Original Research Article

Study of prevalence and antimicrobial susceptibility pattern of blood culture isolates from a tertiary care hospital of North India

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Received: 11 October 2018
Accepted: 01 November 2018

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ABSTRACT

Background: Bacterial bloodstream infections (BSIs) are important causes of morbidity and mortality world-wide. The choice of antimicrobial therapy for bloodstream infections is often empirical and based on the knowledge of local antimicrobial activity profiles of the most common bacteria causing such infections. The objective of the study was to determine the pattern of bacterial isolates from the blood cultures in a teaching hospital and determine their antibiotic resistance and provide guidelines for choosing an effective antibiotic therapy in cases of septicemia.

Methods: The etiological and antimicrobial susceptibility profile of blood cultures over a period of one year at a tertiary care hospital was studied. Blood culture positive isolates were identified by BacT/Alert3D, an automated blood culture system, while as identification of the isolates from these samples and their antimicrobial sensitivity testing was performed with Vitek2 Compact.

Results: There were 2231 blood culture samples, of which 565 (25.3%) were identified to be culture positive. Out of 565 positive cultures, 447 (79.1%) showed bacterial growth; Gram positive were 306 (54.2%) and Gram negative were 141 (24.9%). Candida species were isolated from 118 (20.9%) of positive samples. The most frequently identified Gram-positive bacteria were Coagulase-negative staphylococci 208 (67.9%) and the most common Gram-negative isolates were Acinetobacter species 89 (63.1%). The most sensitive drugs for gram-positive isolates were vancomycin, and linezolid while as gram-negative isolates showed 100% sensitivity to colistin and tigecycline.

Conclusions: This study reveals a significant prevalence of bacterial isolates in blood and it highlights the need for periodic surveillance of etiologic agent and antibiotic susceptibility to prevent further emergence and spread of resistant bacterial pathogens.

Keywords: Blood stream infections, Bacterial isolates, Critical care units, Multi-drug resistance

INTRODUCTION

Bloodstream infections (BSI) are associated with significant morbidity and mortality. The condition can be life threatening in critically ill patients especially in intensive care units (ICUs) of the hospitals. The case fatality rate associated with BSIs is between 35-50%. These infections require rapid identification and also antibiotic susceptibility testing of the causative agent in order to facilitate specific antimicrobial therapy. Bacteremia is defined as the continuous or transient presence of microorganisms within the blood stream, while as septicaemia refers to its dissemination throughout the body with evidence of systemic responses toward microorganisms with variable severity and where contamination has been ruled out. These infections are often classified as primary (no focus) or secondary when associated with clinical or microbiological confirmation of infection at a defined body site. In addition, they have been further traditionally classified as either hospital-acquired or community-acquired.
In developing countries, the steep increase in septicemia cases is a major health problem that creates the biggest challenge for clinicians in the selection of appropriate antimicrobial agents, as it is further complicated by the development of resistance in organisms to antimicrobial agents, which is the mainstay of treatment. Studies have shown that there is a wide range of bacteria, both Gram negative and Gram positive which are associated with these infections.

In India also, septicemia is an important cause of illness and death among hospitalized patients. Early detection of the infectious agent and determination of their susceptibility are essential for the optimization of treatment and represents the best way to reduce hospital stay and improve patient outcomes. Despite recent advances in clinical diagnostics, blood culture remains the gold standard for the detection of bacteremia and fungemia.

The prevalence of antimicrobial resistance in patients with septicemia is increasing, and it varies in accordance with geographical and regional location. This requires the knowledge of common bacterial pathogens prevalent in that area, based on blood culture results, to help clinicians choose the right antibiotic therapy.

Variability between hospitals in different countries is substantial and requires continuous analysis of local trends. Knowledge of resistance patterns in common pathogens associated with these cases is a must before starting the empirical therapy. Therefore, the present study was undertaken to determine the antibiotic resistance of blood culture isolates as it may be a useful guide for clinicians initiating the empiric antibiotic therapy.

METHODS

This prospective study was conducted in the Department of Microbiology on blood samples of patients suspected of having bloodstream infections attending and or admitted in the Associated Hospitals of Government Medical College Srinagar over a period of one year from January 2017 to December 2017. The study was approved by the Ethical Committee of our institute.

Blood collection, organism identification and susceptibility testing

Blood samples were collected after thorough cleaning of the venous site with 70% alcohol and subsequently followed by povidone iodine. Blood samples were collected from the patients before the administration of any antibiotic. For adults, 5-10mL of blood while 1-5mL blood was collected from paediatric patients, and 1-2mL from neonates for blood culture and subsequently incubated in BacTAlert3D (Biomerieux, France), a fully automated blood culture system for detection of growth in blood culture. On getting a positive alarm, the blood culture broth was sub cultured on 5% sheep blood agar and MacConkey agar plates and incubated aerobically at 37°C overnight for bacterial isolation. Isolates were identified by Vitek 2 Compact (Biomerieux) using gram negative, gram positive and yeast identification cards and AST cards for antimicrobial susceptibility testing. The results were interpreted according to CLSI criteria. The patient data that were collected included age, sex, underlying diseases and risk factors. Quality control was performed by testing these same antimicrobials against reference strains of bacteria. The negative results were followed up to 7 days and final report was issued.

Statistical analysis

For statistical analysis SPSS version 17.0 software and MS excel 2007 were used. We also used Chi-square test to know the association between the variables.

RESULTS

This study was carried out from January 2017 to December 2017 on 2231 blood samples received in the Department of Microbiology. Details like medical registration number, laboratory number, age and sex of the patients, and type and place of collection of specimens were recorded.

There were 2231 blood culture samples, of which 565 were identified to be culture positive. Culture positivity was seen in 565 (25.3%) samples, and 1666 (74.6%) samples were sterile as detected with the BACT/ALERT 3D (Biomerieux) blood culture system. The frequency of isolation of Gram-positive bacteria (n=306, 54.2%) was found to be more than that of Gram-negative bacteria (n=141, 24.9%). Also, Candida species were isolated from a significant number of patients (n=118, 20.8%) (Figure 1).

Figure 1: Distribution of culture positive samples from suspected septicemic patients (n=565).

The most frequently identified Gram-positive bacteria were coagulase-negative staphylococci (CoNS) 208...
(67.9%), *Staphylococcus aureus* 75 (24.5%) (out of which 62 (20.2%) were methicillin resistant and 13 (4.2%) were methicillin sensitive) and *Enterococci* species 23 (7.5%) (Figure 2). They were considered significant based on clinical correlation, repeated isolation of same isolate from blood culture and intravenous catheters, host factors like immunosuppression, extremes of age, prolonged use of indwelling devices.

![Figure 2: Distribution of gram-positive isolates (n=306).](image)

The Gram-negative bacteria that were identified were *Acinetobacter species* 89 (63.1%), *Escherichia coli* 19 (13.4%), *Klebsiella pneumoniae* 15 (10.6%) *Salmonella typhi* 9 (6.3%), *Pseudomonas aeruginosa* 9 (6.3%), (Figure 3).

![Figure 3: Distribution of gram-negative isolates (n=141).](image)

The gender distribution of positive samples was found to be 380 (67.2%) males and 185 (32.7%) females with a male: female ratio of 2:1. Out of 565 positive cases, 145 (25.6%) were neonates, 70 (12.3%) were below the age of 20 years, 124 (21.9%) were between the age of 20-40 years, 101 (17.8%) were in the age group of 41-60 years. Number of cases above 60 years was 125 (22.12%).

Antibiotic sensitivity pattern of gram-negative and Gram-positive organisms was studied. The results of antibiotic drug sensitivity of gram-positive bacteria showed that both CoNS and *Staphylococcus aureus* were highly sensitive to vancomycin (100%) and linezolid (100%). Methicillin resistance was higher (31%) in CoNS in contrast with *S. aureus* (20%). This was tested using cefoxitin disc. Among the *Enterococcus species* ampicillin, gentamicin and ciprofloxacin displayed resistance in more than 60% isolates. Maximum sensitivity for *Enterococcus species* was seen with linezolid (100%) followed by vancomycin (85%) (Figure 4).

![Figure 4: Antibiotic resistance pattern in Gram positive isolates.](image)

Antimicrobial resistance levels for the gram-negative organisms most commonly causing blood stream infections were relatively high. Among Gram negative bacterial isolates, *Acinetobacter species*, *Escherichia coli* and *Klebsiella pneumoniae* were dominant species in descending order. Third generation cephalosporins showed a very weak activity against them (<30%) and carbapenem resistance was detected in 83% isolates of *Acinetobacter spp*. 56% cases of *Escherichia coli* and 65% of *Klebsiella pneumoniae* were multidrug resistant (MDR).

For highly resistant bacteria, especially *Klebsiella* species and *Acinetobacter* species, even β-lactam +β-lactam inhibitor combinations and carbapenems were not effective. Amikacin was slightly better. However, all of these strains were sensitive to Colistin (Figure 5).
 DISCUSSION

Despite advances in diagnosis and treatment, bloodstream infections present a challenging problem, and more often they may be life threatening. Therefore, timely detection, identification, and antimicrobial susceptibility testing of blood-borne pathogens are one of the most important functions of diagnostic microbiology laboratory. In this prospective study, an attempt was made to provide information on the distribution of bacterial isolates causing bloodstream infections along with their antibiotic susceptibility pattern as it plays a crucial role in effective management of septicemic cases.

The results of our study demonstrated that 565 (25.3%) out of 2231 blood samples screened were positive for the presence of micro-organisms, out of which 447 (79.1%) were bacteria while as Candida species were isolated from 118 (20.9%) of positive samples. These results are comparable to other studies from India and abroad. Studies from India by Arora et al (20.02%), and Sharma et al (33.9%) have shown comparable results. A study done by Ali et al in Gonder, Ethiopia, in 2008 showed comparable prevalence of 24.2%. In contrast to above reports, the study done by Khanal et al reported high frequency of positive blood cultures accounting for 44%, whereas study by Anbumani et al reported lower frequency of positive blood cultures accounting for 7.89%. This variation may be due to many factors like geographical locations, patient type, timing and number of blood cultures or difference in blood culture system. In India, the variation in isolation rate may also be due to the practice of prescribing antibiotics by the local health practitioners before the patients reach the tertiary care hospital. Also, as reported by Lee et al. The variation in blood culture positivity is also related to the number and amount of blood cultures taken for screen.

In this study, men (67.2%) had high culture positivity as compared with women 32.7%. These results are consistent with the study done by Kaur and Singh who made similar observation of male dominance in their studies.

In our study, the highest rate of prevalence 25.6% was found in neonates followed by 22.1% in the age group 60 years and above. The lowest prevalence rate of 17.8% was found in age group 41-60 years. Sepsis and septic shock occur at all ages but most often in elderly patients. Advanced age is a risk factor for acquiring nosocomial blood stream infection in the development of severe forms of sepsis. Neonates are also vulnerable to infections because of their weak immunological barrier. This result is consistent with other studies done among neonates in Ethiopia (28%), and Cameroon (34.9%).

In the present study, the frequency of isolation of Gram-positive bacteria (n=306, 54.2%) was found to be more than that of Gram-negative bacteria (n=141, 24.9%). It is in accordance with the studies of China et al, Kamga et al, Anbumani et al and Karlowsky et al, who reported similar incidences. However, in contrast some studies like Mehta et al, Mehdinejad et al, Barati et al and Ayobola et al have shown a higher incidence of Gram-negative organisms than Gram-positive organisms. This indicates that infections by Gram-positive organisms constitute a significant threat to septicemia in our locale.

Figure 5: Antibiotic resistance pattern in gram negative isolates.
and the spectrum of organisms is subject to geographical alterations.

Among Gram-positive bacteria, CoNS was the most frequently isolated pathogen followed by *Staphylococcus aureus* and *Enterococcus sp*. CoNS were mainly recognized as mere contaminants till 1970’s; however, several studies have now reported an increasing incidence of infection by this group of bacteria.27,28 This finding is in accordance with various studies conducted in our country.29,30 Some studies however, have reported a higher incidence of *S. aureus* among Gram-positive bacteria causing Blood stream infections.31 The high incidence of CoNS could be because a large number of received samples in our set up were from the intensive care units especially neonatal intensive care unit. According to Souvenir et al, clinical significance of CoNS was defined as at least two blood cultures positive for CoNS within 5 days or one positive blood culture plus clinical evidence of infection, which includes abnormal leucocyte count and temperature or blood pressure CoNS is a well-described pathogen in neonates, especially when associated with prematurity and central venous lines.32 However, as CoNS are also possible skin contaminants, and therefore it is advisable to consider possible risk factors and more importantly their repeated isolation from same patient for deciding future course of therapy.

Analysis of the incidence of Gram-negative bacteria showed that *Acinetobacter* spp., followed by *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Pseudomonas aeruginosa* were the most common causative agents of BSIs among Gram-negative bacteria. The reason for high rate of isolation of *Acinetobacter* spp. among Gram-negative bacteria may be because of acquisition of infection during hospital stay, as it is one of the commonest pathogen seen in nosocomial infections.33 Also, their ubiquitous nature in the ICU environment and inadequate infection control practice have continuously raised the incidence of Acinetobacter infections over the past two decades.34

Fungal septicemia caused by *Candida* species was seen in significant number of patients (20.9%). Candidemia in our study was mainly due to non-albicans species, and all the cases were seen in neonates. According to the surveillance data from US Centre for Disease Control and Prevention (CDC), Candida accounts for 12% of all hospital acquired Blood stream infections.35 This could probably be due to increased use of invasive devices, and broad-spectrum antibiotics in NICU and PICU.36-38

The antimicrobial resistance profile of both Gram Positive and Gram-Negative isolates revealed a high prevalence of resistance among them. Antimicrobial resistance profile of CoNS has demonstrated a higher rate of resistance to beta-lactam antibiotics than other antimicrobials. Methicillin resistance was seen in 31% cases of CoNS. All CoNS isolates were susceptible to vancomycin, teicoplanin and linezolid. These drugs may be included in therapy for patients with methicillin-resistant CoNS infections. However, empirical use must be avoided before recording the results of susceptibility tests as overuse of these antibiotics can promote glycopeptide and oxazolidinone resistance.

*Staphylococcus aureus* was isolated from 24.5% cases out which 20% were methicillin resistant. This is in accordance with other studies.39 All staphylococcal isolates were uniformly sensitive to vancomycin and linezolid, which signifies that high-end drugs are still effective in treatment of multidrug-resistant isolates. CoNS and *Enterococcus species* isolates showed higher level of resistance to beta-lactam antibiotics than *Staphylococcus aureus*, which is similar to other studies.30

Antimicrobial resistance profile of Gram-negative bacteria showed a higher rate of resistance as compared with Gram-positive bacteria as has also been shown in these studies.39,40 Most of the Gram-negative bacteria were multidrug resistant with a very high resistance to beta-lactam antibiotics. Meropenem resistance was seen in 10-30% isolates. This may be due to inappropriate empirical use of meropenem as the first line treatment. Few cases of imipenem resistance were also seen in this study, which is an alarming sign for the clinicians because this leaves a very limited choice of drugs in the form of colistin and tigecycline, which have serious side effects and toxicity.

This study had quite a few limitations. Firstly, though the analysis was confined to blood isolates, which indicate likely infection, no clinical information was included. Secondly, information on variables differentiating community-acquired from healthcare-acquired infections was not available. As only a single blood culture specimen was collected from each patient, hence it was not possible to determine if the patients with CoNS isolation had true bacteremia or the finding was due to skin contamination.

**CONCLUSION**

The present study provided much needed information on the prevalence of bacterial pathogens in blood stream infections and their antibiotic sensitivity patterns. It showed prevalence of multi-drug resistant isolates in critical care patients and this limits the therapeutic options. Non-empirical/inappropriate antibiotic use contributes to the emergence of antimicrobial resistance in bacteria. The key to control of antibiotic resistant pathogens is to strictly adhere to infection control practices and mandates antibiotic policy on a large scale for every hospital. Also, availability of updated epidemiological data on antimicrobial resistance in frequently encountered bacterial pathogens will be useful not only for deciding on empirical treatment strategies, but also devising an effective antimicrobial stewardship program in hospitals. The results warrant continuous
monitoring of antimicrobial pattern so as to build geographical epidemiological data.

Funding: No funding sources  
Conflict of interest: None declared  
Ethical approval: The study was approved by the Institutional Ethics Committee

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