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Microbiological Profile of Blood Stream Infections in a Tertiary Care Hospital, J&K. India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Worldwide, bacteremia is one of the serious infections that cause significant morbidity and mortality in hospitalized patients. A wide range of microorganisms have been implicated in the etiology of BSIs. Early detection of these pathogens along with the determination of antimicrobial susceptibility patterns have been shown to improve treatment outcomes. The present study aimed to determine the microbiological profile of BSIs in a major tertiary care hospital in North India.

Materials and Methods: This is a cross-sectional study conducted in the Department of Microbiology Government Medical College Srinagar. Blood samples submitted in brain heart infusion (BHI) broth for culture and sensitivity over a period of one year from September 2021 to 30th August 2022 were included in the study and processed per standard laboratory protocol techniques for isolating and identifying pathogens causing BSI. The antimicrobial susceptibility of bacterial isolates was determined by the disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

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Results: Out of 4260 blood samples, 1204(28.26%) isolates were obtained. Of these isolates, 575(47.75%) were Gram‑positive bacteria and 468 (38.87%) were Gram‑negative bacteria. Candida species were isolated from 161(13.7%) positive samples and 32 showed contamination. The most commonly identified organism was Coagulase‑negative Staphylococcus (CoNS) (22.59%) followed by *Staphylococcus aureus*(17.69%), *Acinetobacter spp*. (13.70%), *Klebsiella spp* (10.13%) and *Escherichia coli* (6.47%). Among the gram-positive bacteria, maximum resistance was seen with methicillin and azithromycin. No resistance was seen with vancomycin and linezolid. Most of the gram-negative bacilli were multidrug-resistant. Maximum resistance was seen with ampicillin(91.7%), amoxiclav (86.5%), ceftriaxone (88.5%), and gentamicin (60.9%). Higher prevalence of resistance was observed in Gram‑negative bacteria when compared with Gram‑positive bacteria.

Conclusion: Hence, empirical treatment of BSIs should be based on the current knowledge of bacterial resistance profiles as provided by microbiology laboratory reports. The results of this study warrant continuous monitoring of antimicrobial patterns for the clinicians along with judicious antibiotic policy to mandate antimicrobial sensitivity testing against the BSIs in the hospital setup so that appropriate therapeutic measures should be taken at the earliest.

Keywords: Antimicrobial resistance; blood cultures; blood stream infections.

1. INTRODUCTION

“Bloodstream infections (BSIs) are one of the most important infections among hospitalized patients and a significant cause of morbidity and mortality worldwide” [1]. “Approximately 200000 cases of bacteremia and fungemia occur annually with mortality rates ranging from 20-50%” [2]. “Blood cultures also provide essential information for the evaluation of a variety of diseases particularly in patients with suspected sepsis followed by endocarditis, pneumonia, and pyrexia of unknown origin (PUO)” [3]. “Various factors that are responsible for BSIs include prolonged use of in dwelling intravenous catheters, over stay in intensive care units, over use of steroids and immune-modulators as in human immunodeficiency virus (HIV) infections for the treatment and changing patterns of antimicrobial usage” [4,5]. “The detection of microorganisms in a patient’s blood has great diagnostic & prognostic significance” [6]. “The microorganisms implicated in BSIs include Gram-positive bacteria such as Coagulase-Negative Staphylococci (CoNS), *Staphylococcus aureus*, alpha hemolytic *Streptococci* and *Enterococcus spp* and among Gram-negative bacteria such as *Klebsiella spp*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter spp*, *Burkholderia spp* and others. There has been a wide variation of predominant microorganisms detected in blood culture reports among different healthcare facilities” [1]. This bacterial variability prompted the present study, to determine the bacterial isolates involved in bloodstream infections, in adults and children, including neonates, as well as their antibiogram, in order to guide clinicians in the implementation of appropriate empirical antibiotics and to prevent irrational use of antibiotics.

2. MATERIALS AND METHODS

This prospective study was conducted in the postgraduate department of Microbiology, Government medical college and hospital, Srinagar over a period of one year, from September 2021 to August 2022. During this period, 4260 samples received from various departments were processed and relevant findings were noted from patients with prolonged fever or clinical impression of septicemia. Despite antibiotic coverage, patients with prolonged fever in the postoperative period were included in our study. A detailed history was taken to identify the possible risk factors. A history of antibiotic usage empirically either before or after admission was also obtained. Data was collected on a proforma after getting clearance from the institutional ethical committee.

2.1 Statistical Analysis

All the results thus collected were subjected to descriptive statistics. Microsoft Excel 2010 was used for making tables and bar-charts.

2.2 Collection and Processing of the Specimen

Following strict aseptic precautions blood samples for culture were collected. If empirical antibiotics were already started, the collection was timed before the next dose of antibiotic was due or about half an hour before the predicted peak of temperature. A second set from a different venipuncture site was also collected in all patients. Three samples were collected in cases of suspected cases of congenital heart disease and endocarditis. About 1 ml of blood in the case of neonates and about 5 ml in the case of children and adults was collected in each set. The bottle containing 10 ml BHI broth was used in the case of neonates and 50 ml was used for children and adults to allow 1:10 dilution. The culture bottles were incubated at 37°C aerobically for 24hrs. After overnight incubation, the samples were sub cultured onto blood agar, Mac Conkey's agar, and chocolate agar. If no growth was observed on plates by the next day, subcultures were again repeated from the broth on day 3, day 4, and finally on day 7. Antibiotic susceptibility tests were performed by Kirby Bauer disc diffusion method on Mueller-Hinton agar plates as per CLSI (Clinical and Laboratory Standards Institute) guidelines [7].

2.3 Culture Media

The media used in blood culture bottles are Brain heart infusion" (BHI) broth with 0.025% of Sodium Polyanethol Sulphonate (SPS) as anticoagulant.

2.4 General Procedure

The aseptically inoculated blood culture media was incubated at 37°C for 7 days. If any growth appeared during the incubation period, it was preceded with a gram staining. Then based on the gram stain smear, it was sub cultured in the corresponding media. Biochemical tests were performed for further identification as per standard protocol followed in the laboratory as per CLSI guidelines [7]. Antibiotic sensitivity tests were carried out by the Kirby-Bauer disc diffusion method on the Muller-Hinton agar medium. The antibiotics tested on Gram-positive cocci included penicillin, amikacin, cefoxitin, clindamycin, erythromycin, gentamycin, levofloxacin, linezolid and vancomycin. The antibiotics tested on Gram-negative bacilli included amikacin, ampicillin, ampicillin sulbactam, amoxyclav, ciprofloxacin, ceftriaxone, cefixime, cefuroxime, gentamycin, imipenem, levofloxacin, piperacillin tazobactam and colistin. The results were interpreted by measuring the zone of inhibition as per CLSI guidelines. Culture media and antibiotic discs used in the study were obtained from HiMedia Labs Pvt Ltd, India.

3. RESULTS

A total of 4260 blood specimens were sent for culture to the microbiology lab during the period September 2021 to August 2022. Out of 4260 blood samples, 1204(28.26%) positive isolates were obtained [Table 1]. Of these isolates, 575(47.75%) were Gram‑positive bacteria and 468 (38.87%) were Gram‑negative bacteria [Table 2]. Candida species were isolated from 161(13.7%) positive samples and 32 showed contaminations. Maximum blood samples 2890(67.8%) were received from the In patient department IPD followed by 1370(32.1%) from Out patient department OPD patients. From IPD maximum samples were received from medicine ward 1123(38.8%) followed by 742 (25.6%) from patients admitted in Intensive care unit ICU and Neonatal intensive care unit NICU from the pediatric ward with PUO, 516(17.8%) samples from surgery ward mostly with a history of wound ,368(12.7%) from the cardiology ward with a history of infective endocarditis & 516 (12.1%) and 141 (4.8%) from other departments respectively. The most commonly identified organism was Coagulase‑negative Staphylococcus (CoNS) (22.59%) followed by *Staphylococcus aureus* (17.69%), *Acinetobacter spp*. (13.70%), *Klebsiella spp* (10.13%) and *Escherichia coli* (6.97%) [Fig.1]. Among the gram-positive bacteria, maximum resistance was seen with methicillin and azithromycin. No resistance was seen with vancomycin and linezolid except few cases of vancomycin-resistant *Enterococcus*. Among Gram‑negative bacteria, members of Enterobacteriaceae showed more resistance to ampicillin (91.7%), amoxiclav (86.5%), ceftriaxone (88.5%), and gentamicin (60.9%), whereas for imipenem a comparatively lower rate of resistance (21%) was seen. Most of the gram-negative bacilli were multidrug-resistant. Maximum resistance was seen with ceftriaxone, amikacin, cefepime, and ceftazidime. Combination drugs such as piperacillin‑tazobactum were also effective with 8.9% of isolates showing resistance. Other less commonly isolated organisms showed a lesser degree of resistance to the antibiotics used as first-line drugs. Gram‑negative bacteria showed a higher rate of resistance as compared with Gram‑positive bacteria [Table 3,4].

Table 1. Distribution of organisms isolated in blood culture positive cases

S.no	Organism isolated n=1204	Number of isolates n=1204	Percentage (%) n=1204
1.	Coagulase negative *staphylococcus spp*.(CONS)	272	22.59%
2.	*Staphylococcus aureus*	213	17.69%
3.	*Acinetobacter spp*.	165	13.70%
4.	*Candida spp*.	161	13.60%
5.	*Klebsiella spp*	122	10.13%
6.	*Enterococcus spp*	84	6.97%
7.	*Escherichia coli*	78	6.47%
8.	Pseudomonas spp	74	6.14%
9.	*Burkholderia spp*	21	1.74%
10.	*Citrobacter spp*.	06	0.49%
11.	*Proteus spp*	03	0.24%
12.	*Listeria spp*	02	0.16%
13.	*Stenotrophomonas spp*	02	0.16%
14.	*Providentia spp*	01	0.08%

4. DISCUSSION

"BSIs have been a challenge for clinicians due to changing bacterial resistance profiles. The change in the resistance profile may be attributed to the indiscriminate use of antibiotics. Early detection of causative organisms and their antimicrobial susceptibility testing is necessary to decrease the mortality associated with BSIs. Along with that, knowledge of the current trend of bacterial profile and their antimicrobial resistance pattern for a geographical location helps clinicians to decide on appropriate empirical therapy, which ultimately decreases the emergence of resistance. The present study determines the bacterial profile of *4260* blood samples from suspected cases of bacteremia. The blood culture positivity rate was *1204(28.26%)*, which is consistent with the studies conducted earlier were *(20.9%)*, *(20.02%). (33.9%)* and high in comparison to other studies that showed*. (16.4%)* and *10.16%)"* [8-10]. "The positivity rate may be ascribed to the injudicious use of antibiotics not only by clinicians before referring to the tertiary care center but by patients as well" [11]. "The incidence of BSIs caused by Gram-positive bacteria was *575(47.75%),* whereas that of Gram-negative bacteria was *468(38.87%).* It is consistent with other studies conducted in India".[7,12]. "Among Gram-positive bacteria, Coagulase negative Staphylococcus was the most frequently isolated from the blood culture specimen. The high incidence of CoNS could be because a large number of received samples in our setup were from the neonatal intensive care unit and concomitantly CoNS is a well-described pathogen in neonates, especially when associated with prematurity and central venous lines it may be due to CoNS being a common skin contaminant. The other Gram-positive bacteria isolated were *Staphylococcus aureus* and Enterococci. Among Gram-negative bacteria *Acinetobacter spp*. *Klebsiella spp*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Burkholderia spp* were the most common causative agents of BSIs . The reason for the high rate of isolation of *Acinetobacter spp* may be the acquisition of infection during a hospital stay, as it is one of the commonest pathogens seen in nosocomial infections" [13]. "In the present study, *Candida spp*. was isolated in *161(13.60%)* cases, whereas other studies have shown a higher incidence" [8,14].

Table 2. Percentage distribution of gram-positive, gram-negative, and *Candida spp*. isolates among total isolates (n=1204)

Isolates	Total number N=1204
Gram-positive bacteria	575 (47.75%)
Gram-negative bacteria	468 (38.87%)
Candida spp.	161 (13.7%)

"The antimicrobial resistance profile of CoNS has demonstrated a higher rate of resistance to beta-lactam antibiotics than other antimicrobials. Methicillin resistance was seen in *79/272(29.04)%* of cases of CoNS and *81/213(38.02%)*of S. aureus, which implies resistance to beta-lactam antibiotics despite showing sensitivity in antimicrobial susceptibility testing. Also, it may be coupled with increased resistance to other antimicrobials such as aminoglycosides, quinolones, macrolides, and lincosamides" [15]. "All staphylococcal isoates were uniformly sensitive to vancomycin and linezolid, which signifies that high-end drugs are still effective in the treatment of multidrug-resistant isolates. *Enterococcus* isolaltes had shown resistance to vancomycin *(28%),* which is higher in comparison with studies conducted earlier that is (2.4%) and (0%)" [7,16]. "They have also shown resistance to a high level of aminoglycosides *(57.1%),* which implies that they might not act synergistically with cell wall active antibiotics, for example, glycopeptides and beta-lactam antibiotics" [17]. "Moreover, a high prevalence of antimicrobial resistance especially in Gram-negative bacteria was observed in the present study. This might be due to the indiscriminate use of antibiotics in hospitals and over-the-counter sale of drugs, which makes it easy availability of drugs. It may be also attributed due to the higher prevalence of the extended-spectrum beta-lactamase producer Gram-negative bacteria within the hospital environment. The antimicrobial resistance profile of Gram-negative bacteria had shown a higher rate of resistance as compared with Gram-positive bacteria. Most of the Gram-negative bacteria were multidrug resistant with very high resistance to beta-lactam antibiotics. A lower resistance was seen to carbapenems, fluoroquinolones, and combination drugs. Cases of imipenem resistance were seen in this study, which is an alarming sign for the clinicians because thereafter they would have a very limited choice of drugs in the form of colistin and tigecycline, which have serious side effects and toxicity" [18]. Hence, the rational prescription of anti-microbial agent and timely antibiogram in admitted cases for a given healthcare center is the need of hour.

Table 3. Percentage sensitivity of gram negative bacteria

GNB	***Ampicillin***	***Ceftriaxone***	***Ceftazidime***	***Cefepime***	***Ampicillin + Sulbactum***	***Piperacillin + Tazobactum***	***Ciprofloxacin***	***Gentamicin***	***Amikacin***	***Imipenem***	***Tigecycline***
Ecoli	53.2%	61.2%	74.1%	66.1%	75.8%	70.9%	30.64%	43.5%	33.8%	79%	88.7%
Klebsiella	-	21.5%	67.8%	85%	-	91.1%	74.8%	64.3%	65.1%	71.8%	99.5%
Pseudomonas	-	-	33.8%	40%	-	75.3%%	73.8%	78.46 %	-	80%	-
Acinetobacter	-	30.6%	27.2%	-	-	86.7%	39.1%	14.6%	-	55.9%	96.5%
Burkholderia	-	-	-	-	-	79.1%	70.8%	-	-	83.3%	91.3%

Table 4. Percentage sensitivity of gram positive cocci

GPC	***Clindamycin***	***Erythromycin***	***Gentamycin***	***High level Gentamicin***	***Vancomycin***	***Ciprofloxacin***	***Linezolid***	***Tetracyline***
*MRCONS	52.5%	20.5%	33.8%	-	-	55.6%	100%	77.8%
MSCONS	64%	35%	42%		-	62%	100%	84%
*MRSA	45.6%	30.7%	42.2%	-	-	61.1%	100%	73.7%
MSSA	53%	44%	48%		-	66%	100%	77%
ENTEROCOCCUS	-	-	-	39%	72%	56%	100%	-

** All MRSA and MR CONS are deemed to be resistant to all beta lactam drugs including Penicillins, Cephalosporins, (except Ceftaroline and Ceftobiprole), Monobactams and Carbapenems*

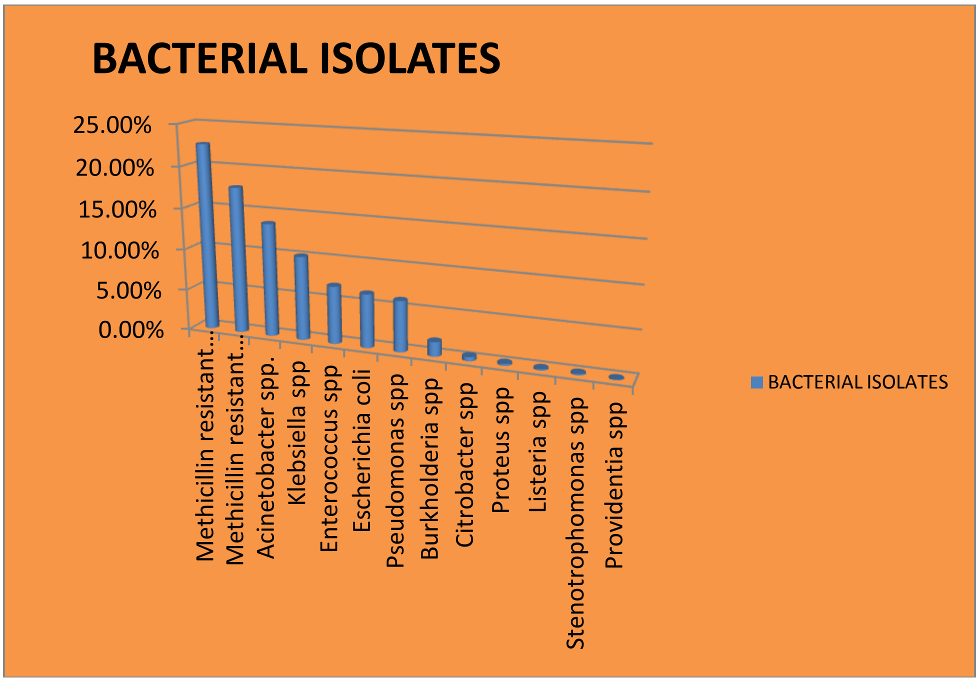

Fig. 1. Distribution of different bacterial isolates in Blood Stream Infection's

5. CONCLUSION

It may be concluded from the study that early diagnosis and antimicrobial susceptibility testing for quick and effective treatment of BSIs should be based on the current knowledge of antibiogram, which should be provided by microbiology laboratories from time to time. This in turn implies that from all the cases of suspected bacteremia and septicemia blood culture and sensitivity testing must be done for effective treatment and early recovery of the patient so that the isolated organisms and their antimicrobial susceptibility pattern is available in real-time for a given health care institution before framing the antibiotic policy.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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