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Neonatal sepsis: Aetiological agents and risk factors

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Abstract:

INTRODUCTION: India accounts for 30% of neonatal deaths globally. Bacterial sepsis is a major cause of morbidity and mortality in newborns. Prompt detection of microorganisms and early institution of therapy are of paramount importance.

MATERIALS AND METHODS: The study was conducted in the Department of Microbiology over a period of one year. Two samples of blood were collected under aseptic precautions, 1 ml each was added to conventional blood culture bottle with biphasic media and paediatric BacT/ALERT bottle. Microorganisms were identified by Gram staining, standard biochemical tests and appropriate antibiograms. The common microorganisms responsible for early- and late-onset neonatal sepsis were identified, and the resistant strains were studied in detail. The main clinical presentations and maternal and neonatal risk factors associated with neonatal sepsis were identified and statistically correlated.

RESULTS: Of the 233 newborns, 44 (18.9%) were culture positive, with higher incidence of sepsis in low birthweight male babies. Of the 44 isolates, 31 (70.5%) were Gram-negative organisms, with *Klebsiella pneumoniae* subspecies *pneumoniae* (45.5%) being the most common isolate. The prevalence of extended-spectrum beta-lactamase production in this study was 54.8%. Screening for AmpC production showed that 25.8% of the isolates were positive. The maternal risk factor of premature rupture of membrane of more than 18 h was seen in the case of 74 babies (31.8%), and 138 (59.2%) babies had prematurity as neonatal risk factor leading to sepsis.

CONCLUSION: As the aetiological agents in neonatal sepsis vary in different circumstances and antimicrobial resistance due to different mechanisms is prevalent, antibiotic usage should be based on culture and sensitivity results.

Keywords:

Blood culture, neonate, sepsis

Introduction

Bacterial sepsis is a major cause of morbidity and mortality in newborns, especially among low birthweight and preterm babies. India accounts for 30% of the neonatal deaths globally. In India, the neonatal mortality rate is 37/1000 live births, and the incidence of neonatal sepsis is around 3.8%.[1]

Microorganisms present in blood are a serious threat to every organ in the body, which can have serious consequences including shock, multiple organ failure, disseminated intravascular coagulation and death. Improvement in neonatal intensive care decreased the morbidity and mortality of early-onset sepsis (EOS) in term infants. However, preterm babies remain at higher risk for both EOS and its sequelae. They are also at risk of late-onset sepsis (LOS). Neonatal survivors of sepsis can have severe neurological sequelae due to central nervous infection as well as from secondary hypoxaemia resulting from septic shock, persistent pulmonary hypertension of newborn and severe parenchymal lung disease.[1]

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In the Western countries, EOS is mostly caused by Group B Streptococcus (GBS) and *Escherichia coli*, whereas in India, most cases are due to Gram-negative organisms, especially Klebsiella, *E. coli and* Enterobacter. In the West, half of the cases of LOS are caused by coagulase-negative staphylococci (CoNS), 22% are caused by other Gram-positive organisms such as *Staphylococcus aureus*, Enterococcus and GBS and 18% by Gram-negative organisms such as Klebsiella, *E. coli* and *Pseudomonas aeruginosa*. In India, about two-third cases of LOS are caused by Gram-negative organisms such as Klebsiella, *E. coli*, *P. aeruginosa* and Proteus spp. *Salmonella typhimurium* has also been reported. The rest are contributed by Gram-positive organisms including *S. aureus* and *Staphylococcus epidermidis*.[2]

Mortality rates associated with neonatal sepsis may be as high as 50% in untreated babies. As bacteraemia frequently results in life-threatening infection, prompt detection and recovery of microorganisms from blood are of paramount importance. Antimicrobial therapy for a baby with suspected sepsis depends on the predominant pathogen and antibiotic sensitivity pattern of a given region. This study was conducted for the early identification of culture positive cases, initiation of appropriate antibiotics at the earliest, thereby reducing the morbidity and mortality.

Materials and Methods

We conducted a prospective study in the Department of Microbiology, Government Medical College, Thiruvananthapuram, and Department of Paediatrics, Sree Avittom Thirunal Hospital, Thiruvananthapuram. The study was conducted during a period of one year from March 2012 to February 2013. Features of neonatal sepsis used for selection of cases included alteration in the established feeding behaviour which is a common and early feature of sepsis, but non-specific. Other features were hypothermia/fever, lethargy, poor cry, hypotonia or absent reflexes, bradycardia or tachycardia, respiratory distress, hypoglycaemia or hyperglycaemia, metabolic acidosis and sclerema.[1] Neonates already started on antibiotics were excluded from the study.

EOS manifests within first three days of life, presents as respiratory distress and pneumonia, and in severe cases, the foetus may be symptomatic *in utero*. LOS presents after three days of age, and neonates usually present with septicaemia or meningitis.

Blood samples were collected from these patients under aseptic precautions. The babies were closely followed up. A pro forma was filled up regarding clinical details, investigations, treatment given and outcome. Two samples of blood (1 ml each) were collected under aseptic precautions. One millilitre of each blood sample was added to conventional paediatric blood culture bottle with biphasic media and paediatric BacT/ALERT bottle. Samples were collected at admission, before the start of antibiotics. Media used for blood culture was a biphasic medium with brain-heart infusion (BHI) broth as the liquid phase and BHI agar as the solid phase.

Biphasic medium was incubated at 37°C. Inversion was done once or twice daily. Agar was observed for any visible growth. If any growth appeared, it was identified by Gram staining; standard biochemical tests and appropriate antibiotic sensitivity tests were done. The results were immediately informed to the clinician. If no growth appeared even after six days, a terminal subculture was done in blood agar and MacConkey agar. If there was no growth in the subculture plates, result was given as culture sterile after six days of incubation.

When the BacT/ALERT machine gave positive signal, Gram stain was done from positive flagged bottle and subculture was done in blood agar and MacConkey agar. The growth was identified by Gram staining; biochemical tests and appropriate antibiotic sensitivity were done.

Antibiotic susceptibility testing of Gram-negative isolates was done on Mueller-Hinton agar plates by the Clinical and Laboratory Standards Institute (CLSI)-recommended Kirby-Bauer disc diffusion method with discs of Ampicillin (10 µg), Gentamicin (10 µg), first-generation Cephalosporin (30 µg), Amikacin (30 µg), third-generation Cephalosporin (30 µg), Co-trimoxazole (1.25/23.75 µg), Ciprofloxacin (5 µg), Cefoperazone-Sulbactam (75 + 30 µg), Piperacillin-Tazobactam (100 + 10 µg) and Meropenem (10 µg).[3,4] The isolates were tested for extended-spectrum beta-lactamase (ESBL) production using double disc approximation test and CLSI phenotypic confirmatory test and also screened for AmpC production using Cefoxitin disc (30 µg). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as the negative and positive controls.

Antibiotic sensitivity tests of staphylococcal isolates were done in Mueller-Hinton agar plates by Kirby-Bauer disc diffusion method. Antibiotics used were Penicillin (10 IU), Gentamicin (10 µg), Erythromycin (15 µg), Cefoxitin (30 µg), first-generation Cephalosporin (30 µg), Amikacin (30 µg), Rifampicin (5 µg), Clindamycin (2 µg), Linezolid (30 µg) and Novobiocin (5 µg) for CoNS. The inocula were standardised by comparing with 0.1 McFarland's opacity standard. The control strain used was *S. aureus* ATCC 25923. The results were interpreted according to the CLSI guidelines.[3,4]

The common microorganisms responsible for early- and late-onset neonatal sepsis were identified, and the resistant strains were studied in detail. Main clinical presentations were analysed. Maternal and neonatal risk factors associated with neonatal sepsis were identified and statistically correlated.

Statistical analysis

Statistical analysis was done with the help of GraphPad Software, Inc. CA USA. Culture positivity rate, male: female ratio, distribution according to onset of disease, most common organisms causing neonatal sepsis, antibiotic sensitivity pattern, prevalence of ESBL and AmpC production were determined. Correlation of culture positivity with maternal risk factors, neonatal risk factors, main clinical presentations and clinical outcome was analysed. *P* values for level of significance were estimated using Fisher's exact test and Chi-square test depending on the circumstance.

Results

Of the 233 newborns with clinical signs of sepsis included in the study, 135 (57.9%) were male and 98 (42.1%) were female. Blood culture was positive in 44 (18.9%) newborns and 189 (81.1%) were culture negative. Of the culture-positive neonates, 28 (63.6%) were male whereas 16 (36.4%) were female. There is a slightly higher incidence of sepsis among males. The male: female ratio was 1.38:1 in the study group, while in culture positive cases, the ratio was 1.75:1.1. Of the total, 167 (71.7%) cases occurred during the first 72 h of life (EOS) whereas 66 (28.3%) occurred after 72 h (LOS). Among the culture-positive cases, 36 (81.8%) were EOS and eight (18.2%) were LOS [Table 1].

Of the 44 isolates, 31 (70.5%) were Gram-negative organisms, and Gram-positive organisms accounted for 13 (29.5%) cases. *Klebsiella pneumoniae* subspecies *pneumoniae* was the most common organism isolated (45.5%) in both EOS -16 isolates (44.4%) and LOS -4 isolates (50%) [Table 2]. Tables 3 and 4 show the antibiotic sensitivity pattern of Gram-positive and Gram-negative isolates, respectively. Among *K. pneumoniae*, 12 (60%) were ESBL producers whereas 8 (40%) were non-ESBL. Out of the seven *E coli*, 4(57.1%) were ESBL producers. The total prevalence of ESBL production in this study was 54.8%. Among the 31 Gram-negative isolates, 8 (25.8%) were positive for AmpC production. The AmpC production in *1umoniae* was 25%, *E. coli*, 28.6% and among *A. baumannii*, it was 50%. The mortality rate was highest among babies with sepsis due to *P. aeruginosa* and *A. baumannii* (50%) followed by babies with sepsis due to *K. pneumoniae* [Table 5].

Table 1: Distribution according to onset of disease

Onset of disease	Culture positive, *n* (%)	Culture sterile, *n* (%)	Total, *n* (%)
<72 h	36 (81.8)	131 (69.3)	167 (71.7)
>72 h	8 (18.2)	58 (30.7)	66 (28.3)
Total	44 (100)	189 (100)	233 (100)

Table 2: Distribution of isolates according to onset of disease

Organism	EOS, *n* (%)	LOS, *n* (%)
Klebsiella pneumoniae	16 (44.4)	4 (50)
Staphylococcus aureus	10 (27.7)	2 (25)
Escherichia coli	6 (16.6)	1 (12.5)
Pseudomonas aeruginosa	2 (5.5)	0
Acinetobacter baumannii	1 (2.7)	1 (12.5)
Staphylococcus epidermidis	1 (2.7)	0
Total	36 (100)	8 (100)

EOS: Early-onset sepsis; LOS: Late-onset sepsis

A comparison was made between culture positive and negative babies with clinical sepsis. The incidence of culture-positive neonatal sepsis is more among low birthweight babies, and the incidence is inversely proportional to the birthweight [Table 6]. Table 7 illustrates the distribution of maternal risk factors. In this study, 74 (31.8%) mothers had premature rupture of membrane (PROM) of more than18 h, 53 (22.7%) had maternal fever and 28 (12%) had maternal UTI as risk factors. Of the 44 culture-positive cases, 26 (59.1%) had PROM as a maternal risk factor. The association of culture positivity with PROM was statistically significant ($P < 0.001$).

The present study revealed the following neonatal risk factors for culture-positive sepsis, in the decreasing order of frequency - prematurity (59.2%), low birthweight (54.5%), meconium stained amniotic fluid (20.2%) and birth asphyxia (18%). The risk factors such as prematurity and low birthweight were statistically significant ($P < 0.05$) [Table 8]. In the culture-positive babies, the main clinical features were respiratory distress (65.9%), poor feeding (36.4%), cyanosis (27.3%), fever (11.4%), jaundice (9.1%), apnoea (9.1%), abdominal distension (6.8%) and bleeding (6.8%). All these clinical features were significantly associated with culture-positive sepsis, except fever and jaundice.

Discussion

Bloodstream infections are one of the most common health-care-associated infections worldwide. The disease can range from self-limiting infections to life-threatening sepsis which requires rapid as well as aggressive antibiotic therapy. A wide variety of organisms have been known to cause sepsis. Increased incidence of antimicrobial resistance is also a worldwide concern.[5]

Table 3: Antibiotic sensitivity pattern of Gram-positive organisms (% sensitivity)

Antibiotic	*Staphylococcus aureus* (12), *n* (%)	*Staphylococcus epidermidis* (1), *n* (%)
Penicillin (10 IU)	5 (41.7)	0
Gentamicin (10 µg)	9 (75)	0
Erythromycin (15 µg)	8 (66.7)	1 (100)
Cephalexin (30 µg)	12 (100)	1 (100)
Clindamycin (2 µg)	12 (100)	1 (100)
Rifampicin (5 µg)	12 (100)	1 (100)
Amikacin (30 µg)	12 (100)	1 (100)
Linezolid (30 µg)	12 (100)	1 (100)
Oxacillin (based on Cefoxitin 30 µg disc)	12 (100)	1 (100)
Novobiocin (5 µg)	NT	1 (100)

NT: Not tested

Table 4: Antibiotic sensitivity pattern of Gram-negative organisms (% sensitivity)

Antibiotic (µg)	*Klebsiella pneumoniae* (20), *n* (%)	*Escherichia coli* (7), *n* (%)	*Pseudomonas aeruginosa* (2), *n* (%)	*Acinetobacter baumannii* (2), *n* (%)
Ampicillin (10)	0	2 (28.6)	NT	0
Gentamicin (10)	6 (30)	3 (42.9)	1 (50)	1 (50)
Cephalexin (30)	2 (10)	2 (28.6)	NT	0
Cotrimoxazole (1.25/23.75)	5 (25)	4 (57.1)	NT	1 (50)
Amikacin (30)	18 (90)	6 (85.7)	1 (50)	1 (50)
Ciprofloxacin (5)	12 (60)	5 (71.4)	2 (100)	1 (50)
Ceftriaxone (30)	7 (35)	3 (42.9)	NT	1 (50)
Ceftazidime (30)	NT	NT	2 (100)	NT
Piperacillin + Tazobactam (100+10)	18 (90)	6 (85.7)	2 (100)	1 (50)
Cefoperazone + Sulbactam (75+30)	17 (85)	5 (71.4)	NT	1 (50)
Meropenem (30)	20 (100)	7 (100)	2 (100)	2 (100)

NT: Not tested

Table 5: Clinical outcome

Status	*Klebsiella pneumoniae*, *n* (%)	*Escherichia coli*, *n* (%)	*Pseudomonas aeruginosa*, *n* (%)	*Acinetobacter baumannii*, *n* (%)	*Staphylococcus aureus*, *n* (%)	*Staphylococcus epidermidis*, *n* (%)
Improved	16 (80)	6 (85.7)	1 (50)	1 (50)	11 (91.7)	1 (100)
Expired	4 (20)	1 (14.3)	1 (50)	1 (50)	1 (8.3)	0
Total	20	7	2	2	12	1

Table 6: Distribution according to birthweight

Birthweight	Culture positive, *n* (%)	Culture sterile, *n* (%)	Total cases, *n* (%)
<2.5	32 (72.7)	114 (60.3)	146 (62.7)
2.5-3.5	10 (22.7)	64 (33.9)	74 (31.7)
>3.5	2 (4.6)	11 (5.8)	13 (5.6)
Total	44 (100)	189 (100)	233 (100)

In this study, which was conducted in 233 neonates, 44 babies (18.88%) were culture positive. This finding is similar to a study from Sikkim (Tsering *et al*. 2011).[6] The studies from other countries reported culture positivity rates ranging from 2.27% to 13.8%.[7,8] According to a study conducted in Amrita Institute of Medical Sciences, Kochi, in 2012, culture positivity was 38%.[9] The incidence of neonatal septicaemia is variable and differs from place to place because it depends on various factors such as gestational age, foetal birthweight, maternal nutrition, perinatal care and hygienic conditions and child health-care facilities.[10]

In the present study, the male babies outnumbered the female babies, both total cases and in culture positivity. This is comparable with studies conducted in India and other countries.[10,11] The reason for male preponderance is unknown, but this could be due to sex-dependent factors. The synthesis of gamma globulins is probably regulated by X-linked immunoregulatory genes, and as males have only one X chromosome, they are more prone for neonatal septicaemia than females.[12]

The organisms isolated in the study are in accordance with the study by Mustafa and Laeeq in Andhra Pradesh in 2014, in which the most common pathogens isolated were *K. pneumonia* (35%) followed by *S. aureus* (24.1%), *E. coli* (22.5%), CoNS (11.2%) and *P. aeruginosa* (6.4%).[10]

Shim *et al*. who conducted a 26-year longitudinal analysis regarding the trends in epidemiology of neonatal sepsis in a tertiary care centre in Korea reported that Gram-positive bacteria accounted for 43.6%, Gram-negative bacteria

Table 7: Distribution according to maternal risk factors

Maternal risk factors	Culture positive, *n* (%)	Culture sterile, *n* (%)	Total, *n* (%)	χ^2	*P*
PROM >18 h	26 (59.1)	48 (25.4)	74 (31.8)	18.696	<0.001
Maternal fever	8 (18.2)	45 (23.8)	53 (22.7)	0.643	0.423
Maternal UTI	6 (13.6)	22 (11.6)	28 (12)	0.135	0.714
No risk factors	4 (9.1)	74 (39.2)	78 (33.5)		
Total	44 (100)	189 (100)	233 (100)		

PROM: Premature rupture of membrane; UTI: Urinary tract infection

Table 8: Distribution according to neonatal risk factors

Neonatal risk factors	Total, *n* (%)	Culture positive, *n* (%)	Culture sterile, *n* (%)	χ^2	*P*
Prematurity	138 (59.2)	32 (72.7)	106 (56.1)	4.12	0.04
LBW	127 (54.5)	30 (68.2)	97 (51.3)	4.09	0.04
MSAF	47 (20.2)	8 (18.2)	39 (20.6)	0.13	0.71
Birth asphyxia	42 (18)	12 (27.3)	30 (15.9)	3.13	0.07

LBW: Low birthweight; MSAF: Meconium-stained amniotic fluid

Table 9: Distribution according to main clinical presentation

Clinical feature	Total, *n* (%)	Culture positive, *n* (%)	Culture sterile, *n* (%)	χ^2	*P*
Respiratory distress	112 (48.1)	29 (65.9)	93 (49.2)	3.99	0.04
Poor feeding	54 (23.2)	16 (36.4)	38 (20.1)	3.98	0.04
Cyanosis	36 (15.5)	12 (27.3)	24 (12.7)	5.80	0.01
Fever	28 (12)	5 (11.4)	23 (12.2)	0.02	0.88
Jaundice	19 (8.2)	4 (9.1)	15 (7.9)	0.06	0.8
Apnoea	7 (3)	4 (9.1)	3 (1.6)	6.896	0.009
Abdominal distension	6 (2.6)	3 (6.8)	3 (1.6)	3.89	0.04
Bleeding tendency	4 (1.7)	3 (6.8)	1 (0.5)	8.36	0.004

for 37.6% and fungus for 18.8% cases. The common microorganisms found were *S. aureus* (25.5%), Candida species (16.8%), *K. pneumoniae* (13.4%), *E. coli* (8.7%) and CONS (8.1%).[11] In a study in the neonatal intensive care unit of a tertiary care hospital in Gujarat by Shah *et al*. in 2012, Gram-negative organisms were isolated in 52% cases, Gram-positive in 45% cases and Candida species in 3% cases.[13] The increased incidence of sepsis by Gram-negative bacteria may be attributed to the fact that there is colonisation of Gram-negative bacteria in the skin of the neonate and the personnel of the neonatal wards. In a study by Kuruvilla *et al*. at Christian Medical College, Vellore, *E. coli* and *Enterococcus faecalis* were the predominant organisms causing EOS, while Klebsiella spp and *E. faecalis* were the predominant organisms in LOS.[14]

According to a study conducted in Egypt in 2008 by Abdel-Hady *et al*., 67% of the Klebsiella isolates were ESBL producers which concurs with the present study.[15] According to a study conducted in 2014 by Srivastava *et al*. in a tertiary care centre in North India, ESBL prevalence was 30.5%.[16] Chandel *et al*. in a study on ESBL-producing Gram-negative bacteria causing neonatal sepsis in India in rural and urban settings in 2011 reported that one-third of Klebsiella (35/113) and *E. coli* (7/21) received were ESBL producers.[17] Vijayakanthi *et al*. in a study from Post-graduate Institute of Medical Education and Research and associated Dr. Ram Manohar Lohia Hospital, New Delhi, India, during December 2009–November 2010, revealed that the frequency of ESBL-producing organisms was found to be 5.3%. Klebsiella (60%) was the most common organism producing ESBL followed by *E. coli* (30%) and *P. aeruginosa* (10%).[18] In a study by Singh *et al*. in 2013, ESBL producers were maximum (45.74%), followed by co-producers of metallo-beta-lactamase (MBL) + AmpC - 18.5%, ESBL + AmpC - 14.8%, ESBL + MBL - 11.12%, AmpC and MBL - 7.4%.[19]

In the present study, 146 (62.66%) of the total cases and 32 (72.72%) of the culture-positive cases were of low birthweight. In a study conducted by Nithin *et al*. in Sree Avittom Thirunal Hospital, Thiruvananthapuram, in 2008, 44% of the neonates had low birthweight as a risk factor.[20] Immature host defence mechanisms make the low birthweight neonate, particularly susceptible to overwhelming infection. In this study, 65.9% of the culture positive neonates had respiratory distress as the main clinical presentation followed by poor feeding (36.4%) [Table 9].

In a study in Mexico, the overall mortality rate of sepsis was 9.5%, which correlates with the present study. Factors associated with mortality in newborns with

sepsis comprised prematurity, low birthweight, low Apgar score, perinatal asphyxia and the requirement of any invasive medical or surgical procedure.[8]

Conclusion

K. pneumoniae subspecies *pneumoniae* was the most common aetiological agent isolated in neonatal sepsis followed by *S. aureus*. The majority of cases occurred during the first 72 h of life. All the staphylococcal strains showed 100% sensitivity to all antibiotics tested except Penicillin, Gentamicin and Erythromycin.

The Gram-negative organisms were 10%–30% sensitive towards first-line antibiotics, 35%–60% sensitive to Aminoglycosides, Cotrimoxazole, third-generation Cephalosporins and quinolones, 85%–90% sensitive to Aminoglycosides such as Amikacin and β-lactam/β-lactamase inhibitor combinations such as Piperacillin + Tazobactam and Cefoperazone + Sulbactam. All (100%) isolates were sensitive to Meropenem.

PROM more than18 h was identified as the most common maternal risk factor. The neonatal risk factors such as prematurity and low birthweight were statistically significant.

The aetiological agents are different in different circumstances. Hence, the antibiotics used should be specific based on culture and sensitivity. Regular antenatal care, special care of at-risk neonates such as pre-term and low birthweight babies, exclusive breastfeeding, proper handwashing, early diagnosis and appropriate management of infection all remain as major pillars in controlling sepsis in neonates.

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Conflicts of interest
There are no conflicts of interest.

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