Original Research Article

Microbiological Aspects and Conventional Methods for Diagnosis of Neonatal Sepsis

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Abstract
Background: septicaemia is a major cause of morbidity and mortality among neonates. Its early diagnosis can save lives of many neonates.
Objective: neonatal septicaemia is very difficult to diagnose due to non specific signs and symptoms. Early diagnosis of septicaemia is very important for saving lives of neonates. Study design: the study is conducted in the department of Microbiology and the department of Pediatrics at Patna medical college, Patna. 120 neonates with signs and symptoms of septicaemia were included in this study. Diagnosis is done by conventional method of culture technique.
Result: result shows that among culture positive cases 68% are male, 33% belong to first week of their lives, and 53% are of low birth weight. Among clinically suspected cases 52% are blood culture positive and the most common organism isolated is Klebsiella pneumoniae.
Discussion and Conclusion: male predominance in neonatal septicaemia shows that there is sex linked factor in host susceptibility. Incidence of septicaemia is highest in first week of life, low birth weight is predisposing factor for septicaemia,. Gram negative bacteria Klebsiella is involved in most of the cases of septicaemia.
Keywords: Sepsis, NICU, SIRS, hypoglycemia, hypothermia, PROM, PPROM, unbooked, leukocytosis, leucopenia, tachypnea, and tachycardia.

Background
The term neonatal sepsis refers to circulation and multiplication of infecting bacteria with their toxic products in the new born within 28 days (4 weeks) of birth.

Sepsis is a common cause of morbidity and mortality among children in developing world. Definitive diagnosis is done by bacteriological culture of blood samples [¹].

Changing bacterial flora and emergence of resistant strains make it imperative to the known
prevailing pattern of antibiotic susceptibility of etiological agent of septicaemia [2]. Neonatal sepsis is difficult to diagnose clinically as it presents with non-specific signs and symptoms like birth asphyxia, intracranial haemorrhage, respiratory distress syndrome, hypoglycaemia, hypothermia etc [3]. Blood stream infections have been quoted as the most common infection in paediatric age group. A very wide spectrum of organisms have been described for cases of neonatal septicaemia and this spectrum is subject to geographical and time variations. The organisms isolated are often resistant to multiple antimicrobials which make the treatment difficult with grave prognosis. Thus the need to monitor bacteriology and its antimicrobial susceptibility pattern becomes a necessity [4].

Etiology & Microbiological aspects
SIRS (Systemic inflammatory response syndrome) - Fever or hypothermia, leukocytosis or leucopenia, tachypnea, and tachycardia are the cardinal signs of the systemic response often called SIRS. Microorganisms involved commonly are a) gram-negative bacteria (non typhoidal Salmonella species, Haemophilus influenza, Enterobacteriaceae and Pseudomonas etc. b) Gram-positive (Staphylococcus aureus, coagulase-negative Staphylococci, Enterococci, Streptococcus pneumoniae, other Streptococci and other gram-positive cocci) [5]. Now a day's gram –negative non-fermenters such as Acinetobacter spp. and Pseudomonas spp. are emerging as frequent causes of neonatal septicaemia [6].

Aims and Objectives
In spite of great advances in antimicrobial therapy, neonatal life support measures and the early detection of risk factors, neonatal septicaemia continues to be a major cause of morbidity and mortality around the world. Thus, the needs for microbial monitoring in neonatal wards cannot be overemphasized. The present study has been undertaken with the following objectives:

1. To study the clinico-etiological profile of neonatal sepsis among neonates admitted to NICU in Patna Medical College, Patna.
2. To isolate and identify the causative organisms of neonatal septicaemia
3. To study the role of various laboratory culture techniques for the identification of bacterial isolates from clinical samples from patients of septicaemia.

Materials and Methods
The present study was conducted in department of Microbiology, Patna Medical College & Hospital, Patna. Blood samples were collected from patients admitted in the NICU in Department of paediatrics, Patna Medical College & Hospital, Patna. One hundred twenty neonates up to 4 weeks of age, with clinically suspected septicaemia were studied prospectively.

Selection of cases
1. Maternal risk factors
Prolonged rupture of membranes (PROM, rupture of membranes for >18 hours before delivery), preterm prolonged rupture of membranes (PPROM, rupture of membranes <37 weeks of gestation and >18 hours before delivery), unbooked mother (less than three antenatal check-ups of the mother during pregnancy), outside hospital delivery, delivery by untrained personnel, meconium stained amniotic fluid and vaginal delivery.

2. Associated perinatal risk factors
Low birth weight (LBW, <2.5 kg), Preterm (<37 weeks), gestational age, birth asphyxia, presence of intravascular catheter, congenital abnormality, non-breast feeds.

3. Signs and symptoms
Feeding intolerance, refusal of feed, lethargy, temperature instability, icterus, apnea, respiratory distress, poor perfusion, seizures, bleeding diathesis.
Blood culture

Materials

- Blood culture bottles containing 2.5 ml Brain–Heart Infusion Broth (BHI).
- 70% Alcohol for swab.
- 2% Tincture of iodine for swab.
- Tourniquet.
- Sterile disposable syringe and 21-23 G needle.
- Sterile gauze.

Methods

1) Two blood culture bottles were labelled with the name and patient identification and hospital detail.
2) A peripheral vein on the hand or foot of the neonate was selected. The skin over the venipuncture site was cleansed with 70% alcohol–soaked sterile gauze, starting in the centre of a circle, approximately 5 cm in diameter, rubbing vigorously. The alcohol was allowed to air dry.
3) Starting in the centre of the circle, 2% tincture of iodine, or, povidone-iodine was applied in ever widening circles until the entire circle had been saturated with iodine.
4) The screw top of the blood culture bottle was removed and the diaphragm top was swabbed with 70% alcohol. The alcohol was allowed to air dry.
5) With the help of a sterile disposable syringe and needle, 0.5 to 1.0 ml of blood was withdrawn from the selected venipuncture site. The blood culture bottle used in the process was Mc-Cartny blood culture bottle. The ratio of blood to B.H.I broth should be 1:5 to 1:10.
6) Using another sterile, disposable syringe and needle, a second sample of blood was collected at the same time, from the selected venipuncture site and inoculated as described above into the second BHI broth.
7) The venipuncture sites were cleansed with 70% alcohol again, after withdrawing blood.

Culture

1) The inoculated Brain-Heart Infusion broth was incubated aerobically at 37°C for 18 hours. Growth was indicated by haemolysis of red blood cells (RBC’S), gas bubbles in the medium, or, turbidity.
2) Gram stained smear of an air dried drop of the medium, on a sterile glass slide, was performed when macroscopic evidence of growth was apparent.
3) In addition to daily visual examination, subcultures were performed after the first 6-12 hours of incubation by aseptically removing few drops of the well-mixed medium and spreading this inoculum onto a Blood agar, MacConkey agar and Nutrient agar plate.
4) The isolates on subcultured plates were identified using standard methods.
5) Culture-negative bottles were reincubated for 7 days.

Methods of Identification of Organism From Blood Culture

Identification of micro-organism: - Identification was done on the following grounds

a) Colony characters: Among colony characters size, shape, margin, surface, consistency, haemolysis, appearance, swarming of the colony were seen.

b) Gram’s staining: Smear was prepared from isolated colony on culture plate and Gram’s staining was done. Size, shape, arrangement and morphological characters are seen under high power of microscope.

c) Motility test: Hanging drop preparation was done for motility test.

d) Biochemical tests: Series of biochemical tests were required for identification of different bacterial isolates as per method described by Collee et al. (1996).
FLOW CHART SHOWING IDENTIFICATION OF GRAM POSITIVE COCCI.

GRAM POSITIVE COCCI

Positive → Catalase

Staphylococcus
Slide coagulase/Tube coagulase

Positive
Negative

S. aureus
CONS

Novobiocin susceptibility

Sensitive
Resistant

S. epidermidis
S. saprophyticus & other CONS

α
β
γ
S. viridans
Enterococcus spp.

Bacitracin susceptibility

Group A streptococci
Resistant

S. pyogenes
Non group A Streptococci

FLOW CHART SHOWING IDENTIFICATION OF GRAM NEGATIVE BACTERI.

GRAM NEGATIVE BACILLI

Growth on MacConkey agar

L.F.

INDOLE TEST

Positive
Negative

E. coli
Klebsiella & Enterobacter spp.

OXIDASE TEST

Positive
Negative

pseudomonas spp.
PPA

Oxidase test

Positive
Negative

Swarming +nt
Smear & motility

Proteus spp.
Nonmotile gram-ve cocobacilli

Citrate

Positive
Negative

Providencia spp.
Morganella spp.

Acinetobacter spp.
Results and Observation

Table -1 Sex wise distribution of cases (total no. of cases-120)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Suspected cases</th>
<th>Culture Positive case</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>Males</td>
<td>82</td>
<td>68 %</td>
</tr>
<tr>
<td>Females</td>
<td>38</td>
<td>32 %</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Table 1 showed out of 120 suspected cases 82 were males (68%) and 38 were females (32%). Among 62 culture positive cases 46 (57.09%) were males and 16 (42.10%) were females. So males were higher in number compared to females.

Table-2 Age wise distribution of cases (total no. of cases-120)

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Suspected cases</th>
<th>Culture Positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>1-7 (1st week)</td>
<td>40</td>
<td>33 %</td>
</tr>
<tr>
<td>8-14 (2nd week)</td>
<td>28</td>
<td>23 %</td>
</tr>
<tr>
<td>15-21 (3rd week)</td>
<td>27</td>
<td>23 %</td>
</tr>
<tr>
<td>22-28 (4th week)</td>
<td>25</td>
<td>21 %</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Table 3 showed out of 120 suspected cases 40 (33%) belonged to first week, 28 (23%) belonged to second week, 27 (23%) belonged to third week and 25 (21%) belonged to fourth week. Among 62 culture positive cases 22 (55.00%), 15 (53.57%), 14 (51.85%) and 11 (45.83%) belonged to first, second, third and fourth weeks respectively. So the incidence was highest in first week followed by second, third and fourth weeks.

Table-3 Distribution of cases according to the birth weight

<table>
<thead>
<tr>
<th>Birth weight</th>
<th>Suspected cases</th>
<th>Culture Positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>Low birth weight (&lt;2.5kg)</td>
<td>63</td>
<td>53 %</td>
</tr>
<tr>
<td>Normal birth weight (≥2.5kg)</td>
<td>57</td>
<td>47 %</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Table 4 showed out of 120 suspected cases 63 (53%) were of low birth weight and 57 (47%) were of normal birth weight. Among 62 culture positive cases 38 (60.30%) were of low birth weight and 24 (42.10%) were of normal birth weight. So incidence of neonatal sepsicaemia was higher in low birth weight neonates compared to normal birth weight.

Table -4 Results of Blood Culture

<table>
<thead>
<tr>
<th>Blood culture</th>
<th>cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>62</td>
</tr>
<tr>
<td>Negative</td>
<td>58</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
</tr>
</tbody>
</table>

Table 6 showed out of 120 clinically suspected cases of neonatal sepsicaemia 62 (52%) were blood culture positive and 58 (48%) were blood culture negative.
Table 5 Organism isolated by blood culture

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Organism</th>
<th>Isolates</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Klebsiella pneumonia</td>
<td></td>
<td>21</td>
<td>34.50 %</td>
</tr>
<tr>
<td>2</td>
<td>Staphylococcus aureus</td>
<td></td>
<td>16</td>
<td>25.80 %</td>
</tr>
<tr>
<td>3</td>
<td>Escherichia coli</td>
<td></td>
<td>10</td>
<td>18.00 %</td>
</tr>
<tr>
<td>4</td>
<td>Coagulase negative Staphylococci</td>
<td></td>
<td>11</td>
<td>15.41 %</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td>03</td>
<td>04.83 %</td>
</tr>
<tr>
<td>6</td>
<td>Proteus mirabilis</td>
<td></td>
<td>01</td>
<td>01.61 %</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>62</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Table 7 showed commonest organisms isolated was Klebsiella pneumoniae (34.50%) followed by Staphylococcus aureus (25.80%), Escherichia coli (18.00%), Coagulase negative Staphylococci (15.41%), Pseudomonas aeruginosa (04.83%), Proteus mirabilis (1.61%).

MacConkey’s agar & Nutrient Agar Showing Klebsiella pneumoniae

Biochemical Tests For Klebsiella pneumoniae

(INDOLE, METHYL-RED, CITRARE, UREASE, & T.S.I)
Figure I. Pie Chart Showing Organism Isolated by Blood Culture

- **Klebsiella pneumoniae (34.50%)**
- **Staphylococcus aureus (25.80%)**
- **Escherichia coli (18.00%)**
- **Coagulase Negative Staphylococci (15.41%)**
- **Pseudomonas aeruginosa (04.83%)**
- **Proteus mirabilis (01.61%)**

### Discussion

Patna Medical College & Hospital is a 1700 bedded tertiary care centre in Patna, Bihar. It provides health care services to the vast population of whole Bihar, Eastern U.P, and Nepal. This study was conducted in the department of Microbiology and Paediatrics at Patna Medical College & Hospital, Patna. One hundred neonates with clinical suspicion of septicaemia on the basis of clinical features and associated perinatal risk factors and maternal risk factors were included in the study. Blood culture, was done by the conventional method. In the present study an attempt was made to know the various bacterial flora responsible for neonatal septicaemia, through blood culture.

### Sex

In our study neonatal septicaemia was more common in males (57.09%) as compared to females (42.10%). Similar reports are reported by other workers. Khatua et al., reported 70.7% cases of neonatal septicaemia to be males. In the study conducted by U. Vaidya et al., Male: Female ratio was 1.6:1. Anitha Sharma et al., also reported male predominance, that is out of 50 cases 37 (74%) were males. In a study conducted by Anuradha De et al., out of 200 suspected cases of neonatal septicaemia 114 (57%) were males and 86 (43%) were females. The percentage of females in present study with neonatal septicaemia were 42.10%. The usual male predominance in neonatal septicaemia has suggested sex linked factor in host susceptibility.
Age
In our study, incidence of septicaemia was highest in first week of life (55.00%) followed by second week (53.57%), third week (51.85%), and fourth week (45.83%). According to Barbara J Stoll et al., 1975 neonates are more susceptible for infection in the first week of life. High incidence of Gram negative bacteraemia was noted between the age of 6 days to 17 days.[13] K.K. Anand et al.,[11] reported 58.6% of neonates were less than 10 days in the study conducted in Safdar Jang Hospital, New Delhi.[12]

Birth Weight
In our study septicaemia was more common in low birth weight neonates (60.30%) as compared to the normal weight neonates (42.10%). Sinha et al 1986 reported 64.9% incidence in low birth weight neonates. K.K. Anand reported 81.3% of neonatal septicemia cases were below 2200gms. According to study conducted by K.Chug et al., the mean birth weight of septicaemic neonates was 1.84 kgs. G.G. Christo et al., 1990 reported high rate of septicaemia among low birth weight neonates.[7] According to Barbara J. Stoll et al., 1975 rate of infection is inversely proportional to birth weight.[13]

Blood culture
Total 120 cases of clinically suspected neonatal septicaemia were selected for the present study, 62 cases were positive by blood culture and 58 cases were culture negative. So the blood culture positivity was 52%. Madhubala Parikh and Nandan Singh in 1995 reported out of 254 cases, 199 were blood culture positive (47%).[16] U. Vaidya et al., in 1991 reported out of 381 cases, blood culture was positive in 156 cases (41%).[14]

In the study conducted by Khatua et al., 1986, culture was positive in 59.8%, Namdeo et al., 1987 showed 50%, P.P.Sharma et al., 1987 reported 56%, P.S. Rao’s study showed 40.0%, Marina Thomas 1999 showed 40% and S.G. Joshi in 2000 reported 25% culture positivity respectively.[17]

### Table – 6 Results of Blood Culture by other workers[8,14,16]

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Author</th>
<th>Percentage of positive blood culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Khatua et al. 1986</td>
<td>59.8%</td>
</tr>
<tr>
<td>2.</td>
<td>Namdeo et al. 1987</td>
<td>50.0%</td>
</tr>
<tr>
<td>3.</td>
<td>P.P. Sharma 1987</td>
<td>56.0%</td>
</tr>
<tr>
<td>4.</td>
<td>Madhubala Parikh et al. 1995</td>
<td>47.0%</td>
</tr>
<tr>
<td>5.</td>
<td>Vaidya U et al. 1991</td>
<td>41.0%</td>
</tr>
<tr>
<td>6.</td>
<td>Rao P.S et al. 1993</td>
<td>40.0%</td>
</tr>
<tr>
<td>7.</td>
<td>Joshi S.G et al. 2000</td>
<td>25.0%</td>
</tr>
<tr>
<td>8.</td>
<td>Sriparna Basu et al. 2012</td>
<td>41.36%</td>
</tr>
<tr>
<td>9.</td>
<td>B. Patel, R. Prasad, et al. 2013</td>
<td>47.5%</td>
</tr>
<tr>
<td>10.</td>
<td>Present study</td>
<td>52.0%</td>
</tr>
</tbody>
</table>

Organism isolated
*Klebsiella pneumoniae* 21 (34.50%), *Staphylococcus aureus* 16 (25.80%), were commonest organisms isolated in our study followed by *Escherichia coli* 10 (18.00%), CONS 11 (15.41%), *Pseudomonas aeruginosa* 3 (04.83%), and *Proteus mirabilis* 01 (1.61%). In 62 isolates gram negative organisms were 35 (56.45%) and gram positive organisms were 27 (43.54%).
So gram negative septicaemia was commonest. Anitha sharma et al., reported 85% of culture positive cases to be gram negative organism.[15] Where as in another study by Narang et al., 61.1% neonates had gram negative septicaemia. Anuradha De et al., 1995 reported 72.4% of gram negative septicaemia. [11] Sinha et al., 1986 reported *Pseudomonas* as most common isolates (34.5%), then *Klebsiella* and *Escherichia coli* as 16.4% each, *Staphylococcus aureus* as 14.5% of isolates. Chug et al., 1988 reported *Staphylococcus aureus* as a most common isolates 20.1%, then *Escherichia coli* and *Klebsiella* as 14% each, *Pseudomonas* 06.2%, CONS as 07.5% and *Streptococcus faecalis* as 06.7% of isolates. Marina Thomas et al., 1999 reported *Klebsiella* 08.0%, *E. coli* as 04.0%, *pseudomonas* 12.0%, *Staph. aureus* 50.6%, *Streptococcus faecalis* 09.3%. S.G Joshi et al., In 2000 reported *Klebsiella* 30.4%, *E. coli* 15.6%, *Pseudomonas* 38.3%, etc.
Table – 15 | Organisms isolated by other workers [8, 9, 16, 17]

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella</td>
<td>16.4%</td>
<td>14.2%</td>
<td>77.3%</td>
<td>14.2%</td>
<td>08.0%</td>
<td>30.4%</td>
<td>27.8%</td>
<td>16.84%</td>
<td>34.5%</td>
</tr>
<tr>
<td>E.coli</td>
<td>16.4%</td>
<td>14.1%</td>
<td>6.5%</td>
<td>41.1%</td>
<td>04.0%</td>
<td>15.6%</td>
<td>13.9%</td>
<td>09.47%</td>
<td>18.0%</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>34.5%</td>
<td>6.2%</td>
<td>6.5%</td>
<td>06.7%</td>
<td>12.0%</td>
<td>38.3%</td>
<td>19.4%</td>
<td>05.26%</td>
<td>04.8%</td>
</tr>
<tr>
<td>Staph. Aureus</td>
<td>14.5%</td>
<td>20.1%</td>
<td>9.7%</td>
<td>20.1%</td>
<td>50.6%</td>
<td>-</td>
<td>5.6%</td>
<td>11.58%</td>
<td>25.8%</td>
</tr>
<tr>
<td>CONS</td>
<td>-</td>
<td>07.5%</td>
<td>-</td>
<td>07.5%</td>
<td>-</td>
<td>2.8%</td>
<td>10.53%</td>
<td>15.4%</td>
<td></td>
</tr>
<tr>
<td>Strepto. Faecalis</td>
<td>-</td>
<td>06.7%</td>
<td>-</td>
<td>06.7%</td>
<td>09.3%</td>
<td>-</td>
<td>3.16%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>-</td>
<td>03.3%</td>
<td>-</td>
<td>03.3%</td>
<td>15.9%</td>
<td>15.6%</td>
<td>36.1%</td>
<td>01.6%</td>
<td></td>
</tr>
</tbody>
</table>
(25.80%), Pseudomonas aeruginosa 3 (04.83%), and Proteus mirabilis 1 (1.61%).

9) Out of 62 blood culture positive cases 15 (24.19%) expired. In our study mortality rate was 24.19% among culture positive cases.

10) Coagulase-negative Staphylococcus were the predominate isolates from neonates with intravenous catheter and prolonged rupture of membranes.

11) Escherichia coli were the predominant isolate in neonates with maternal preterm prolonged rupture of membranes.

Conclusion
Neonatal septicaemia is leading cause of mortality and morbidity in developing countries like India. Neonatal septicaemia presents with non specific signs and symptoms. It is more common in males, low birth weight, and preterm neonates. A positive blood culture is the only definitive method of confirming a case of septicaemia, which helps in prompt and timely administration of antibiotics which could be life saving.

References