DOI: IJNMR/2014/8934.2002

Original Article

Microbiology Section

Clinico-bacteriological Profile of Neonates Born with Risk Factors of Septicemia

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ABSTRACT

Background: Septicaemia is the single most important cause of neonatal morbidity and mortality in the world, accounting for over half of the cases.

Aim: This study was conducted to establish the clinical and bacteriological profile in newborns with risk factors of septicaemia.

Subjects and Methods: One hundred and fifty consecutive neonates delivered with risk factors of septicaemia were recruited after obtaining informed consent. Blood culture was done using BACTEC PEDS PLUS/F CULTURE vials as a part of sepsis screen used to diagnose septicaemia. In addition acute phase reactants like total leucocyte count and C-reactive protein measurements were also documented.

Statistical analysis: Correlation of all risk factors with laboratory findings was obtained by using Pearson chi-square test and Fisher's exact test. p-value of less than 0.05 was considered as significant.

Results: Blood culture positivity was found in 28% neonates born with risk factors of septicaemia out of which 57.1% were gram-positive and 42.9% were gram-negative isolates respectively. *Staphylococcus aureus* being the most common among the gram-positive bacteria, was isolated in 19 (45.2%) of cases. Among the gram-negative bacteria, *Acinetobacter baumannii* was the most common being present in 13 (30.9%) of cases. Among all the risk factors studied, fever in the mother was significantly correlating with positive sepsis screen.

Conclusion: Blood culture positivity increases with increase in number of risk factors in neonatal septicaemia. Hence screening tests for septicaemia must be done routinely in such cases.

Keywords: Neonate, Risk factors, Septicaemia, C-reactive protein, Fever in mothers

INTRODUCTION

Neonates hospitalized with septicaemia in the Neonatal Intensive Care Unit (N.I.C.U.) is a global problem and a significant contributor to morbidity and death. World Health Organization (WHO) estimates about 3.3 million neonatal deaths a year, majority of them occurring in developing countries [1]. Septicaemia has been found to occur in 2.3% of intramural live births [2].

A high index of suspicion is important in the management of neonatal septicaemia because the diagnosis is hampered by vague, nonspecific or nonexistent clinical manifestation. However, it is imperative that treatment is instituted early in these cases because of the high mortality associated with neonatal septicaemia [2].

Early empiric antibiotic treatment of neonates suspected of having septicaemia is the standard practice. Nonetheless, the problem of unnecessary exposure to antibiotics in this vulnerable population remains, creating an environment for emerging bacterial resistance and the potential for poor outcomes [3]. Also definitive culture results takes at least 48-72 hours resulting in treatment delays. Hence, a battery of tests called sepsis screen which includes acute phase reactants, X-ray chest, urine examination and blood culture, is used to diagnose septicaemia early and initiate a presumptive treatment. Acute phase reactants include CRP, IL-6, micro-ESR, total leucocyte count and absolute neutrophil count. If any two of the tests in sepsis screen are positive, it is taken to be positive as per A.I.I.M.S. N.I.C.U. protocol 2008 [4]. Isolation of bacteria from a central body fluid (usually blood) is the standard and the most specific method to diagnose neonatal septicaemia [4]. Despite the fact that a majority of the cytokine markers have high negative predictive values these have not been adopted for general medical use [5], mainly because of their relatively high cost and lack of validated studies.

There is insufficient data available to correlate the risk factors of neonatal septicaemia with the clinical and bacteriological profile of these neonates. Through this study, an attempt was made to establish the clinical and bacteriological profile of newborns with risk factors of neonatal septicaemia.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Microbiology and Pediatrics (N.I.C.U level III) of a 1500-bedded government hospital in Delhi, India for 18 months. Ethical approval certificate for the study was obtained from the institutional ethical committee.

Inclusion criteria

All consecutive neonates delivered in the hospital with at least one of the following risk factors of sepsis mentioned below [4] were recruited after obtaining informed consent from the parents.

1. Preterm premature rupture of membranes
2. Amnionitis
3. Meconium stained liquor
4. Low birth weight (<2.5 kg)
5. Preterm (<37 weeks)
6. ≥3 per vaginal examinations during labour
7. Active resuscitation required in labour room
8. Dai handling
9. Fever in the mother during labour (temperature of 38ºC or more)
10. Urinary tract infection in the mother

Exclusion criteria

Babies born to mothers who had received antenatal antibiotic therapy within 48 hours prior to the delivery or had major congenital anomaly.

METHODOLOGY

Hundred and fifty consecutive neonates born with risk factors of septicaemia were studied. Of them 90 were males and 40 were females. After recruitment 1.5-2 mL venous blood sample was collected within six hours of birth using aseptic technique, 0.5-1mL of which was directly transferred into the BACTEC PEDS PLUS/F Culture Vials (Becton Dickinson Biosciences). The remaining portion was used for estimation of C-reactive protein (CRP) and total leukocyte count (TLC). All the samples were transported at room temperature as soon as possible to Microbiology laboratory for further processing.

The inoculated BACTEC vials were placed in BACTEC 9120 system. A positive result was indicated by an audible alarm and yellow illumination of the positive indicator lamp at the site of positive vial. On the computer instrument status display the station number was showed by flashing green in case of a positive vial. The bottles were incubated for five days before being reported as negative. A Gram stain and a subculture on blood agar and MacConkey agar were performed from each presumptive positive vial. After incubation at 37ºC the bacterial isolates were identified by gram staining, colony characteristics and a battery of biochemical tests, following a standard protocol [6].

Leucocyte count

Total leucocyte count (TLC) was done by automated analyser-Coulter L H 500. 5000-25000 leucocytes/µl was taken as normal.

C-reactive protein (CRP)

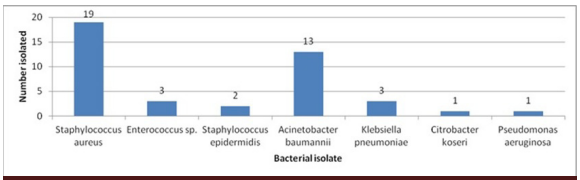
CRP latex slide test (SmarTest Diagnostics) was used for the semi-quantitative measurement of C-reactive protein in human serum. The threshold value was taken as 0.8 mg/dL.

STATISTICAL ANALYSIS

Correlation of all risk factors with laboratory findings was obtained by using Pearson chi-square test and Fisher's exact test. p-value of less than 0.05 is considered as significant.

RESULTS

[Table/Fig-1] shows the number of each bacterial species isolated from the blood samples. *Staphylococcus aureus* being the commonest was isolated in 19 of the



[Table/Fig-1]: Profile of bacterial isolates from blood samples (n=42)

cases. Among the gram-negative bacteria *Acinetobacter baumannii* was the most common being present in 13 of the cases.

The frequency of risk factors among newborns recruited for study along with blood culture positivity is shown in [Table/Fig-2]. Low birth weight was the most evident risk factor present among the neonates (87%). *Staphylococcus aureus* was the commonest isolate recovered from the septicemic neonates (45.2%) followed by *Acinetobacter baumannii* (31%), *Enterococcus* sp. (7.1%), *Klebsiella pneumoniae* (7.1%), *Staphylococcus epidermidis* (4.8%), *Citrobacter koseri* (2.4%) and *Pseudomonas aeruginosa* (2.4%). *Staphylococcus aureus* was the most common isolate in neonates born with maternal fever during labour. Only *Acinetobacter baumannii* could be isolated in neonates with history of dai handling and amnionitis. *Staphylococcus aureus* was the only isolate in neonates born to mothers with preterm premature rupture of membranes. It was also the commonest isolate in neonates born with meconium-stained liquor, preterm and low birth weight neonates. *Acinetobacter baumannii* was isolated in neonates in whom active resuscitation was done.

The blood culture positivity related to the number of risk factors present is shown in [Table/Fig-3]. Maximum numbers of neonates were born with two 79 (52.7%) risk factors followed by three (29.3%) and one (18%) risk factors. The blood culture positivity increased with rising number of risk factors. It was 45.5%, 26.6% and 3.7% with ≥ 3, 2 and 1 risk factors respectively.

The CRP and TLC values among the blood culture positive and negative samples are compared in [Table/

Fig-4]. Of 42 positive blood cultures, 15 (35.7%) had CRP more than 0.8 mg/dL, whereas amongst the 108 blood culture negative neonates 20 (18.5%) had CRP more than 0.8 mg/dL. Significant association was present between CRP and blood culture result as shown by the p-value. Abnormal leucocyte counts were seen in 2 (4.8%) of 42 positive blood cultures, whereas in all the blood culture negative neonates, leucocyte count was normal. Insignificant association was found between leucocyte count and blood culture results as shown by the p-value.

Out of the 42 culture positive neonates, 25 (59.5%) died within seven days, of whom 16 (64%) were males and 9 (36%) were females. There was no mortality amongst the culture negative neonates.

Mean birth weight of the neonates who died was 1.624±0.568 SD kg, and their mean gestational age was 30.12±0.544 weeks.

DISCUSSION

The bacterial species responsible for sepsis vary by geographical location. In [Table/Fig-5] recent studies on

S. No.	Frequency of Risk factors	Blood culture positivity	Isolates	p-value*
1	Fever in the mother during labour (n=22)	15	*Staphylococcus aureus* n=7 *Acinetobacter baumannii* n=6 *Enterococcus* sp. n=2	0.000
2	UTI in the mother (n=1)	1	*Staphylococcus epidermidis* n=1	0.28
3	Dai-handling (n=2)	1	*Acinetobacter baumannii* n=1	0.483
4	Amnionitis (n=2)	1	*Acinetobacter baumannii* n=1	0.483
5	Preterm premature rupture of membranes (n=5)	3	*Staphylococcus aureus* n=3	0.134
6	Meconium-stained liquor (n=38)	7	*Staphylococcus aureus* n=4, *Acinetobacter baumannii* n=2, *Staphylococcus epidermidis* n=1	0.128
7	Preterm (n=108)	35	*Staphylococcus aureus* n=16, *Acinetobacter baumannii* n=9, *Enterococcus* sp. n=3, *Klebsiella pneumoniae* n=3, *Staphylococcus epidermidis* n=2 *Citrobacter koseri* n=1, *Pseudomonas aeruginosa* n=1	0.084
8	Low birth weight (n=131)	40	*Staphylococcus aureus* n=18, *Acinetobacter baumannii* n=12, *Enterococcus* sp. n=3, *Klebsiella pneumoniae* n=3, *Staphylococcus epidermidis* n=2 *Citrobacter koseri* n=1, *Pseudomonas aeruginosa* n=1	0.069
9	Active resuscitation given (n=9)	1	*Acinetobacter baumannii* n=1	0.446

[Table/Fig-2]: Correlation of risk factors of septicaemia with blood culture positivity. *p-value of less than 0.05 is considered as significant

Number of risk factors present	Number of neonates n(%)	Number of positive blood cultures n(%)	Number of negative blood cultures n(%)
1	27(18%)	1(3.7%)	26(96.3%)
2	79(52.7%)	21(26.6%)	58(73.4%)
≥3	44(29.3%)	20(45.5%)	24(54.5%)
Total	150	42	108

[Table/Fig-3]: Blood culture positivity according to the number of risk factors present (n=150)

	CRP				TLC		
	<0.8 mg/dl (normal) n(%)	0.8-1.5 mg/dl (abnormal) n(%)	1.6-3.1 mg/dl (abnormal) n(%)	≥3.2 mg/dl (abnormal) n(%)	(Abnormal) <5000/µL	(Normal) 5000-25000/µl	(Abnormal) >25000/µL
Blood culture positive (n=42)	27 (64.3%)	1 (2.3%)	7 (16.7%)	7 (16.7%)	1 (2.4%)	40	1 (2.4%)
Blood culture negative (n=108)	88 (81.5%)	20 (18.5%)	0	0	0	108 (100%)	0
p-value*	0.025	0.011	0	0	p-value=0.077		

[Table/Fig-4]: Correlation of C-reactive protein and total leucocyte count with blood culture positivity.
*P value of less than 0.05 is considered as significant

Study	% of isolation of gram-positive bacteria	% of isolation of gram-negative bacteria	Commonest bacterial isolate (%)
Khanal et al., [8]	74	26	*Staphylococcus aureus* (60)
Muhammad et al., [9]	45.4	54.6	*Staphylococcus aureus* (27)
Agnihotri et al., [10]	41.5	58.5	*Staphylococcus aureus* (35)
Chacko et al.,[16]	20	80	*Pseudomonas sp.* (60)
Pereira et al., [17]	59.8	33.3	*Coagulase negative Staphylococcus* (35.5%)
Kayange et al., [18]	38.9	61.1	*Klebsiella pneumoniae* (33.6%)
Kumhar et al.,[19]	37.5	60	*Klebsiella* sp.(33.8%)
Sriram R. [20]	41.4	56.9	*Klebsiella pneumoniae* (43.1%)

[Table/Fig-5]: Bacteriological profile of neonatal septicaemia obtained in various studies

causes of neonatal septicaemia have been elaborated along with their findings. In the United States Group B *Streptococcus* is the leading cause of neonatal septicaemia [7]. However according to the recent (2002-2003) national neonatal perinatal database in India *Klebsiella* sp. is the commonest bacteria causing neonatal septicaemia, followed by *Staphylococcus aureus* [2]. In the present study *Staphylococcus aureus*, being present in 19(45.2%) of the positive cases, was the most common isolate. The difference probably reflects variations in population characteristics and in predisposing factors. In this study Gram-positive bacteria were isolated in 24(57.1%) of neonates whereas gram negative bacteria were isolated in 18(42.9%) of neonates. In a study done by Khanal B et al., [8] blood samples from 1567 neonates with suspected septicaemia were cultured. Gram-positive bacteria (74%) were more common than gram-negative bacteria (26%), *Staphylococcus aureus* (60%) being the most common bacterial isolate. The results in this study were comparable to the present study as gram-positive bacteria were more commonly responsible for neonatal septicaemia than gram-negative bacteria, with *Staphylococcus aureus* as the most common bacterial isolate. Our findings are corraborated by two more studies on neonatal septicaemia conducted by Muhammad Z et al., [9] and Agnihotri N et al., [10] respectively. In both studies *Staphylococcus aureus* was the commonest isolate being present in 27% cases in the former and 35% in the latter.

Among the gram-negative bacteria obtained in this study, *Acinetobacter baumannii* was the commonest isolate. *Acinetobacter* species are gaining importance as a potential pathogen in neonatal septicaemia because of frequent isolation in the recent years [11]. In this study *Acinetobacter baumannii* was isolated in 30.9% of the total septicaemia cases, which is in tune with the incidence of *Acinetobacter* sepsis reported from India (6.5 to 31.5%) [11]. *Acinetobacter* septicaemia is common in babies with intravenous catheterization and artificial ventilation [11,12]. The most important neonatal factor predisposing to infection is prematurity and/or low birth weight [11,13], since they often require prolonged intravenous access, endotracheal intubation or other invasive procedures that provide a portal of entry for infection. In this study, *Acinetobacter baumannii* was isolated from majority of the premature and low-birth weight neonates suffering from gram-negative septicaemia [Table/Fig-2]. *Acinetobacter baumannii* was also the only isolate obtained from neonates that had undergone active resuscitation [Table/Fig-2]. A recent study from Pakistan has reported that prematurity and LBW are associated with increasing risk of *Acinetobacter* infection in neonates [14]. Also, use of both central venous catheter and mechanical ventilation were identified as significant risk factors for neonatal *Acinetobacter baumanii* septicaemia in a Brazilian study [15].

Blood culture positivity in our study was related to the number of risk factors present. In the presence of a single risk factor, blood culture positivity was present in 3.7% cases. In the presence of two risk factors, blood culture positivity increased by more than seven times to 26.6%, while in the presence of ≥3 risk factors, blood culture positivity increased by more than 12 times to 45.5%. It may be concluded that as the number of risk factors increase, risk of septicaemia in the neonate also increases. Hence, screening tests for septicaemia must be done routinely in such cases. In the present study, among all the maternal and neonatal risk factors, only maternal fever during labour had a statistically significant association with septicaemia (p-value 0.000) [Table/Fig-2]. Maternal fever during labour is defined as a temperature of 38ºC or more. Maternal temperature elevation during labour can be a nonspecific indicator of maternal and/or fetal infection. Much of the fever during labour might not be so much a result of infection, but rather a consequence of the use of epidural analgesia. However, some evidence suggests that epidural analgesia is associated with fever during delivery only in the presence of placental inflammation [21]. Similar findings were also present in a study done by Nili F et al., [22] where sepsis was present in all (100%) cases with fever in the mother during labour as the risk factor which was a significant finding (p-value =0.000),and in a retrospective cohort analysis done in the United States for the period 1995-1997 [21], wherein strong association (p-value <0.05) was found between intrapartum fever and neonatal sepsis and mortality.

In this study significant association was found between CRP and positive blood culture results (p-value 0.025 [Table/Fig-3]. Similar results were obtained in a study by Caldas JPS et al., [23] where elevated CRP levels were associated with culture-positive cases (p-value<0.05).

This indicates the importance of CRP as a significant marker of septicaemia. No significant association was found between leucocyte counts and neonatal septicaemia in this study. Similar result was obtained in a study done in Philippines by Mayuga WAB et al., [24]. In a study by Ottolini MC et al., [25] it was found that total leukocyte counts are of limited value in the diagnosis of septicaemia in newborns. Total leukocyte counts are particularly unreliable indicator of infection during the first several hours of early-onset (within 48h of birth) sepsis because these are normal at the time of initial evaluation in more than one third of infants with proven bacteremia. Conversely, among neonates evaluated for suspected sepsis, far less than half of neonates with reduced (<5000 cells/mm3) or elevated (>20,000 cells/mm3) cell counts are ultimately identified to be infected [25].

Gram-negative (GN) bacteria have often been implicated in the pathogenesis of severe sepsis and septic shock, although the exact mechanism is uncertain [26].There is evidence to support two different theories on how GN bacteria induce harmful systemic responses. The intravascular stimulus hypothesis states that bacteria invade through a normal or damaged epithelium and enter the bloodstream, inducing systemic inflammatory responses (for example, increased vascular permeability, leukocyte–endothelial adhesion, and activation of complement and clotting pathways) resulting in multiorgan failure. A second theory suggests that the multiorgan dysfunction and shock result from neuroendocrine dysregulation and mediators released into the bloodstream from the infected tissues; circulating bacteria or endotoxin are not needed as direct stimuli for intravascular inflammation [27]. In the present study gram-negative bacteria, being present in 60% of the non-survivors outnumbered the gram-positive bacteria that were present in 40% of the non-survivors, Similar result was seen in the study by Trotman et al., [28] study where gram-negative bacteria were present in 67% of the non-survivors and gram-positive bacteria were present in 33% of the non-survivors.

CONCLUSION

The findings of the study suggest that neonatal septicaemia can be diagnosed by blood culture and also there is significant association of risk factors of septicaemia. Hence irrespective of appearance of clinical features of septicaemia, sepsis screening is warranted in the presence of risk factors of the same. In addition, knowledge of the likely causative organisms would contribute towards a more rational and appropriate use of antibiotics, thus minimizing the irrational use and emergence of multidrug resistant bacteria in neonatal units.

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FINANCIAL OR OTHER COMPETING INTERESTS:
None.

Date of Publishing: **Jul 30, 2014**