

A hospital based study on clinico microbiological profile of neonatal septicemia



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ABSTRACT

Background: Globally, neonatal septicaemia is one of the common causes of neonatal death making it a grave problem. Timely diagnosis and adequate treatment is crucial for managing this burning issue. **Aims and Objectives:** Early detection of neonatal septicaemia along with its infective etiological agent(s) and assessment of antimicrobial sensitivity pattern. **Materials and Methods:** After getting approval from institutional ethics committee, an institution based observational, epidemiological study was conducted in ninety two neonates, clinically suspected for sepsis, admitted in neonatal intensive care unit (NICU) were included in the study. Written consent was obtained from their guardian. Blood was drawn from the neonates for estimating routine parameters and cultured for isolation of causative agents using standard protocol. Isolates were identified by staining and biochemical parameters. **Results:** The present study had 92 neonates, out of which 53 were male and 39 were female. Bad obstetric history (BOH) was present in 23 mothers. Low to very low birth weight was seen in more than two thirds culture positive neonates. All neonates (100%) had poor cry, sucking and reflex problems. 51 (55.43%) were culture positive of which bacterial pathogens was detected in 27 (52.94%) and fungal agents in 24 (47.05%) cases. Bacterial sepsis was predominantly caused by different gram negative organisms (66.66%). Klebsiella sp. and Staphylococcus sp. were the principal isolates. Candida was the commonest fungus reported. Klebsiella isolates were most sensitive to cefotaxime and amikacin while Staph. epidermidis isolates were sensitive to Amoxicillin clavulanic acid. **Conclusion:** This study was of immense help to the clinicians to minimise the neonatal mortality rate by early detection of causative agents and formulation of effective treatment.

Key words: NICU, Septicemia, Neonates, Blood culture, Anti-microbial sensitivity

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INTRODUCTION

Infant mortality is still a major problem in the developing and underdeveloped countries around the world. Neonatal mortality rate (NMR) is a component of Infant mortality Rate (IMR) and it is universally regarded not only as an important indicator of community health status, but also as quality of life of people in general. In addition, neonatal death is a reflection of assessment of the maternal and child health facilities.¹ Neonatal death accounts for about two thirds of infant mortality in India, making it a condition of public health importance. The present prevalence in India is about 33/1000 live

births.² Neonatal sepsis is described as a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections. The common causes of neonatal mortality are different types of infections (32%) like septicemia, meningitis, respiratory infections, diarrhea, neonatal tetanus followed by birth asphyxia and injuries (29%) and prematurity (24%).³ Neonatal septicemia is the most common reason in the developing country accounting for about 30-50%. As per the data of National neonatal and perinatal data base

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(NNDB, 2002-03), the incidence of neonatal sepsis in India is 30/1000 live births and it contributes to nearly 19% of total neonatal deaths.⁴ Reported literatures showed bacteria as the main causative agent of neonatal septicemia.⁵ Among bacteria, gram negative organisms are shown to be more commonly involved etiological agents and *Escherichia coli*, *Klebsiella*, *Pseudomonas* and *Salmonella* sp. topped the chart whereas among the gram positive organisms, *Staphylococcus aureus*, coagulase negative staphylococci (CONS), *Streptococcus pneumoniae* and *Streptococcus pyogenes* are the principal isolates.⁶ Septicemia due to fungal sepsis are attributed to long stay in hospital, multiple invasive procedures and inadvertent use of antibiotics.⁷ Various types of *Candida* sp. are the most common fungal isolates though a few cases may be due to *Malassezia* sp.⁸ Classically, *Candida albicans* has been reported as the causative agent in half of the cases of fungal neonatal sepsis.⁹ Non-*albicans Candida* infections have become more frequent in last few years.¹⁰ As a reason of insidious and nonspecific nature of presentation, neonatal sepsis is very difficult to diagnose despite its high incidence, till date. Blood culture is still considered as the gold standard for the diagnosis of neonatal septicemia.¹¹ Forty-one percent of all deaths every year in children below 5 years are due to deaths occurring in the neonatal period. In developing countries, every year, one million of such deaths occur due to different infections including neonatal sepsis, meningitis and pneumonia.¹² Timely diagnosis and adequate treatment is of utmost importance to manage this universal problem. For rational management of these cases, treatment should be guided by prompt isolation of etiological agents and appropriate antibiotic sensitivity report at the earliest. In many situations, initiation of empirical treatment becomes essential to save the lives of the neonates. That is why knowledge of the trend of the prevalent strains of organisms in a particular set up and their antibiotic sensitivity pattern is absolutely essential for the clinician to plan the effective treatment protocol and management of the patients.

Timely diagnosis and adequate treatment is of utmost importance for the management of this universal problem. Treatment should be guided by prompt isolation of etiological agents and appropriate antibiotic sensitivity report for rational management.

MATERIALS AND METHODS

This clinico microbiological study was conducted in the Department of Microbiology, School of Tropical Medicine, Kolkata in collaboration with Neonatal Intensive Care Unit (NICU) under the Department of Pediatrics of Calcutta Medical College. The study was conducted on ninety two clinically suspected neonates admitted in neonatal intensive

care unit of Calcutta Medical College throughout a year after obtaining approval from the institutional ethical committee. Early onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS) were defined as per standard guidelines.^{13,14} The newborns of serologically HIV negative mothers, born within 28 days of birth were included in the study and blood culture was done only among the clinically suspected neonates prior to any antibiotic administration. McCartney bottle containing 10 ml of Brain Heart Infusion (BHI) medium was used for blood culture. One to two ml of blood from neonate was collected maintaining the standard protocol for blood culture. After collection, the sample was sent to the Department of Microbiology for further processing. For the processing of the sample, the inoculated blood culture bottle was kept in the incubator at 37°C for 24 hrs. Subculture was done from the next day till the fifth day of incubation before declaring it to be negative. Subculture was done on Sheep Blood Agar, Mac Conkey Agar and Sabouraud Dextrose Agar (SDA) medium. This was followed by gram staining for the growth of micro-organism. Further processing was done according to the stain we found. For gram negative organisms, Indole Triple Sugar Iron Agar (TSI) urease, citrate arginine ornithine lysine decarboxylase test was done and for gram positive organism catalase and coagulase test was carried. Germ tube test, growth on corn meal agar and chrome agar was done for candida growth which was the sole fungus isolated. Antibigram of various bacterial isolates were determined by disc diffusion method (Kirby-Bauer method).¹⁵ Antimicrobial sensitivity and interpretations were done as per the protocol of Clinical and Laboratory Standards Institute (CLSI) standards.¹⁶ For *Candida*, species identification done with the help of VITEK.¹⁷ The weight of the baby was categorised as per guidelines laid down by World Health Organization.¹⁸

Ethics

The present study proposal was first presented before Institutional ethics committee (IEC). As per their directive, the proposal was modified and again sent to IEC to get the final permission for conducting the research.

Statistics

Results were analyzed with help of Microsoft excel for windows 2010. Some of the responses were coded and analyzed. For descriptive statistics proportion was used.

RESULTS

The present study was conducted among 92 neonates, clinically suspected of septicemia. Study subjects had 53(57.6%) male and 39 (42.4%) female neonates. Religion wise, 31 were Muslim and 61 were Hindu. Among 92

neonates, 48 (52.18%) neonates delivered normally and 44 (47.82%) had undergone cesarean section. In the study mothers, bad obstetric history (BOH) was present in 23 cases. Among the 51 culture positive cases, 25(49.0%) were low birth weight whereas 12 (23.52%) neonates were very low birth weight (Table 1).

*Responses overlapped

On analyzing the clinico microbiological profile of the ninety two clinically suspected neonatal sepsis cases, it was seen that, 51 out of 92 (55.43%) neonates showed positive results on culture while the remaining 41(44.56%) were culture negative. Bacterial and fungal pathogens each contributed nearly half, 52.94 % and 47.05% respectively. Sex distribution of 51 culture positive cases showed that 27(52.94%) were male, 24 were (47.05%) female. Among the culture negative cases 26(63.41%) were male and 15(36.58%) were female. Among the bacterial infection, 9 out of 27 cases (33.33%) were caused by gram positive organisms whereas 18 (66.66%) were due to gram negative organisms (Table 2).

Table 1: Background information (n=92)

| Parameters | Frequency (%) |
|-------------------------------------|---------------|
| Onset of sepsis | |
| Early onset neonatal sepsis | 35 (38.04) |
| Late onset neonatal sepsis | 57 (61.95) |
| *Clinical presentation | |
| Poor suck | 92 (100) |
| Poor cry | 92 (100) |
| Poor reflex | 92 (100) |
| Fever | 6 (85.71) |
| Convulsion | 7 (76.09) |
| Birth weight | |
| Normal(>=2.5 kg) | 43 (46.73) |
| Low birth weight (1.5 kg to<2.5 kg) | 34 (36.97) |
| Very low birth weight (< 1.5 kg) | 15 (16.30) |
| Mode of delivery | |
| Normal | 48 (52.18) |
| Cesarean section | 44 (47.82) |

Table 2: Microbiological profile of the study subjects*

| Parameters | Frequency (%) |
|---------------------------|---------------|
| Etiology (n=51) | |
| Bacterial | 27 (52.95) |
| Fungal | 24 (47.05) |
| Bacterial etiology (n=27) | |
| Gram positive | 9 (33.33) |
| Gram negative | 18 (66.66) |
| Sex wise etiology (n=51) | |
| Bacterial | |
| Male | 12 (23.52) |
| Female | 15 (29.41) |
| Fungal | |
| Male | 15 (29.41) |
| Female | 9 (17.64) |

*n varies

The present study reported various types of gram positive organisms in the culture. These were Staphylococcus epidermidis, Streptococcus pyogenes, Staphylococcus aureus, and many more. Staph.epidermidis was the most common of all bacterial isolates. Among the Gram negative organisms Klebsiella pneumonia was the most commonly encountered gram negative bacteria followed by Esch.coli and they accounted for 48.18% and 14.81% respectively. One case of Acinetobacter species was also reported. CSF sample from two neonates were examined but in both the cases, CSF were negative for Gram stain and India ink. None of the two CSF sample show any growth in any of the commonly used culture media (Table 3).

Table 4 depicted the frequency distribution of candida species. With special reference to candidemia, it was seen that, non albicans Candida were mostly encountered as the etiological agents among the twenty four fungal septicemia cases. Eleven (11) cases of C. lipolytica, five cases of C. albicans, four (4) cases of C. rugosa, two (2) cases of each C. krusei and C. norvegensis was isolated on fungal culture of sepsis cases. Total white blood cell count of 51 septicemic neonates revealed that, 43 had WBC count in between 5000 - 20000/cmm, 3 had more than 20000/cmm and 5 had less than 5000/cmm.

The antibiotic sensitivity tests showed the sensitivity pattern of bacterial pathogens to the commonly used antibiotics like amoxicillin clavulanic acid, cefotaxime and amikacin. Among 13 Klebsiella. pneumoniae, 2 sensitive and 11 resistant isolates were seen to Amoxicillin clavulanic acid regimen. In case of Cefotaxime and Amikacin, the

Table 3: Distribution of organisms among positive bacterial cases (n=27)

| Bacterial pathogens | N (%) |
|---------------------|------------|
| Staph.aureus | 1 (3.70) |
| Staph.saprophyticus | 1 (3.70) |
| Staph.epidermidis | 5 (18.51) |
| Staph.haemolyticus | 1 (3.70) |
| Strep.pyogenes | 1 (3.70) |
| Kleb.pneumoniae | 13 (48.14) |
| Esch.coli | 4 (14.81) |
| Acinetobacter | 1 (3.70) |

Table 4: Distribution of organisms among positive fungal cases (n=24)

| Fungal isolates | Frequency (%) |
|-----------------|---------------|
| C.lipolytica | 11 (45.83) |
| C.albicans | 5 (20.83) |
| C.rugosa | 4 (16.67) |
| C.krusei | 2 (8.3) |
| C.norvegensis | 2 (8.3) |

sensitive isolates were eight (8) and seven (7) respectively. Staph. epidermidis and E. Coli both were most sensitive to cefotaxime (Table 5)

DISCUSSION

The present study was conducted in 92 clinically suspected septicemic neonates admitted in the neonatal intensive care unit of tertiary hospital of West Bengal. The present study had 57.6% male and 42.4% female neonates. The ratio of male is to female in our study was similar to Massod et al (2011). Their study had 57% boys with male to female ratio of 1.32:1.0.¹⁹ Our study had shown that, out of ninety two (92) neonates, 35 (38.04%) had clinically susceptible early onset neonatal sepsis (EONS) and 57 (61.95%) suffered late onset neonatal sepsis (LONS). The findings differed from a Bangladesh study by Hafsa et al.(2011), where out of 104 culture positive neonates, EONS accounted for 68 cases (65.38%) and LONS accounted for 36cases (34.62%).¹² Babylon study also differed with our findings, in their study, a total of 29 (58%) neonates were presented with EONS and 21 (42 %) neonates were presented with LONS.¹⁵ The results of a study carried by Dawodu et al in 1997 was quite similar with the current study findings.¹⁴ A study done by Karthikeyan G and Premkumar K showed similar proportions of both EONS and LONS.²⁰ The birth weight of the neonates in the present study varied. Out of 51 septic neonates, 25 (49.0%) were low birth weight and 12 (23.52%) were very low birth weight. Fourteen neonates were normal birth weight of more than equals to 2.5 kg. In a study carried out by Naher HS and Khamael AB had 33 (66%) neonates with low birth weight (<2500 g) while 7(14%) neonates with very low birth weight (< 1500 g). The total count profile of the study subjects showed that, 84.31 % septicemic neonates had normal white blood cell (WBC) count (5000- 20,000/mm³), 35.88% had high WBC count (>20,000/mm³) and 9.80% had low WBC count (<5000/mm³). This study results differed from study by Naher et al where about half of neonates (56%) had normal white blood cell count,16% had high WBC count and 28% had low WBC count.¹³ Our research had 51 (55.43%) culture positive septicemic neonates that was similar with the study done by Ako-Nai et al in Nigeria. In the Nigeria study, coagulase-negative staphylococci also contributed for 21 % cases while Staphylococcus epidermidis for 5 %

of the isolates. *Listeria monocytogenes* was cultured from 8.4 % of septic neonates, *Pseudomonas aeruginosa* from 3 %, *Klebsiella pneumoniae* from 14 % and *Escherichia coli* from 7 % isolates. The bacterial isolates were resistant to traditional antibiotics that were employed to treat septicemia. The study showed a high prevalence of neonatal sepsis of bacterial origin.²¹ A study from Hubli, Karnataka reported slightly higher positive results where the blood culture positivity was found in 64.87% cases.²² In contrast, much lower positive results were reported from studies in Turkey and Saudi Arabia (15.8% and 5%).^{14,23} These variations could be attributed to various factors like the antibiotic therapy prior to the laboratory diagnosis that might had the most important impact on the culture results. The present study found that, among the bacterial pathogens, 9 out of 27 cases (33.33%) were due to gram positive organisms whereas 18 cases (66.66%) were due to gram negative organisms. The findings were compatible to the Georgia study where gram-negative organisms were recovered from 78% isolates and gram-positive organisms from 22% neonates. *Klebsiella pneumoniae* was the most common gram negative bacterial pathogen, similar with the present study.²⁴ In contrast to our study, *Staphylococcus aureus* (61.5%) was the predominant pathogen in the study by Karthikeyan et al.²⁰ In the present study, among the gram negative organisms, *Klebsiella pneumoniae* was most common followed by *Esch.coli*, accounted for 48.18% and 14.81% respectively. One case each of *Acinetobacter* sp, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Staph. Haemolyticus* were also found. This result was similar to the study done by Tallur (2000) et al where *Klebsiella pneumoniae* was the most common organism.²² In reference to candidemia, our study differed from the study by Ariff et al where *C. albicans* was the leading causative organism (55%) followed by *C. tropicalis* (21%) and *C. glabrata* (9%).¹⁰ Non albicans candida was also common in the Femitha (2013) et al study but there *C.glabrata* was the leading offending agent in 16 (44.4%), followed by *C. albicans* in 9(25%) cases.⁷ Similar findings were evoked by Goel et al study where emergence of non albicans *Candida* (80.59%) was seen as a major cause of neonatal candidemia. *C. tropicalis* (61.19%) was the most common species in their study followed by *C. albicans* (19.40%) and *C. glabrata* (11.94%).²⁵ Roy A et al at Calcutta Medical College, Kolkata found bacterial isolates in 34 (61.8%) cases and *Candida*

Table 5: Distribution of antibiotic sensitivity pattern

| Antibiotics | Pattern of sensitivity | Klebsiellapneumoniae | Staph. epidermidis | Esch. coli |
|-----------------|------------------------|----------------------|--------------------|------------|
| Amoxycillin | Sensitive | 2 | 3 | 1 |
| Clavulinic acid | Resistance | 11 | 2 | 3 |
| Cefotaxime | Sensitive | 8 | 4 | 3 |
| | Resistance | 5 | 1 | 1 |
| Amikacin | Sensitive | 7 | 2 | 2 |
| | Resistance | 6 | 3 | 2 |

species from 9 cases (16.4%) as pure culture. Twelve (12) samples did not show any growth. The distributions of different *Candida* species were *C. albicans*, *C. parapsilosis*, and *C. guilliermondii*.²⁶ Antibiogram in our study revealed that gram-negative organisms were highly resistant to ampicillin and amoxicillin/clavulanate that was similar to Macharashvili study where the gram-negative organisms showed a high degree of resistance to commonly used antibiotics such as ampicillin and amoxicillin/clavulanate.²⁴ In the study by Karthikeyan et al, 66% of *Staph. aureus* were methicillin resistant. Antibiotic resistance was common with the sensitivity to various antibiotics like to ampicillin 19%, gentamicin 21.6%, cefotaxime 32.8%, amikacin 50%, chloramphenicol 59.6% and ciprofloxacin 90.3%.²⁰

From this study it can be concluded that proper and timely treatment of neonatal sepsis can only be possible when an adequate blood culture facility is available. Late onset neonatal sepsis was more common that might be due to improper hand hygiene of the mother or the health care workers. Higher number of fungal sepsis was a striking finding in this study. It may be due the rampant use of broad spectrum antibiotics or due to proper infrastructural facility available for the detection of fungal sepsis. Non *albicans Candida* were more commonly diagnosed with some less commonly encountered species. Follow up study of patient could not be possible due to time constrain. In this study, fungal drug sensitivity could not be done due to due lack of antifungal disk and standardization.

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