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Original Research Article

Spectrum of aerobic bacteria and their antimicrobial pattern in blood stream infections of hospitalised patients: a retrospective study

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ABSTRACT

Background: Bacteria associated with blood stream infections are an important public health problem which results in morbidity and mortality globally. Emergence of multidrug resistant isolates in hospitalized patients is a major problem. Automation techniques play a major role in early identification of the isolate and its drug susceptibility testing which is important for better outcome of the treatment. This study was aimed to detect the blood stream isolates and their drug susceptibility pattern in hospitalized patients.
Methods: This was a retrospective study conducted from 377 records of automated blood culture (bact/alert) and drug susceptibility testing (vitek) results. Positive blood culture bottles were sub cultured to different culture media and the isolates were identified and screened for drug susceptibility testing on Vitek II.
Results: Around 20.68% of samples were positive for blood stream infections caused by different pathogens. A total of 78 microorganisms were isolated from 377 samples. Among which gram negative bacilli was observed in 52.56%, gram positive cocci in 44.87% and yeast in 2.56% samples. Coagulase negative *staphylococci* and *Klebsiella pneumoniae* were the predominant isolates of the study.
Conclusions: 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, 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.[1,2] As case fatality rate is high it requires appropriate and immediate antimicrobial therapy. Different bacteria were associated with (BSI) from time to time at different geographical areas. These bacteria play an important role in causing mortality, increasing the length of hospital stay and also the health care cost.[3]

Drug resistance of these bacteria is an important issue of public health concern. Since many studies have reported that gram negative and gram positive bacteria are associates with these infections which are often drug resistant. Empirical antibiotic therapy is initiated in almost all cases before the blood culture reports are available. Choice of right empirical therapy is important. An early blood culture report may help in selection of appropriate antibiotics.[2,4]

Minimal time is required to get a blood culture report using automated systems. The etiology and antimicrobial pattern of (BSI) may vary at different times in the same region hence a continuous update is essential for epidemiological purpose and also for rational and accurate use of antibiotics by clinicians. The present

study aimed to determine the etiology and antibiotic resistance pattern in blood stream infections.

METHODS

Study design

A retrospective study was conducted from the records of automated blood culture (bact alert) and drug susceptibility testing (vitek) results in the clinical microbiology laboratory from June, 2016 to July, 2018 at Ananta institute of medical sciences and research centre, Rajsamand. This data includes 377 records of hospitalised patients who were admitted to different units of hospital during the study period.

Sampling technique and data collection

Blood cultures were performed for different age groups up to 90 years. Samples were collected by phlebotomist from the patients after disinfection of vein puncture site with 70% alcohol. 3-4ml of blood was inoculated in 30ml BacT/Alert blood culture bottles for paediatrics and 4-5ml blood was inoculated in 30ml BacT/Alert blood culture bottles for adults. These bottles were incubated in BacT/Alert automated system. The bottles which showed positive signal for growth were removed from the automated system and subculture was done on Nutrient agar, blood agar and MacConkey's agar. Smears from the colony of different agar plates were prepared and stained with Gram stain to identify the growth (i.e. gram positive or gram negative bacteria). Then the growth of the bacteria was run on VITEK II automated system for identification of the organism and Antibiotic Sensitivity. If there is no growth of bacteria within five days of inoculation of blood sample into BacT/Alert blood culture bottle then the sample is considered to be negative.

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 377 samples were screened for blood culture from hospitalized patients of different units. 20.68% of samples were positive for blood stream infections caused by different pathogens (Table 1).

A total of 78 microorganisms were isolated from 377 samples. Among which gram negative bacilli was observed in 52.56%, gram positive cocci in 44.87% and yeast in 2.56% samples. The leading isolate among the gram positive cocci were CONS (coagulase negative *staphylococci*) and among the gram negative bacilli, *Klebsiella pneumoniae*. Details of each isolate were mentioned in Table 1. Only two samples were found to be positive for yeast i.e. *Candida tropicalis*.

Table 1: Bacteria and yeast isolated from blood culture samples.

	Isolate	Positive %
Gram negative bacilli (A)	*Acinetobacter baumannii*	4 (9.75%)
	Acinetobacter iwoffii	1 (2.44%)
	Burkholderia cepaciae	1 (2.44%)
	Escherichia coli	10 (24.39%)
	Enterobacter cloacae Complex	2 (4.87%)
	Klebsiella pneumoniae	17 (41.46%)
	Pseudomonas aeruginosa	3 (7.31%)
	Spingomonas paucimobilis	1 (2.44%)
	Stenotrophomonas maltophilia	1 (2.44%)
	Serratia marcescenes	1 (2.44%)
Total		41 (100%)
Gram positive cocci (B)	*Staphylococcus aureus*	3 (8.57%)
	Staphylococcus cohnii#	2 (5.71%)
	Staphylococcus epidermidis#	6 (17.14%)
	Staphylococcus haemolyticus#	4 (11.48%)
	Staphylococcus hominis#	3 (8.57%)
	Staphylococcus lentus#	2 (5.71%)
	Staphylococcus pseudintermedius#	2 (5.71%)
	Enterococcus faecalis	3 (8.57%)
	CONS	10 (28.57%)
Total		35 (100%)
Yeast (C)	*Candida tropicalis*	2(100%)
Total (A+B+C)		78/377* (20.68%)

*Total number of samples tested, CONS-coagulase negative *staphylococcus*, # CONS

Isolates were predominant in males (62.82%) as compared to females (37.18%) (Table 2). Paediatric patients were more (31.57%) exposed to BSI than adult and elderly patients (23.52%). Statistical analysis showed a P value of 0.03. CONS were the leading pathogens in paediatric patients. *Klebsiella pneumoniae* was the leading pathogen among the adult and elderly patients. 83.3% (65) of samples were culture positive in <24hours, 15.4% (12) in between 24 <48hours, 1.3% (1) between 48-72hours.

Table 2: Sex wise and age wise distribution of positive and negative blood culture samples.

Variable	Blood culture result			P value
	Positive	Negative	Total	
Male	49	191	240	X^2=0.03 P=0.86
Female	29	108	137	
Age				
<5 years	25	58	83	X^2=6.88 P=0.03
5 <15 year	5	37	42	
>15 years	48	204	252	

Among the gram-negative isolates, *Escherichia coli* showed 100% sensitivity to colistin and tigecycline, 80% to ertapenem. *Acinetobacter* species showed 80% sensitivity to colistin and 70% to amikacin. *Enterobacter cloacae* showed 100% sensitivity to tigecycline,

Klebsiella pneumoniae showed 100% sensitivity to colistin and 88.3% to tigecycline. *Pseudomonas aeruginosa* showed 66.7% sensitivity to colistin (Table 3). Among gram positive isolates, CONS showed 93.1% sensitivity to linezolid, 89.7% to tigecycline and 86.2% to vancomycin. *Staphylococcus aureus* showed 100% sensitivity to oxacillin, gentamicin, linezolid, vancomycin, tetracycline and tigecycline. E*nterococcus faecalis* showed 100% sensitivity to gentamicin, linezolid and vancomycin (Table 4).

Very low positivity 1 (2.44%) was observed for *Spingomonas paucimobilis, Stenotrophomonas maltophilia, Burkholderia cepaciae* and *Serratia marcescenes.*

Table 3: Antibiotic resistance of gram negative bacilli isolated from blood culture.

Antimicrobial agent	Resistance percentage of gram negative bacilli				
	***Eschericia coli* (n=10)**	***Acinetobacter species* (n=05)**	***Enterobacter cloacae* (n=02)**	***Klebsiella pneumoniae* (n=17)**	***Pseudomonas aeruginosa* (n=03)**
Ampicillin	100	100	NT	100	NT
Amoxicillin/clavulanate	80	100	100	94.1	NT
Piperacillin/tazobactam	80	80	100	94.1	66.6
Cefuroxime	100	100	100	100	NT
Cefuroxime axetil	100	100	100	100	NT
Ceftriaxone	100	80	100	100	NT
Cefaperazone/sulbactum	70	80	100	94.1	100
Ertapenem	20	NT	NT	NT	NT
Cefepime	70	80	100	100	100
Imipenem	50	70	100	35.3	66.6
Meropenem	60	70	100	58.8	66.6
Amikacin	30	30	100	88.2	66.6
Gentamicin	30	80	100	100	66.6
Nalidixic acid	80	80	100	82.3	NT
Ciprofloxacin	80	80	100	82.3	66.6
Tigecycline	0	60	0	11.7	100
Trimethoprim/ sulphamethoxazole	60	80	100	52.9	NT
Colistin	0	20	NT	0	33.3

NT - Not Tested

Table 4: Antibiotic resistance of gram-positive cocci isolated from blood culture.

Antimicrobial agent	Resistance percentage of gram-positive cocci		
	Cons* (n=29)	***Staphylococcus aureus* (n=03)**	***Enterococcus faecalis* (n=03)**
Benzylpenicillin	96.5	100	100
Oxacillin	82.7	0	100
Gentamicin	31.3	0	0
Ciprofloxacin	65.5	33.3	50
Levofloxacin	65.5	33.3	50
Erythromycon	89.6	66.6	100
Linezolid	6.9	0	0
Clindamycin	86.2	66.6	100
Tecoplanin	27.5	80	50
Vancomycin	13.8	0	0
Tetracycline	27.6	0	50
Tigecycline	10.3	0	100
Trimethoprim	51.7	66.6	50

DISCUSSION

The overall frequency of blood culture isolates in present study was (20.68%). This is comparable with studies conducted in India by Pal et al, 2016 (22.3%) and Gill et al, 2016 (24.8%).[5,4] However, some studies have reported high frequency of bacterial pathogens from blood cultures (24.2%-37.1%).[6,7] 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. Incidence of gram negative bacilli (GNB) was 52.56% and gram positive cocci (GPC) were 44.87%. Similar findings with high frequency of GNB as compared to GPC were previously reported by an Indian study.[8]

In our study, coagulase negative *staphylococcus* was the leading blood culture isolates (37.1%). Similar results were reported from India (61%) and globally (42%).[9,10] They often occur as skin contaminants during the collection of blood. Cross infections in ICU'S with multidrug resistant CONS can be prevented by use of appropriate antimicrobial agents. There is a need for differentiation between true pathogen and contaminant which can be achieved by correlating clinically the blood culture isolate and time taken for positivity of CONS.[11] Among the GPC group CONS showed high frequency of antimicrobial resistance as compared to others (Table 4). *Staphylococcus* aureus was 100% sensitivite to oxacillin, gentamicin, linezolid, vancomycin, tetracycline and tigecycline. Similarly, an earlier study reported 100% sensitivity to linezolid and vancomycin.[8]

Enterococcus faecalis was observed in (8.5%) of gram positive cocci. Similar findings (8.4%) were reported by an earlier Indian study.[11] It is a normal flora of female genitourinary tract and gastrointestinal tract. Though vancomycin resistance was reported since a decade, in the present study no resistance to vancomycin was observed for *Enterococcus faecalis*. This may be due to differences in the circulating strains. However, an earlier study from north India reported 23% of vancomycin rersistant enterococci.[11]

Gram negative bacteria accounted for more than fifty percent among the total isolates of the blood culture. This is consistent with an earlier study, though there is difference in the range of isolates.[1] Among the non fermenters, *Acinetobacter* species and *psuedomonas aeruginosa* showed high level resistance to cephalosporins and carbapenems. There is increase in the trend of carbapenem resistance to *Acinetobacter* species.[11] This may be because of extensive use of these antimicrobials. The overall antimicrobial resistance of gram negative bacteria and gram positive bacteria varied from 0% to 100% in our study. This is different when compared to a previous study which reported a higher resistance in gram negative bacteria (20-100%) as compared to gram positive bacteria (23.5%-58.8%).[12] Among the *Klebsiella pneumoniae* isolates high level sensitivity was shown by colistin 100% followed by tigecycline 88.3%, imipenem 64.7% and meropenem 41.2%. Singh et al, 2014 reported 100% and 71.4% sensitivity for imipenem and meropenem respectively each.[8] *Eschericia coli* showed high level sensitivity to colistin and tigecycline 100%, followed by ertapenem 80%, amikacin and gentamicin 70%. The sensitivity of gentamicin in our study was much higher as compared to an earlier Indian study 35%.[13]

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

Among the yeast isolates *Candida tropicalis* was isolated in two blood culture samples. Both the isolates were 100% sensitive to fluconozole, voriconazole, caspofungi, micafungin, amphotericin-B, and flucytosin. However, studies from different parts of India reported the emergence of *nonalbicans Candida* and resistant to widely used antifungal agents.[14,15]

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, vancomycin and tigecycline are the choice of antibiotics for gram positive cocci. Good antibiotic policy and strict hospital infection control measures may help to curb the emergence of multidrug resistant pathogens. There is a need for continuous monitoring and updating the BSI isolates and their antimicrobial patterns for an early effective approach to treatment.

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