DOI: http://dx.doi.org/10.18320/JIMD/201603.0280

Microbiology- Original article

Journal of International Medicine and Dentistry 2016; 3(2): 80-87

JOURNAL OF INTERNATIONAL MEDICINE AND DENTISTRY

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JIMD

p-ISSN: 2454-8847

e-ISSN: 2350-045X

Bacteriological profile and antimicrobial resistance of blood culture isolates among paediatric patients from tertiary care hospital

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Abstract:

Bloodstream infection ranges from self limiting infection to life threatening sepsis and requires rapid and aggressive antimicrobial treatment. A total of 928 blood samples were collected from suspected cases from August 2014 to July 2015, they were processed aerobically and antibiogram pattern was studied . Cultures were positive in 252 (27.16%) cases. All the positive cultures were obtained after 48 hours of incubation. Gram negative bacteria were 84(36.20%) of the total isolates and Gram positive were 108 (46.55%), Candida species were isolated in 40(17.24%) of cases. In gram negative isolates, highest resistance was seen for Ticarcillin- Clavulanic acid 22 (39.29%), Cefoperazone – sulbactum 22 (39.29%), Cefuroxime 22 (39.29%), Cefazolin 22 (39.29%) and Amoxicillin-Clavulanic acid 22 (39.29%); in contrast gram positive isolates showed resistance to Ciprofloxacin 40(100%). Blood cultures provide a valuable guide in identifying etiological agent and selecting appropriate antibiotic. This helps to achieve a high level antibiotic activity against the offending bacterial organism.

Key words: Bloodstream infection, paediatrics, isolates, antibiogram

Introduction:

Bloodstream infections are an important cause of mortality and morbidity and are among the most common healthcare associated infections[1]. Septicemia, a symptomatic bacteraemia is a common condition in children with a resultant high morbidity and mortality.[2] Illness associated with bloodstream infection which ranges from self-limiting infection to life threatening sepsis and requires rapid and aggressive antimicrobial treatment.[3] It has been seen that microbial culture of blood is the only available means for diagnosis and provides essential information for the evaluation of broad range of diseases like endocarditis, pneumonia, pyrexia of unknown origin and helpful particularly in patients with suspected sepsis and it allows for successful recovery of bacteria in 99% patient with bacteraemia.[4,5] The predominance of gram positive or gram negative bacterial isolates is influenced by the geographical location and changes with time; so also is the antimicrobial susceptibility pattern influenced by location and time[6]. With the increase in antibiotic resistance among the organisms which is of worldwide concern, it has become more important to know the microbial profile and its susceptibility pattern for treatment and specific therapy. The antibiogram of the isolate will improve the therapeutic outcome. The present study was taken to know the microbial profile of bacteraemia in paediatric patients and describe the antibiotic resistance of blood culture isolates which may guide in infection control and rational use of antibiotics in this region.

Materials and Methods:

The present study was carried out from August 2014 to July 2015 in the Department of Microbiology, Karwar Institute of Medical Sciences Hospital. After the approval from Institutional Ethics Committee and obtaining a written

informed consent from the patient, a total of 928 samples were collected from suspected septicemic cases with detailed history taken to identify the possible risk factors. History of antibiotic usage empirically either before or after admission was also obtained. Blood sample for culture was collected following aseptic precautions. The venous site was cleansed with 70% alcohol and allowing it to dry for 1-2 minutes, a set of two samples were collected giving a one hour interval from different anatomical sites. About 1ml blood in case of neonates, 5 ml in case of children were collected and inoculated into blood culture bottles containing 10 ml and 50 ml brain heart infusion broth with 0.025 % of sodium polyanethol sulphonate as anticoagulant (Himedia, a commercial firm) respectively.

The blood culture bottles were then incubated at 37^0C aerobically. After overnight incubation, the bottles were observed for turbidity and broths were subcultured with aseptic precautions onto blood agar, MacConkey agar and chocolate agar. The plates were incubated at 37^0C overnight. If there was no growth observed on the plate next day, the sample was further incubated and subcultured subsequently till seventh day. If there was any growth, it was identified by gram staining, colony characteristics and standard biochemical tests[7] and antibiotic susceptibility test was performed according to the standard protocol. The culture reports were issued and patients were followed up for clinical impact and the modification of therapy.

Results:

A total of 928 blood culture samples were collected among which 580 were males and 348 females. Positivity of 142 (24.48%) was seen in males and 110(31.61%) was seen in females as seen in **Table I**. Highest blood culture positivity was found in the age group of < 1 month 150(59.52%) and least in 1-3 years 22(8.73%) age group as seen in **Table II**. Among the positive cases, 79(31.35%) were in intensive care unit (ICU) as seen in **Table III**. Thirty eight (48.10%) of the ICU patients were on ventilators, 17(21.52%) on invasive lines and 24(30.38%) of the children were on broad spectrum antibiotics.

Table I: Distribution based on gender

Gender	Positive	Negative	Total
Male	142(24.48%)	438	580
Female	110(31.61%)	238	348
	252	676	928

Table II: Frequency distribution in various age groups

Age	Positive	Negative	Total
< 1 Month	150(59.52%)	218	368
1 Month – 1 Year	34(13.49%)	122	156
1 Year – 3 Year	20(8.73%)	82	104
>3 Year	40(18.25%)	254	300

Table III: Distribution characteristics

Location	Percentage (n= 252)
Intensive care unit	79(31.35%)
Non – intensive care unit	120(47.62%)
Out patients	53(21.03%)
Total	252

Single isolate blood stream infection (BSI) was found in 232(92.06%) whereas mixed BSI observed in 20(7.94%) cases. The combination of organisms isolated in mixed BSI were *Staphylococcus epidermidis* and *Enterococccus fecalis* in 12 (4.76%), *S.epidermidis* and *Bacillus species* 4(1.58%) and *Streptococcus pneumoniae* and Diphtheroides 4(1.58%) as seen in **Table IV.**

Gram negative bacteria were 84(33.33%) of the total isolates and gram positive were 108 (42.86%), Candida species were isolated in 40(17.24%) of cases. Most common gram negative isolate was

Klebsiella species 32(13.79%) followed by *Pseudomonas speci*es 28(12.07%), *Acinetobacter species* 12(5.17%), *Escherichia coli* 8(3.45%) and *Enterobacter species* 4 (1.72%). Among Gram positive bacteria, *Coagulase negative Staphylococcus species* (CoNS) were the commonest isolates 52(22.41%) followed by *Staphylococcus aureus* 40(17.24%) and *Enterococcus species* 16(6.90%) as seen in **Table IV**.

Klebsiella spp and *S.aureus* were common isolates among children below 1 month of age as shown in **Table V.** The *Staphylococcus species* isolated were resistant to Ciprofloxacin 40(100%), Erythromycin 24(60%), Cotrimoxazole 16(40%) and Amoxicillin – Clavlulanic acid 16(40%). Enterococcus was resistant to Penicillin 8(25%), Erythromycin 8(25%), Ampicillin 4(12.5%) and Teicoplanin 4(12.5%) **Table VI.**

On the other hand, gram negative bacilli (GNB) oxidase negative showed 100% sensitivity to Polymyxin B (300) and Colistin. It was resistant to Ticarcillin-Clavulanic acid 22 (39.29%), Cefoperazone–sulbactum 22 (39.29%), Cefuroxime 22 (39.29%), Cefazolin 22 (39.29%) ,Amoxicillin- Clavulanic acid 22 (39.29%), Piperacillin – Tazobactum 20(35.71%),Aztreonam,20(35.71%),Ampicillin 20(35.71%), Cefepime 20(35.71%), Gentamicin 18(32.14%), Amikacin 17(30.36%), Ciprofloxacin 16(28.75%)and Cotrimoxazole12(21.43%).

Least resistance was recorded with Meropenem 3.57%. GNB oxidase positive showed highest resistance with Ceftazidime 10 (35.71%) followed by Ciprofloxacin 4 (14.29%), Amikacin 3(10.71%) and least resistance with Gentamicin 2 (7.14%) and Cotimoxazole 2.(7.14%). No resistance was found against Aztreonam, Cefaperazone-Sulbactum, Cefepime, Meropenem, Piperacillin- Tazobactum, Ticarcillin - Clavulanic acid, Polymyxin B and Colistin**.**

Table IV: Frequency of isolation

Isolate	**Percentage positive (n=252)**		
	Male	Female	Total
Gram Negative Bacilli (Oxidase Negative)			
Escherichia coli	4	4	8 (3.17%)
Enterobacter species	4	0	4 (1.59%)
Klebsiella species	16	16	32 (12.70%)
Acinetobacter species	8	4	12 (4.76%)
Gram Negative Bacilli (Oxidase positive)			
Pseudomonas species	16	12	28 (11.11%)
Gram Positive Cocci			
Staphylococcus aureus	20	20	40 (15.87%)
Coagulase negative Staphylococcus species(CoNS)	32	20	52 (20.63%)
Enterococcus species	4	12	16 (6.35%)
Fungus			
Candida species	28	12	40(15.87%)
Total	132	100	232
Polymicobial isolates			
Staphylococcus epidermidis + Bacillus species	3	1	4(1.58%)
Staphylococcus epidermidis +Enterococcus fecalis	5	7	12(4.76%)
Streptococcus pneumoniae +Diphtheroides	2	2	4(1.58%)
Total	142	110	252

Table V: Percentage isolation in neonates

Isolates	Percentage positive(n=150)
Gram negative bacilli (oxidase negative)	
Klebsiella species	25(16.67%)
Acinetobacter species	4(2.67%)
Escherichia coli	4(2.67%)
Enterobacter	4(2.67%)
Gram negative bacilli (oxidase positive)	
Pseudomonas species	17(11.33%)
Gram positive cocci	
Staphylococcus aureus	26(17.33%)
Coagulase negative Staphylococcus Species	25(16.67%)
Enterococcus species	8(5.33%)
Candida species	37(24.67%)
Total	150

Table VI: Resistance patterns of Gram positive cocci

Antibiotic	Staphylococcus species	Enterococcus species
Amoxicillin–Clavulanic acid	16(40%)	-
Ampicillin	4(10%)	4(12.5%)
Chloramphenicol	8(20%)	Nil
Ciprofloxacin	40(100%)	Not tested
Co-trimoxazole	16(40%)	Nil
Erythromycin	24(60%)	8(25%)
Gentamicin	Nil	Nil
Penicillin	8(20%)	8(25%)
Clindamycin	8(20%)	Nil
Rifampacin	Nil	Nil
Linezolid	4(10%)	Nil
Teicoplanin	Nil	4(12.5%)

Discussion:

Septicemia is a clinical condition associated with considerable morbidity and mortality. The timely detection of bacteraemia can have a profound influence on the final clinical outcome.[8] In our study, a total of 252(27.16%) positive cultures was seen which is in accordance with some of the previously conducted studies by DS Murthy et al (24.3%), Madhu et al (22.9%), Atul Garg et al (20.5%) and AlaaH Al et al (23.48%).[9,10,11,12] This is quite low compared to studies conducted by Jain et al which shows a positivity of 52.63%.[13] The reason for low rate of positive culture could be due to prior empirical antibiotic therapy before collection of samples for culture which may result in negative blood cultures and one of the other reasons could be due to anaerobic infections which cannot be detected by routine aerobic cultures.

Most studies on BSI have concentrated on the neonatal period, since this age group is most vulnerable for infection due to their developmental status and physical examination findings are less reliable in neonates[14,15,16,17,18] and neonates are particularly vulnerable to infections because of their weak immunological barriers. In the present study, highest blood culture positivity was found in neonates that is 150(59.52%) and similar high isolation rates in neonates

are quoted in other studies by DS Murthy et al (52.63%) and AlaaH Al et al (23.4%).[9,12]

In our study, the predominance of gram positive organisms was seen compared to gram negative bacilli. Recent reports have revealed that gram positive organisms are now assuming greater significance in bacteraemia concomitant with increasing incidence of nosocomial blood stream infection.[19,20] Such changes happened parallel to the evolution of medical care, more so with increasing number of critically ill and immunocompromised patients requiring aggressive medical support and indwelling devices[21] and in the present study, it was also seen that out of the total positive cultures, 79(31.35%) were from intensive care unit.

CoNS previously considered as a contaminant has been recognised increasingly as a cause of bacteraemia. The ascendance of this group of Staphylococci has created increased interpretative difficulties for the clinician since the great majority of CoNS isolates continue to represent contamination rather than true bacteraemia.[22] Rate of isolation of CoNS in our study is 52(20.63%) which is similar to the rate of isolation in study conducted by DS Murthy et al[9] , but is low when compared to study conducted by Hanan A et al[23] which shows a positive isolation rate of 55.4%.

CoNS isolated from blood are often considered contaminants[24] as it is a common skin inhabitant and may indeed contaminate poorly collected blood cultures making it difficult to differentiate bacteraemia from contamination.[25] Haimi Cohen et al suggested qualitative culture to aid interpretation and determining whether it has vascular relation.[26] However, antibiotic sensitivity testing was performed to know the sensitivity pattern of the doubtful pathogens causing septicemia and was seen that it was resistant to Ciprofloxacin 40(100%) and no resistance was seen for Gentamicin, Rifampacin and Teicoplanin.

S. aureus 40(15.87%) was the second most common gram positive cocci isolated and isolation of *S. aureus* from blood usually signifies infection. According to one study, upto 57%of cases where *S.aureus* was repeatedly isolated will have a cardiac pathology and all such patients with *S.aureus* bacteraemia should be thoroughly evaluated for the presence of any cardiac pathology as the cardiac vegetation serves as an important source of persistant *S.aureus* bacteraemia.[27] Friedland et al reported 36% of children with silent endocarditis had staphylococcal septicemia or pericarditis and therefore echocardiography should be considered in cases of staphylococcal BSI without a focus.[28]

Although *E.coli* is the most common etiological agent of gram negative bacteraemia as seen in other studies, our study revealed that *Klebsiella spp* was the predominant isolate 32(12.70%) which correlates with other studies conducted by AlaaH Al et al 46.8% and DS Murthy 35%.[9.12]

In the current study, among the various antibiotics used for the antibiotic susceptibility testing for gram negative oxidase negative organisms, Meropenem and Cotrimoxazole were more effective as they showed least resistance 2(3.5%) and 12(21.43%) respectively, where as for Non-fermenters like *Pseudomonas spp.*, no resistance was seen for few antibiotics like Aztreonam, Cefoperasone – Sulbactum, Cefepime, Meropenem, Piperacillin – Tazobactum and Ticarcillin – Clavlulanic acid.

Gentamicin a relatively cheap and an easily available antibiotic was seen resistant to gram negative bacilli oxidase negative isolates in 18 (32.14%), showed further less resistance for non-fermenters oxidase positive 2(7.14%) and in case of gram positive cocci isolates, no resistance was seen. None of the antibiotics used singly showed high susceptibility to all GNB, so a combination of two or more drugs is recommended to cover the broad

range of pathogens. This may prevent the emergence of resistance as they have additive or synergistic antimicrobial activity.[29]

In 20 (7.94%) polymicrobial growths were seen with isolates such as *S.epidermidis*, *Bacillus spp.*, diptheroides; these are considered as contaminants in the absence of clinical features of sepsis. Diptheroides are the normal flora of the mucous membrane and gastrointestinal tract and some as well have environmental origin.[30, 31] *Bacillus spp.* may have originated from environment.[30] These contaminants are noticed commonly in paediatric population since it is difficult to take blood samples due to much skin manipulation leading to contamination.[23] In addition, only single samples are usually taken and this adds to difficulty of interpretation . A number of publications have associated diptheroides with human infection including bacteraemia.[32, 33]

Enterococcus spp. was isolated in 16(6.35%) cases and it was reported earlier that enterococcal BSI is more common in older age group children with instrumentation and prior to or with current antimicrobial therapy.[34]

In the recent years, an increased incidence of systemic fungal infections has been reported in hospitalised intensive care unit patients and systemic candidiasis is commonest among them. With an increased use of broad spectrum antibacterials, invasive lines, endotracheal tubes etc. in these patients, it is easy for Candida to bypass the natural barrier of infection and contribute to deep seated infection. Maternal factors also contribute to neonatal septicaemias and it was seen that babies on broad spectrum antibacterials contribute to invasive candidiasis. In the present study, Candida species was isolated in 40 (15.87%) children and out of those, 37(24.67%) isolates were from neonates. Antifungal susceptibility testing was not done due to lack of facilities.

Among the positive cultures, we have observed that all of the cultures were positive by second subculture (48 hours). Reller et al suggested that incubation beyond 7 days is generally unnecessary with relatively few clinically significant isolates detected.[35]The predictive value of blood cultures that were negative at day 4 was similar to that waiting for 7 days of processing before discarding.[36]

Conclusion:

In conclusion, BSI is an important cause of morbidity and mortality in our paediatric patients. Blood cultures provide a valuable guide to the clinician in identifying etiological agent and selecting appropriate antibiotic .There should be an effective and rational use of antibiotic especially in tender age group in order to achieve a high level antibiotic activity against the offending bacterial organism.

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Conflict of interests: Nil
Source of funding: Nil

Date of submission: 20-03-2016
Date of acceptance: 16-06-2016

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