Original article

Bacteriological profile and antibiotic susceptibility patterns in neonatal septicemia in view of emerging drug resistance

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Article history

Received 22 October 2013
Accepted 01 January 2014
Early online 25 February 2014
Print 28 February 2014

Abstract

The objective of this study was to isolate pathogenic bacteria in neonatal septicemia cases, and to know their antibiograms. Under aseptic precautions, blood was drawn from 140 neonates with suspected septicemia and inoculated in BHI broth. Isolates obtained were identified as per standard protocol and antibiotic susceptibility was done by Kirby Bauer disc diffusion method (as per CLSI guidelines). A total number of 62 (44.2%) patients had positive blood cultures. The most common pathogens isolated were Klebsiella pneumoniae (n=22, 35%) followed by Staphylococcus aureus (n=15, 24.1%), Escherichia coli (n=14, 22.5%), CONS (n=7, 11.2%) and Pseudomonas aeruginosa (n=4, 6.4%). The Gram negative organisms showed high resistance to commonly used antibiotics and were highly sensitive to Meropenem. The Gram positive bacteria showed high resistance to Ampicillin, Erythromycin and Amoxycillin; but they were highly susceptible to Linizolid and Vancomycin. As the Gram negative organisms were the most common isolates in neonatal septicemia, their resistance pattern should be considered essential for deciding the empirical treatment. Prompt treatment of neonatal sepsis with judicious use of appropriate antibiotics can minimize the morbidity and mortality, besides reducing the emergence of multi-drug resistant organisms in ICU’s.

Key words: coagulase negative Staphylococcus (CONS), drug susceptibility, early onset septicemia, late onset septicemia, neonatal septicemia

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Key words: coagulase negative Staphylococcus (CONS), drug susceptibility, early onset septicemia, late onset septicemia, neonatal septicemia

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eonatal sepsis refers to systemic and generalized bacterial infection of the newborn documented by a positive blood culture in the first 4 weeks of life, and is one of the four leading causes of neonatal mortality in India1.

It is characterized by variable systemic physiologic changes triggered by infection, which continues to provide an extraordinary challenge to clinicians who manage critically ill neonates and children2.

Not surprisingly, sepsis is the commonest admitting diagnosis among neonates at referral facilities4, and is a medical emergency that requires urgent rational antibiotic therapy4, so as to minimize the risk of severe morbidity and mortality besides reducing the emergence of multi-drug resistant organism in ICU’s.

The gold standard for diagnosis of septicemia is the isolation of bacterial agent from blood culture6.
Many infections in the neonatal and pediatric age group can only be established on the basis of etiological agent recovered from blood, but a negative blood culture does not rule out the possibility of neonatal sepsis.

The prevalence of bacterial profile of blood cultures and their susceptibility patterns in an area, provide guidance to start empirical treatment which is the cornerstone in the management of sepsis.

Therefore, the present study was aimed at determining the bacteriological profile and their antimicrobial susceptibility patterns in neonatal septicemia cases.

**Materials and methods**

**Study centre, design and period**

This study involved 140 clinically suspected cases of septicemia among 4300 neonates admitted and born during the study period 2012-2013 in a teaching hospital at Hyderabad. The cases were categorized depending upon the time of presentation, within 72 hours of life as early onset septicemia (EOS) and after 72 hours of life as late onset septicemia (LOS).

Institutional Ethics Committee Clearance and informed consent from all parents of the neonates under study were obtained.

**Subject selection**

**Inclusion criteria:**

Neonates having features suggestive of systemic inflammatory response syndrome (SIRS), with no localizing source of infection.

Neonates with poor activity, fever, refusal of feed, lethargy, tachypnea, tachycardia, birth asphyxia, prematurity, low birth weight, or delivered with PROM, foul smelling liquor, etc.

**Exclusion criteria:**

Neonates having extreme prematurity (less than 30 weeks of gestational age), birth weight less than 1000 gm, gross congenital anomalies, and all children who had received antibiotics before admission, were excluded from the study.

**Sample collection and processing**

With all aseptic precautions about 1-2 ml of blood was drawn from each neonate. About 1 ml of blood was inoculated into 10 ml of brain heart infusion (BHI) broth and processed as per the protocol and incubated for one week at 37°C and was checked daily for evidence of bacterial growth. For positive broth cultures, subcultures were done next day on blood agar, Mac Conkey’s agar and chocolate agar and were incubated at 37°C for 24 hours. If no growth occurred on plates after 24 hours, subsequent cultures were done on 2nd, 3rd, 5th and 7th day. The grown bacteria were identified by colony morphology, Gram stain and standard biochemical tests. A second blood culture was processed in cases where coagulase negative Staphylococcus (CONS) was isolated.

The antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method for the bacterial isolates, as per Clinical and Laboratory Standards Institute guideline (CLSI). ATCC control strains were used accordingly as per standard procedures.

The antibiotic disks (Himedia Co., Mumbai, India) and their concentrations per disk (μg) comprised: Ampicillin (10), Cefotaxime (30), Gentamicin (10), Amikacin (30), Ciprofloxacin (5), Vancomycin (30), Piperacillin (100), Meropenem (10), Ceftriaxone (30), Ceftazidime (30), Amoxycillin (30), Erythromycin (15), Cefoperazone (75), Cefoxitin (30), Colistin (10), Linezolid (30), Cefpodoxime (10).

Gram negative bacteria resistant to third generation cephalosporins were tested for ESBL production by disc potentiation test using Ceftazidime/Clavulanic acid (30μg/10μg) discs with plain Ceftazidime (30μg) disc and interpreted as per the standard protocol.

**Data analysis**

The data of all neonatal septicemia cases was tabulated using Microsoft Office computer software. The Chi-square test was used in assessing the association between variables. A p-value of 0.05 or less was considered statistically significant.

**Results**

**Table 1: Blood culture positivity**

<table>
<thead>
<tr>
<th>Blood cultures</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture Positive</td>
<td>38(61.2%)</td>
<td>24(38.7%)</td>
<td>62(44.2%)</td>
</tr>
<tr>
<td>Culture Negative</td>
<td>34(43.5%)</td>
<td>44(56.4%)</td>
<td>78(55.7%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>72</strong></td>
<td><strong>68</strong></td>
<td><strong>140</strong></td>
</tr>
</tbody>
</table>

The total number of culture positive cases was found to be 62 giving the culture positivity rate of 44.28% (Table 1). Among the culture positives, 38 (61%) were male and 24 (38.7%) were female.
neonates, giving the male to female ratio of 1.58:1 (Table 1).

**Table 2: Distribution of cases according to age of onset and culture positivity**

<table>
<thead>
<tr>
<th>Age of onset</th>
<th>Culture positive</th>
<th>Culture negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOS</td>
<td>36 (58%)</td>
<td>30 (38.5%)</td>
<td>66</td>
</tr>
<tr>
<td>LOS</td>
<td>26 (42%)</td>
<td>48 (61.5%)</td>
<td>74</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>62</strong></td>
<td><strong>78</strong></td>
<td><strong>140</strong></td>
</tr>
</tbody>
</table>

Table 3: Bacterial isolates in blood cultures

<table>
<thead>
<tr>
<th>Organisms</th>
<th>EOS</th>
<th>LOS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>14</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>9 (25%)</td>
<td>6 (23.1%)</td>
<td>15</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>10 (27.7%)</td>
<td>4 (15.4%)</td>
<td>14</td>
</tr>
<tr>
<td>CONS</td>
<td>2 (5.6%)</td>
<td>5 (19.2%)</td>
<td>7</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1 (2.8%)</td>
<td>3 (11.5%)</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>36</strong></td>
<td><strong>26</strong></td>
<td><strong>62</strong></td>
</tr>
</tbody>
</table>

The age of culture positive neonates ranged from 12 hours to 28 days. Among culture positive neonates, 36 (58%) were having EOS and 26 (42%) were having LOS (Table 2, Fig1).

The age of culture positive neonates ranged from 12 hours to 28 days. Among culture positive neonates, 36 (58%) were having EOS and 26 (42%) were having LOS (Table 2, Fig1).
Fig 3A. Showing antibiotic sensitivity pattern of the Gram positive bacterial isolates

Table 4B: Showing antibiotic sensitivity pattern of the Gram negative bacterial isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>Klebsiella pneumonia</em> (22) N (%)</th>
<th><em>Escherichia coli</em> (14) N (%)</th>
<th><em>Pseudom. aeruginosa</em> (4) N (%)</th>
<th>Total (40) N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>2(9%)</td>
<td>1(7.1%)</td>
<td>0</td>
<td>3(7.5%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>15(68.1%)</td>
<td>10(71.4%)</td>
<td>2(50%)</td>
<td>27(68%)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>11(50%)</td>
<td>8(57%)</td>
<td>1(25%)</td>
<td>20(50%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10(45.4%)</td>
<td>7(50%)</td>
<td>1(25%)</td>
<td>18(45%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>16(72.7%)</td>
<td>10(71.4%)</td>
<td>2(50%)</td>
<td>28(70%)</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>9(41%)</td>
<td>7(50%)</td>
<td>1(25%)</td>
<td>17(42.5%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>12(54.5%)</td>
<td>8(57%)</td>
<td>2(50%)</td>
<td>22(55%)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>10(45.4%)</td>
<td>7(50%)</td>
<td>1(25%)</td>
<td>18(45%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>22(100%)</td>
<td>14(100%)</td>
<td>4(100%)</td>
<td>40(100%)</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>11(50%)</td>
<td>7(50%)</td>
<td>1(25%)</td>
<td>19(47.5%)</td>
</tr>
<tr>
<td>Colistin</td>
<td>22(100%)</td>
<td>14(100%)</td>
<td>4(100%)</td>
<td>40(100%)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>10(45.4%)</td>
<td>7(50%)</td>
<td>1(25%)</td>
<td>18(45%)</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
<td>20</td>
<td>270</td>
</tr>
</tbody>
</table>
Gram negative organisms were more than Gram positive organisms, constituting about 65% of total isolates (Fig 2 and Table 3).

The isolated Gram positive bacteria showed high resistance to Ampicillin (n=3, 13.6%) and considerable resistance towards Gentamicin (n=10, 45%). They showed moderate susceptibility to third generation Cephalosporin (Cefotaxime-73%, Ceftriaxone-68%, Cefoperazone-63%), Amikacin-68%, and Ciprofloxacin-63%. They were highly susceptible to Linezolid (n=22, 100%) and Vancomycin (n=21, 95%) (Table 4A, Fig 3A).

Among the Gram negative bacteria, many of them were resistant to ampicillin (7.5%), gentamicin (45%), third generation cephalosporins (cefotaxime-55%, ceftriaxone-50%, cefoperazone-45%) and piperacillin (45%). They were moderately susceptible to Amikacin (68%) and ciprofloxacin (70%), but highly susceptible to Colistin (100%) and Meropenem (100%) (Table 4B, Fig 3B). The ESBL producers were detected in 6 (27%) of Klebsiella pneumoniae and 3 (21%) of Escherichia coli isolates.

**Discussion**

There were 4300 live births during the study period, of which 140 neonates were clinically diagnosed with septicemia. Among them 62 (44.2%) were blood culture positive. This was in concordance with the other studies by Roy I et al.\(^\text{11}\) and Kayange N et al.\(^\text{12}\). The incidence of culture-proven neonatal septicemia was 14.4 per 1000 live births. This was comparable to the study done by Karthikeyan et al.\(^\text{13}\). The incidence of neonatal septicemia is variable and differs from place to place, because it depends on various factors like gestational age, fetal birth weight, maternal nutrition, perinatal care and hygienic conditions, child health care facilities, etc.

In the present study, males were more affected than females, and the male to female ratio was 1.58:1. This is comparable to the other studies by Begum S et al.\(^\text{14}\) and Shrestha NJ et al.\(^\text{15}\). The reason for male preponderance is unknown, but this could be due to sex-dependent factors.\(^\text{16}\) The synthesis of gamma globulins is probably regulated by X-linked immunoregulatory genes and as males are having one X chromosome, they are more prone for neonatal septicemia than females.\(^\text{17}\)

In our study, early onset septicemia (58%) was more than late onset septicemia (42%), which is consistent with the studies of Aletayab et al.\(^\text{16}\), Waseem R et al.\(^\text{18}\) and Al-Shamahy et al.\(^\text{19}\). This could be due to prematurity, low birth weight and unhy-
The organisms causing neonatal septicemia differ from area to area and also change with respect to time even in the same area, which may be due to different life conditions.

Gram negative bacterial isolates (65%) were more than Gram positive isolates (35%) in our study. This is in contrast to developed countries, where Gram positive bacteria were more commonly reported. This was in concordance with National Neonatal Perinatal Database (NNPD) (2003)22, Aletayeb SMH et al16, and Sundaram V et al23.

In this study, the most frequent isolate was Klebsiella pneumonia 22 (35.4%) in both EOS and LOS. This was in accordance with other Indian studies NNPD 200322, Kumar GD et al24, Roy I et al11 and Kayange N et al12. Escherichia coli was the second most common Gram negative organism followed by Pseudomonas aeruginosa.

Staphylococcus aureus was the commonest Gram positive organism and was second most common organism among all isolates. Among the isolates, a considerable percentage (11%) was CONS as pathogen, which could be due to immature immune system development, and a large population of premature and debilitated infants.

In this study, Pseudomonas aeruginosa isolates were found to be highly resistant to routinely used antibiotics, followed by Klebsiella pneumoniae and Escherichia coli. This increasing resistance could be due to irrational use of antibiotics. All Gram negative isolates were having considerable sensitivity to Amikacin and Ciprofloxacin; but were highly susceptible to Meropenem (100%) and Colistin (100%). Our study findings correlated well with the findings of others viz. Aletayeb SMH et al16, Wasem R et al16, Mane AK et al3 and Mullu M et al26.

The ESBL producers were detected in 27% of Klebsiella pneumoniae and 21% of Escherichia coli isolates. Our findings were comparable to studies by Vinod Kumar CS et al27 and Malakan R E et al28.

The Gram positive isolates were having better susceptibility to Amikacin, Cephalosporins and Ciprofloxacin; but were more resistant to Ampicillin and Gentamicin in the present study. They showed high susceptibility to Linezolid and Vancomycin. Our findings correlated with the studies by Aletayeb SMH et al16, Mane AK et al3, Roy I et al11 and Bhat R et al29.

Conclusion
From our study we noticed that Gram negative bacteria were more commonly the cause of septicemia in neonates, and Klebsiella pneumoniae was the predominant pathogen. We also noticed that these Gram negative bacteria were resistant to routinely used antibiotics, hence their resistant pattern should be considered essential before deciding the empirical treatment. The higher antibiotics such as Colistin and Meropenem should be reserved for multi-drug resistant Gram negative bacteria, where as Linezolid and Vancomycin should be reserved for drug resistant Gram positive isolates.

The positive blood culture with antibiotic sensitivity of the isolated organism(s) is the best guide to antimicrobial therapy, as resistance to antibiotics is a worldwide problem that causes ineffectiveness of empirical treatment. More so, strict infection control practices combined with judicious use of antibiotic therapy are the main solutions to this problem. However, it will be important to continue the surveillance of neonatal septicemia in order to closely follow changes in trends and identify risk factors, to obtain information for empiric antibiotic therapy and to act rapidly in case of major changes in susceptibility patterns.

Acknowledgments: None

Conflict of interest: None

References
Bacteriological profile in neonatal septicemia

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