

Early Onset of Neonatal Sepsis: Analysis of the Risk Factors and the Bacterial Isolates by Using the BacT Alert System

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ABSTRACT

Objective: To evaluate the risk factors and the bacterial isolates of early onset of neonatal sepsis and to study their resistance patterns.

Methods: Neonates who were suspected of early onset sepsis (EOS) based on the presence of risk factors, and/or clinical features that were consistent with bacterial infections, with the positivity of two or more sepsis screen parameters during the first 72 hours of life were studied prospectively. The demographic data, risk factors, clinical features, haematological screening parameters and CRP were studied. Bacterial isolates from a single blood culture and their resistance patterns were analyzed by using the BacT alert system. .

Results: A total of 52 neonates with early onset sepsis were studied. The male: female ratio was 1.36: 1. The mean (SD) birth weight and gestation were 2169 ± 878 g and 35.9 ± 3.6 weeks

respectively, 19 were inborn. The estimated incidence of EOS among the inborn neonates was 11 per 1000 live births. One or more risk factors were present in 34.6 % of the cases. A significantly higher incidence of EOS among the premature (OR, 15.8) and low birth weight (OR, 10.2) neonates was observed. Two or more sepsis screen parameters were positive in 67% cases. The blood culture yielded bacterial growth in 28.8 % cases. The common organisms which were isolated were Klebsiella, followed by Pseudomonas and MRSA. A high resistance to the commonly used antibiotics like ampicillin and gentamicin was observed. The case fatality rate was 19.2 %.

Conclusions: The premature and low birth weight neonates were at an increased risk of developing EOS. Gram negative organisms, especially Klebsiella and Pseudomonas remained as common pathogens. A high resistance of the blood culture isolates to the commonly used antibiotics was observed.

Key Words: Early onset sepsis, Neonates, Blood culture, Antibiotics

INTRODUCTION

Early onset bacterial sepsis (EOS) remains a major cause for neonatal morbidity and mortality [1-5]. Apart from the obstetric risk factors, prematurity and low birth weight are associated with increased bacterial infection rates [6,7]. The incidence of the sepsis, the bacterial profile and the resistance pattern vary from a developed country to a developing country and from nursery to nursery. An overall incidence of the culture proven sepsis of 1-8 cases per 1000 live births from the developed countries and 4.8-20.7 per 1000 live births from India has been reported. The common aetiological agents include Group B Streptococcus (GBS) and Escherichia coli (E.coli) following the preventive strategies for GBS in the developed countries [1, 2, 6] and Klebsiella pneumoniae and Staphylococcus in India [5]. Another Indian study identified E.coli as the most common causative bacteria [8]. Recently, BacT/Alert microbial detection systems are being used for blood cultures [9]. Such systems have also reported a better yield and an early detection of microorganisms.

The case fatality rate in early onset neonatal sepsis ranges from 16.7% to 19.4% [2, 4, 8]. Early and appropriate antibiotic therapy is likely to improve the outcome. The knowledge of the sensitivity and the resistance pattern of the microorganisms would help in choosing the empirical therapy. We aimed to evaluate the risk factors which were associated with EOS and the bacterial profile by using the BacT alert system and their resistance patterns.

METHODS

This prospective study was carried out at a neonatal nursery which was attached to a teaching hospital, between July 2006 and December 2007. The study included neonates who were suspected of having sepsis, based on the presence of risk factors and/or clinical features that were consistent with bacterial infections, with the positivity of two or more sepsis screen parameters during the first 72 hours of life. The entire inborn population was scrutinized for EOS during the study period. The risk factors such as prematurity, low birth weight, maternal fever, chorioamnionitis, foul smelling liquor, prolonged rupture of membranes (PROM) and maternal urinary tract infection (UTI) were collected. We did not study vaginal colonization. Obstetric data was collected from the obstetric case sheets and/or by interviewing the mothers. The presence of meconium stained amniotic fluid (MSAF) and perinatal asphyxia were noted in the neonates. The total leucocyte count, the platelet count, the C-reactive protein (CRP) and the band: neutrophil (B: N) ratio were obtained in all the newborns. A single blood sample was collected by taking sterile precautions (0.5 ml) and inoculated into the culture bottle. The BacT alert microbial detection system (bioMerieux, Inc., Durham, North Carolina, USA) was used for the blood culture.

A leucocyte count (TLC) of $<5000/\text{cumm}$ or $>20000/\text{cmm}$ [10], an absolute neutrophil count of $<1750/\text{cumm}$, a B: N ratio of ≥ 0.2 [11], a platelet count of $<150000/\text{cmm}$, and a CRP value of ≥ 12 mg/L

were considered as suggestive of sepsis. A chest X-ray was obtained on indication. CSF analysis was carried out whenever meningitis was suspected or when the blood culture grew microorganisms. The clinical features, the risk factors, the blood culture isolates and their sensitivity patterns were documented and analyzed. The data was analyzed by using the SPSS data editor and the proportions were analyzed by using the Fisher's exact test or the Chi square test.

RESULTS

A total of 52 neonates with early onset sepsis were studied. The male: female ratio was 1.36:1. The mean (SD) birth weight and gestation were 2169 ± 878 g and 35.9 ± 3.6 weeks respectively. The characteristics of the study neonates are shown in [Table/Fig-1]. Of the total 52 neonates, 19 were inborn. The estimated incidence of EOS among the inborn neonates was 11 per 1000 live births. Among the inborn neonates with EOS, 73.6 % had low birth weight and 46.2% were preterm.

One or more risk factors were present in 18 (34.6 %) cases [Table/Fig-2]. Seven neonates had both MSAF and perinatal asphyxia as the risk factors. There were no cases with maternal fever or foul smelling liquor. Among the inborn, 13 out of 228 preterm neonates and 6 out of 1577 term neonates had EOS. Of 398 low birth weight neonates, 14 and of 1407 normal birth weight neonates, 5 had EOS. The odds ratio for prematurity and low birth weight for early sepsis were 15.8 (5.95-42.09. $p < 0.05$) and 10.2 (3.65-28.55; $p < 0.05$) respectively.

The most common clinical feature which was observed was lethargy (80.7%), followed by respiratory distress (57.6%) and feed intolerance (53.8%). Temperature instability was present in 34.5% of the neonates. CSF analysis was done in 16 cases and 3 had meningitis (5.8%). Among them, the blood culture grew microorganisms in all and CSF grew microorganisms in one.

Characteristics	n	%
Sex		
Male	30	57.7
Female	22	42.3
Mode of delivery		
Vaginal	22	42.3
Vaginal assisted	2	3.8
Emergency caesarian section	24	46.2
Elective caesarian section	4	7.7
Gestation		
Term AGA	21	40.4
Term SGA	7	13.5
Preterm AGA	19	36.5
Preterm SGA	5	9.6

[Table/Fig-1]: General characteristics (n=52)

AGA, appropriate for gestational age; SGA, small for gestational age.

Risk factors	n
Prolonged rupture of membranes > 18 hours	4
Meconium stained amniotic fluid	8
Maternal UTI	2
Perinatal asphyxia	12

[Table/Fig-2]: Obstetrics risk factors (n=18)

UTI, urinary tract infection.

Organisms	n
Klebsiella	4
Pseudomonas	3
CONS	2
MRSA	3
Enterobacter	1
Acinetobacter	1
Streptococci	1

[Table/Fig-3]: Bacterial isolates (n=15)

CONS = Coagulase negative Staphylococcus aureus, MRSA = Methicillin resistant staphylococcus aureus.

An abnormal total leucocyte count (TLC) was seen in 36.4% neonates; it was low in 5.7% and high in 30.7% neonates. All the 3(5.7%) cases with a TLC of less than $5000/\text{mm}^3$ also had a low ANC value of $< 1750/\text{mm}^3$. An elevated CRP value was observed in 69 % neonates and the band: neutrophil ratio was ≥ 0.2 in 53%. A low platelet count was seen in 52% neonates. Two or more sepsis screen parameters were positive in 35(67%) cases.

The blood culture yield in the current study was 28.8 % (15/52). The common organisms which were isolated were Klebsiella, followed by Pseudomonas and MRSA. The antibiotic susceptibility pattern is shown in [Table/Fig-4]. Klebsiella was sensitive to the extended spectrum penicillins like piperacillin and the carbapenems. Pseudomonas was sensitive to most of the third generation cephalosporins. CONS and Streptococci were sensitive to the first line antibiotics like ampicillin, while MRSA was sensitive to amikacin, ciprofloxacin, linezolid and vancomycin. The case fatality rate in the present study was 19.2 % (10/52). Co morbid conditions were present in 25 cases; 12 had hyaline membrane disease, 2 had meconium aspiration syndrome, 2 had congenital diaphragmatic hernia and 12 had perinatal asphyxia.

DISCUSSION

The incidence of EOS varies widely. Western studies have reported 1-8 EOS cases per 1000 live births [1, 2] Indian studies have reported 4.8 to 20.7 EOS per 1000 live births [3-5, 8]. In the present study, out of 1805 inborn neonates, 19(1.1%) had early onset sepsis with an estimated incidence of 11 per 1000 live births. A slight male preponderance in the present study agreed with the findings of earlier studies [6, 8].

Obstetric risk factors were present in 34.6 % of the cases in the present study. Similar observations were reported in a previous study [8]. A much higher obstetric risk factor association of 77.8% was reported by Betty and Sohi [4]. Schuchat et al [6] reported an obstetric risk factor-preterm association in 49% GBS and in 79% of other EOS species. PROM, meconium stained amniotic fluid, maternal UTI and perinatal asphyxia were associated with EOS. A significant association of these factors with EOS and a negligible risk of EOS in the absence of these factors have been reported earlier [4]. We observed significantly higher incidences of EOS among the premature (OR, 15.8) and low birth weight (OR, 10.2) neonates. In a study which involved 1743 live births, Betty and Sohi reported significantly higher incidences of EOS among the low birth weight and preterm infants [4]. In their series of 65 infants with EOS, 83.3% were low birth weight and 80.6% were preterm. Soman et al also observed that 83% of those with sepsis were low birth weight neonates [12]. Bizzarro et al [13] reported that 43% of the mothers of the EOS group had preterm labour.

	Pseudomonas (n=3)	Klebsiella (n=4)	CONS (n=2)	MRSA (n=3)	Enterobacter (n=1)	Acinetobacter (n=1)	Beta hemolytic streptococci (n=1)
Amikacin	3	2	0	0	1	1	NT
Augmentin	3	1	0	3	1	1	0
Ampicillin	3	4	0	3	1	1	0
Cefotaxime	0	4	0	3	1	1	0
Ceftriaxone	NT	NT	NT	NT	1	1	0
Ceftazidime	0	4	NT	NT	1	1	NT
Cefuroxime	3	NT	NT	NT	1	1	NT
Cefaperazone	0	NT	NT	NT	0	0	NT
Ciprofloxacin	1	3	0	0	NT	1	NT
Cotrimoxazole	2	3	1	0	0	1	1
Cloxacillin	NT	NT	0	3	NT	NT	NT
Erythromycin	NT	NT	0	3	NT	NT	0
Gentamycin	3	3	0	1	1	1	1
Netilmycin	3	2	0	NT	NT	0	NT
Piperacillin	0	4	NT	0	0	1	NT
Imepenam	0	NT	NT	0	0	NT	NT
Linezolid	NT	NT	0	0	NT	NT	NT
Vancomycin	NT	NT	1	0	0	0	NT

[Table/Fig-4]: Antibiotic resistance pattern of bacterial isolates.

CONS-coagulase negative Staphylococcus; MRSA-methicillin resistance staphylococcus aureus; NT-not tested. The number in the table represents resistant strains.

The National Neonatal Perinatal Database (NNPD) reports [5] of 2002-2003 and few other studies [2-4] identified respiratory distress as the commonest presentation of early onset sepsis. Respiratory distress and hyperbilirubinaemia were the common presenting features in a study which was done by Doud et al [14]. We observed that lethargy, respiratory distress and feed intolerance were the common manifestations. Meningitis was observed in 5.8% neonates. A higher rate of 8.3% was reported by Betty and Sohi [4]. Two or more sepsis screen parameters were positive in 67% neonates in the present study. Sharma et al [15] and Varsha et al [16] reported two or more tests to be positive in 80% of the neonatal septicaemia cases.

The blood culture yield by using the BacT/alert system in the current study was 28.8 %. This was similar to the 26% which was reported by Ahmed [17] et al. A lower rate of about 20% yield was suggested by Baltimore et al [2], Gladstone et al [18] and Sharma et al [15]. d Haens et al, [19] by using the BacT system, also got a yield of 20%. Much higher culture positive rates of 51%, as observed by Karthikeyan and Prem Kumar [20], and of 64%, as observed by Tallur et al [21], were reported from India.

In the west, Group B streptococcus is the microorganism which is most frequently isolated in EOS. Schuchat et al [6] reported GBS (1.4/1000 birth) and *E.coli* (0.6/1000) as the most common aetiological agents of EOS. Bizzaro et al [13] also reported these two organisms as common for EOS in their review of 75 years of neonatal sepsis. Hyde et al [22] also reported these two as the common organisms. The current study isolated Klebsiella mainly, followed by Pseudomonas and MRSA. This finding agreed with the reports of Sharma et al [15]. NNPD 2003 [5] reported Klebsiella pneumoniae and Staphylococcus as the most frequent causative organisms for neonatal sepsis in India. In contrast, Kuruvilla et al [8] reported *E.coli* as the most common cause.

Klebsiella was sensitive to piperacillin and the carbapenems. Pseudomonas was sensitive to ceftazidime. CONS and Streptococci were sensitive to the first line antibiotics like ampicillin, while MRSA was sensitive to amikacin, ciprofloxacin, linezolid and vancomycin. These organisms were resistant to the commonly used antibiotics like ampicillin and gentamicin. Thaver et al [24] reported the resistance of all the Klebsiella species to ampicillin in their study. An increasing ampicillin resistance among *E.coli* of EOS has been reported by Hide et al [22], especially in prematurely born infants. Ganatra et al [25] discussed a similar growing antibiotic resistance of neonatal pathogens in developing countries in their review on an international perspective on early onset neonatal sepsis.

The case fatality rate in the present study was 19.3 %, which was closer to 19.4%, as reported by Betty et al [4]. A lower mortality rate of 10.2 % was reported by Bizzaro et al [13]. A higher mortality rate of 37% was reported by Tallur et al [21] from India. Out of 10 deaths, 4 cases had positive blood cultures.

CONCLUSION

The EOS incidence varied widely. The prematurity and low birth weight neonates were particularly at a higher risk. The obstetric risk factors contributed significantly to the EOS. The culture yield which was obtained by using the BacT system was about 29%. Gram negative organisms, especially Klebsiella and Pseudomonas predominated. Gram positive organisms were responsible for at least a third of the cases. Pseudomonas remained sensitive to ceftazidime. The resistance of pathogens to the commonly used antibiotics remained high.

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