In vitro Activities of 6 Antimicrobial Agents against Bacterial Isolates from Cases of Neonatal Meningitis in Kano, Nigeria

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Abstract
Meningitis is inflammation of meninges, which affects all age groups from the newborn to elderly and occurs more commonly during the first. It is usually caused by viral, bacterial or fungal pathogens. The objective of the study was to determine the antimicrobial susceptibility of bacterial isolates from neonatal meningitis cases at Murtala Muhammad Specialist Hospital (MMSH), Kano, Nigeria. Sixty neonates with suspected meningitis were enrolled of whom 10 were excluded due to contraindications to lumbar puncture (LP) or failed LP. Microscopy and culture were performed on all the fifty samples. The antibiotic susceptibility testing of the bacterial isolates from cases of neonatal meningitis to ampicillin (10µg), amoxycillin (25µg), ceftriaxone (30µg), ceftazidime (10µg), chlorampenicol (30µg) and gentamicin (10µg) was performed using the modified disc diffusion method (modified Kirby-Bauer technique). Bacteria were isolated in cerebrospinal of six neonates (12.0%), of which three isolates were Streptococcus pneumoniae, and the other 3 isolates were Escherichia coli, Pseudomonas aeruginosa, Klebsella pneumoniae and Acinetobacter. All isolated bacteria were resistant to ampicillin, amoxycillin, chlorampenicol and gentamicin but were sensitive to ceftriaxone and ceftazidime except for one Pseudomonas aeruginosa isolate which was resistant to ceftriaxone. It is recommended that neonates in Kano and its environment presenting with specific signs/symptoms of bacterial meningitis should be empirically treated with cephalosporins as first line therapy as confirmatory bacteriological tests are undertaken.

Key words: Prevalence, aetiologic agents, neonates, meningitis antibiotics, resistance,
neonatal meningitis continue to contribute substantially to neurological disability (Thaver and Zaidi, 2009).

African experiences a disproportionately large burden of meningitis due to its young population (Bell et al., 1989). Bacterial meningitis in Africa is associated with high case fatality and frequent neuropsychological sequelae. Neonatal meningitis remains a serious problem with the high mortality of 60% (Bell et al., 1989). This study evaluates antimicrobial susceptibility profile of bacterial agents causing neonatal meningitis in Kano, Nigeria.

**MATERIALS AND METHODS**

**Study design**

Sixty neonates admitted with suspected neonatal sepsis at Murtala Muhammad specialist Hospital over the period of March 2015 and April 2015 was enrolled into the study. Among these 60 neonates that satisfied the inclusion criteria, 10 of them were excluded for different reasons (6 had failed lumbar puncture and 4 had clear contraindications to lumbar puncture), therefore, 50 neonates were analyzed and reported in results.

**Collection of Cerebrospinal Fluid**

Sixty samples of Cerebrospinal Fluid were collected using a lumbar puncture between L4-L5 with Spinal needle gauge 22 or 23 by the Medical Officers. CSF containing bottles were transported to Medical Microbiology laboratory of Murtala Muhammad specialist Hospital as soon as possible and not later than 30 minutes after the lumbar puncture. The appearance of the CSF was recorded even before taking it to the laboratory. CSF was termed as turbid if one could not read well a letter through the CSF bottle.

**Isolation of Bacteria from Neonatal CSF**

Fresh CSF was centrifuged using bench centrifuge for 10 minutes at 3000 revolutions per minute to get the sediment of centrifuged CSF. At least 20-50μL of the sediment was inoculated with a sterile pipette on to chocolate and blood agar plates (Oxoid, UK). The inoculated culture media of chocolate and blood agar were incubated for at least 24 hours at 37°C in candle extinction jars to provide 5-8% carbon dioxide. Growth was checked after 24 hours. The chocolate and blood agar plates were prepared according to the manufacturers’ instruction.

**Antibacterial Susceptibility Testing**

Antimicrobial susceptibility testing was performed using the modified disc diffusion method (modified Kirby-Bauer technique). This method used Müller-Hinton agar. Antibiotics tested in this study included ampicillin, amoxycillin, ceftriaxone, ceftazidime, chloramphenicol and gentamicin. Results were interpreted based on criteria of National committee on clinical laboratory standards (CLSI, 2007).

**Preparation of turbidity standard equivalent to 0.5 McFarland scale**

Approximately 85 ml of 1% sulfuric acid (H₂SO₄) was added to a 100ml volumetric flask. Using a volumetric pipette, 0.5ml of 1.175% anhydrous barium chloride (BaCl₂) was added drop wise to the 1% sulfuric acid (H₂SO₄) while constantly swirling the flask. The volume was brought to 100 with 1% H₂SO₄, after which it was stirred or mixed for approximately 3 to 5 minutes while examining visually, until the solution appears homogeneous and free of clumps (Cheesbrough, 2010).

**Antibiotic Discs**

The susceptibility testing of isolates to some conventional antibiotics was carried out by the disk diffusion method according to the clinical laboratory guideline (NCCLS, 2006). The antibiotics to be tested include; ampicillin (10µg), amoxycillin (25µg), ceftriaxone (30µg), ceftazidime (10µg), chloramphenicol (30µg) and gentamicin (10µg). Staphylococcus aureus (ATCC 25923) was used as control in every tests run. Results were interpreted based on criteria of National committee on clinical laboratory standards (CLSI, 2007).

**Modified Kirby-Bauer disc diffusion method**

A bacterial suspension adjusted to 0.5 McFarland standard was inoculated onto Muller Hinton agar using sterile cotton swab. Filter paper discs containing the antibiotics above were then aseptically placed on the inoculated Muller Hinton agar. All plates were incubated at 35°C for 24 hours. The diameter of zone of inhibition was then measured according to the National committee on clinical laboratory standards (CLSI, 2007).

**Ethical Considerations**

This research work was approved by ethical and review committee of Murtala Muhammad specialist Hospital, Kano. All essential ethical considerations to ensure the confidentiality of the identity of the patients were taken. The parents/guardians of the patients had the details of the study fully explained to them before recruitment followed by consent through signing of the written informed consent form.

**RESULTS**

Of the 50 neonates enrolled, bacterial culture of CSF was positive in six neonates (12%). Three of these isolated bacteria were Streptococcus pneumoniae while the other three were Escherichia coli, Pseudomonas, Klebsiella pneumoniae with one isolate each (Table 1).
Among these isolates, the three *Streptococcus pneumoniae* were isolated from newborns that had late onset meningitis (age 8-28 days). *Escherichia coli* and *Pseudomonas aeruginosa* were isolated from neonates who had early onset meningitis (age 0-7 days) while *Klebsiella pneumoniae* was isolated from preterm neonates (Table 2).

The result of the antibiotic susceptibility test showed that all the three isolates of *Streptococcus pneumoniae* were sensitive to the two cephalosporins used [ceftriaxone (30µg) and ceftazidime (10µg)] but resistant to ampicillin (10µg), amoxycillin (25µg), chloramphenicol (30µg), and gentamycin (10µg). All the Gram-negative bacteria isolated were sensitive to cephalosporins and resistant to ampicillin (0µg), amoxycillin (25µg), gentamycin (10µg), and chloramphenicol (30µg). The only one *Pseudomonas aeruginosa* recovered was found to be resistant to ceftriaxone (30µg) (Table 3).

### Table 1: Distribution of bacterial isolates among suspected neonatal meningitis

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>No. of isolates (n=6) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-Positive</strong></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>3(50.0)</td>
</tr>
<tr>
<td><strong>Gram-Negative</strong></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1(16.7)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>1(16.7)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1(16.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6 (100)</td>
</tr>
</tbody>
</table>

### Table 2: Distribution of bacterial isolates according to Neonate Age

<table>
<thead>
<tr>
<th>Neonate Age</th>
<th><em>Streptococcus pneumoniae</em></th>
<th><em>Escherichia coli</em></th>
<th><em>Klebsiella pneumoniae</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>&lt;7 days</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8-28 days</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 3: Antibiotic susceptibility of the bacterial isolates from cases of neonatal meningitis

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>Streptococcus pneumoniae</em> (n=3)</th>
<th><em>Escherichia coli</em> (n=1)</th>
<th><em>Klebsiella pneumoniae</em> (n=1)</th>
<th><em>Pseudomonas aeruginosa</em> (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (10µg)</td>
<td>S 0 R 0</td>
<td>S 0 R 0</td>
<td>S 0 R 0</td>
<td>S 0 R 0</td>
</tr>
<tr>
<td>Amoxycillin (25µg)</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Ceftriaxone (30µg)</td>
<td>3 0</td>
<td>1 0</td>
<td>1 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Ceftazidime (10µg)</td>
<td>3 0</td>
<td>1 0</td>
<td>1 0</td>
<td>1 0</td>
</tr>
<tr>
<td>Chloramphenicol (30µg)</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Gentamicin (10µg)</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

R= Resistant S= Sensitive

### DISCUSSION

The prevalence and distribution of the isolates from cerebrospinal fluid were similar to those reported previously (Morena et al., 2006). The most common clinical features observed in this study were similar to those found in study done by Laving et al. (2003) on neonatal bacterial meningitis in Kenya. In this study, no group B streptococci were isolated; this corresponds to what is known that group B streptococcus appears to be much less frequent cause of neonatal meningitis in developing countries (Jones et al., 2004).

In this study, *Streptococcus pneumoniae* accounted for 50% of organisms isolated. For the confirmed early onset neonatal bacterial meningitis pseudomonas and *Escherichia coli* were the enteric Gram-negative organisms, which were isolated. In two subjects, *Streptococcus pneumoniae* was isolated in late onset. These finding are consistent with the study done by Heath et al. (2003) which have reported that gram negative enteric organisms appeared to account for the majority of early onset meningitis.
onset bacterial meningitis and Streptococcus pneumoniae for late onset meningitis in developing countries (Heath et al., 2003). Both Escherichia coli and Klebsiella pneumoniae were isolated in this prospective study and this finding is in agreement with previous study done in Ethiopia (Gebremariam, 1998).

All bacterial isolates in this study were susceptible to ceftriaxone and ceftazidime except for one Pseudomonas aeruginosa isolate which was resistant to ceftriaxone, this is consistent with the finding reported by Laving et al in their study done in Kenya where by the majority of gram negative isolates were highly resistant to the first line antibiotics, ampicillin and gentamycin (Laving et al., 2003) however in another study, ampicillin and gentamycin were prescribed as a treatment for majority of neonatal bacterial meningitis cases as the report by the previously done local retrospective study (Melese, 2011) and still now the practice is the same. The findings also agrees with those reported by Andargachew et al in their study in Ethiopia, which reported resistance to commonly prescribed antibiotics ampicillin and gentamycin for bacterial isolates from CSF (Andargachew et al., 2005).

CONCLUSION
Bacteria were detectable in 12.0% of neonates with clinical meningitis and the isolated bacteria were predominantly Streptococcus pneumoniae. Both gram-positive and gram-negative isolates were susceptible to the two cephalosporins (ceftriaxone and ceftazidime) and resistant to penicillins (amoxycillin and ampicillin) and aminoglycosides (chloramphenicol and gentamycin) tested.

RECOMMENDATION
From the findings of this study it is recommended that neonates in Kano and its environment presenting with specific signs/symptoms of meningitis should be empirically treated with any of the two cephalosporins (ceftriaxone and ceftazidime) as first line therapy as confirmatory bacteriological tests are undertaken.

REFERENCES


