Original Article

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Aerobic Bacteriological Profile and its Antimicrobial Sensitivity Pattern From Blood Culture Specimens in A Tertiary Care Hospital.

Santosh A. Kotgire*, Sunil Hatkar S

Department of Microbiology, Indian Institute of Medical Sciences, Jalna, Maharashtra, India

ABSTRACT

Background : Blood stream infections (BSIs) are important cause of mortality and morbidity and are among most common health-associated infections.Blood stream infection constitutes one the most serious situations with increased cost of care, morbidity, mortality and thus, timely detection and identification of blood stream pathogen is important.Sothe present study was undertaken to describe aerobic bacteriological profile and its antibiotic sensitivity pattern from blood culture specimen in a tertiary care setting.

Method: The study was carried out in the Department of Microbiology, Indian Institute of Medical Sciences, Jalna, Maharashtra for a period of 8 months (July 2015 to February 2016). A total 1920 samples were evaluated from clinically suspected cases of bacteremia. Blood was collected depending upon age group with aseptic precaution and inoculated on brain heart infusion broth (BHIB).Subculture were made on blood agar and Mac-conkey agar plates. Organisms were identified and antibiotic sensitivity test of isolates were performed.

Result : During 8 month study period out of 1920 blood culture, 369(19.21%) yielded growth of different organisms. Out of this 369 organisms gram positive bacteria 199 (53.9%) were isolated more often than gram negative bacteria 170 (46.1%). *Staphylococcus aureus* 49.05 % was leading pathogens isolated followed by enterobactericae group 21.00% (*Escherichia coli* and *Klebsiella spp)* and *Salmonella typhi* & *S. Paratyphi A* 13.82%.

Conclusion: This study provides information on antibiotic sensitivity pattern of blood isolates which may be useful to guide clinicians to initiate empiric therapy and will help in formulation of antibiotic therapy strategy in this part of country.

Keywords: *Blood Culture, Antimicrobial Sensitivity Pattern, Bacteremia.*

Introduction

Blood stream infections (BSIs) are important cause of mortality and morbidity and are among most common health-associated infections [1].Bacteremia signifies the presence of bacteria in the blood stream[1].Bacteremia may be transient, continuous or intermittent. Micro-organisms present in the circulating blood are a threat to every organ in the body[1].

It can have serious consequences like shock, multiple organ failure, disseminated intravascular coagulation, etc. thus, the blood stream infection constitutes one the most serious situations with increased cost of care, morbidity, mortality and , as a result,timely detection and identification of blood stream pathogen is important. Blood culture plays an integral role in the evaluation of sepsis[1,2].

Increasing antimicrobial resistance is worldwide concern. The prevalence of resistance in both out-patients and hospitalised patients with septicaemia is increasing, and its varies in accordance with geographical and regional location. In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available[3].

Selecting appropriate antimicrobial for treating BSI is multifaceted, including possible cause and source of infection, in vitro activity of drug according to microbiological susceptibility testing results, pharmacokinetics and adverse effects of the drug[2].

However, before considering these aspects, the choice of antibiotic mainly relies on knowledge of the pathogen likely involved.Monitoring and analysing the antimicrobial suscepitibility pattern of most frequently isolated micro-organisms according to local epidemiology which helps clinicians to choose empirical therapies and develop rational prescription policy for antibiotics[4,5].

Therefore, the present study was undertaken to describe aerobic bacteriological profile and its antibiotic sensitivity pattern from blood culture specimen in a tertiary care setting to guide clinicians to initiate empiric antibiotic therapy and to formulate antibiotic policy.

Materials and Methods

Study Design: The present study is prospective type of study and was carried out at Department of Microbiology,

Indian Institute of Medical Sciences Badnapur , Jalna, Maharshtra after approval from institutional ethics committee.

Sample Size: The study was carried out during the period of July 2015-February 2016; a total 1920 samples were evaluated from clinically suspected cases of bacteremia.

Inclusion Criteria: All patients with unexplained fever/ undiagnosed fever, whose blood culture specimens were sent to department of microbiology were included in the study.

Exclusion Criteria: Contaminated, duplicate and repeat specimens from patients were excluded from study.

Collection of Sample and Processing : In adults 5 ml of blood for culture was drawn in sterile syringe after skin preparation by a two step process with 70% alcohol andpovidone iodine application and then dried for 1 min. Blood collected was aseptically incubated into blood culture bottle containing 50 ml of Brain Heart Infusion Broth (BHIB). In paediatric cases 1-2ml of blood was inoculated in 5-10 ml of BHIB.These bottles will be incubated at 37° C temperature under aerobic conditions in the incubator maximum for 7 days. Subculture will be made on Blood agar and MacConkey's agar daily from 1st to 7th day. However if the growth was observed further sub cultures were not done. Growth was processed according to standard microbiological techniques which includes Gram staining, colony characteristics and biochemical properties described in WC. Koneman's Colour Atlas and text book of Diagnostic Microbiologyand Bailey and Scott's Diagnostic Microbiology[6,7]. Blood culture broth which showed no microbial growth after 7 days were reported as culture negative.

Antimicrobial Sensitivity Testing Criteria for antimicrobial sensitivity testing used was carried out as per Clinical Laboratory standard institute (CLSI) [8].Antimicrobial sensitivity testing was done on Muller Hinton Agar (MHA) by Kirby Bauer disc diffusion method . Commercially available discs (Hi-media) were be used. Concentration of discs used were Erythromycin (15 mcg), Vancomycin (30mcg), Co-trimoxazole (25mcg), Ciprofloxacin (5mcg), Linezolid (30mcg), Ampicillin (30mcg), Piperacillin+Tazobactum (100/10mcg), Ceftazidime (30 mcg), Amikacin (30 mcg), Ofloxacin (5mcg), Gentamicin (10mcg) & high level (30mcg), Furazolidone(300mcg), Azetronam(30mcg), Chloramphenicol (30mcg), Ceftriaxone (30mcg), Amoxicillin (20mcg) and Imipenem (10mcg).

Methicillin resistance in *Staphylococcus aureus* (MRSA) was tested using Muller Hinton Agar with Cefoxitin disc (30mcg) by Kirby-bauerdisc diffusion methods as per CLSI guidelines[8]. Suspected extended- spectrum beta lactamases (ESBLs) producing Enterobactericae were confirmed by double disk synergy test as as per CLSI guidelines[8].

Staphylococcus aureus (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruoginosa* (ATCC 27853) were used as quality control throughout the study for culture and antimicrobial susceptibility testing.

Statistical analysis The results were expressed as percentages for analysis of various epidemiological details and for analysing the distribution of different bacterial isolates and their sensitivity pattern. Microsoft excel was used for the interpretation of these results.

Results

During 8 month study period 1920 blood culture were analysed. 369(19.21%) yielded growth of different organisms. Of all isolates 291(76.8%) were isolated from hospitalised patients while 78 (23.2%) were from those who attended out-patients department. Majority of the patients were males 285(77.23%); male to female ratio was 3.4:1.

Out of this 369 organisms, gram positive bacteria 199 (53.9%) were isolated more often than gram negative bacteria 170 (46.1%). In our study *Staphylococcus aureus* 49.05 % was leading pathogens isolated followed by Enterobactericae group 21.00% (*Escherichia coli* and *Klebsiellaspp)* and *Salmonella typhi* & *S. paratyphi A* 13.82%.

The detailed microbiological data of pathogens and their antimicrobial susceptibility causing blood stream infection is shown in table 1, 2, 3 &4

Discussion

The varying microbiological pattern of bacteremia/ septicaemia warrants the need for an ongoing review of causative organisms and their antimicrobial susceptibility pattern.

Out of 1920 suspected cases of bacteremia, in our study, 369 were culture positive with blood culture positivity rate of 19.21%. Similar positivity rates were reported by other studies[9,10].

Our study highlights that gram positive septicaemia was encountered in 53.9% culture positive cases which was in concordance with studies done by China et al; and Gupta et.al; which shows increase incidence of gram positive bacteria especially *Staphylococcus aureus* in producing BSIs[11,12].

Table 1 Distribution of isolates

Organisms	OPD	Med	Paeds	ICU	Surg	Others	Total (n=369)
Staphylococcus aureus	25	36	29	15	20	56	181
Salmonella typhi and S. paratyphi A	16	12	09	03	01	10	51
E.coli	08	18	10	08	0	02	46
Klebsiellaspp	05	10	05	07	02	02	31
Pseudomonas aeruginosa	04	03	05	10	04	03	25
Acinetobacterspp	02	02	02	07	03	01	17
Enterococci spp	06	05	05	01	0	01	18

OPD-out patient department, Med-medicine ward, Paeds-paediatric ward, ICU-intensive care unit, Surg-surgery ward, Others-obstetrics &gynaecology ward, orthopaedic ward, etc

Table 2:Distribution of organisms isolated from blood culture.

Name of organism	Numbers (n=369)	Prevalence%
Staphylococcus aureus	181	49.05
E.coli	46	12.46
Klebsiellaspp	31	8.54
Salmonella typhi/Para typhi A	51	13.82
Pseudomonas aeruginosa	25	6.77
Enterococci spp	18	4.87
Acinetobacterspp	17	4.60
Total	**369**	**100.00**

Table 3: Antimicrobial sensitivity pattern of Gram positive organisms.

Antibiotics	Staphylococcus aureus(n=181)	Enterococci spp (n=18)
Penicillin	41.4% (75)	44.44%(08)
Cotrimaxozole	49.72%(90)	Not tested
Ciprofloxacin	49.72%(90)	Not tested
Erythromycin	55.24%(100)	66.66%(12)
Gentamicin	70.16%(127)	Not tested
Cefoxitin	77.34%(140)	Not tested
Vancomycin	100%(181)	100%(18)
Linezolid	100%(181)	100%(18)
Gentamicin (high level)	Not tested	83.33%(15)
Ampicillin	Not tested	88.88%(16)

Table 4: Antimicrobial sensitivity pattern of Gram negative organisms

Antibiotics	*E.coli*(n=46)	*Klebsiellaspp*(n=31)	*Salmonella typhi/ paratyphiA*(n=51)	*Pseudomonas aeruginosa*(n=25)	*Acinetobacterspp* (n=17)
Cotrimaxozole	43.47%(20)	48.38%(15)	80.39%(41)	48.00%(12)	52.94%(09)
Ciprofloxacin	50.00%(23)	54.83%(17)	Not tested	76.00%(19)	70.58%(12)
Ceftazidime	54.34%(25)	58.06%(18)	Not tested	80.00%(20)	76.47%(13)
Amikacin	65.21%(30)	64.51%(20)	Not tested	88.00%(22)	82.35%(14)
Gentamicin	82.60%(38)	80.64%(25)	Not tested	92%(23)	58.82%(10)
Ampicillin-sulbactam	89.13%(41)	93.54%(29)	88.23%(45)	Not tested	Not tested

Antibiotics	*E.coli*(n=46)	*Klebsiellaspp*(n=31)	*Salmonella typhi/ paratyphiA*(n=51)	*Pseudomonas aeruginosa*(n=25)	*Acinetobacterspp* (n=17)
Azetronam	73.91%(34)	83.87%(26)	Not tested	56.00%(14)	47.05%(08)
Pipercillin-tazobactam	Not tested	Not tested	Not tested	100%(25)	100%(17)
Chloramphenicol	Not tested	Not tested	76.47%(39)	Not tested	Not tested
Furazolidone	Not tested	Not tested	62.74%(32)	Not tested	Not tested
Imipenem	100%(46)	100%(31)	Not tested	100%(25)	100%(17)
Ceftriaxone	Not tested	Not tested	100%(51)	Not tested	Not tested
Amoxicillin	Not tested	Not tested	82.35%(42)	Not tested	Not tested

Gram negative septicaemia was encountered in 46.1% of culture positive cases which is lower as compared to other studies which had reported increased incidence of gram negative bacteria ranging from 50% to 78%[12,13]. Commonest gram negative bacteria isolated in our study was *E.coli , Klebsiella spp* and followed by *Salmonella spp* and non-fermenters (*Pseudomonas* and *Acinetobacter spp*) which was in concordance with other studies carried out in different parts of India[14,15].

The gram positive organism especially *Staphylococcus aureus* showed 41.4% and 55.24% sensitivity to penicillin and erythromycin respectively but were 100 sensitive to Vancomycin and Linezolid similar sensitivity pattern were seen in other studies[11,14,15]. 41(22.65%) were detected as methicillin resistant *Staphylococcus aureus* (MRSA). Studies by Indian researchers[16] , reported a similar prevalence of 41% MRSA. Our study also point out increase isolation of *Enterococci spp* as many studies had not isolated any single case of *Enterococci* spp except for few[17,18]. All *Enterococci Spp* were 100% sensitive to Vancomycin and showed 88.88% and 83.33% sensitivity to Ampicillin and Gentamicin (high level) respectively.

Most of gram negative bacteria especially Enterobactericae (except *Salmonella spp*)showed 100 sensitivity to imipenem and high resistance to ciprofloxacin and ceftazidime , similar trends has been reported by an Indian study[19] and many other researchers[11,12] highlighting higher resistance to third generation cephalosporins. Our study found out around 30% Enterobactericae isolates were ESBL producers which is alarmingly on higher side as compared with other studies[13].

While member of nonfermenter (*Pseudomonas* and *Acinetobacter spp*) shown 100% sensitivity to Imipenem and Pipercillin-tazobactam this results are comparable to work done at other centers[19,20] and also revealed better sensitivity to third generation Cephalosporin and Gentamicin as compared with other studies[18,20].

Conclusion

The detection, identification and susceptibility testing of causative species of bacteria are essential for proper treatment and better prognosis of patient in case of BSIs. Blood culture still remains as one of the most important microbiological investigation available to clinicians for diagnsosis of BSIs. Gram positive organisms were predominant in our setup for producing BSIs with higher incidence of MRSA. Our study also showed alarmingly higher incidence of ESBL producing Enterobacterciae group which points towards judicious uses of third generation Cephalosporins.

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***Corresponding author:**
Santosh A. Kotgire, Department of Microbiology, Indian Institute of Medical Sciences, Jalna, Maharashtra, India
Phone: +91 9922867558
Email: santosh_kots2001@yahoo.com

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