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BACTERIOLOGICAL ANALYSIS OF BLOOD CULTURE ISOLATES WITH THEIR ANTIBIOGRAM FROM A TERTIARY CARE HOSPITAL

R. Sharma *[1], R. Sharma [2] and S. Gupta [1]

Department of Microbiology [1], Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, India.
Department of pharmacology [2], Himalayan Institute of Medical Sciences, Dehradun, Uttaranchal, India.

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Correspondence to Author:
Dr. Rajani Sharma

Assistant Professor, Department of Microbiology, Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, India.

E-mail: rajanidhaundiyal@gmail.com

ABSTRACT: Introduction: Bloodstream infections (BSI) are a serious cause of morbidity and mortality worldwide. Emerging antimicrobial drug resistance among bacterial pathogens causing BSI can limit therapeutic options and complicate patient management. **Objective:** To encourage the prudent use of appropriate antibiotics in our tertiary care Hospital, we studied the prevalence and antibiogram patterns of blood culture isolates from March 2013 to February 2014. **Results:** Of 829 blood cultures examined, 116 (13.9 %) were positive for bacterial growth. The frequency of Gram-positive bacteria isolated was 52.5% (61 of 829) and that for Gram-negatives was 47.4% (55 of 829). The most common gram-positive organism isolated was *Staphylococcus aureus* (28.5%), followed by CoNS (13.3%) and *Enterococcus* spp. (5.7%). *Staphylococcus aureus*, had exhibited least resistance to tetracycline, doxycycline, vancomycin, daptomycin, and linezolid. The rates of methicillin (oxacillin) resistance in *Staphylococcus aureus* were 32%. Among the Gram-negative isolates, the predominant isolates were *Acinetobacter* and *Salmonella typhi* (36.3%) followed by *Escherichia coli* (10.9%), *Klebsiella* species (9%) and *Pseudomonas* species (7.27%). ESBL and MBL production was seen in 25 (45.5%) and 13 (23.6%) isolates, respectively. **Conclusions:** Our findings underscore the need to monitor blood culture isolates and their antimicrobial resistance patterns to observe resistance trends that would influence appropriate empiric treatment and infection control strategies for bactaeremic cases.

INTRODUCTION: Bacteremia and other bloodstream Infections (BSIs) are a major cause of morbidity and mortality worldwide [1, 2]. Usually, the bloodstream is sterile. Individuals with bacteremia may develop Septicemia, a life-threatening condition in which multiplying bacteria release toxins into the bloodstream. Microorganisms present in circulating blood whether continuously or intermittently are a threat to every organ in the body. Approximately 200,000 cases of bacteraemia and fungemia occur annually with mortality rates ranging from 20-50% [3].

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Blood culture remains the highly specific indicator of bacteremia and, antimicrobial susceptibility test assist a great deal in precise identification of the most appropriate choice of drug to be administered [4]. However, blood culture techniques take a minimum of 7 days for definitive results- a time span that may not be available for the desperately sick patient. This is because, early administration of drugs to patients with BSI drastically reduce cases of mortality [5].

It is therefore necessary to periodically document research results obtained from analysis of blood culture, to assist clinicians have the needed idea to commence treatment for desperately sick patients, awaiting blood culture results.

This study reports the prevalence, as well as antibiotic resistance profile of isolates obtained from the blood of adults and children/neonates

attending the Mahatma Gandhi Hospital, a tertiary health care facility.

MATERIAL AND METHODS:

In this study, a total of 829 blood samples from the clinically suspected cases of bacteraemia were reviewed for a period of one year from March 2013 to February 2014. All the samples were collected at Mahatma Gandhi Hospital a 1000 bedded, tertiary care, teaching hospital providing a full range of medical, surgical and superspeciality facilities.

Processing of samples was done at the department of Microbiology. Five ml of blood was collected from each adult patient by nursing personnel or physicians, using strict aseptic precautions, and inoculated immediately into 50 ml of 'Brain Heart Infusion' (BHI) broth with 0.025% of sodium polyanethol sulphonate as anticoagulant (Himedia, a commercial firm). In paediatric cases 1 - 2 ml of blood was inoculated in 5 - 10 ml of BHI broth. The broths were subcultured on 5% sheep blood agar and MacConkey agar after overnight incubation. A negative result was followed-up by examining the broth daily and doing a final subculture at the end of second and seventh day. Positive growth was identified by Gram staining, colony characteristics, and standard biochemical tests.

Antimicrobial Susceptibility Test:

The antimicrobial susceptibility test was performed for isolated organisms by Kirby-Bauer's disk diffusion technique according to the Clinical and Laboratory Standards Institute (CLSI, 2013) guideline [6]. The routine antimicrobial susceptibility tests were put for following antibiotics:

For Gram Positive Cocci: Amoxycillin, Linezolid, Cotrimoxazole, Doxycycline, Vancomycin, Ciprofloxacin, Imipenem, Amikacin, Gentamicin, Augmentin, Cefuroxime, Tetracycline, Erythromycin, Daptomycin.

For Gram Negative Bacilli: Doxycycline, Augmentin, Ciprofloxacin, Ceftriaxone, Cefuroxime, Co-trimoxazole, Chloramphenicol, Gentamicin, Amikacin, Piperarcillin, Imipenem, Piperacillin/tazobactum, Amoxycillin, Colistin, Polymyxin B, Tigecycline.

RESULTS:

During the study period, 829 blood cultures were analyzed of which 116 (13.9%) microorganisms were isolated. Of all the isolates, 72.41% were isolated from hospitalized patients while the remaining 27.58% were from those who attended out-patients departments. During study period we did not observe multiple positive blood cultures from any patient. The distribution and percentage of various bacterial isolates are shown in **Table 1**.

TABLE 1: DISTRIBUTION AND PERCENTAGE OF VARIOUS BACTERIAL ISOLATES

Micro-organism	Total no. isolated (%)	IPD	OPD
Staphylococcus aureus	30 (25.8)	18	12
Coagulase Negative Staphylococci	23 (21.5)	15	8
Enterococci	8 (6.8)	6	2
Salmonella typhi / paratyphi	20 (17.2)	15	5
Acinetobacter spp.	20 (17.2)	20	Nil
Escherichia coli	6 (5.1)	2	4
Klebsiella pneumonae	5 (4.3)	4	1
Pseudomonas spp	4 (3.4)	4	Nil
	116	**84**	**32**

Among the Gram-positive isolates, the predominant isolate was *Staphylococcus aureus*, which exhibited least resistance to tetracycline, doxycycline, vancomycin, daptomycin, and linezolid, as shown in **Table 2**. Oxacillin resistance (MRSA) was 32% in these strains. Vancomycin resistance in *Staphylococcus aureus* isolates was not found. Other Gram-positive isolates coagulase negative staphylococcal strains (CONS) showed least resistance to tigecycline, linezolid, vancomycin, gentamicin, quinolone, tetracycline and co-trimoxazole. Enterococci showed least resistance to tetracycline, teicoplanin, vancomycin and tigecycline.

Among the Gram-negative isolates, the predominant isolates were *Acinetobacter* and *Salmonella typhi* (36.3%) followed by *Escherichia coli* (10.9%), *Klebsiella* species (9%) and *Pseudomonas* species (7.27%). Amongst gram negative isolates, 25 (45.5%) were ESBL producers and 13 (23.6%) were MBL producers. The present study showed that in case of Salmonella typhi,

most effective antibiotics were found to be ceftriaxone (92%) followed by chloramphenicol (86%) and ciprofloxacin (83%). Nonfermenters showed highest sensitivity to Polymixins, tigecycline, imipenem followed by amikacin.

2: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM BLOOD

Antibiotics	Disc contents (ug/ml)	Enterobacteriaceae	Nonfermenter	Gram positive cocci
Amikacin	30	73.8	67.3	83
Augmentin	30	46.2	-	60
Ciprofloxacin	5	50	52.6	80
Ceftriaxone	30	53.8	52.6	-
Ceftazidime	30	54	57.8	-
Gentamicin	10	50	47.3	87
Imipenem	100	83	77	-
Erythromycin	30	-	-	53.3
Vancomycin	20	-	-	100
Co-trimoxazole	25	46.2	-	69
Ceftazidime/Clavulanic acid	30/10	56.4	43	-
Cefuroxime	30	39	-	62
Ampicillin	20	52	-	58
Tetracycline	30	48	-	74
Linezolid	30	-	-	100
Piperacillin	100	60	55	-
Piperacillin/Tazobactam	100/10	75	67	-
Colistin	10	100	100	-
Polymixin B	330 U	100	100	-
Tigecycline	15	100	100	100

DISCUSSION: This study revealed that 116 (13.9%) out of 829 total samples screened were positive for the presence of bacteria which is quite lower to other studies done in India [7, 8, 9, 10]. The low incidence in our study is due to various reasons. Majority of the patients reported to us are referred by other specialists or hospitals and these patients were offered antibiotics elsewhere before they reached our hospital. On the other hand, the variation in blood culture positivity depends on several other factors such as the amount of blood taken for screening [11], formulation of the blood culture medium used for bacterial detection or administration of antibiotic therapy prior to taking blood for culture [12]. Our study showed that the rate of isolation of Gram positive bacteria is higher (52.5%) than Gram negative bacteria (47.4%) quite similar to others [9, 13].

The commonly isolated organisms from this study include: *Staphylococcus aureus*, members of the Enterobacteriaceae and *Pseudomonas* species [14]. Most of the cultures in the present study yielded monomicrobial growth. The polymicrobial growth could mean contamination or a severe infection with bad prognosis [15, 16, 17].

The high occurrence of non-lactose fermenters especially *Pseudomonas* spp. and *Acinetobacter* spp. is of concern. Both of these bacteria are associated with a high degree of resistance to antibiotics. Blood stream infections with *Pseudomonas* spp. have been associated with increased morbidity in some studies. Another significant finding of the study is the isolation of *Salmonella* species in 15% of the cases. An increasing incidence of *Salmonella* species has also been reported in some recent studies [18, 19].

The present study showed that in case of *Salmonella typhi*, most effective antibiotics were found to be ceftriaxone (100%) followed by chloramphenicol (100%) and ciprofloxacin (88%) in agreement with others [7, 20]. Our study showed that in case of non fermenters (*Acinetobacter* species and *Pseudomonas* species), most effective antibiotics were found to be imipenem (86.6%) followed by amikacin (73.3%). *E. coli* isolates showed least resistance to carbapenems, aminoglycosides, and tigecycline and moderate resistance to beta-lactam beta-lactamase inhibitors. *Klebsiella* showed least resistance to carbapenems, tigecycline and moderate resistance to

aminoglycosides, and beta-lactam beta-lactamase inhibitor combination.

The most common gram-positive organism isolated was *Staphylococcus aureus* (25.8%), followed by CoNS (21.5%) and *Enterococcus* spp. (6.8%) of the total bacterial isolates (n=116). We suspect that the observed low isolation of CoNS in our study could be due to or related to strict aseptic practices of collection method followed for blood sampling of blood culture. *Staphylococcus* seems to be emerging as the dominant organisms in blood stream infections with 32% MRSA isolates. Nosocomial infection due to *Staphylococcus aureus* constitutes a major part of the total annual nosocomial infections [20]. Erythromycin and ampicillin were found to be the highly resistant antibiotics among gram positive organisms. The study also showed that *Staphyloccocus aureus* was found to be highly sensitive to vancomycin, linezolid, tigecycline, gentamicin and ciprofloxacin as reported by other investigators [21].

CONCLUSION: The present study provided much needed information on the prevalence of bacterial pathogens in blood stream infections and their antibiotic sensitivity patterns. The study identified both gram positive and gram negative bacteria were responsible for blood stream infections and most of them were multi drug resistant. The main forces driving the increase in antimicrobial resistant bacteria are poor infection control practices and inappropriate use of antibiotics. Specific antibiotic utilization strategies like antibiotic restriction, combination therapy and antibiotic recycling may help to decrease or prevent the emergence of resistance and antibiotic usage according to the standard antimicrobial susceptibility testing may reduce the incidence of blood stream infections.

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