Antimicrobial susceptibility pattern of blood culture isolates from patients with suspected neonatal sepsis in a tertiary care hospital in Pakistan

T Ghafoor¹, A Hussanain¹, MM Qureshi¹, T Mehmood¹, L Ali¹

Sri Lankan Journal of Infectious Diseases 2020 Vol.10 (1):30-37
DOI: http://dx.doi.org/10.4038/sljid.v10i1.8244

Abstract

Introduction: Neonatal septicemia is one of the commonest reasons for neonatal morbidity and mortality in developing countries. Knowledge of microbial flora and their susceptibility will help paediatricians decide on empirical treatment of neonatal sepsis.

Objective: To determine the antimicrobial susceptibility of blood culture isolates from patients with suspected neonatal sepsis in a tertiary care hospital in Pakistan.

Study design: A laboratory based prospective study was carried out in the Department of Pathology, Combined Military Hospital, Sialkot, from March 2017 to February 2019.

Methods: Blood culture and sensitivity testing was carried out on patients admitted for suspected neonatal sepsis from March 2017 to February 2019. Antimicrobial susceptibility testing of blood culture isolates was carried out by disk diffusion method using the Clinical Laboratory Standard Institute (CLSI) guidelines.

Results: During the study period, 345 blood cultures from 345 neonates (age range 1 day-28 days; mean age 13 days) were received by the laboratory, of which 76 were culture positive. Gram negative bacilli were isolated from 44 cultures and Gram positive cocci from the remaining 32. Staphylococcus aureus was the commonest isolate (n=28) followed by Escherichia coli (n=10), Klebsiella pneumoniae (n=9) and Acinetobacter spp. (n=8). Methicillin resistant S. aureus (MRSA) constituted (n=19) 25% of culture positive isolates. Pseudomonas aeruginosa was isolated from six patients. Serratia spp., Enterobacter spp. and Enterococcus spp. from four patients each and Proteus spp. isolated from three patients. Linezolid was the most effective antimicrobial against the isolated Gram positive cocci, with 93.7% sensitivity demonstrated by MRSA, methicillin sensitive S. aureus and enterococcus spp. followed by sensitivity to clindamycin at 92.8%. Among Gram negative isolates, more than 80% of E. coli, K. pneumonia

¹Combined Military Hospital (CMH) Sialkot/ National University of Medical Sciences (NUMS) Pakistan

Address for correspondence: Dr. Tahir Ghafoor, Consultant Microbiologist. Department of Pathology, Combined Military Hospital (CMH) Sialkot, Pakistan. Telephone: +923317013950 Email: drtahirghafoor@gmail.com

https://orcid.org/0000-0002-0848-763X
and *Acinetobacter* spp. were multidrug resistant. Susceptibility to polymyxin-B and tigecycline was shown by 88% and 71% of these isolates.

**Conclusion:** A high degree of antibiotic resistance was observed in both Gram positive and Gram negative isolates which reflects a dire need for culture facilities and antimicrobial susceptibility testing in hospitals in Pakistan. No group B β hemolytic streptococci were isolated, most likely due to predominantly late onset neonatal sepsis in this cohort.

**Keywords:** Neonatal sepsis, Antimicrobial susceptibility, Neonatal Intensive Care Unit (NICU)

**Introduction**

The presence of a positive blood culture historically constitutes the “gold standard” for diagnosis of neonatal sepsis.¹ Neonatal sepsis is a clinical condition showing systemic signs and symptoms due to bactereemia in the first month of life. Neonatal sepsis may also be a nosocomial infection which, by definition, is established within 48 to 72 hours after admission to a hospital.² Neonatal sepsis is frequently seen in neonatal intensive care units (NICU) and involves multiple factors including birth weight, sex, gestational age, parenteral nutrition, severity of disease, use of antimicrobials, repeated invasive procedures such as central venous catheterization, assisted ventilation as well as immunodeficiency during the neonatal period.³⁻⁵ The World Health Organization (WHO) estimates 1.6 million neonatal deaths per year occur globally due to sepsis including 40% of all neonatal deaths that occur in developing countries.⁶

Neonatal sepsis is life threatening if appropriate treatment is not given in time. Moreover, due to its nonspecific signs and symptoms, clinical diagnosis of this disease can be challenging. Microorganisms causing neonatal sepsis may show diversity in different countries and in hospitals of the same region and are of concern due to the presence of multidrug resistance.⁷ Antibiotics are among the most frequently used medications in the NICU.⁸ Use of antibiotics varies in different NICUs which necessitates implementation of antibiotic stewardship.⁹,¹⁰ Pediatricians and neonatologists must be made aware that starting antibiotics in certain circumstances may be more harmful than beneficial.¹¹ While prompt antibiotic therapy for possible infections in this vulnerable population is crucial for a good outcome, inadequate or inappropriate use of antibiotics results in a rising trend of multidrug resistant bacteria.¹²,¹³ Antibiotic susceptibility patterns vary geographically depending upon the prevalent local pathogens and common antibiotics being used in neonatal units.¹⁴ Emergence of antimicrobial resistance has become an alarming public health concern with non-responsiveness to available antibiotics.

Effective management of neonatal sepsis requires an understanding of the pathogenesis of neonatal sepsis along with up-to-date information on possible aetiological agents and their antibiotic sensitivity profile. For prompt diagnosis of neonatal sepsis, bacteriological culture of blood and representative samples plays an important role. A wide range of pathogens including *S. aureus, E. coli, K. pneumoniae, Proteus mirabilis, Enterobacter* spp., *P. aeruginosa* and *Enterococcus* spp. may cause neonatal sepsis. The wide variety of resistance mechanisms utilized by these bacteria has led to the emergence of multidrug resistant (MDR) bacteria which has forced clinicians to switch over to carbapenems, vancomycin and colistin.

Knowledge of common pathogens causing neonatal sepsis and their antimicrobial susceptibility profiles, nationally as well as in individual health care institutions, is of utmost importance to select
appropriate empirical antibiotic therapy and decrease neonatal morbidity and mortality. This study aimed to provide the antibiogram of pathogens causing neonatal sepsis in our setting. This would help in rational use of antibiotics as well as contribute to national data, assisting in formulating effective and up-to-date antibiotic guidelines for local and national use in neonatal sepsis.

Methods

This laboratory-based study was carried out at the Microbiology Department of the Combined Military Hospital (CMH) Sialkot, Pakistan. All blood cultures received from patients admitted to the NICU from March 2017 to February 2019 with suspected neonatal sepsis, regardless of gestational age and weight were processed and culture positive specimens were included in this study. Repeat samples from the same patient showing growth of similar microorganism and similar sensitivity pattern were excluded.

One to two ml of blood was collected aseptically before starting antimicrobial therapy into quality-controlled Brain Heart Infusion broth (BHI) at a blood:BHI ratio of 1:5 and incubated at 35 °C ± 2 for 24 hours. Blood cultures were then subcultured on Sheep Blood Agar (SBA), MacConkey agar and chocolate agar daily for 7 days. S. aureus (ATCC 25923), E. coli (ATCC 25922) and P. aeruginosa (ATCC 27853) were used as control strains for quality control of SBA, MacConkey agar, chocolate agar and Mueller-Hinton agar (MHA) plates. Blood agar and MacConkey agar plates were incubated aerobically, while chocolate agar plates were incubated in a CO₂ enriched humid atmosphere using a candle jar at 35 °C ± 2 for 24-48 hours. Blood culture bottles which showed no growth after incubation for 7 days were reported as negative.

Identification of the microorganisms was done using Gram staining, biochemical tests and serology. Analytical profile index API-20E (Biomerieux, France) was used to identify Enterobacteriaceae and associated organisms and API-20NE (Biomerieux, France) to identify Acinetobacter spp. and P. aeruginosa according to the manufacturer's directions. For the confirmation of S. aureus, gram staining, catalase test, DNase test and coagulase test were performed. Enterococcus spp. were identified using the Gram stain, catalase test, arabinose (1%), bile esculin (40%) and NaCl broth (6.5%) tests.

Antibacterial susceptibility of the isolates with 0.5 McFarland standards was done on prepared MHA (MAST Diagnostics, UK) using the Kirby-Bauer disk diffusion method as per the CLSI protocol. Commercially available standard antibiotic discs (Oxoid UK) were used. The zones of inhibition were measured and recorded according to the CLSI guidelines. Amikacin (30 µg), imipenem (10 µg), gentamicin (30 µg), ceftriaxone (30 µg), amoxicillin / clavulanic acid (20/10 µg), doxycycline (30 µg), ciprofloxacin (5 µg), tigecycline (15 µg), trimethoprim / sulfamethoxazole (1.25/23.75 µg), ampicillin (25 µg), linezolid (30 µg), piperacillin / tazobactam (100/10 µg), cefepime (30 µg), and cefoperazone / sulbactam (75/30 µg) were used for susceptibility testing while cefoxitin (30 µg) was used for MRSA screening. Vancomycin could not be tested due to non-availability of e-strips for measuring MICs.

The data obtained was entered in Statistical Package for Social Sciences (SPSS) version 17 for statistical evaluation. Descriptive statistics including mean and standard deviation for age, percentages of variables including gender and antimicrobial susceptibility pattern of bacteria isolated in samples were calculated.
Results

During the two year study period, 345 samples were submitted. Excluding coagulase negative staphylococci (CONS), 76 (22%) yielded growth, of which 30 (39.5%) were from female patients while 46 (60.5%) were from males giving a male to female ratio of 1:1.5. The mean age of patients was 13 days, ranging from 1 to 28 days. Gram negative bacilli constituted the majority of isolates (n=44; 57.9%) with Gram positive cocci isolated in 32 (42.1%) of patients. E. coli was the most common Gram negative isolate (n=10; 13.1%). S. aureus was the commonest Gram positive isolate (n=28; 36.8%). The frequency of culture positive isolates in patients with suspected neonatal sepsis is shown in Table 1.

More than 90% of Gram positive isolates were susceptible to linezolid and clindamycin while Gram negative isolates, especially the Enterobacteriaceae group exhibited excellent susceptibility to polymyxin-B, tigecycline, carbapenems and amikacin. The sensitivity pattern of all blood culture isolates is listed in Tables 2 and 3.

Table 1: Frequency of bacteria isolated from blood culture in patients with neonatal sepsis (n=76)

<table>
<thead>
<tr>
<th></th>
<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>No</td>
<td>10</td>
<td>9</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>%</td>
<td>13</td>
<td>11.8</td>
<td>5.3</td>
<td>7.9</td>
<td>5.3</td>
<td>5.3</td>
<td>10.5</td>
<td>3.9</td>
<td>25</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Table 2: Susceptibility pattern of Gram positive isolates (n=32)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MRSA 19</th>
<th>MSSA 9</th>
<th>Enterococcus spp 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>S NT</td>
<td>R NT</td>
<td>S R</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>6 NT</td>
<td>13 NT</td>
<td>6 3</td>
</tr>
<tr>
<td>Amikacin</td>
<td>9 NT</td>
<td>10 NT</td>
<td>9 0</td>
</tr>
<tr>
<td>Trimethoprim/ sulfamethoxazole</td>
<td>6 NT</td>
<td>13 NT</td>
<td>2 7</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>12 NT</td>
<td>7 NT</td>
<td>4 5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6 NT</td>
<td>13 NT</td>
<td>2 7</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>17 NT</td>
<td>2 NT</td>
<td>9 0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2 NT</td>
<td>17 NT</td>
<td>2 7</td>
</tr>
<tr>
<td>Linezolid</td>
<td>18 NT</td>
<td>1 NT</td>
<td>9 0</td>
</tr>
</tbody>
</table>

n = number of isolates, S=sensitive, R= resistant; NT- Not tested as per CLSI guidelines
Discussion

Pakistan is the 7th most populous country in the world and number three among those countries in neonatal deaths with 298000 deaths occurring annually. In Pakistan, the neonatal mortality rate is 49/1000 live births and we account for 7% of global neonatal deaths. Among developing countries like Pakistan where high-tech diagnostic facilities are sparse, newer mutant bacterial strains are also emerging making it more difficult to control infections. Effective management of patients suffering from neonatal sepsis requires identification of the bacterial isolate along with selection of an appropriate and effective antibiotic for appropriate treatment.

Microbial resistance to antibiotics is an ongoing serious problem in the treatment of neonatal sepsis. This study highlights the high rate of antimicrobial resistance among bacterial pathogens isolated from blood cultures of neonates admitted to the NICU and confirms that Sialkot and surrounding area is no exception to progressive antimicrobial resistance in major bacterial pathogens. Surprisingly, Group B β hemolytic streptococci were not isolated in our study. One possible reason is that most of the patients in our study were probable late onset neonatal sepsis.

Table 3: Susceptibility pattern of Gram negative isolates (n=44)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
<th>Serratia spp.</th>
<th>Proteus spp</th>
<th>Enterobacter spp</th>
<th>Acinetobacter spp</th>
<th>Pseudomonas spp</th>
<th>Overall sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>S 0</td>
<td>R 10</td>
<td>NT 2</td>
<td>NT 6</td>
<td>NT 3</td>
<td>NT 4</td>
<td>NT 2</td>
<td>0/10</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2/40</td>
</tr>
<tr>
<td>Amikacin</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>5/38</td>
</tr>
<tr>
<td>Amoxicillin / clavulanate</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>9</td>
<td>NT</td>
<td>NT</td>
<td>1</td>
<td>2/44</td>
</tr>
<tr>
<td>Imipenem</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1/17</td>
</tr>
<tr>
<td>Meropenem</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2/44</td>
</tr>
<tr>
<td>Cefoperazone / sulbactam</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1/34</td>
</tr>
<tr>
<td>Piperacillin / tazobactam</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2/44</td>
</tr>
<tr>
<td>Trimethoprim / sulfamethoxazole</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2/44</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>NT</td>
<td>3/35</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>1/35</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>2/34</td>
</tr>
<tr>
<td>Cefepime</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>6/38</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>7</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>NT</td>
<td>NT</td>
<td>3/36</td>
</tr>
<tr>
<td>Polymyxin-B</td>
<td>10</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>4/35</td>
</tr>
</tbody>
</table>

NT - Not tested as per CLSI guidelines
Similar to our results, most previous studies also emphasized the major role of *E. coli*, *K. pneumoniae* and *S. aureus* as the main microorganisms causing neonatal sepsis.\textsuperscript{18} The causative agents of neonatal sepsis change over time and may vary from place to place. In the current study, among Gram negative organisms, *E. coli* was the most commonly isolated followed by *K. pneumoniae* while among Gram positive organisms *S. aureus*, especially MRSA was the most frequent. This finding is consistent to a previous study in a Pakistani population.\textsuperscript{19} Shrestha et al. also identified *E. coli* as the predominant organism causing neonatal sepsis.\textsuperscript{20} Resistance of sepsis related pathogens to routinely used antibiotics has been reported widely. The high levels of resistance to gentamicin in this study are comparable to a Pakistani study by Aftab et al.(2006) and an Iranian study by Marzban A et al.(2010).\textsuperscript{19,21} In our study, isolates showed considerable resistance to ceftriaxone. This emerging resistance to the third generation cephalosporins is also supported by studies from Pakistan\textsuperscript{22} and Nepal.\textsuperscript{23} Our study showed that 84% of Gram negative rods were resistant to ceftriaxone as compared 66.9% observed in another Pakistani study done in 2017.\textsuperscript{24} This rising trend of ceftriaxone resistance by common pathogens may be due to the irrational use of this drug in Pakistan. In our study, amikacin was the most effective aminoglycoside for both Gram positive and Gram negative microorganisms which correlates with the findings of Muley et al.\textsuperscript{25} Not unexpectedly, our study demonstrated a high level of resistance to commonly used antibiotics like ampicillin, amoxicillin clavulanic acid and gentamicin in both Gram positive and Gram negative pathogens causing neonatal sepsis. A Nepalian study by Nepal HP et al. reported more than 90% resistance of Gram negative rods to cotrimoxazole which is also in conformity to our study.\textsuperscript{26}

An Indian study done in 2017 showed 100% sensitivity of *Pseudomonas* spp to colistin and 54% sensitivity to cefepime, while *S. aureus* showed more than 90% sensitivity to linezolid and clindamycin, which was also confirmed by our study.\textsuperscript{27} Another study from Pakistan also showed 100% susceptibility of *S. aureus* to linezolid.\textsuperscript{28} However, in contrast to our results, the Indian study showed less resistance of *Enterobacteriaceae* to carbapenems and aminoglycosides.\textsuperscript{26} Our study results on *Acinetobacter* spp. sensitivity to carbapenem and gentamicin are also consistent to those of a study done in Paraguay.\textsuperscript{29} A South African study reported 100% piperacillin-tazobactam and 80% meropenem resistance in *Acinetobacter* spp which is also comparable to our study results.\textsuperscript{30} The main reason for variation in antibiotic susceptibility pattern might be due to variant pattern of antimicrobial use in different hospitals or to the emergence of multidrug resistant strains as a result of widespread inappropriate use of antibiotics.

In the current study, a larger number of the *Enterobacteriaceae* were susceptible to tigecycline polymyxin (Table 3) and it is imperative that these drugs should be used with caution and according to susceptibility results in our hospitals.

**Conclusion**

*S. aureus*, *E. coli*, *K. pneumoniae* and *Acinetobacter* spp were the predominant causes of neonatal sepsis in our study which serves as a surveillance of antimicrobial resistance in the NICU for a 2 year period. The study demonstrates a high level of resistance to commonly used first line antibiotics in the hospital and provides information for use and further investigation by the hospital infection control team. Continuing surveillance of antimicrobial resistance is recommended to get
regular updates on the susceptibility of pathogens causing neonatal sepsis to the broader spectrum antibiotics available for clinical use in Pakistan.

**Limitations of study:** This study covered only Sialkot and surrounding rural areas. Blood samples could not be segregated on the basis of early and or late onset suspected neonatal sepsis.

**Funding:** This work was completely supported by Combined Military Hospital (CMH) Sialkot, Pakistan.

**Ethical clearance:** Permission was obtained from the Hospital Ethics and Research Committee.

**Conflict of interest:** The material for research purpose was made available by the hospital. However, there were neither conflict of interest of authors with the material provider companies nor any financial or other gains from the companies.

**References**


