Neonatal septicemia: Blood culture bacterial isolates and their antimicrobial susceptibility pattern

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Abstract
Introduction: Neonatal septicemia is defined as “a clinical syndrome of bacteremia with systemic signs and symptoms of infection in the first four weeks of life”. Blood culture is the gold standard method for diagnosis of septicemia. Isolation of organism in blood culture and its antimicrobial sensitivity pattern carries a great role.

Aim: To provide prevalence and antimicrobial susceptibility pattern of bacteria isolated from suspected cases of neonatal septicemia at a tertiary care hospital, Valsad.

Materials and Methods: Retrospective study is conducted at a tertiary care hospital, Valsad from January 2017 to June 2018. Processing of blood culture samples, Isolation and identification of bacteria were done using standard microbiology techniques. Antimicrobial susceptibility testing was performed using modified Kirby-Bauer disk diffusion method as per Clinical laboratory Standard Institute guideline. ATCC E. coli 25922, ATCC S. aureus 25923, ATCC P. aeruginosa 27853 were used as standard strains.

Results: Out of 452 blood cultures, organisms were isolated in 127(28.09%). Commonly isolated bacteria are Coagulase Negative Staphylococci (42.52%) followed by Klebsiella spp. (18.11%), Acinetobacter spp. (15.75%), Enterococcus spp. (9.45%) and others (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa). Coagulase Negative Staphylococci strains have highest sensitivity to Vancomycin (100%), Linezolid (100%), Teicoplanin (100%), followed by Chloramphenicol (92.6%), Levofloxacin (90.7%), Cefoxitin (83.3%), and Tetracycline (77.8%). 16.7% isolates were Methicillin Resistant Staphylococci (MRS). Klebsiella spp. strain have highest sensitivity to Meropenem (73.9%), Imipenem (73.9%) and Amikacin (69.6%) followed by Chloramphenicol (92.6%), Levofloxacin (90.7%), Cefoxitin (90.7%), Ceftazidime (83.3%), and Piperacillin Tazobactum (52.2%). 78.3% strains were Extended spectrum Beta-Lactamase (ESBL) producers.

Conclusion: Antimicrobial resistances are growing among bacteria and it differs according to different areas. So, proper surveillance system should be established to guide antimicrobial policy at local level.

Keywords: Neonatal septicemia, Blood culture, Antimicrobial susceptibility.

Introduction
Neonatal septicemia is defined as “a clinical syndrome of bacteremia with systemic signs and symptoms of infection in the first four weeks of life”.¹ Blood culture is the gold standard method for diagnosis of septicemia.²

According to WHO data, in 2016, Neonatal mortality rate (NMR) per 1000 live births globally is 18.6, whereas the same of South-East Asia region is 22.6 and of India is 25.4. Under five mortality rate among children of India is 43 per 1000 live births.³ So, developing countries like India still need proper measures to reduce it. Major causes of neonatal mortality worldwide are infections (36%, which includes sepsis/pneumonia, tetanus and diarrhea), pre-term (28%), and birth asphyxia (23%).⁴ Neonatal septicemia is important factor responsible for morbidity as well as mortality. Isolation of organism in blood culture and its antimicrobial sensitivity pattern carries a great role.

Antimicrobial resistance among bacteria is growing world-wide and the same scenario exist even in developing countries like India.³ Proper surveillance system is required for better understanding of the situation and to provide guidance in making health policies at local, regional, national and international level, which is lacking in India.⁵

Aim of this study is to provide prevalence and antimicrobial sensitivity pattern of bacteria isolated from suspected cases of neonatal septicemia at tertiary care hospital, Valsad.

Materials and Methods
Retrospective study is conducted taking data of blood cultures received in microbiology laboratory at a tertiary care hospital, Valsad, Gujarat, India from suspected cases of neonatal septicemia from January 2017 to June 2018. Blood cultures were received in Brain-Heart Infusion broth, incubated aerobically at 37°C and repeated subcultures were done on Blood agar and MacConkey agar. Isolated organisms were identified using standard microbiology techniques.⁷ Antimicrobial sensitivity testing (AST) were performed on Muller Hinton agar using modified Kirby-Bauer disk diffusion method as per Clinical laboratory Standard Institute guideline 2017 and 2018.⁸ ATCC E. coli 25922, ATCC S. aureus 25923, ATCC P. aeruginosa 27853 were used as standard strains. Data was analyzed using Microsoft Excel 2016.
Results

**Result of culture (%)**

![Result of culture](image)

**Fig. 1: Result of culture**

**Table 1: Frequency and percentage positivity of bacterial isolates**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Frequency (out of 127)</th>
<th>Percentage positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase Negative Staphylococci</td>
<td>54</td>
<td>42.52</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>12</td>
<td>9.45</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>8</td>
<td>6.3</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>2</td>
<td>1.57</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>23</td>
<td>18.11</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>20</td>
<td>15.75</td>
</tr>
<tr>
<td>E.coli</td>
<td>5</td>
<td>3.94</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>3</td>
<td>2.36</td>
</tr>
</tbody>
</table>

**Table 2: Age wise distribution of samples**

<table>
<thead>
<tr>
<th>Age</th>
<th>Total</th>
<th>Positive</th>
<th>Percentage positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8 days</td>
<td>367</td>
<td>99</td>
<td>26.97%</td>
</tr>
<tr>
<td>8 days or more</td>
<td>85</td>
<td>28</td>
<td>32.94%</td>
</tr>
</tbody>
</table>

(p value: 0.42 > 0.05, Difference is statistically not significant)

**Table 3: Sex wise distribution of samples**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total</th>
<th>Positive</th>
<th>Percentage positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>185</td>
<td>51</td>
<td>27.56 %</td>
</tr>
<tr>
<td>Male</td>
<td>267</td>
<td>76</td>
<td>28.46 %</td>
</tr>
</tbody>
</table>

(p value: 0.87 > 0.05, Difference is statistically not significant)

**Fig. 2: AST pattern of Coagulase Negative Staphylococci (n=54)**
Discussion

Result shows that the common blood culture bacterial isolates from NICU are Coagulase Negative Staphylococci and Klebsiella spp. with blood culture positivity rate of 28.09%.

Out of 452 blood cultures included in the study, organisms were isolated in 127(28.09%) as shown in Fig. 1. Frequency and percentage positivity of all isolated organisms is shown in Table: 1. Gram positive and Gram Negative bacterial isolates were 76(59.84%) and 51 (40.16%) respectively. Similar studies conducted by Hitesh J Assudani et al.10, Patel D et al.11 and Shah Manisha et al.12 in Gujarat shows positivity rate of 35.34%, 43.36% and 20% respectively with Klebsiella spp. and Coagulase Negative Staphylococci or Staphylococcus aureus as commonly isolated organism.

Positivity rate depend on many factors like non bacterial causes of septicemia, anaerobic bacterial infection, ongoing antibiotic therapy, less volume of blood etc.10 Klebsiella spp. and Coagulase Negative Staphylococci are commonly isolated organism in different studies14,18,19 may be because of their nature as saprophyte or commensals,22 environmental factors, low birth weight or prematurity which makes them more susceptible to surrounding infections.10,23 Some studies also found E.coli and Staphylococcus aureus as commonly isolate organisms16,17,20,21 may be because of different geographical area.10,23 Age and sex wise distribution is shown in Table: 2 and Table: 3. Difference is statistically not significant. (p value < 0.05).

Coagulase Negative Staphylococci strains have highest sensitivity to Vancomycin (100%), Linezolid (100%), Teicoplanin (100%), followed by Chloramphenicol (92.6%), Levofloxacin (90.7%), Cefoxitin (83.3%), and Tetracycline
(77.8%) as shown in Fig. 2. 16.7% isolates were Meticillin Resistant Staphylococci (MRS). Similar study conducted by Patel D et al.11 shows 33% prevalence of MRS strains. Klebsiella spp. strains have highest sensitivity to Meropenem (73.9%), Imipenem (73.9%) and Amikacin (69.6%) followed by Chloramphenicol (65.2%), Tetracycline (60.9%) and Piperacillin Tazobactum (52.2%) as shown in Fig. 3. All strains shows higher resistance to Cephalosporins. 78.3% strains were Extended Spectrum Beta-Lactamase (ESBL) producers. Similar study conducted by Patel D et al.11, Hitesh J Assudani et al.10 and Shah Manisha et al.12 shows 94.87%, 37.5% and 43.47% ESBL Klebsiella spp. prevalence respectively. Only 4 strains (17.39%) were Multi drug resistance. (MDR) Similar study conducted by Mamatha P. Samaga et al.13 shows 83.3 % MDR gram Negative bacteria.

Acinetobacter spp. strains shows highest sensitivity to Levofloxacin (100%) and Polymyxin B (100%) followed by Tetracycline (80%), Amikacin (70%), Piperacillin-Tazobactum (60%), Imipenem (60%) and Meropenem (60%). Different cephalosporins shows sensitivity varying from 20-40%.

All strains of Staphylococcus aureus (n=8) were sensitive to Cefoxitin, Teicoplanin, Linezolid and Vancomycin along with fluoroquinolones. All strains of Enterococcus (n=12) were sensitive to Teicoplanin, Linezolid and Vancomycin.

Low prevalence of MRS strains and MDR organisms in our study suggest good hygienic practices among health care workers. Still lower susceptibility of Penicillin group and Cephalosporins among all bacteria create alarming sign for developing Anti Microbial resistance as they are commonly used drugs for Outpatient based treatment. Spread of these resistance strains leads to physical, financial and emotional burden to patients as well as community. There should be Antimicrobial usage guidelines based on local antimicrobial resistance data to reduce further development of resistance.

Conclusion

Klebsiella spp. and Coagulase Negative Staphylococci are common isolates in NICU. Resistance to frequently used Penicillin and Cephalosporin group is high among all bacterial isolates. Antimicrobial stewardship program should be implemented to make antibiotic policy at local level to deal the current scenario.

Conflicts of Interest: None.

References

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17. Vrishali Avinash Muley et al., Bacteriological Profile of Neonatal Sepsicemia in a Tertiary Care Hospital from Western India, J glob infect dis:2015.7(2):75-77.