ORIGINAL RESEARCH

Identification of clinically important aerobic microorganism by automated blood culture system and antimicrobial resistance pattern

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ABSTRACT

Background: Blood stream infections are important public health problem can lead to life threatening sepsis and require rapid antimicrobial treatment. Emergence of multidrug resistant isolates in hospitalized patients is a major problem. **Aim**: This study was aimed to detect the blood stream isolates by automated blood culture system and their antimicrobial resistance pattern. **Methods:** A cross sectional study was conducted in the department of Microbiology. During the study period, blood samples collected from all age group OPD, IPD and ICU patients suspected of bacteremia and septicemia were analyzed. All Gram-negative bacilli, Gram-positive cocci and Yeast were investigated. Identification of isolates and antibiotic susceptibility testing was done by using standard CLSI methods. **Result:** Around 18.6% of samples were positive for blood stream infections caused by different pathogens. Among Gram positive cocci, coagulase negative staphylococci (CoNS) is commonest followed by Staphylococcus aureus whereas in Gram negative bacilli, Klebsiella pneumoniae was commonest organism followed by E. coli. Majority of the gram positive cocci were most resistance to penicillin and was most sensitive to vancomycin and linezolid. Gram negative isolates of Enterobacteriaceae were least sensitive to cephalosporins while it was most sensitive to Colistin. **Conclusion:** Early diagnosis of blood stream infections in hospitalized patients is life saving. Hence a continuous monitoring of isolates and their drug susceptibility is the need of the day

Keywords: Blood stream infections, blood culture, Antibiotics, Resistance, Coagulase negative staphylococcus, Klebsiella pneumoniae

INTRODUCTION

Blood stream infection (BSI) remains one of the foremost important causes of morbidity and mortality globally. The infection may range from self limiting to life threatening sepsis. As case fatality rate is high it requires appropriate and immediate antimicrobial therapy [1-2]. Early identification of microorganisms and their antimicrobial resistance properties in patients with bloodstream infections (BSI) has been shown to be crucial in the clinical management and initiation of the appropriate antimicrobial therapy. .Blood culture is currently the gold standard for detecting and identifying microorganisms causing BSI [3-4]. Blood cultures of patients have isolated a wide range of organisms in BSIs. These include Acinetobacter spp., Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae among Gram-negative bacteria and Staphylococcus aureus, coagulase-negative staphylococci (CoNS), enterococci and alpha-hemolytic streptococci among Gram-positive bacteria [5-6]. Candida species are the fourth most common cause of nosocomial bloodstream infections worldwide [7]. The change of prevalence and antimicrobial resistance pattern among bloodstream pathogens is a significant problem worldwide with severe consequences including increased cost of care, morbidity, and mortality [8]. The surveillance of etiological agents in these infections is essential for their prevention and treatment. Microbial invasion of the bloodstream can

have serious immediate consequences i.e., shock, multiple organ failure, disseminated intravascular coagulation (DIC) and death [9]. Sensitive bacterial strains are now being replaced by multi-drug resistant (MDR) strains of Klebsiella, Pseudomonas, Acinetobacter, and Citrobacter species. Increase in incidence is also seen among Gram-positive isolates such as methicillin resistance in Staphylococcus aureus (MRSA) and vancomycin resistance in Enterococci. This increasing antimicrobial resistance is a worldwide concern and is subjected to regional variation [10-11].

Awareness of the baseline microbial resistance specific to a hospital prevents irrational use of antibiotics in that hospital. Thus helps progress a step forward in the prevention of spread of antibiotic resistance and antibiotic stewardship.

AIMS & OBJECTIVES

Determine the aerobic microbial profile and antimicrobial resistance pattern in blood stream infections.

MATERIALS AND METHODS

This cross sectional study was carried out in the Department of Microbiology, a tertiary care centre, central India, from January 2016 to December 2016 (01 years).

During the study period, blood samples collected from all age group OPD, IPD and ICU patients suspected of bacteremia and septicemia were analyzed. All samples were collected in BacT/ALERT FA plus and BacT/ ALERT PF plus bottle and BD Bactec Media plus Aerobic/F bottle irrespective of antibiotics administration. Quantity of blood sample from adult and children was 5-10 ml and 1-5 ml respectively collected with all aseptic precautions.

Samples were incubated in the automated BacT/ALERT 3D system (BIOMERIEUX, USA) and BACTEC 9050 (BD Bactec™ 9050 Blood Culture System) for 7 days. The negative results were followed up to 7 days and final report was issued. The preliminary signal of bacterial growth was detected and displayed on the 3D monitor of BacT/ALERT system and BD Bactec™ 9050 Blood Culture System mentioning the detection time. Further identification of all blood culture positive samples was accomplished by sub-culture on Blood agar, Chocolate agar and MacConkey agar media and direct Gram's staining from positive blood culture.

Identification and antimicrobial susceptibility testing done as per Clinical Laboratory Standards Institute (CLSI) guidelines [12]

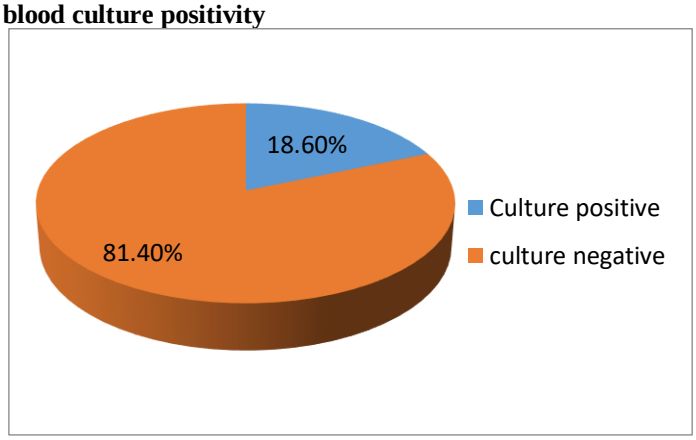
STATISTICAL ANALYSIS

Data regarding the age, sex, isolate and its antimicrobial pattern was collected and statistical analysis of the data was done by Chi square test to study the P value using social science statistics online software.

RESULTS

A total of 1560 blood cultures were analyzed during the study period out of which 290 (18.6%) were found to be positive [Figure: 1]. Out of positive cases, 188 (64.8%) were male and 102 (35.2%) were female.

Figure 1: incidence of blood culture positivity



Isolates were predominant in males (64.8%) as compared to females (35.2%). Pediatrics patients were more (24.2%) exposed to BSI than adult and elderly patients (Table 2).

Table 1: Age and gender wise distribution of positive and negative blood culture samples

Variable	Blood culture Positive	Blood culture Negative	Total	P value
Male	188	782	970	**0.302**
Female	102	488	590	
Age group				
<15 years	70	290	360	0.053
15-30 years	44	191	235	
31-45 years	56	264	320	
46-60 years	59	336	395	

>60 years	61	190	250	

The leading isolate among the gram positive cocci were CONS (53.7%) followed by Staphylococcus aureus (12.7%) and among the gram negative bacilli, Klebsiella pneumoniae (44.8%) followed by (26.2%). Details of each isolate were mentioned in Table 2.

Table 2: Microorganism isolated from blood culture samples

Types of isolates	Isolate	Positive %
Gram positive cocci (A)	Staphylococcus aureus	37 (12.7%)
	CONS	156 (53.7%)
	Enterococcus faecalis	21 (7.3%)
Gram negative bacilli (B)	Klebsiella pneumoniae	130 (44.8%)
	Escherichia coli	74 (25.5%)
	Pseudomonas aeruginosa	76 (26.2%)
	Acinetobacter species	14 (4.8%)
	Salmonella typhi	23 (7.9%)
	Serratia marcescenes	11 (3.7%)
	Burkholderia species	17 (5.8%)
Yeast (C)	Candida species	65 (22.4%)

Among the gram positive cocci were most resistance to penicillin (90-96%) and was most sensitive to vancomycin and linezolid (90-95%). Gram negative isolates of Enterobacteriaceae were least sensitive to cephalosporins (90-100%) while it was most sensitive to Colistin. Details description showed in table 3 & 4.

Table 3: Antibiotic resistance (in percentage) of gram-positive cocci

Antibiotics	CONS	Staphylococcus aureus	Enterococcus faecalis
Penicillin	96.2	91.9	90.5
Oxacillin	82.7	54	88.3
Gentamicin	44	27.1	76.2
Ciprofloxacin	32	52.3	50
Levofloxacin	65.5	39.3	50
Erythromycin	88.6	66.6	90.5
Linezolid	6.4	5.4	9.5
Clindamycin	86.2	66.6	100
Vancomycin	12.8	10.8	4.7
Tetracycline	23.1	29.7	52.3
Cotrimoxazole	51.7	47.8	33.3

Table 4: Antibiotic resistance of gram negative bacilli isolated from blood culture

Antibiotics	Escherichiacoli	Klebsiella pneumoniae	Pseudomonas aeruginosa
Piperacillin/tazobactam	80	94.1	66.6
Cefuroxime	100	100	33
Ceftriaxone	100	100	52
Cefaperazone/sulbactum	70	94.1	100
Cefepime	70	100	100
Imipenem	50	35.3	66.6
Meropenem	60	58.8	66.6
Amikacin	30	88.2	66.6
Gentamicin	30	91.2	66.6
Nalidixic acid	80	82.3	24
Ciprofloxacin	80	82.3	66.6
Tigecycline	0	11.7	100
Colistin	0	0	33.3

DISCUSSION

The overall frequency of blood culture isolates in present study was 18.6%. This is comparable with studies conducted in India by Pal et al [13], and Gill et al [14], blood culture positivity were 22.3% & 24.8% respectively. However, some studies have reported high frequency of bacterial pathogens from blood cultures (24.2%-37.1%) [15-16]. This may be due to use of different blood culture systems, different sample size, variations in study design and protocols, different geographical locations, variations in

causative agents and the policies adopted for infection control between countries.

In our study majority of the blood culture positive patients were < 15 years male, whereas statistically no significant difference in relation to age and gender, concordance finding also reported by Swamy MA et al [17] and Ramana KV et al [18]

Incidence of gram negative bacilli (GNB) was higher than gram positive cocci (GPC), Similar findings with high frequency of GNB as compared to GPC were previously reported by an Indian study [19-20].

Coagulase negative staph was the most common organism isolated (53.7%) of case followed by Klebsiella species (44%) which is in accordance to Vatkar et al [21] and Dash et al [22]. CoNS being the most common contaminant, 2 sets of blood culture from different sites were taken or clinically correlated positive samples were considered clinically significant. Cross infections in ICU'S with multidrug resistant CONS can be prevented by use of appropriate antimicrobial agents

Klebsiella was the most common Gram negative bacilli isolated which was in accordance to Parihar et al [7] and Fayyaz M et al [23] while in discordance to Vasudev et al [24] and Gupta et al [8] where E. coli was the most common organism isolated.

Candida species were isolated in 22.4% of cases, mostly in neonatal ICU setting which is in higher frequency than other studies and is probably due to sporadic surge of cases and their spread to others [25]. The isolation of patients and proper barrier nursing were done to prevent the same in the future.

In the present study, bacterial isolates were tested for antimicrobial susceptibility by Modified Kirby Bauer Disc Diffusion technique according to CLSI guideline 2017.

Among the GPC group CONS showed high frequency of antimicrobial resistance as compared to others. Staphylococcus aureus was most sensitive to gentamicin, linezolid, vancomycin, and tetracycline. Similarly, an earlier study reported by Singh AK et al [26] and Manjunath et al [27].

Among the non fermenters, Acinetobacter species and pseudomonas aeruginosa showed high level resistance to cephalosporins and carbapenems. There is increase in the trend of carbapenem resistance to Acinetobacter species [28]. This may be because of extensive use of these antimicrobials.

Klebsiella species were most resistant to all cephalosporins group which is similar to Chowdhury et al [29], whereas most sensitive to Tigecycline which is concordant to a similar study [30].

Current study found Pseudomonas aeruginosa was most sensitive to colistin and Nalidixic acid, similar finding reported by Rout et al [31] and Laer A et al [32].

Differences in antimicrobial resistant pattern in different studies may be due to circulation of different strains in different regions at different times.

CONCLUSION

Coagulase negative staphylococcus and Klebsiella pneumoniae were the predominant isolates of the study. High level multidrug resistance was observed in both GPC and GNB. Tigecycline and colistin remains the choice of antibiotics for gram-negative bacilli. Gentamicin, linezolid and vancomycin are the choice of antibiotics for gram positive cocci. Rapid isolation and identification of pathogens by automated blood culture system and antibiogram with minimum inhibitory concentration (MIC) value provides early and appropriate treatment to the seriously ill patients leading to reduce mortality and reduce duration of hospitals.

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CONFLICT OF INTEREST

None declared

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