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Pattern and antimicrobial sensitivity of pathogens in acute bacterial meningitis beyond neonatal period at Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria

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Abstract The causative agents of acute bacterial meningitis vary from time to time and from place to place. In addition, changes in pathogen's sensitivity to antibiotics in use occur. The study was undertaken to identify the pattern and susceptibility of pathogens to antibiotics among children beyond neonatal period for prompt empirical treatment of this important cause of morbidity and mortality especially in developing countries.

Objective. To identify the pattern and susceptibility of pathogens in acute bacterial meningitis among children aged one month to 12 years at ABUTH Shika, Zaria.

Method: This was a prospective study carried out in children presenting to Emergency Pediatric Unit of ABUTH Shika, Zaria, from October 1st 2005 to September 30th, 2006. Children who met the inclusion criteria for lumbar puncture were consecutively recruited. Cerebrospinal fluid (CSF) samples were collected for microscopy, culture, sensitivity, and Latex Particle Agglutination (LPA) test. Sixty-six (66) patients satisfied the criteria for acute bacterial meningitis and were studied.

Results: The prevalence of acute bacterial meningitis in this study was 6.9%. Sixty-six patients with acute bacterial meningitis were identified using 3 diagnostic methods (Gram stain, culture and LPA). The 3 commonest organisms were *Streptococcus pneumoniae* 34 (51.5%), *Haemophilus influenzae* 18 (27.3%), and *Neisseria meningitidis* 12 (11.2%). The sensitivity of the isolates to three commonly used non-cephalosporin antibiotics was chloramphenicol 74.4%, penicillin 38.5%, and ampicillin 2.6%. The sensitivity to the cephalosporin was cefotaxime 97.4%, and ceftriaxone 89.7% while that to Ofloxacin, a quinolone, was 100%.

Conclusion: The three commonest bacterial agents causing post neonatal meningitis were *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*. There is an increasing resistance to the three commonly used non-cephalosporin antibiotics (chloramphenicol, ampicillin and penicillin) and hence, cefotaxime or ceftriaxone should be used in the empirical treatment of children with suspected acute bacterial meningitis beyond neonatal period.

Introduction

Acute bacterial meningitis is an important cause of morbidity and mortality throughout the world.[1] It is among the commonest neurological disorders in children in the tropical countries.[2] it is of note that the disease remains a significant cause of morbidity and mortality in many developing countries despite advances in medical treatment.[2,3] it has been observed that many pathogens can cause meningitis, but however, *Haemophilus influenzae* was reported to be the common cause in infancy and toddler-age group in pre-Hib (*Haemophilus influenzae* type b) vaccine era.[4,5] The predominance of Hib in infancy and toddlers in pre Hib vaccine era was more in developed countries,whereas reports from African studies including Nigeria found *Streptococcus pneumoniae* and *Neisseria meningitidis* as the two most important pathogens.[6]

The causative agents of meningitis varies from place to place and alsotheir antibiotic susceptibility and therefore knowledge of the locally predominant organisms in different age groups and their sensitivity pattern is essential for effective chemotherapy. Furthermore,for a poten-

tially fatal infection like childhood meningitis,the need for periodic assessment of the continuing sensitivity of the causative agents to antimicrobial in use is necessary. This is particularly necessary especially where non- existenceof rapid investigative modalities leave the clinician with no choice but to initiate empirical treatment. The objectives of the current study were therefore, to identify the pattern and antimicrobial sensitivity of pathogens in acute bacterial meningitis beyond neonatal period at ABUTH Shika, Zaria.

Subjects and Methods

The study was conducted in the Emergency Pediatric Unit (EPU) of ABUTH Shika, Zaria from October 1st 2005 to September 30th, 2006. The approval of ABUTH Shika, Zaria Ethical Committee was sought for and obtained before the commencement of the study. Also informed parental/Care giver consent was sought before lumbar puncture (LP).Children aged one month to 12 years with suspected acute bacterial meningitis had LP. The criteria for LP for children < 18 months are as follows: presence of fever and convulsion with or without either vomiting, irritability, loss of consciousness, and/ or bulging anterior fontanelle while for children > 18 months, LP was done in the presence of: (a) fever and convulsion plus presence or absence of typical signs of meningeal irritation (defined by neck stiffness, positive Kernig's and/ or Brudzinski's sign), (b) fever without convulsion plus typical signs of meningeal irritation. For the purpose of this study, acute bacterial meningitis was defined as the presence of at least one of the following features: bacterial growth on CSF culture, positive CSF Latex Particle Agglutination (LPA) or an organism seen on Gram stain of CSF.[7] The CSF samples collected in the sterile bottles were subjected to standard Microbiology culture, sensitivity, and Gram staining technique.[8] CSF culture was regarded as the gold standard method for the diagnosis of acute bacterial meningitis. The inclusion of LPA in the diagnostic methods in this study was done to improve the diagnostic yield as culture of some of the bacterial agent from CSF can be difficult because of their fastidious nature. Also LPA was included in the diagnostic method because the results of CSF Gram stain and culture may be altered by prior use of antibiotics, a common problem in developing countries.[9]The LPA test was performed on CSF supernatant using PASTOREX MENINGITIS according to the manufacturer's instruction (BIO-RAD France). The test kits can identify *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus group B*, and *Escherichia coli.*The sensitivity of the test kits reagent was 70% to 90% while specificity was 95%. The CSF supernatant was heated for 3 minutes at 100^{O}C in order to denature CSF proteins and to prevent non-specific agglutination and was then centrifuged at 17000 revolutions per minute (rpm) for five minutes. The reagents were shaken and one drop (30 microlitre) of each dispensed to corresponding reaction fields on the latex agglutination card and in the two wells of the two plastic reactions slides. Each card has seven reaction fields corresponding to the reagents. Forty microlitre of test specimen was dispensed to each field and thoroughly mixed using the stirrer (supplied with the kits) for each field the mixture covered the entire field completely. For the plastic reaction slides, it was ensured that the latex-sample mixture migrated into the capillary of each slide. The result was read within 10 minutes. A uniform white precipitate appearing in only the reaction field was considered positive, indicating the presence of antigen of the corresponding organism. The test was considered negative if the mixture remained homogenous.

Data collected were entered into computer and Epi-info version 3.3.2 was used for analysis. Simple tables were drawn for the frequencies and percentages. Chi –square test was used for comparison of proportions. A p-value < 0.05 was considered statistically significant.

Results

Sixty-six patients who met the inclusion criteria were studied. During the study period, 956 children aged one month to 12 years were admitted into EPU. The prevalence of bacterial meningitis was 6.9% (66 of 956). Thirty-nine (59.1%) patients were males and 27 (40.9%) females. The ages ranged from one month to 12 years with a mean age (+_1 SD) of 42.7 +_ 41.3 months. The male to female ratio (M: F) was 1.4:1.

Infections and age

The distribution of patients with acute bacterial meningitis by age is shown in table 1. Twenty-seven (40.9%) of the 66 patients with acute bacterial meningitis were infants, 20 (30.3%) were aged 13 months to four years while 19 (28.8%) were above four years of age.

Table 1: Distribution of cases of acute bacterial meningitis in 66 patients by age.

Age in months	Frequency	Percentage
1-2	6	9.1
3-12	21	31.8
13-24	6	9.1
25-48	14	21.2
49-144	19	28.8
Total	66	100

Pathogens and age

The distribution of pathogens in CSF was *Streptococcus pneumoniae* 34 (51.5%), *Haemophilus influenzae* 18 (27.3%), *Neisseria meningitidis* 12 (18.2%), Escherichia coli 1 (1.5%), and Enterobacter specie 1 (1.5%). Eleven (32.4%) of pneumococcus and 12 of *Haemophilus influenzae* occurred during infancy while only 2 (16.7%) cases of *Neisseria meningitidis* were present during infancy.

Table 2 The distribution of pathogens in CSF of 66 patients with meningitis

Organisms	Age groups (months) 1-2	3-12	13-24	25-48	49-144	Total(%)
Streptococcus pneumoniae	1	10	5	10	8	34(51.5)
Haemophilus influenzae	3	9	1	3	2	18(27.3)
Neisseria meningitidis	-	2	-	1	9	12(18.2)
*Escherichia coli**	1	-	-	-	-	1(1.5)
*Enterobacter species***	1	-	-	-	-	1(1.5)
Total	6	21	6	14	19	66(100)

*Isolated in a 6- week old infant
** Isolated in a 2- month old infant

Laboratory diagnosis

The result of the three methods used to identify the aetiological agent in CSF of 66 patients is shown in table 3. LPA detected 53 (80.3%) of 66 agents in CSF, Gram stain identified 47 (71.2%) and CSF culture isolated 39 (59.1%) organisms. Among the 13 (19.7%) agents missed by LPA, Gram stain detected 11 organisms while culture yielded two. The 27 (40.9%) cases that are negative by CSF culture were positive by LPA (18) and Gram stain (9).

Table 3: Results of the 3 methods used to identify the aetiological agents in the CSF of 66 patients.

Method	Positive No.(%)	Negative No.(%)
Gram stain	47 (71.2)	19 (28.8)
LPA	53 (80.3)	13 (19.7)
Culture	39 (59.1)	27 (40.9)

Relationship between CSF culture, Gram stain, LPA, and the use of antibiotics before presentations

A history of use of antibiotics before presentation was obtained in 30 (45.5%) of 66 patients.Among the 30 patients on antibiotics before presentation 13, 12 and 24 were positive for culture, Gram stain and LPA respectively. There was a statistically significant relation between the use of antibiotics before presentation and negative CSF culture(13/39 (33.3%) versus 17/27 (62.7%); $x^2 = 4.52$, $p = 0.03$) and Gram stain (12/47 (25.5%) versus 18/19 (94.7%); $x^2 = 14.04$, $p = 0.00$) while LPA was not affected by prior use of antibiotics (24/53 (45.3%) versus 6/13 (46.2%); $x^2 = 0.06$, $p = 0.79$).

Sensitivity of the pathogens to antibiotics

The sensitivity pattern of the 39 bacterial isolates from CSF culture to antibiotics is shown in table 4.

Table 4: CSF bacterial isolates and their in-vitro antibiotic sensitivity rates.

Organisms	Antibiotics CHL	PEN	AMP	CEF	CEFO	OFL
Streptococcus pneumoniae (n=22)	18 (81.8)	11 (50)	0 (0)	21 (95.5)	22 (100)	22 (100)
Haemophilus influenzae (n=9)	6 (66.7)	0 (0)	0 (0)	7 (77.8)	9 (100)	9 (100)
Neisseria meningitidis (n=6)	4 (66.7)	4 (66.7)	0 (0)	6 (100)	6 (100)	6 (100)
Escherichia coli (n=1)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)
Enterobacter species (n=1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
All isolates (n=39)	29 (74.4)	15 (38.5)	1 (2.6)	35 (89.7)	38 (97.4)	39 (100)

Figures shown as no. (%) sensitive.
CHL – Chloramphenicol PEN – Penicillin CEF – Ceftriaxone
CEFO – Cefotaxime AMP – Ampicilin OFL – Ofloxacin

The sensitivity rate was highest to Ofloxacin 39 (100%) followed by cefotaxime 38 (97.4%) and ceftriaxone 35 (89.7%). It was moderate to chloramphenicol 29 (74.4), and lowest to ampicillin one (2.6%) and penicillin 15 (38.5%). The sensitivity of pneumococcus to penicillin and chloramphenicol were 50% and 81.8% respectively while that to ceftriaxone and cefotaxime were 95% and 100%. The isolates of *Neisseria meningitidis* had a sensitivity rate of 66.7% to both chloramphenicol and penicillin. Thirty-eight (97.4%) of the total isolates were resistant to ampicillin. All the isolates of *Haemophilus influenzae* were resistant to ampicillin and penicillin while sensitivities to cefotaxime, ceftriaxone and chloramphenicol were 100%, 77.8, and 66.7% respectively.
Outcome: Eight (12.1%) of 66 patients died while 16 (24.2%) had neurologic sequelae.

Discussion

The prevalence of acute bacterial meningitis in this study was 6.9%. This is similar to the prevalence of 6.2% reported from Ibadan.[10]However, the prevalence was higher than the ones reported from Shagamu (2.8%), [3] Maiduguri (3.5%), [9] Enugu (3.1%),[11]and more recently from Ilesa (1.6%).[12]The lower prevalence in Shagamu as well as in others [10, 11,12] may be due to the differences in methodology. The prevalence of childhood bacterial meningitis over a time frame in Nigeria seems to be decreasing. The exact reason for the relative decrease in the prevalence of childhood meningitis in Nigeria is not known. In developed countries there is a decline in the overall prevalence of meningitis as a result of reduction in the prevalence of Hib infection due to Hib vaccine introduction and this vaccine was yet to be available widely in Nigeria.This study has shown that meningitis is commoner in males than females. The

male preponderance (1.4:1) in this study agrees with the finding of other workers in developing countries. [3] Okoroma and Izuora, [13] found a male to female ratio of 1.3:1. Olanrewaju *et al* in Shagamu found a male to female ratio of 2.1:1. The male preponderance may be due the relative absence of a gene locus for elaboration of immunoglobulin, which was said to be located on the x chromosomes.[14]

Infants accounted for 27 (40.9%) of the total patients with meningitis in this study. This finding is similar to what has been reported previously.[10, 15, 16] The incidence of meningitis in this age group may be partly due to the vulnerability of their choroid plexus to penetration by bacteria during the septicaemic process, and to low immunological status.

The major organism detected in the present study was *Streptococcus pneumoniae*, which accounted for 51.5% while *Haemophilus influenzae* was the second most common pathogen accounting for 27.5% and *Neisseria meningitidis* accounted for 18.2%. This pattern is in contrast to that reported[9] from Maiduguri in northeastern Nigeria were *Neisseria meningitidis* was the most common cause of sporadic cases of meningitis accounting for 36% of the total isolates while *Streptococcus pneumoniae* occurred in 29.3% of cases. However, the pattern of predominance of *Streptococcus pneumoniae* in this study was not different from reports from other centres in Nigeria[3, 10,12,15] and elsewhere.[5, 6,16] Cerebrospinal fluid culture is the gold standard for the diagnosis of bacterial meningitis but use of antibiotics prior to presentation can significantly reduced the positivity of both CSF culture and Gram stain as shown in this study. Latex particle agglutination test may overcome the limitation of CSF culture, as bacterial antigens in CSF are not significantly affected by prior antibiotics use. LPA test detected more cases (80.3%) than culture (59.1) or Gram stain (71.2%). Despite its limitations of intra specie and inter specie cross reactions,[17] the test has utility value as result can be obtained within 15 minutes. There is high rate of in-vitro resistance to 2 (ampicillin and penicillin) of the three (ampicillin, penicillin and chloramphenicol) commonly used antibiotics. Overall, the sensitivity of isolates to ampicillin was 2.6%, penicillin 38.5%, chloramphenicol 74.4%, cefotaxime 97.4%, ceftriaxone 89.7%, and Ofloxacin 100%.

The relatively free access to antibiotics from patent medicine store might be contributory to the high rates of resistance to penicillin and ampicillin. The 74.4% susceptibility rate of isolates to chloramphenicol in this study was lower than the 89.3% reported from Maiduguri.[9]The high rates sensitivity to cefotaxime (97.4%) and ceftriaxone (89.7%) agrees with previous report from Libya.[16] The low resistance to the cefotaxime and ceftriaxone might be due to their restricted use owing to their high cost which majority of parents could not afford. The high rate of susceptibility (100%) of organisms to Ofloxacin, a quinolones in this study may be because they are rarely used in children in view of their potential to cause damage to the growing cartilage in these age groups. The high rate (50%) of pneumococcal resistance to penicillin found in this study is comparable to that reported previously from Nigeria[9,12]and Kenya.[18]The sensitivity rate of *Neisseria meningitides* to penicillin in this study is low (66.7%). The 81.8% pneumococcal sensitivity to chloramphenicol is also similar to that in previous reports from Nigeria.[3,10,12,15] There was an occasional report from Nigeria, describing a low (30.8%) sensitivity of pneumococcus to chloramphenicol.[19] The sensitivity rate of both *Haemophilus influenzae* and meningococcus to chloramphenicol was 66.7% each.

The mortality rate of 12.7% in this study compares favourably with what was obtained in some reports from Nigeria[3]and Libya.[16] The overall neurologic sequelae rate of 24.2% is similar to that reported by Olanrewaju et al[3] from Shagamu, Nigeria, but however, much higher than the report from Enugu[11] and more recently from Ilesa.[12]

Conclusion

The finding of high rate of resistance to ampicillin and penicillin in this study does not support the continued use of the combination of chloramphenicol with either penicillin or ampicillin for empirical treatment of acute bacterial meningitis and hence, cefotaxime or ceftriaxone should be commenced pending the outcome of CSF culture result. Also LPA should be included in the initial evaluation of CSF of children with suspected meningitis.

Limitation of the study

The sample size was too small to describe the sensitivity pattern.

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