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

Kumurya, A. S.

Department of Medical Laboratory Science, Faculty of Allied Health sciences, Bayero University, P.M.B. 3011, Kano-Nigeria

Correspondence: askumurya.med@buk.edu.ng; Phone: +2347032153561

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 cases neonatal meningitis 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,

INTRODUCTION

Meningitis is inflammation of meninges usually caused by viral, bacterial or fungal pathogens. Bacterial meningitis is potentially a life-threatening infection that is associated with high rates of morbidity and mortality (Gebremariam, 1998; Andargachew *et al.*, 2005). The burden of bacterial meningitis in developing countries ranges between 1.1 –1.9 cases per 1000 live births higher than 0.2-0.5 cases per 1000 live births in Europe (Laving *et al.*, 2003). Meningitis affects all age groups from the newborn to the elderly. Until recently, up to 50% of patients who survived acute meningitis infection were left with permanent sequelae such as mental retardation and hearing loss (Melese, 2011).

Meningitis occurs more commonly during first month of life (Roberton, 2012). Signs and symptoms are non specific and indistinguishable from those of septicemia and other non-infective causes such as respiratory distress syndrome, birth asphyxia, and hypoglycaemia. In developed countries, group B *Streptococci* are found to be the most common aetiology of bacterial meningitis. Therefore, identifying and treating maternal genitourinary infection is being used as a prevention strategy. In the developing countries, gram-negative bacilli are more common than Group B *Streptococcus*. The mortality varies based on the treatment, with survival rates being 17% to 29% and complication rates being 15% to 68%. Despite the preventive measures and the availability of medicines, the incidence of newborn bacterial meningitis for the last 30yrs has remained constant (Laving *et al.*, 2003; Luzia *et al.*, 2007).

Bacterial meningitis is a serious often disabling and fatal infection, which causes 170,000 deaths worldwide each year (Thaver and Zaidi, 2009). It is a common infection often unrecognized and partially treated with sepsis.

Due to immaturity of their immune systems, young infants are particularly vulnerable to bacterial meningitis and poor outcomes may occur. Despite the development of effective vaccines, useful tools for rapid identification of pathogens and potent antimicrobial drugs,

neonatal meningitis continue to contribute substantially to neurological disability (Thaver and Zaidi, 2009).

Africa experiences a disproportionally 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 contra indications 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 10minutes 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 24hours at 37^0C 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 Müeller-Hinton agar. Antibiotics tested in this study included ampicillin, amoxycillin, cefriaxone, ceftazidime, chlorampenicol 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 (H_2SO_4) was added to a 100ml volumetric flask. Using a volumetric pipette, 0.5ml of 1.175% anhydrous barium chloride ($BaCl_2$) was added drop wise to the 1% sulfuric acid (H_2SO_4) while constantly swirling the flask. The volume was brought to100 with 1% H_2SO_4, 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), chlorampenicol (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 24hours. 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 (12.0%). 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

	Bacterial isolates	**No. of isolates** (n=6) (%)
Gram-Positive	*Streptococcus pneumoniae*	3(50.0)
Gram-Negative	*Escherichia coli*	1(16.7)
	Klebsiella pneumoniae	1(16.7)
	Pseudomonas aeruginosa	1(16.7)
Total		**6 (100)**

Table 2: Distribution of bacterial isolates according to Neonate Age

Neonate Age	**Bacterial isolates**			
	Streptococcus pneumoniae	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
Preterm	0	0	1	0
<7 days	0	1	0	1
8-28 days	3	0	0	0

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

Antibiotics	*Streptococcus pneumoniae* (n=3)		*Escherichia coli* (n=1)		*Klebsiella pneumoniae* (n=1)		*Pseudomonas aeruginosa* (n=1)	
	S	R	S	R	S	R	S	R
Ampicillin (10µg)	0	0	0	0	0	0	0	0
Amoxycillin (25µg)	0	0	0	0	0	0	0	0
Ceftriaxone (30µg)	3	0	1	0	1	0	0	0
Ceftazidime (10µg)	3	0	1	0	1	0	1	0
Chloramphenicol (30µg)	0	0	0	0	0	0	0	0
Gentamicin (10µg),	0	0	0	0	0	0	0	0

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 bacterial meningitis and *Streptococcus pneumoniae* for late onset meningitis in developing countries (Heath *et al*., 2003). Both *Escherichia coli* and *Klebsiella pneumonia* 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.

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