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Characterization and Antibiotic Susceptibility Pattern of Gram Positive Cocci from Blood Stream Infections in Children in a Tertiary Care Hospital, Punjab

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Abstract: ***Purpose:*** *Blood stream infections are very common in the paediatric age group and these are one of the common causes of morbidity and mortality in neonates and children. The incidence of bacteraemia in children varies widely. The rate of blood stream infections in children is about 20-50% in developing countries.*
Objectives: *Therefore purpose of this study was to characterize various gram positive cocci and to know their antibiotic susceptibility pattern causing blood stream infections in children.*
Materials and Methods: *This retrospective study was conducted in the Microbiology department of a tertiary care hospital, in Punjab. A total of 2498 paediatrics blood cultures were received from suspected cases of septicaemia in the laboratory. All the blood samples were processed in the Microbiology Laboratory by RAPID BD BACTEC PLUS system and organisms were identified as per standard microbiological techniques. Antimicrobial susceptibility testing was done using Kirby Bauer disc diffusion method as per CLSI guidelines.*
Results: *Out of the 2498 samples from paediatrics patient's 200 (8.01%) blood cultures were positive. Gram negative bacilli were the predominant organism 134 (67%) followed by Gram positive cocci 66(33%).*
Conclusion: *BSI is a challenging problem, and sometimes, it may be life-threatening; therefore, timely detection, identification, and antimicrobial susceptibility testing of blood-borne pathogens are one of the most important functions of diagnostic microbiology laboratory. This study is an attempt to analyze the bacterial profile of blood culture isolates, assess antimicrobial trends, correlate bacteremic source, and their impact which may help us devise the best ways of managing BSIs.*
Key words: *Bacteraemia, Gram positive cocci, MRSA.*

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I. Introduction:

Blood stream infections are very common in the paediatric age group and these are one of the common causes of morbidity and mortality in neonates and children. The incidence of bacteraemia in children varies widely. The rate of blood stream infections in children is about 20-50% in developing countries.[1,2] Bacteremia refers to a state in which bacteria circulate through the vascular system.[3] Bacteraemic episodes may be transient, intermittent or continuous reflecting several mechanisms by which bacteria enter the blood stream.[4]

Blood stream infections especially sepsis is a major challenge in medicine.[5] The problem is more severe in infants and young children with high fever and no apparent focus of infection. They cause substantial morbidity and mortality. Changing patterns of the isolates, increasing rates of antimicrobial resistance, wide application of new medical technologies like rampant usage of indwelling devices, may change the epidemiology and outcome of BSIs.[6] It is therefore important to continually review and update the epidemiology of BSIs mainly with respect to the antibiotic susceptibility pattern of the common pathogens, so that it is useful for prompt treatment of patients.[6,2] In the present study an attempt was made to know the bacterial profile of clinically suspected BSIs and antibiotic susceptibility pattern of the isolates. The results obtained in the present study were analyzed and compared with other studies.

A wide variety of bacteria are involved in blood stream infections, the majority belong to groups of bacteria, such as Streptococci, Enterococci, Staphylococci or members of family Enterobacteriaceae.[7]

The successful recovery of microorganism from blood by possible types of bacteremia depends upon specimen collection methods, blood volumes, the number and timing of blood cultures, interpretation of results and the type of patient's population being served by the laboratory.[8] There is a wide variation in the incidence and clinical characteristics of invasive infections caused by different species of bacteria. Identifying the causative agents and characterizing the clinical significance in a particular age group is essential for the prevention and treatment of these infections.[9] Sepsis related mortality is largely preventable with rational antimicrobial therapy and aggressive supportive care. Now a days the use of antibiotics has become a routine practice.[10] The varying microbiological pattern of blood stream infections in children warrants the need for an ongoing review of the causative organisms and their antimicrobial susceptibility pattern. The gold standard for diagnosis is positive blood culture.[11] Antimicrobial testing was performed for all the isolates along with detection of Methicillin resistance in Staphylococcus aureus (MRSA) strains in Gram positive bacterial isolates that will ensure better therapeutic success and improved antibiotic efficacy.

II. Objectives :

Therefore purpose of this study was to characterize various gram positive cocci and to know their antibiotic susceptibility pattern causing blood stream infections in children

III. Material & methods :

This retrospective study was conducted in the Microbiology Department of a tertiary care hospital in Punjab. A retrospective analysis was conducted from September 2012 to Feburary 2014. During this period 2498 paediatrics blood cultures were received from suspected cases of septicaemia in the laboratory. All the blood samples were processed in the Microbiology Laboratory by RAPID BD BACTEC PLUS system and organisms were identified as per standard microbiological techniques[12].Every positive vial flagged by the Bactec 9120 (Becton Dickinson, USA) was removed for sub culturing in the safety cabinet. The lid of the positive blood culture vial was disinfected with an alcohol swab and 1 ml of blood was aseptically removed from the vial and plated on 5% Sheep blood agar, Chocolate agar and MacConkey's agar. The Sheep blood agar and Chocolate agar were incubated in 5% CO_2 at 37°C for 24 hours while the MacConkey's agar was incubated aerobically at 37°C for 24 to 48 hours and then observed for growth of bacteria. Following subculture from each positive vial smear was prepared from the contents of that vial, heat fixed and Gram stained for microscopy. All the plates showing growth were processed according to standard microbiological techniques including Gram staining.[12] Gram positive cocci were identified based on their Gram reaction and other microscopic features. All bacterial isolates were identified using standard microbiological techniques.[13] The biochemical tests include Catalase, Oxidase, Coagulase, Bile solubility, Sugar fermentation, Indole production, Methyl red, and other test for identification to the genus or species level.[14] Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 12228, and Streptococcus pneumoniae ATCC 49619 were inoculated along with the test organisms..Antimicrobial susceptibility testing of all the isolates using Kirby-Bauer disc diffusion method[15] was carried out with discs containing and the results were interpreted as per clinical and laboratory standards institute (CLSI) recommendation[16]. However for Pneumococci Mueller Hinton sheep blood agar was used. Drug resistant strains in primary screening were further processed for the detection of Methicillin resistance in Staphylococcus aureus (MRSA) strains.Plates were incubated aerobically at 37°C for 24 hours. Results were scored as susceptible, moderately susceptible or resistant, according to the Clinical and Laboratory Standard Institute (CLSI, 2013) criteria.[16]

Methicillin Resistant Staphylococcus Aureus (Mrsa) Testing :

Testing for Methicillin resistant Staphylococcus aureus (MRSA) was done using Cefoxitin disc (30µg).[17] An inhibition zone of 21 mm or less around Cefoxitin disc indicates MRSA.

IV. Results:

A total of 2,498 blood samples were collected from paediatric patients clinically suspected of bacterial BSIs. The study was done in the Microbiology department of a tertiary care hospital, in Punjab.

TABLE 1: COMPARISON OF BLOOD CULTURE POSITIVITY AND GENDER

SEX	NO. OF CASES INVESTIGATED	POSITIVE BLOOD CULTURE
MALE	1,629(65.21)	138(69.0)
FEMALE	869(34.79)	62(31.0)
TOTAL	2,498(100)	200 (100)

Percentage in parentheses represent out of total isolates (n=2,498)

It is evident from table-1, out of the total 2,498 samples that were received, 1,629 samples, accounting for 65.21 % were obtained from males while 869 samples (34.79%) were from females. Out of total 2,498 cases,

200 yielded positive blood culture giving a positive rate of 8.01%. It is also evident from table-1 that out of 1,629 males, 138 (69.0%) yielded positive blood culture, while 62 (31.0%) of 869 females were positive.

TABLE 2: COMPARISON OF CULTURE POSITIVITY AND TOTAL BLOOD CULTURES

BLOOD CULTURE	NUMBER OF CASES
POSITIVE	200(8.01)
NEGATIVE	2,298(91.99)
TOTAL	2,498(100)

Percentage in parentheses represent out of total isolates (n=2,498))
Out of the total 2,498 blood samples processed, culture yielded bacterial growth in 200 samples. The culture positivity rate was 8.01%.

TABLE 3: PREVALENCE OF GRAM NEGATIVE AND GRAM POSITIVE BACTERIAL ISOLATES

GRAM NEGATIVE	134(67.0)
GRAM POSITIVE	66(33.0)
TOTAL	200(100)

Percentage in parentheses represent out of total isolates (n=200)

Out of the 2498 samples from paediatrics patient's 200 (8.01%) blood cultures were positive. Gram negative bacilli were the predominant organism 134 (67%) followed by Gram positive cocci 66(33%).Table-3 shows the distribution of Gram negative organism to be 67.0% (134/200) as against 33.0% (66/200) Gram positive organism.

TABLE 4: PERCENTAGE OF GRAM POSITIVE ORGANISMS ISOLATED FROM BLOOD CULTURE

SI.N	NAME OF ORGANISM	NUMBER
1	Staphylococcus aureus	45(22.50)
2.	Enterococcus spp	9(4.50)
3.	Coagulase negative Staphylococcus(CONS)	9(4.50)
4.	Streptococcus pnumoniae	3(1.50)
	TOTAL	66

Percentage in parentheses represent out of total isolates (n=200),

Table 4 describe the pattern of isolates. Out of total 66 Gram positive cocci, there was the predominance of Staphylococci aureus 22.50% (45/200) followed by Enterococcus spp 4.50% (9/200), Coagulase negative Staphylococcus 4.50% (9/200) and Streptococcus pnumoniae 1.50% (3/200).

TABLE 5: AGE WISE DISTRIBUTION OF POSITIVE BLOOD CULTURE ISOLATES

ORGANISM	0-1M	1M-1YR	1YR-5YR	5YR-10YR	10-18 YR	TOTAL
Staphylococcus aureus	21	2	9	7	6	45
Enterococcus spp	8	-	-	-	1	9
Coagulase Negative Staphylococcus	6	1	1	-	1	9
Streptococcus pnumoniae	-	3	-	-	-	3

Staphylococcus aureus (46.66%) was most commonly isolated organism in the 0-1 month age group.

S.aureus showed high level resistance to Penicillin (80.0%), Erythromycin (51.11%) Cotrimoxazole (40.0%) and Clindamycin (37.78%) but the 80.0% isolates were sensitive to Cefoxitin and Amoxicillin-Clavulanic acid, sensitive to Ciprofloxacin (71.11%).All the Staphylococcus aureus isolates showed 100 % sensitivity to Vancomycin, Linezolid and Teicoplanin. The present study identified 9 (20.0%) Methicillin resistance Staphylococcus aureus (MRSA) isolates.

It was found that Coagulase Negative Staphylococcus (CONS) showed high level resistance to Penicillin (100%), Erythromycin (66.67%) Cotrimoxazole (44.44%) and Ciprofloxacin (44.44%) but the 50.0% isolates were sensitive to Cefoxitin , Amoxicillin-Clavulanic acid Clindamycin. All the Coagulase Negative Staphylococcus (CONS) isolates showed 100 % sensitivity to Vancomycin, Linezolid and Teicoplanin.

In case of Enterococcus the two species were identified: E. faecalis (n = 6), E. faecium (n = 3), Enterococcus spp showed high level resistance to Penicillin (66.67%) and Erythromycin (55.56%) but the 88.89% isolates were sensitive to Vancomycin, Teicoplanin and 66.67% isolates were sensitive to Ciprofloxacin, High level Gentamicin and Ampicillin. All the Enterococcus spp showed 100 % sensitivity to Linezolid.

Streptococcus pneumoniae showed high level resistance to Erythromycin (66.67%), Cotrimoxazole (66.67%) and (66.67%) isolates were sensitive to Penicillin. All the Streptococcus pneumoniae isolates showed 100% sensitivity to Ciprofloxacin, Chloramphenicol, Ceftriaxone and Cefotaxime.

V. Discussion:

Out of the 2,498 samples that were received with clinically suspected BSI, 200 (8.01%) cases were positive for bacterial growth. The rate of bacteria isolation from the blood culture of patients in the current study was relatively low (8.01%) compared to some previous studies done. It was in accordance with the different studies done via these workers, in Nigeria, 4.1% (Alaa H et al)[18], 4.2% (Karki S et al)[19], 6.1% (Jambo GTA).[20] The culture positivity rates shown by other authors were 48.9% (Meremikwu MM et al)[2], 47.5% (Roy I et al)[21], 29.2% (Hugonnet S)[22], 20.2% (Arora U and Devi P)[23], 18.7% (Murthy DS and Gyaneshwari M)[24], 16.6%(Qureshi M and Aziz F)[25] and 13.8% (Ameen MK et al).[26] The variation in blood culture positivity is related to different factors such as the number and amount of blood cultures taken for screening as reported by (Lee et al).[27] They commented that for achieving a detection rate of >99% as many as four blood cultures may be needed. Similar comment was made by other investigators that more than three blood cultures are needed for 99% test sensitivity. The system and type of blood culture medium formulation used for bacterial detection were other factors affecting the final bacterial yields.[27] In India, variation might be due to the fact that most of the patients are given antibiotics before they come to the tertiary care hospital and other reason is that in most of the cases self medication is very common as the medicines are available at the counter. The variations in culture positivity rates may be due to prior treatment with antibiotics.[26] Further our study showed that prevalence of Gram negative organisms was high 67.0% (134/200) as compared to that of Gram positive organisms 33.0% (66/200).

(Siegman-Igra Y et al)[28] in their study isolated (68%) GNB and (30%) GPC out of 1,113 isolates. (Decousser J-W et al)[29] showed that GNB (54.2%) were isolated more than GPC (45.8%). (Son JS et al)[30] documented (59.2%) GNB and (38.9%) GPC. Similarly (Ameen MK et al)[26] showed that GNB (79.6%) were more isolated than GPC (20.3%). 60% of the isolates were GNB and 40% GPC in a study done by Qureshi M and Aziz F.[25] In a study done by (Rahbar M et al) [31], of the 85 isolates both GNB and GPC were equally isolated 42.3% each, the rest 14.3% was reported as contaminants. However, in few studies, GPC were the predominant isolates – (78.1%) GPC and (21.9%) GNB (Karlowsky JA et al)[32], (54.1%) GPC and (38.2%) GNB (Riedel et al)[33] and (61%) GPC and (39%) GNB (Hana A. Babay).[34] Thus, wide variations were observed in the isolation patterns. Our study correlates well with the studies done by (Siegman-Igra Y et al)[28] and (Son JS et al).[30] It is well known that the number of infections resulting in bacteraemia or septicaemia by various microorganisms varies from one country to another, one place to another, and from time to time in same institution.

In this study among the GPC S.aureus, CoNS, Enterococcus spp and Streptococcus pneumoniae were the isolated. In a study conducted by (Martin M Meremikwu et al)[2] in Nigeria, they found that Staphylococcus aureus was isolated in 48.7% as the most frequent isolates. Aziz et al[35] also reported Staphylococcus aureus 25% as the most pathogenic bacteria recovered from the blood samples.

In certain other studies done by (Karlowsky JA et al)[32] and (Riedel S et al)[33] even though GPC were the predominant isolates. Haddon RA et al[36] showed that Streptococcus pneumoniae were the most commonest isolated organism.

The isolation of (22.50%) Staphylococcus aureus observed in the present study agrees with the (18.30%) reported by (Hill et al)[37] in a study conducted in West Africa. However, reports from other African countries including Ghana; (Evans et al)[38] 29%, (Komolafe et al)[39] 43.6% and (Meremikwu et al)[2] 48.7% identified high incidence of Staphylococcus aureus infant bacteraemia. However low incidence of this organism 8% was obtained (Maitland et al)[40] in a study conducted in Kenya.

In the present study it was found that S.aureus showed high level resistance to Penicillin (80.0%), Erythromycin (51.11%), Cotrimoxazole (40.0%) and Clindamycin (37.78%) but these isolates were sensitive to Cefoxitin (80.0%), Amoxicillin- Clavulanic acid (80.0%) and Ciprofloxacin (71.11%). All the Staphylococcus aureus isolates showed (100%) sensitivity to Vancomycin, Linezolid and Teicoplanin in the present study. In the study it was found that CONS showed high level resistance to Penicillin (100%), Erythromycin (66.67%), Cotrimoxazole (44.44%) and Ciprofloxacin (44.44%) but the (50.0%) isolates were sensitive to Cefoxitin, Amoxicillin-Clavulanic acid Clindamycin.

All the CONS isolates showed (100%) sensitivity to Vancomycin, Linezolid and Teicoplanin in the present study. Arora U and Devi P in their study also found maximum resistance to Ampicillin (74.61%) and Erythromycin (69.67%) amongst the GPC.[23] In their study, Fluit et al[41] reported that all Staphylococci remained fully susceptible to Vancomycin. Similar result was obtained in the study done by Roy et al.[21] Rahbar M et al found S.aureus were resistant to Penicillins (82.6%) but sensitive to Cotrimoxazole and Vancomycin.[31]

The present study identified 20.0% (9/45) Methicillin resistance Staphylococcus aureus (MRSA). The incidence of MRSA varies from 25 per cent in western part of India[42] to 50 per cent in South India.[43] MRSA is resistant to β-lactam antibiotics which include Penicillin and Cephalosporin (Klein et al).[44] This means patients with MRSA cannot be treated with readily available drugs such as the Penicillin and Cephalosporin. MRSA bacteraemia is associated with higher mortality rate, longer hospital stay and is a significant independent

risk factor for death (Khairulddin et al).[45] The emergence of MRSA will put lots of pressure on health facilities to stock much more expensive drugs such as Vancomycin and Teicoplanin which are currently used in the treatment of MRSA infections (Schentag et al).[46] To prevent the spread of MRSA in hospitals, employers should ensure the availability of adequate facilities and supplies that encourage workers to practice good hygiene (Khairulddin et al).[45] Non-adherence to infection control practices, such as hand hygiene, is the most potentially modifiable cause of health care-associated infections in hospitals (Asare et al).[47] In our study 6 isolates of Enterococcus faecalis and 3 isolates of Enterococcus faecium were identified. Studies from various parts of India[48-50] and elsewhere[32] have shown E.faecalis as the predominant species isolated in humans. A study of hospitalized patients from the United States reported E.faecalis to be two times more common than E.faecium among blood culture isolates.[32] In the study it was found that Enterococcus spp showed high level resistance to Penicillin (66.67%), Erythromycin (55.56%). But the (89.89%) isolates were sensitive to Vancomycin and Teicoplanin (66.67%) isolates were sensitive to Ciprofloxacin, High level Gentamicin and Ampicillin. All the Enterococcus isolates showed (100%) sensitivity to Linezolid in the present study. Multidrug-resistant enterococci are being increasingly reported from all over the world.

In the present study (1.50%) Streptococcus pneumoniae bacteraemia obtained compares with the studies conducted in Nigeria (Komolafe et al)[39] and (Ghana Evans et al)[38] identified (0.5%) to (2.0%) Streptococcus pneumoniae infant bacteraemia respectively. However (11%) reported by (Bronzan et al)[51] in a prospective study involving 1388 children conducted in Malawi. However, these results differ from figures obtained in the current study. Contrary to the present study, (Maitland et al)[40] obtained 35% Streptococcus pneumoniae in a study involving 920 children in Kenya. This high rate could be due to the fact that there were more malnourished children in the Kenya study who are mainly prone to invasive bacteria infections (Eddleston et al).[52] In the study it was found that Streptococcus pneumoniae showed high level resistance to Erythromycin (66.67%) and Cotrimoxazole (66.67%). (66.67%) isolates were sensitive to Penicillin. All the Streptococcus pneumoniae isolates showed 100% sensitivity to Ciprofloxacin, Chloramphenicol, Ceftriaxone and Cefotaxime.

The present study observed that Streptococcus pneumoniae isolates were susceptible to Penicillin 66.67% by disc diffusion. Penicillin has been an important drug in the treatment of Streptococcus pneumoniae infections. This finding was in agreement with a study conducted in the (Gambia Hill et al).[37] However, another study in Ghana reported an increase in resistance to Penicillin (19.4%) (Donkor et al).[53] This increase in resistance could be due to the emergence of Penicillin resistance strains. Improved commitment to rational use of these antibiotics is needed to sustain this relatively high level of susceptibility to Penicillin.

VI. Conclusions

Staphylococcus aureues were the commonestgram positive cocci isolated in our study. The different species of gram positive cocci have shown a varied sensitivity pattern in our study. Therefore identification of Gram positive cocci and monitoring their susceptibility pattern are important for proper management of these infections caused by them.. This would avoid unnecessary usage of antibiotics and emergence of drug resistant strains.

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