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Original Research Article

Ordinarily Isolated Organisms from Blood Cultures of Neonates and Children: A Hospital Based Study

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ABSTRACT

Background: Sepsis is a serious medical condition caused by an overwhelming immune response to infection. Neonatal sepsis specifically refers to the presence in a newborn baby of a bacterial blood stream infection (BSI) along with an underlying condition such as meningitis, pneumonia, pyelonephritis, or gastroenteritis in the setting of fever. Bloodstream infections affect approximately 2% of all hospitalized patients and up to 70% patients admitted in the Intensive Care . Mortality is high, ranging from 14% to 57% .The aim of present study to evaluate the aerobic bacterial profile of isolates causing blood stream infection.

Materials and Methods: The present hospital based study was conducted in the Department of Microbiology of the Institute during a period of 6 months. Based on the standard formula sample size of 80 was selected. In this study all the clinically suspected cases of blood stream infection admitted in intensive care unit were included. Blood cultures were done by following ways. First by BACTEC blood culture and by Traditional blood culture.

The data thus obtained was arranged in a tabulated form and analysed by SPSS software. Chi square test was used for analysis wherever required.

Results: Out of 41 blood samples of male, 25(52%) were culture positive rests 23(48%) were culture negative. Out of 80 blood samples, 75(93.7%) were collected in a pediatric Bactec blood culture bottle and 5 (6.25%) samples were collected in a traditional blood culture bottle. 51.25% blood cultures were positive. A total of 32 isolates were obtained in 24hours, 4 isolates were obtained in 36 hours while 8 isolates were found in 48 hours. Out of 41 organisms, 21 were gram positive cocci, 9 were gram negative bacilli and 8 were Candida.

Conclusion: Gram positive microorganisms are most frequently isolated from the blood cultures majority of them being staphylococci aureus.

Keywords: Blood Culture, Candida, Staphylococcus, Sepsis.

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INTRODUCTION

Bacteremia is defined as the presence of organisms in the blood, documented by at least one positive blood culture. Sepsis is defined as bacteremia with systemic inflammatory response syndrome (SIRS). Sepsis is a serious medical condition caused by an overwhelming immune response to infection.

Neonatal sepsis specifically refers to the presence in a newborn baby of a bacterial blood stream infection (BSI) along with an underlying condition such as meningitis, pneumonia, pyelonephritis, or gastroenteritis in the setting of fever. Neonatal sepsis is the single most important cause of neonatal death in hospital as well as community in developing country.

The current practice in newborns less than 30 days old is to perform a complete workup that includes blood culture and treat empirically for serious bacterial infection for at least 48 hours until cultures are demonstrated to show no growth.

Over all studies have reported the most common gram-positive organism isolate from blood culture to be Staphylococcus aureus (70-95%), followed by Staphylococcus Coagulase Negative (CoNS)[1] & member of Enterobacteriaceae family species such as Klebsiella, Citrobacter, Enterobacter followed by the Pseudomonas species. The risk of specific infections is related to the age of the child.[2]

Sepsis is one of the major causes of mortality and morbidity in hospitals. Bloodstream infections affect approximately 2% of all hospitalized patients and up to 70% patients admitted in the Intensive Care.[3,4] Mortality is high, ranging from 14% to 57%.[5] The aim of present study to evaluate the aerobic bacterial profile of isolates causing blood stream infection.

MATERIALS AND METHODS

The present hospital based study was conducted in the Department of Microbiology of the Institute during a period of 6 months. Based on the standard formula sample size of 80 was selected. In this study all the clinically suspected cases of blood stream infection admitted in intensive care unit were included. Ethical committee clearance was obtained from the institutional ethical committee and a written informed consent was obtained from the subjects.

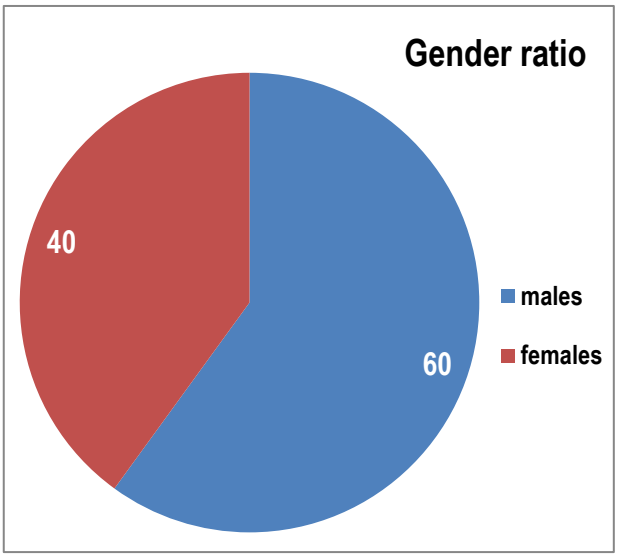
The study included neonates and children with any clinical signs and symptoms of blood stream infection. Patient's demographic details were filled in a predesigned proforma. After following universal precautions and under complete aseptic conditions blood samples were withdrawn by venipuncture. Blood cultures were done by following ways. First by BACTEC blood culture and by Traditional blood culture.

The principle behind BACTEC blood culture is that the sample to be tested is inoculated into the vial which is entered into the BACTEC 9050 instrument for incubation . Each vial has a sensor which responds to the concentration of CO2 produced by the metabolism of microorganisms needed for the growth. The sensor is monitored for an increase in its fluorescence, which is proportional to the increasing amount of CO2 present in the bottle. In case of positive sample, BACTEC 9050 instrument gives a beep sound. The positive samples were inoculated on Blood agar, Cystine Lactose Electrolyte Deficient (CLED) agar for the differentiation of coliforms, staphylococci. The culture plates was examined after overnight incubation at 370C for 18-24 hrs, the relative numbers and types of the colonies was noted and further tests for their identification and determination was done.If culture was negative then subculture was done at least twice during first 2-3 days & final subculture was done after 5 days.[6]

Afterwards Microscopic examination was done by making a smear from the colony and examined under oil immersion after the gram staining. After culture the bacteria obtained in pure subculture, identification of isolates was done on the basis of colony morphology, motility, catalase, oxidase, coagulase test and biochemical tests such as – Indole, Methyl red, Voges-Proskauer, Urease, Citrate, Nitrate reduction tests, Triple sugar iron agar, Hydrogen sulphide test, Phenylalanine deaminase test, Carbohydrate fermentation tests.[6]

The data thus obtained was arranged in a tabulated form and analysed by SPSS software. Chi square test was used for analysis wherever required.

Graph 1: Showing male and female ratio



RESULTS

A Total 80 patients were enrolled for the study. Out of 80 no. of subjects, 48 male and 32 female subjects enrolled in this study. (Graph 1)

Table 1 shows the age wise distribution of male patients. Majority of patients were between 1 to 14 years of age i.e. 18 patients followed by 1 to 5 hours ie 14 patients. Out of 41 blood samples of male, 25(52%) were culture positive rests 23(48%) were culture negative. Table 2 shows the age wise distribution of female patients. Majority of patients were between 1 to 5 hours of age i.e 19 patients and only 9 patients were between 1 to 14 years of age. Out of 32 blood samples of female subjects, 15(46.87%) were culture positive while 18(56.25%) were culture negative.

Out of 80 blood samples, 75(93.7%) were collected in a pediatric Bactec blood culture bottle and 5 (6.25%) samples were collected in a traditional blood culture bottle. Graph 2 illustrates the percentage of positive blood cultures amongst the study. 51.25% blood cultures were positive. Table 3 shows the amount of isolates in relation to the incubation period in Bactec cultures. A total of 32 isolates were obtained in 24hours, 4 isolates were obtained in 36 hours while 8 isolates were found in 48 hours.

Table 4 shows the APAGAR scores of the neonates. APGAR SCORE of the neonates subjects at 1minute and at 5minutes after birth. Maximum APGAR SCORE at 1 minute is 15 in 5/10 whereas at 5 minutes the maximum score is 14 in 7/10 as well as in 8/10.

Table 1: Age wise distribution in male subjects.

MALE	1HR-5HR	1DAY-1MONTH	1MONTH-1YR	1YR-14YR
48	14	10	6	18

Table 2: Age wise distribution in female subjects.

FEMALE	1HR-5HR	1DAY-1MONTH	1MONTH-1YR	1YR-14YR
32	19	1	3	9

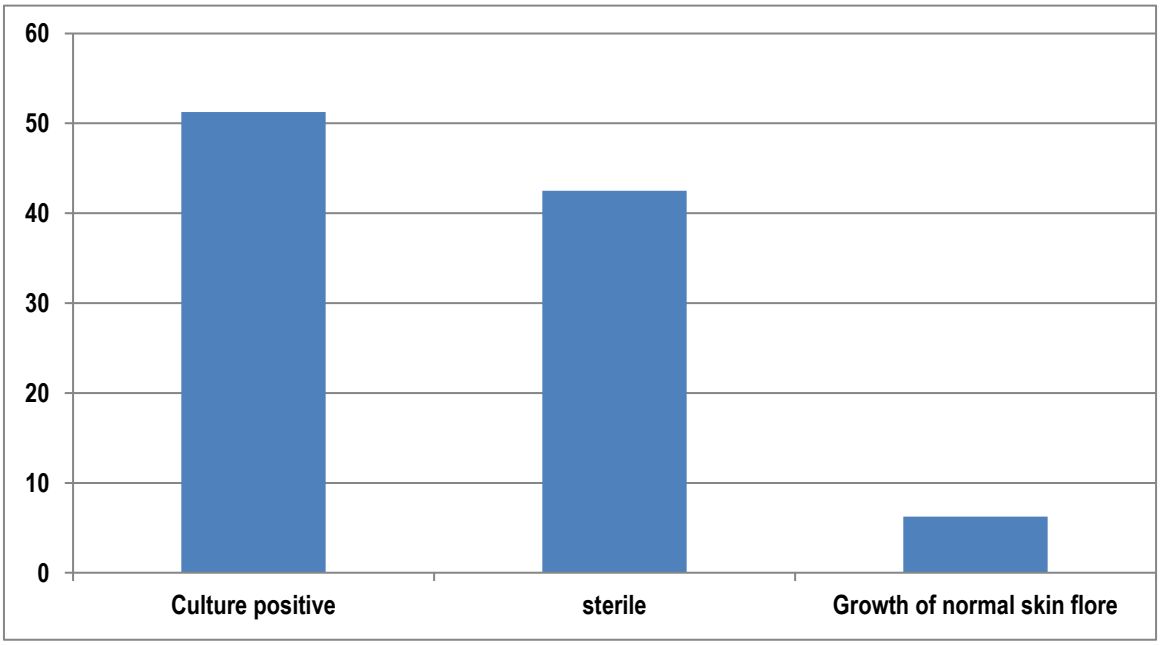
Table 3: Timeline of culture positivity in Bactec culture

Incubation period	24 hours	36hours	48 hours
Isolates	32	4	8

Table 4: APGAR SCORE of neonates subjects.

APGAR SCORE	NO.OF NEONATES SUBJECTS				
1 minute	3/10=1	5/10=10	6/10=15	7/10=12	8/10=2
5minutes	5/10=1	6/10=5	7/10=14	8/10=14	9/10=6

Graph 2: Graphical distribution of culture results



Graph 3: Graphical distribution was according to organisms.

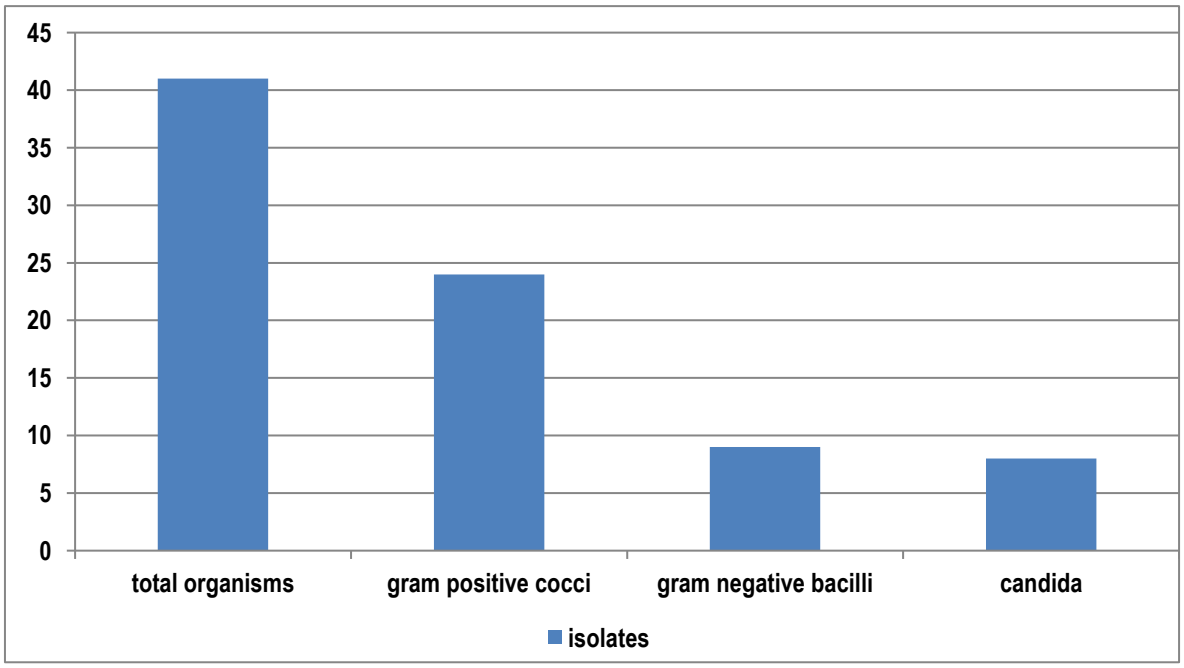
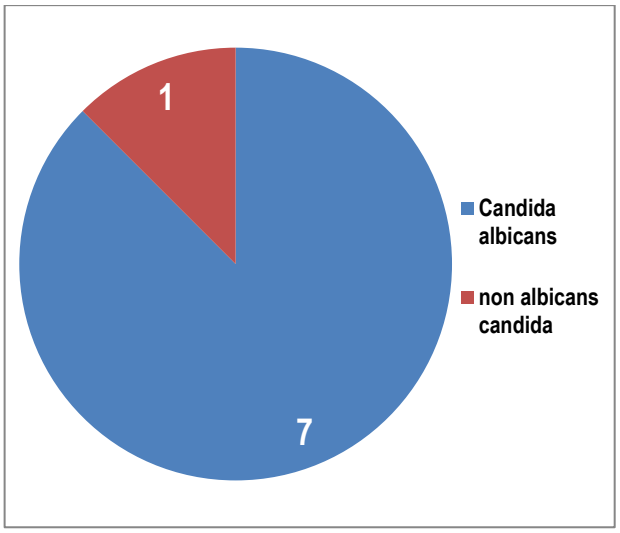


Table 5: Types of isolates in Gram positive cocci

GRAM POSITIVE COCCI	S.aureus	CONS	Enterococcus
24	16	5	3

Table 6: Types of isolates in Gram negative bacilli

GRAM NEGATIVE BACILLI	E.coli	Klebsiella	Acinetobacter	Citrobacter
9	4	3	1	1



Graph 4: Types of isolates in Candida

Graph 3 shows the Organisms isolated from blood culture. Out of 41 organisms, 21 were gram positive cocci, 9 were gram negative bacilli and 8 were Candida. Table 5 and 6 shows the isolates amongst gram positive and gram negative organisms.Out of 24 isolates, 16 ioslates were Staphylococcus aureus, 3 isolates were Coagulase negative staphylococci while 3 isolates were enterococcus. Out of 9 isolates, 4 isolates were E.coli, 3 isolates were Klebsiella while 1-1 isolates were Acinetobacter and Citrobacter in each.

Graph 4 shows the isolates of candida species. Out of 8 isolates of Candida, 7 isolates were Candida albicans and only one isolate was Non- albicans Candida.

DISCUSSION

Severe sepsis remains one of the leading causes of death in children. Physical signs and symptoms, though useful in identifying possible cases, have limited specificity. Definitive diagnosis is by bacteriologic culture of blood samples to identify organisms and establish antibiotic susceptibility. Sepsis is one of the important causes of neonatal morbidity and mortality.[7] The causative organisms in neonatal sepsis vary from place to place and the frequency of the causative organisms was different in different hospitals and even in the same hospital at different time.[8,9] In our study, there was not a single case of sepsis by GBS. A possible explanation might be due to the fact that GBS has a tendency to colonize the cervix and sexual partners of infected women can harbor GBS in their urethra and possibly act as reservoirs in certain societies.[10]

A total 80 blood samples (Bactec/ Blood culture bottle) were sent for culture to the microbiology lab during the period of January 2016 to June 2016. There were 80 blood culture samples of which 41 (51.25%) were identified as a culture positive samples. Out of 80 cases studied maximum blood samples 44 (55%) were received from NICU and 36 (45%) from PICU. Blood culture positivity in our study was 51.25%, while 4.76% positivity were reported in a study Mehmood et al[11], 46% found in study by Roy et al.[12] Shaw et al[13] reported 54.64% positivity whereas in a study by Bhattacharjee et al 48% positivity were reported.[14]

A preponderance of male infants was apparent in almost all studies of sepsis in newborns.[9] In our study, culture positivity was more in male patient 48 (60%) compared to female patients were 32(40%). The male: female ratio was 3:2 while in a study by Galhotra, the male: female ratio was 2.1:1.[15]

In present study out of 80 blood samples growth were obtained in 44 samples, among them 8 isolates obtained after 48 hours of incubation, 4 isolates after 36 hours and 32 isolates after 24hrs of incubation and also 5 isolates showed growth of skin contaminants. Out of 80 samples 41(51.25%) had positive cultures and the following bacteria were found in this study- Staphylococcu aureus, Coagulase-negative staphylococci, Enterococcus, E.coli, Klebsiella, Citrobacter & Acinetobacter were isolated. Candida was isolated in 8 cases in which 7 isolates were Candida albicans and only one isolate was Candida non-albicans and which was confirmed by germ tube test.Among them 24 (30%) were Gram positive bacteria, 9(11.25%) were Gram negative bacilli. In gram-positive bacteria,Staphylococcus aureus in 16 (20%) and which was the most common organism found in this study, Coagulase negative Staphyolococci in 5 (6.25%) of cases and Enterococcus were present in 3 (3.75%) blood cultures. Incase of gram negative bacteria out of 9(11.25%) positive cultures, E.coli were obtained in 4 (5%) of cases, 3 (3.75%) Klebsiella were obtained in blood cultures & Acinetobacter, Citrobacter 1 in each.

In comparison with other study, a study by Waseem et al in 2005, out of total 100 cases, 64 were positive. Gram negative organisms were isolated from more than 80% of the cases. Escherichia coli was the commonest isolate (n=34), followed by Klebsiella (n=30) and Pseudomonas (n=13), involving both early and late onset group.[16] In a study by Dias et al[17], out of 303 cases of neonatal septicemia, 140 (46.20%) organisms were isolated. These included Klebsiella (66, 47.14%), Staphylococcus aureus (35, 25%), Coagulase negative staphylococci (CONS) (53.57%), E. coli (15, 10.71%), Proteus (5, 3.57%), Acinetobacter (3, 2.14%), Pseudomonas (6, 4.28%) and Candida (5, 3.57%). Majority of organisms isolated were resistant to commonly used antibiotics. Maximum sensitivity was seen by Cefoperazone-sulbactum (97%) & Piperacillin-Tazobactum (98%) for gram negative organisms & Vancomycin (100%) for gram positive organisms.[17] But here, we did not find any Proteus & Pseudomonas.

Various drawbacks of this study that can be put forth are, the sample size of the study was very small. Had the sample size being more the results might have varied a bit. And the study was conducted in a single hospital setting, not taking into consideration the various other hospital environments that could affect the percentage of microorganisms.

CONCLUSION

Blood stream infection is the leading cause of mortality amongst newborns and neonates. Strict asepsis measures needed to be ensured during the entire hospital stay. Gram positive microorganisms are most frequently isolated from the blood cultures majority of them being staphylococci aureus.

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