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Original Research Article

Bacteriological Profile of Neonatal Sepsis in Outborn Neonates in a Tertiary Hospital in New Delhi

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Abstract

Background: Neonatal sepsis is a leading cause of neonatal morbidity and mortality in India. However, limited data is available on the causative pathogens for community acquired sepsis. The aim of this study was to identify causative bacteria of neonatal sepsis in outborn neonates so that empirical treatment in the periphery may be initiated accordingly.

Material and Methods: In this prospective observational study 145 consecutively admitted outborn neonates with symptoms of sepsis were enrolled. After initial resuscitative measures, a detailed history and examination was done and all relevant investigations including blood culture was sent. Lumbar puncture was done in the presence of a positive blood culture or if the clinical picture was consistent with meningitis in early onset sepsis and in all cases of late onset sepsis.

Result: There were 36(24.8%) neonates with Early onset (EOS) and 109(75.2 %) with Late onset (LOS). The septic screen was found to be positive in 115(79.3%) and negative in 30(20.7%) of cases. The blood culture was found to be positive in 42(28.9%) cases. Among the culture positive cases, most common organisms found were *Klebsiella pneumoniae* 14(33.3%) and *Staphylococcus aureus* 14(33.3%) followed by *CONS* 9(20.4%), *Streptococcus species* 3(7.1%) of cases. Categorical variables were presented as number and percentage and SPSS 21.0 was used for statistical analysis.

Conclusion: Empirical therapy for suspected neonatal septicemia should cover both Gram-negative bacilli and Gram-positive bacteria.

Keywords: Neonatal sepsis, Bacteraemia, *Klebsiella pneumoniae, Staphylococcus aureus*, Neonatal mortality.

Introduction

Neonatal sepsis is a clinical syndrome characterised by signs and symptoms of infection with or without accompanying bacteraemia in the first month of life. Neonatal sepsis is a major cause of mortality and morbidity, accounting for approximately 22% of global annual neonatal deaths [1] It is responsible for 30-50%of the total neonatal deaths in developing countries. [2] The Sustainable Development Goals (SDGs) target a reduction of neonatal mortality in all countries to less than 12 deaths per 1,000 live births by 2030 [3]

Neonatal sepsis may be classified according to the time of onset of the disease into Early Onset Sepsis (EOS) and Late Onset Sepsis (LOS). Early Onset Sepsis is defined as infections presenting within the first 72 hours of life. These are vertically transmitted and perinatally acquired. Late onset sepsis is defined as infection presenting after 72 hours of age and are nosocomial or community acquired. Various factors that predispose to an increased risk of nosocomial sepsis include low birth weight, prematurity, invasive procedures, parenteral fluid therapy, ventilation and use of stock solutions; factors that may increase the risk of community acquired sepsis include poor hygiene, poor cord care, and bottle feeding and prelacteal feeds.

The organisms causing neonatal sepsis vary over geographies and with time. Group B Streptococcus, *E. coli* and *Listeria monocytogenes* have been the organisms isolated most frequently in developed countries. [4] Gram negative organisms such as Klebsiella sp, *E. coli* and Acinetobacter are more common in less developed countries. [5,6]

Many studies have been conducted on neonatal sepsis in developing countries but they are hospital based. The majority of babies in developing countries are born at home, and hospital based information from these regions, have little relevance to the community settings, particularly for late onset sepsis. The reason behind lack of etiological data from community setting are numerous and includes the lack of laboratory and culture facilities in most primary and secondary health facilities and rural areas, as well as delay in, and reluctance of families to seek care, resulting in most babies succumbing to serious infection with in their homes without coming to medical attention.

Therefore, community based studies are needed to be carried out in order to know the prevailing pathogens in the community and to plan proper treatment regimens for peripheral health set ups which are the first and sometimes only point of contact. This will help in avoiding misuse of antibiotics and curtail development of bacteriological resistance. The present study is an attempt to formulate a treatment protocol to reduce the morbidity and mortality related to neonatal sepsis in the community.

Material and Methods

This was an observational prospective study conducted on neonates brought to the outborn nursery of Dr. Baba Sahib Ambedkar Hospital, with clinical features and risk factors of sepsis. The study was approved by the institute ethical committee.

Inclusion criteria:

Out born neonates presenting with any two or more of the following signs and symptoms were included into study:

1. Hypothermia(<95^{0}F) or fever(>99^{0}F)
2. Lethargy, poor cry, refusal to suck
3. Hypoglycaemia (<40mg/dl)/ Hyperglycemia(>125mg/dl)
4. Poor perfusion, prolonged capillary refill time
5. Hypotonic, absent neonatal reflexes
6. Bradycardia (<100/min)/ tachycardia (>160/min)
7. Respiratory distress, apnoea and gasping respiration
8. Bulging anterior fontanel, vacant stare, high-pitched cry, excess irritability, stupor/coma, seizures, neck retraction
9. Feed intolerance, vomiting, diarrhoea, abdominal distension
10. Bleeding, petichae , purpura
11. Multiple pustules (>10), abscess, sclerema, mottling, umbilical redness and discharge.

Exclusion criteria

Neonate with obvious congenital malformation, born to HIV positive mother, already received antibiotics, with history of birth asphyxia or TORCH infection.

The present study was conducted from 1st June 2016 for a period of one year. Neonates with symptoms and risk factors for sepsis and satisfying the inclusion and exclusion criteria who came for admission to the outborn nursery were enrolled for the study after written informed consent from the parents. A total of 200 outborn neonates were considered for the study and out of these 153 satisfied the inclusion criteria. Parents of 8 neonates refused consent after detailed explanation about the study. A total of 145 outborn neonates thus were enrolled after written informed consent.

The neonates were assessed and emergency resuscitative measures were given and subsequently appropriate antibiotic treatment was given as per the NICU management protocol. Following this, a questionnaire was filled. A detailed history including chief complaints, risk factors, past and family history obstetric history and treatment details were taken.

Subsequently a detailed examination was performed including a general physical examination, anthropometry and systemic examination.

Following investigations were done for rapid screening of neonate:

1. Total Leukocytes Count
2. Absolute Neutrophil Count
3. Immature to Total neutrophil Ratio
4. m-ESR
5. C-Reactive Protein
6. Blood culture and sensitivity
7. Peripheral smear to look for band cells

Other investigations based on indication:

Chest-X-Ray: in patient with history of respiratory distress

X-Ray abdomen : for necrotising enterocolitis

Liver function test

Kidney function test

Serum electrolyte

ABG

Cranial Ultrasonography

CSF examination etc.

Collection of Sample:

Blood Culture:

A sterilized, sealed pack blood culture bottle of volume 40 ml containing BACTEC Peds plus TM/F culture vial was used. After aseptically injecting the blood, the inoculated aerobic vials were placed in the BACTEC fluorescent series instrument as soon as possible for incubation and monitoring. [At the end of the testing period, a negative vial appears visually positive (i.e. chocholatized blood, bulging septum, lysed and/or very darkened blood)] .Positive vials were sub cultured and gram stained smear prepared and treated as presumptive positive.

From the sensor positive culture bottles, aspirate was taken out and cultured on blood agar or Maconkey agar at 30 degree Celsius for 18-24 Hrs and if growth was detected then it was further subjected to anti-microbial antibiotic sensitivity testing.

Lumbar Puncture:

In Early onset sepsis lumbar puncture was done in the presence of a positive blood culture or if the clinical picture was consistent with meningitis. In cases of Late onset sepsis, lumber puncture was done in all infants prior to starting antibiotics. The CSF was obtained by lumbar puncture performed under all aseptic precaution. The sample was collected in three separate sterile vials each for cytological, biochemistry and microbiological examination.

While awaiting test results, based on clinical suspicion intravenous antibiotics were started. This was later modified/discontinued based on test results. The duration of antibiotics depended upon the diagnosis i.e. for pyogenic meningitis (3wks), for definitive sepsis (2wks), for probable sepsis (7days) and for no laboratory evidence of sepsis (3-5 days). After completion of course of antibiotics the baby was discharged with follow-up advice.

The outcome measures noted were:

- Septic screen positive or negative
- Organism isolated on blood culture
- Clinical outcome of the neonate
- CSF culture

Categorical variables were presented as number and percentage and SPSS 21.0 was used for statistical analysis. The statistical significance of categorical variable between No laboratory evidence of sepsis, Probable sepsis and definitive sepsis groups was determined by Chi-square or Fischer-Exact test. The level of statistical significance was taken as P value < 0.05. The data was analysed by using SPSS Statistical Software Version 21.0.

Results

According to the age of onset of infection, neonates were classified as EOS (≤ 3 days) and LOS (>3 days). There were 36(24.8%) neonates admitted as EOS and 109(75.2 %) admitted as LOS.

All the neonates underwent rapid screening which included TLC, ANC, ITR, CRP, m-ESR and Peripheral smear to look for band cells. The rapid screen was found to be positive in 114(78.6%) and negative in 31(21.4%) of cases.

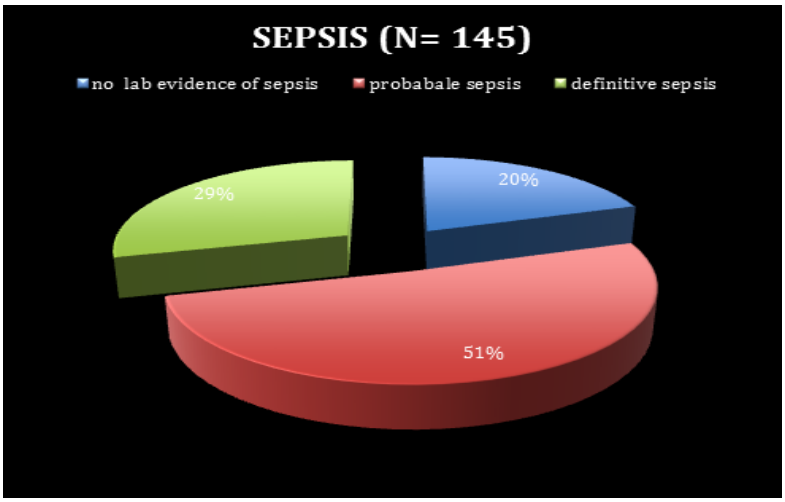

Figure 1: Distribution of cases in subgroups of sepsis

Only 42(29%) of neonates showing clinical signs and symptoms of sepsis were culture positive (definitive sepsis) where 74(51) % were probable sepsis (culture negative, rapid screen positive) and 29(20%) were no laboratory evidence of sepsis (rapid screen negative, blood culture negative).

Table 1: Bacteriological profile of blood culture positive neonates

Organisms In Blood Culture	No of Class (N=42)	Percentage (%)
Staphylococcus aureus	14	33.3
Klebsiella pneumonia	14	33.3
CONS	9	21.4
Streptococcus species	3	7.1
Staphylococcus hemolyticus	1	2.4
Ralstonia paucula	1	2.4

The blood culture was found to be positive in 42(28.9%) cases. Among the culture positive cases, most common organisms found were *Klebsiella pneumoniae* 14(33.3%) and *Staphylococcus aureus* 14(33.3%) followed by *CONS* 9(20.4%), *Streptococcus species* 3(7.1%), *Staphylococcus hemolyticus* 1(2.4%), *Ralstonia paucula* 1(2.4%) of cases.

Table 2: Comparison of common isolated organisms by type of onset of sepsis (n=42)

Organisms In Blood Culture	EOS sepsis No= 12 (28.6%)	LOS sepsis No =30 (71.4%)	Total cases No=42(100%)	P value (0.5)
Staphylococcus aureus	4(28.5%)	10(71.5%)	14 (33.3%)	1.0
Klebsiella pneumoniae	3(27.2%)	11(72.8%)	14 (33.3%)	0.46
CONS	4(44.4%)	5(65.6%)	99 (21.4%)	0.23
Streptococcus species	1(33.3%)	2(66.7%)	3(7.1%)	0.84
Staphylococcus hemolyticus	0(0%)	1(100%)	11 (2.4%)	0.5 0.52
Ralstonia paucula	0(0%)	1(100%)	11 (2.4%)	0.52

There was no statistically significant correlation between the type of organism isolated and the time of onset of sepsis as seen from table 2.

Table 3: Outcome of cases based on different group of sepsis

Sepsis	Outcome			Total
	Discharged	LAMA	Expired	
No lab evidence	25(86.2%)	4(13.8%)	0(0%)	29(100%)
PROBABLE	63(85.1%)	10(13.5%)	1(1.4%)	74(100%)
SEPSIS	32(76.2%)	4(9.5%)	6(14.2%)	42(100%)

NT: Not Tested

CONS - Coagulase negative staphylococcus

Table 4: Shows the antibiotics susceptibility pattern in gram positive and gram-negative bacteria. But overall culture sensitivity among gram negative bacteria for Ceftriaxone (100%) and for Vancomycin among Gram positive bacteria (100%)

Antibiotic sensitivity of organism isolated						
Organism in blood culture	Staphylococcus aureus	Klebsiella pneumoniae	CONS	Streptococcus species	Staphylococcus hemolyticus	Ralstonia paucula
	n=14	n=14	n=9	n=0	n=1	n=1
Antibiotic						
Ampicillin	0	0	0	0	0	0
Amikacin	0	5	0	1	0	0
Gentamicin	3	3	2	1	1	0
Cloxacillin	5	NT	4	0	1	NT
Ceftriaxone	6	14	8	3	1	1
Ceftazidine	6	NT	7	2	1	NT
Vancomycin	14	NT	10	NT	1	NT
Ciprofloxacin	5	8	8	NT	NT	NT

Result of CSF analysis done in 119 cases (12 EOS + 107 LOS) revealed that 8(5.5%) cases were pyogenic meningitis. All the 8cases were of LOS group. CSF culture was sterile in all the cases while blood culture was positive in 4(50%). Out of these 8 cases, 2(16.6%) expired, 2(16.6%) went LAMA and 4(66.6%) were given treatment for 3 weeks and then discharged with follow-up advice.

Discussion

In our study, 36(24.8%) neonates had early onset sepsis and 109(75.2 %) had late onset sepsis. This is contrast to Lamba et al [7] and Basavaraj et al [8] who found that EOS (61.41%,74.8%)was more common than LOS (38.59%,25.2%).In a multicentre cohort study in NICUs in Delhi [6] it

was found that nearly two-thirds of total episodes of neonatal sepsis occurred at or before 72 h of life. Our study was on out born neonates and this may be a reflection of the post-natal practices in the community like pre-lacteal feeds, bottle feeding and poor cord care.

In the present study, positive culture in blood was observed in 42(29%) of cases. Lamba et al[7] and Rajendraprasad et al [8] reported a higher blood culture positivity rate of 37.8% and 47.5% respectively while in studies by Basavaraj et al [9]. and Upadhyay et al [10] the culture positivity was lower at 19.2% and 18.6%respectively. Muley et al [11] found that out of 180 blood samples, septicemia could be confirmed by culture in 26.6% (48 out of 180) cases which is similar to our study.

In our study Klebsiella and *Staphylococcus aureus* were the most common organisms isolated, 33.3% each among the culture positive group. Other organisms were CONS (20.4%), Streptococcus species (7.1%) and *Staphylococus hemolyticus* and *Ralstonia paucula* (2.4% each).

National Neonatal Perinatal Database (NNPD). [12] found Klebsiella to be the most common pathogen 27.5%, *S. aureus* 14.8% and *E. coli* 11.6%. Klebsiella was found to be the predominant pathogen in study by Muley et al [11] followed by *S. aureus* accounting for 35.4% and 22.9% cases respectively. Similarly, Klebsiella was found to be the most common pathogen in studies by Basavaraj et al [9], Mustafa et al [13], Mane et al [14] and Wen et al. [15] *S. aureus* as a major pathogen of neonatal septicemia has been reported by Lamba et al [6] and Thakur et al. [16]

Chaurasia et al [6] in a metanalysis of data (n=24 273) from South Asia found that Gram negative organisms (63%) were the most common, with Klebsiella spp (23%), Escherichia coli (14%), and Acinetobacter spp (8%) being the top three. The most common Gram positive organisms were *Staphylococcus aureus* (20%) and Coagulase negative Staphylococci (9%). This list of causative agents is in stark contrast to data from western countries with a predominance of group B streptococci [4] as compared to a high prevalence of Gram negative pathogens (>60%) in India. [5]

Antibiotic susceptibility of all causative agents of neonatal sepsis was done in the present study. We observed that all organisms, gram positive and gram negative, were 100% resistant to Ampicillin. Gram negative organisms were 100% sensitive to ceftriaxone whereas among gram positive bacteria, staphylococcus Aureus was 100% sensitive to Vancomycin. Basvaraj et al [9] also found that among gram negative isolates 97% were resistant to ampicillin which is like our study. However Basvraj et al observed that gram negative isolates were least resistant to imipenem whereas in our study gram negative isolates were least resistant to ceftriaxzone.(Table 4)

The present study found no statistically significant correlation between the type of organism isolated and the time of onset of sepsis. However, in another study from South Asia *E. coli* were isolated more in both early onset and late onset sepsis, however, *S. aureus* was more common in late onset sepsis as compared with early onset sepsis and was statistically significant ($p = 0.046$). This was albeit a hospital based study which may explain the difference with our study on outborns which are a reflection of the community [17].

In the present study of total 145 neonates, 120(82.4%) cases were discharged whereas 7(4.8%) expired and 18(12.8%) left against medical advice. Among all deaths, 4(0.8) was in EOS and 20(4.0%) were in LOS group. So, with 7 neonatal deaths, case fatality rate was low at 4.8%. This low case fatality rate could be a result of early diagnosis, timely and proper management of the sick outborn babies in our institution. In a systematic review neonatal mortality was reported to vary from 11% in developed to 19% in the developing countries. [18]

In settings with evolving health system infrastructures that are yet insufficient to ensure success, an unmet need for improving the treatment of neonatal infections exists. By treating uncomplicated cases of presumed neonatal sepsis in the home or at community-based clinics, the potential burden to the family of accessing hospital care, and the costs associated with hospitalization could also be alleviated. The present study is a small step in this direction as by identifying the predominant pathogens in the community, appropriate antibiotic regimen may be charted reducing misuse and emerging microbial resistance.

Conclusion

A high degree of clinical suspicion for neonatal sepsis should be there in sick neonates referred from the community. Empiric therapy for suspected neonatal septicemia should cover both Gram-negative bacilli and Gram-positive cocci particularly *Klebsiella pneumonia* and *Staphylococcus aureus*.

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