organism	Antimicrobial agent	n (%)
Pseudomonas aeruginosa (n=18)	Amikacin	17 (94.44)
	Imipenem	15 (83.33)
	Meropenem	15 (83.33)
Acinetobacter spp. (n=8)	Minocycline	8 (100)
	Gentamicin	7 (87.50)
	Doxycycline	7 (87.50)
E. coli (n=10)	Minocycline	8 (80)
	Doxycycline	6 (60)
	Chloramphenicol	9 (90)
K. pneumoniae (n=15)	Minocycline	13 (86.67)
	Doxycycline	11 (73.33)
	Chloramphenicol	14 (93.33)
Gram-positive isolates (n=18)	Linezolid	18 (100)
	Vancomycin	18 (100)
	Doxycycline	15 (83.33)

[Table/Fig-4]: Antimicrobial susceptibility pattern of bacterial isolates.

DISCUSSION

The diagnostic microbiology laboratory performs its most important work through the process of identifying and isolating BSI samples, which undergo antimicrobial susceptibility testing [20]. The BSIs remain a major clinical obstacle to the lives of individuals who present a weakened immune system and pose a life-threatening danger. This is attributed to the detection of a delay in both the process of their diagnosis and the inappropriate nature of antimicrobial therapy. The progression of current challenges is exacerbated by evolving resistance patterns and updates to empirical therapy recommendations [14]. Despite this limited information about the frequency and patterns of antibiotic susceptibility of bloodstream pathogens in immunocompromised people, especially in developing nations. One of the most enduring challenges to the successful treatment of BSIs is antimicrobial resistance [16].

In the current investigation, 75 (30.49%) of patients with impaired immune systems had positive blood cultures. Reports from comparable high-risk populations, whose culture-positive rates range from 20% to 35%, are consistent with the present study. There was a 45 (60%) male and 30 (40%) female prevalence among culture-positive cases. Furthermore, BSIs were more common in people under 60 years, with the 41-50 years age group accounting for the largest percentage, 20 (26.67%). Dagnew M et al., have reported similar demographic distributions [5]. The changes that occur in middle-aged male patients from their increased exposure to healthcare services, their growing number of existing health conditions, and their accumulated medical treatments, as opposed to their natural biological tendencies [21].

The two most prevalent predisposing factors among study participants with positive culture results were immunosuppression 11 (14.66%) and chronic liver disease 26 (34.66%). The research conducted by Nanyunja D et al., shows that chronic kidney disease increases the likelihood of BSIs occurring. The combination of immune dysfunction, dialysis-related procedures, and frequent vascular access in these patients may allow opportunistic microorganisms to enter the circulation, which could account for the consistency of these findings across investigations [22].

The study found that Gram-negative bacteria outnumber Gram-positive bacteria 57 (76%) of the time, which only makes up 18 (24%) of the population, similar to Dagnew M et al., [5]. *Pseudomonas aeruginosa and Klebsiella pneumoniae* were the most common pathogens according to studies by Fayyaz M et al., which showed that these two Gram-negative isolates occurred at rates of 31.57% and 26.31%, respectively [23]. The medical equipment that remains inside patients' bodies, together with their prolonged hospitalisation and their treatment with broad-spectrum antibiotics, leads to the development of these bacterial infections [24]. The data show that MRSA constituted 7 (38.89%) of Gram-positive cocci, which demonstrates that MRSA remains a critical bacteremia pathogen for patients with weakened immune systems. Vasudeva N et al., showed similar findings [14].

The antimicrobial susceptibility profile demonstrated that *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* exhibit extreme resistance against multiple drugs. Bonomo RA et al., documented instances of Gram-negative bacteria that demonstrated the same pattern of multidrug resistance, which has been observed in other studies [25]. The increasing use of antibiotics as a last treatment option creates a risk because it may lead to the development of new resistance patterns. Fayyaz M et al., found that Gram-positive isolates remained completely susceptible 18 (100%) to linezolid and vancomycin, which

demonstrates their ongoing therapeutic value and necessitates their judicious usage [23].

The antimicrobial sensitivity patterns that have been observed in the past will not continue to exist in the future. Therefore, to direct the prudent use of antibiotics, it is essential to routinely develop regional antibiograms while staying informed on local sensitivity-resistance trends [12]. The research study shows how common bacterial pathogens cause BSIs and which antibiotics they are resistant to, because both Gram-positive and Gram-negative bacteria show significant patterns of multidrug resistance [16]. The rising problem of antimicrobial resistance occurs because people misuse and overuse antibiotics, while infection control methods remain ineffective. The implementation of antimicrobial stewardship programs, which include antibiotic restriction rules and proper combination treatment methods and periodic assessment of initial treatment protocols, will help decrease the emergence and spread of drug-resistant bacteria. The implementation of local antibiogram data and individual antimicrobial susceptibility results for antibiotic selection will lead to improved treatment outcomes, decreased BSI rates, and severity [9].

Limitation(s)

One of the major constraints of this study was that it was conducted within a single unit and over a very short period of time, limiting its applicability to other health care settings. Advanced molecular methods for detecting various mechanisms of resistance were not used, and bacterial isolates of cultures with positivity were considered for the study, excluding fungi and viruses.

CONCLUSION(S)

According to this study, multidrug-resistant Gram-positive and Gram-negative bacteria are the main cause of BSIs, which are common in immunocompromised people. The results show that antibiotic resistance in this vulnerable population is becoming a greater therapeutic concern. The most important clinical pathogens that researchers identified during their study were *Pseudomonas aeruginosa, Klebsiella pneumoniae, and Acinetobacter baumannii*. The antimicrobial drugs remain effective at present; however, their continued development of resistance threatens to end their future effectiveness. The study emphasises that healthcare providers need to monitor patients continuously while using evidence-based antibiotic treatment to achieve better clinical results for patients who have immune system deficiencies.

Authors' contribution: JR and DSP: Helped with the first draft; MP and TKS: Collaborated to develop the conceptual framework; DSP and MP were responsible for collecting the information. The final evaluation was completed by JR. The integrity and correctness of each contributor's individual contributions to the analysis are their own personal responsibility. The work and its contents have been reviewed and approved for publication by all authors.

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Data availability statement: Upon reasonable request, all data related to the present study are available with the corresponding author, Dr. Jaishriram Rathored.

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