NEONATAL SEPSIS- ORGANISMS RESPONSIBLE AS DETECTED BY BLOOD CULTURE

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ABSTRACT**BACKGROUND**

Neonatal sepsis refers to bacteraemia and clinical symptoms of infection caused by microorganisms and their toxic products in the first four weeks of life. It is the major cause of mortality and morbidity in neonates and accounts for 30% - 50% of neonatal deaths in developing countries. In India incidence reported as 30 per 1000 live births for the year 2002 - 2003. Blood culture is the gold standard for diagnosis of neonatal sepsis apart from utility of routine haematological, biochemical and radiological tests. Now BacT/ALERT 3D microbial detection system are being used for blood culture.

This study will enable us to have important data regarding bacteriological spectrum associated with neonatal sepsis in suspected sepsis cases admitted in our rural hospital in Bihar.

MATERIALS AND METHODS

Our study included suspected cases of sepsis among all neonates delivered at this hospital and also included cases of neonatal sepsis referred from outside. We included 341 cases of neonates, which were suspected to have sepsis and in whom blood cultures were done.

RESULTS

Out of total 341 cases of suspected sepsis, 146 (42.8%) showed positive isolates confirmed by blood culture. We had total 62 (42.3%) Gram positive, 70 (48.1%) Gram negative and 14 (9.6%) fungal isolates out of total 146 positive blood culture isolates.

CONCLUSION

Our study showed that gram negative bacteria was the predominant cause of infection among neonates in our hospital. Coagulase negative Staphylococcus and Klebsiella were commonly isolated organisms among outborn and inborn neonates respectively.

KEYWORDS

Neonatal Sepsis, Organisms.

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BACKGROUND

Neonatal sepsis refers to bacteraemia and clinical symptoms of infection caused by microorganism and their toxic products in the first four weeks of life.⁽¹⁾ It is the major cause of mortality and morbidity in neonates and accounts for 30% - 50% of neonatal deaths in developing countries.⁽²⁾ In India, incidence reported as 30 per 1000 live births for the year 2002 - 2003.⁽³⁾

Since neonates is a relatively compromised host unable to localise infection, pathogenic bacteria gain access into blood causing generalised overwhelming infection (Septicaemia) or predominantly localised to lung (pneumonia) or involving meninges (Meningitis).⁽⁴⁾ So systemic infections like septicaemia, pneumonia, meningitis, arthritis and osteomyelitis are considered as part of neonatal sepsis, but superficial infections like conjunctivitis or oral thrush are excluded from it.⁽⁵⁾

Based on time of onset of symptoms, neonatal sepsis is divided into two types, i.e. Early Onset Sepsis (EOS) if sepsis occurs in first 72 hours of life and Late Onset Sepsis (LOS) if it occurs after 72 hours of life.⁽⁶⁾ Usually vertical transmission of pathogens causes EOS and horizontal transmission of pathogens causes LOS.⁽⁷⁾ EOS is commonly present with respiratory distress and pneumonia, and source of infection is usually maternal genital tract. The risk factors of EOS include low birth weight (< 2500 gms), prematurity, prolonged rupture of membrane, prolonged labour, frequent unclean vaginal examination, perinatal asphyxia (Apgar < 4 at 1 minute). LOS is usually present with pneumonia or meningitis or both. The risk factors of LOS include low birth weight, prematurity, prolonged admission in NICU, poor hygiene, bottle feeding, etc.

The common symptoms and signs of neonatal sepsis are hypo/hyperthermia, refusal to feed, lethargy, poor cry, respiratory distress, brady/tachycardia, hypoglycaemia, hypotonia, metabolic acidosis, poor or absent neonatal reflex.⁽⁵⁾ The bacterial agents implicated in early sepsis include E. coli; Group B streptococcus; coagulase negative staphylococcus; staphylo-aureus; Listeria monocytogenes.^(7,8) The organisms commonly associated with late onset sepsis include staphylo-aureus, coagulase negative staphylococcus, Klebsiella pneumonia, E. coli, Enterobacter spp., Pseudomonas aeruginosa and Acinetobacter spp.⁽⁸⁾

Blood culture is the gold standard for diagnosis of neonatal sepsis apart from utility of routine haematological,

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biochemical and radiological tests.⁽⁹⁾ Now BacT/ALERT 3D microbial detection system are being used for blood cultures.⁽¹⁰⁾ This advanced technique can detect bacteria even at a reduced concentration (1 - 2 CFU per mL) very early.⁽¹¹⁾ Along with sending blood culture, all neonates suspected of having sepsis must undergo sepsis screening which are total leukocyte count, absolute neutrophil count, immature to total neutrophil ratio, micro ESR and C-reactive protein. But these groups do not all require antimicrobial therapy.^(12,13,14) Two or more abnormal sepsis screen parameters is considered positive sepsis screen and it warrants starting antibiotics.⁽¹⁵⁾ Negative sepsis screen along with persistence of clinical symptoms require repetition of sepsis screen after 12 hours. Further negative result almost exclude sepsis.

The bacteriological profile of causative agents of neonatal sepsis are different among developed and developing countries.⁽⁸⁾ Even there is extensive regional variation in prevalence of bacterial agents within developing countries themselves.⁽⁸⁾ Pattern of bacterial pathogens also changes with time.⁽⁷⁾

This study will enable us to have important data regarding bacteriological spectrum associated with neonatal sepsis in suspected sepsis cases admitted in a rural hospital in Bihar. It will help setting guidelines in choosing empirical therapy at this neonatal setup. This will be of immense help in reducing cost burden of treatment and lowering neonatal mortality from septicaemia in proposed geographic region.

Aims and Objectives

To find out the blood culture positivity of all suspected cases of neonatal sepsis among newborns delivered in this hospital and newborn referred from other health facilities and to isolate the organisms accountable for neonatal sepsis.

MATERIALS AND METHODS

Study Type and Design

This study was a cross-sectional study conducted in a rural medical college of Bihar in a span from March 2015 to March 2016.

Study Area

This study was carried out at level 2 and level 3 neonatal care units and post-partum ward of this hospital.

Study Population

Our study included suspected cases of sepsis among all neonates, which were delivered at this hospital. The study population also included suspected cases of neonatal sepsis referred to this hospital from outside.

Sample Size

We included 341 cases of neonates, which were suspected to have sepsis and in whom blood cultures were done. Parents and relatives were explained about nature of study and procedures. They were also assured about confidentiality of information and its anonymity.

Exclusion Criteria

Patient's refusal to participate in the study.

Ethical Clearances

Written permission from Institutional Ethics Committee was obtained prior to beginning of the study. Parents or relative of the patients were explained in their own languages about nature of study and procedure. They were also assured about confidentiality of information and its anonymity.

Study Tools

1. Proforma for data collection.
2. Clinical examination.
3. Assessment of age by using New Ballard Score.
4. The blood culture was done by BacT/ALERT 3D and VITEK-2 Compact, which is an automated rapid culture system. It is proven to enhance recovery of organism from blood even if there has been prior antibiotic administration, which happened among neonates referred in this hospital from outside. Culture bottle contains peptone-enriched Tryptic Soy Broth (TSB) with Brain Heart Infusion (BHI) solid and activated charcoal. It detects 95% pathogens within 24 hours using specific colorimetric technology with visual positive signal.

Study Technique

Blood culture was taken from all neonates suspected to have sepsis. Sepsis was suspected in the following parameters-

1. All neonates of mother with fever during and before delivery. H/O prolonged rupture of membrane for more than 24 hours, H/O foul smelling or meconium stained liquor, H/O more than 3 vaginal examinations.
2. Neonates having severe prematurity, H/O severe birth asphyxia with active resuscitation at time of birth.
3. Neonates with poor sucking reflex, respiratory distress, convulsion, hypo/hyperthermia, abdominal distension, bleeding manifestations with any of the above conditions, pathological jaundice diagnosed by clinical and laboratory features.

Parents and guardian of the patient were explained about the procedure and rationality of blood culture to be done in their own vernacular languages. They were explained about their right to opt out from the study at any time during the study after informing them about the blood culture to be an important component of treatment.

After location of vein selected accessible venepuncture site was scrubbed with 70% alcohol for minimum 30 seconds. Then Povidone iodine solution was applied in concentric circles centring the puncture site and then the site was allowed to dry.

Then the flip top overlap of the bottle was removed and it was cleansed with 70% alcohol. The bar code labelling of the bottle was kept intact; 3 mL of blood (in a ratio of 10 to 1 in culture bottle of 30 mL volume) was collected in a 5 mL syringe after inserting a intravenous cannula and the blood was directly inoculated into the bottle after inserting the needle into it. As contamination was highly possible during collection of blood for culture, utmost aseptic precautions were taken.

Parameters Studied

1. Positivity of culture.
2. Isolated organisms.
3. Organism pattern among inborn and outborn neonates.

Statistical Analysis

We entered data in Microsoft Excel sheet 2010 and tabulated later. Frequency tables and cross tables have been constructed. Frequency along with percentage have been shown in tables. These are presented in Pie charts and Bar charts.

RESULTS

We had total 341 cases of neonates suspected of sepsis in our study, out of which 146 (42.8%) showed positive isolates confirmed by blood culture and 195 (57.2%) showed no growth. Out of 242 outborn 110 (45.6%) showed positive growth, while 132 (54.4%) had no growth in blood culture. Among 99 inborn 36 (36.2%) showed positive growth, while 63 (63.8%) had no growth in blood culture (Chart 1).

Chart 1. Showing Distribution of Blood Culture Results among Outborn/Inborn Neonates among total suspected Cases of Sepsis

Distribution of Isolates

We had total 62 (42.3%) Gram positive, 70 (48.1%) Gram negative and 14 (9.6%) fungal isolates out of total 146 positive blood culture isolates. Among outborn showing positive blood cultures there was 53 (48%) gram positive, 53 (48%) gram negative and 5 (4%) fungal isolates. Among inborn neonates showing positive growth in culture, there were 9 (25%) cases Gram positive, 17 (48.6%) gram negative and 9 (26.4%) fungal isolates (Chart 2).

Chart 2. Distribution of various Organisms in Positive Blood Culture among Inborn and Outborn Neonates

We found 19.5% (29 out of 146 isolates) early onset sepsis and 80.5% (117 out of 146 isolates) late onset sepsis among all culture confirmed cases. In outborns, early and late onset sepsis were 17.6% and 82.4% respectively confirmed by blood culture. Among inborn showing growth in culture, there were 25% and 75% respective cases of early and late onset sepsis.

Chart 3. Distribution of Blood Culture Positive Early Onset Sepsis (Age 1 - 3 Days) and Late Onset Sepsis (Age >= 4 days) and Outborn/Inborn neonates

Positive Blood Culture Outborn, Inborn

Age Group in Days (1 - 3 days) 17.6%, 25%

Age Group in Days (> 4 days) 82.4%, 75%

Sepsis (Age = > 4 days) and Outborn/Inborn neonates

Pattern of Distribution of Different Organisms

Coagulase negative staphylococcus (CONS) 34 cases (23.2%) was the predominant isolates followed by Klebsiella 33 cases (22.2%), E. coli 17 cases (11.6%), Staph. aureus 15 (10.2%), Candida 14 (9.6%), Enterococcus 13 (8.9%), Acinetobacter 8 (5.1%), Burkholderia 7 (4.8%) and Pseudomonas 7 (4.5%) respectively. Out of all isolates among outborn babies, most predominant was CONS 28 cases (25.3%) followed by Klebsiella 18 (16.7%), E. coli 15 (14%), Staph. aureus 13 (11.8%), Enterococcus 12 (10.9%), Acinetobacter 7 (6.3%), Burkholderia 7 (6.3%), Pseudomonas 5 (4.5%) and Candida 5 (4%). Among isolated organisms in Inborn, the most common was Klebsiella 15 (38.9%) followed by Candida 9 (26.3%), CONS 6 (16.7%), Staph. aureus 2 (5.6%), E. coli 2 (4.2%), Pseudomonas 2 (4.2%), Enterococcus 1 (2.8%) and Acinetobacter 1 case.

Chart 4. Showing Pattern of Distribution of Different Organisms

DISCUSSION

Neonatal blood culture positivity rate varies among various studies done at different times and in different centres in the range 20% to 64%.^(16,17,18,19,20) Culture positivity around 20% was found in study by Baltimore et al, Haens et al,⁽²¹⁾ Sharma et al⁽⁹⁾ using BacT/ALERT system. On the other hand culture positivity rate of as high as above 50% was found in study by Karthikeyan et al⁽²²⁾ and Tallur et al⁽²³⁾ found culture positivity as high as 64% in his study in India.

In our study, we found blood culture positivity rate of 42.8% among all suspected neonates, which was similar to study by Thakur S et al⁽²⁴⁾ 42% in a study in North India and 41.6% culture positivity in a study in South India by Zakariya BP et al. But a wide variation of result was found in a study in central India, where Meher V et al⁽²⁵⁾ found only 22.1% culture positivity and Jyothi P et al⁽¹⁷⁾ found only 19.2% culture positivity in their study in South India.

In our study out of total neonates with suspected sepsis 70.9% were outborn and 29.1% were inborn neonates which corroborates with study by Deepandra G et al⁽¹⁸⁾ and by Samiya NK et al⁽⁷⁾ in their studies as 77% and 75.4% outborn and 75.4% and 24.6% inborn neonates respectively.

The magnitude of neonatal sepsis in inborn neonates could be due to increased incidence of nosocomial sepsis from invasion of hospital flora colonising the skin into the bloodstream, uses of central and peripheral lines and indwelling catheters of the neonates during prolonged hospital stay, requirements of invasive ventilation, etc.

We found gram positive organism in 42.3% cases, gram negative in 48.1% cases and growth of *Candida* sp. in 9.6% cases out of total blood culture isolates. Predominance of growth of gram negative organisms also found by Lamba M et al⁽²⁶⁾ as 60.7% and Mehar V et al as 56.7% in their studies with suspected neonatal sepsis. Predominance of gram negative organism as high as 70% was found in a study by Rahaman et al.⁽²⁷⁾

Fungal growth mainly *Candida* was found in our study as 9.6% of total blood cultures. But increasing trend as high as 13.4% was found by study by Mehar V et al⁽²⁷⁾ and 22.3% among positive isolates in a study by Rao YK et al.⁽²⁸⁾ This increasing trend may be due to inappropriate use of antibiotics in hospital admitted neonates, prolonged hospital stay of low birth weight and very low birth weight neonates due to increased morbidity.

In our study, coagulase negative *Staphylococcus* was the most isolated pathogen (23.2%) followed by *Klebsiella pneumonia* (22.2%), *Escherichia coli* (11.6% cases), *Staphylococcus aureus* (10.2% cases), *Candida* species (9.6% cases), *Enterococcus faecium* (8.9% cases), *Acinetobacter baumannii* (5.1% cases) and *Pseudomonas* species (4.4% cases). So most frequently encountered pathogens were coagulase negative *Staphylococcus* and *Klebsiella pneumonia* in our study, which is similar to a study in North India by Lamba M et al (52). But Movahedian AH et al⁽²⁸⁾ in their study found *Pseudomonas aeruginosa* (36% cases) to be the most common isolated organism, which was in contrast to 4.4% of total isolates in our study. Shrestha S et al⁽¹⁶⁾ and Jyothi P et al⁽¹⁸⁾ in their studies found *Klebsiella pneumonia* was the predominant organism among the suspected cases. *Escherichia coli* (66.7%) was the predominantly isolated organism in a study by Monsef A et al, which in contrast to our study where only 11.6% of total isolates were *E. coli*.

In our study, coagulase negative *Staphylococcus* and *Klebsiella pneumonia* were found in 25.3% and 16.7% cases among outborn neonates with suspected sepsis. These were two most frequently isolated organisms among outborn neonates, which is similar to studies by Samiya NK et al⁽⁷⁾ and Deepandra et al.⁽¹⁸⁾ But in contrast study by Amin AJ et al (8%) found *Klebsiella pneumonia* (38.9%) was the most frequently isolated organism among outborn neonates. *Klebsiella pneumonia* (38.9%) and *Candida* (26.3%) were the most frequently isolated organisms in inborn neonates in our study, which is similar to findings in the study by Anand JA et al⁽⁵⁾ and Samiya NK et al.⁽⁷⁾ Significant growth of *Candida* among inborn neonates in our study corroborates with study by Rao YK et al,⁽²⁷⁾ where they found *Candida* species as commonest organism in 22.3% of suspected sepsis cases.

The increased incidence of coagulase negative *Staphylococcus* among all the neonates suspected of having sepsis (23.2% of total isolates and 25.3% among culture positive outborn neonates) may be of equivocal significance. Because it is indicative of either contamination with skin flora or true bacteraemia. It is important that all the cases showing growth of coagulase negative *Staphylococcus* were also having increase in CRP level more than 10 mg/dL and most of them were outborn received in hospital after being treated outside with incomplete course of multiple antibiotics in most cases making neonates more susceptible to various blood stream infections with antibiotics resistant microorganisms. So the increased incidence of coagulase negative *Staphylococcus* in our study was probably true and it was not caused by contamination from skin flora.

CONCLUSION

Our study showed that gram negative bacteria was the predominant cause of infections among neonates in our hospital. Coagulase negative *Staphylococcus* and *Klebsiella* were commonly isolated organisms among outborn and inborn neonates respectively.

So continuous analysis of blood culture isolates and the pattern of their antimicrobial susceptibility by periodic epidemiological survey has become mandatory to recognise most frequently encountered organisms and to initiate safe and appropriate empirical antimicrobial therapy in a particular neonatal unit.

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