

Bacterial Isolates and Antibigram of Cerebrospinal Fluid of Paediatric Patients as Seen at the University of Port Harcourt Teaching Hospital.

Dr Yaguo Ide Lucy E.¹, Dr Awopeju Abimbola Temitayo O.²

¹Dept. of Paediatrics and child health, University of Port Harcourt Teaching Hospital,
Port Harcourt, Nigeria.

²Dept. Microbiology and Parasitology, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria.

ABSTRACT

AIMS AND OBJECTIVES: Bacterial meningitis is a potent cause of morbidity and mortality in the paediatric age group, with a very high incidence of neurological complications. The aim of this study was to identify the pathogens isolated from the cerebrospinal fluids of pediatric patients and their sensitivity patterns.

METHODS: Descriptive retrospective study of six hundred and fifty seven cerebrospinal fluid culture reports taken from Paediatrics patients with presumed bacterial meningitis at the University of Port Harcourt Teaching hospital between January 2009 and December 2014

The samples were subjected to macroscopic and microscopic examination, Gram's stain and culture tests. Organisms isolated were characterized by standard procedure and antibiotic susceptibility tests done according to CLSI guidelines. Data were retrieved from laboratory record books and entered into a Microsoft® excel spreadsheet and analysed using SPSS

RESULTS: Six hundred and fifty seven samples from children aged between 1 day to 14380 days, 381 males (58%) and 267 females (40.6%) and 9 not indicated (1.4%). Neonates were 113(17.20%) while children aged above the neonatal age group constituted the remaining 82.80%. Male female ratio was 1.4:1. Only 12 (1.83%) samples were positive for culture. Of these 12, Gram positive cocci were the most common organisms of which *Staphylococcus aureus* was the most prevalent 8 (66.67%), Beta haemolytic *Streptococcus* 1 (8.33%), *Escherichia coli* was isolated in 2 (16.67%), *Cryptococcus neoformans* in 1(8.33%). The organisms all exhibited resistance to amoxicillin-clavulanic acid 100%. Cefazidime exhibited 100% sensitivity against *Escherichia coli* isolates. Ceftriaxone, cefotaxime and ciprofloxacin exhibited good sensitivity among all the bacteria isolates that were cultured.

CONCLUSIONS: There was a low yield of organisms in cerebrospinal fluid culture of Paediatric patients at the University of Port Harcourt. The third generation cephalosporins are still effective in bacterial meningitis in paediatric patients.

KEY WORDS: CSF; Meningitis; Paediatric.

INTRODUCTION

Meningitis is an inflammation of the meninges, the membranes surrounding the brain and spinal cord. It is a major cause of morbidity and mortality in many parts of the world [1].

The aetiological agent can be viral, bacterial or fungal pathogens. Bacterial meningitis is one of the most potentially serious infections occurring in infants and older children and is associated with a high incidence of acute complications and risk of long-term morbidity [2].

Antibiotics are usually administered before the laboratory results of CSF culture and sensitivity are available. To ensure appropriate therapy, current knowledge of the organisms that cause septic meningitis and their antibiotic susceptibility pattern in a particular setting or region is of utmost importance. Information obtained from laboratory based surveillance is important in determining the most common aetiology of meningitis pathogens. This information is also necessary for improving the clinical management of cases, guiding therapeutic decisions and for designing preventive strategies. [3]

This study was therefore undertaken to determine bacterial isolates in the cerebrospinal fluids of paediatric patients as seen at the University of Port Harcourt Teaching Hospital.

METHODOLOGY

The study was carried out at the University of Port Harcourt Teaching Hospital (UPTH) Rivers State, Nigeria. The teaching hospital apart from taking care of patients located in the state, also attends to referral cases from the entire South-south region of the country.

*Corresponding Author: Dr Yaguo Ide Lucy E., Dept. of Pediatrics and child health, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria.

A retrospective analysis of 657 clinically suspected cases of meningitis, admitted during a 5 years period from January 2009 to December 2014 was undertaken. All the clinically suspected cases that were from the patients' sample entry points into the laboratory were included in this study.

The study reviewed CSF samples of children that were aged 0day to 17years with presumed meningitis.

Data were retrieved from laboratory record books and entered into a Microsoft® excel spreadsheet. Demographic data, clinical diagnoses, cerebrospinal fluid (CSF) appearance, WBC cell count, type of isolated organisms, and antibiotic susceptibility profile of organisms were collected.

Analyses of the samples were done following standard Microbiology guideline [4].

Physical evaluation of the CSF samples were done and were assessed as Clear and Colourless, Turbid, Blood stained, Xanthochromic but clear, and Xanthochromic and Cloudy. White cell count was done using the Neubauer counting chamber. Wet mount and direct Gram staining of the samples were done. Indian ink staining was done for patients with HIV and other immunosuppressive conditions.

The samples were centrifuged at 5000 rev/min for 5 minutes and the sediment incubated in Blood agar, Chocolate agar and MacConkey agar for bacteria culture and in Sabourand agar for fungal culture. Plates were incubated at 35-37°C over night in CO₂ enriched atmosphere and in air for the fungal culture.

Plates were examined for growth after overnight incubation and re-incubated for another 24 hours if no growth was observed.

Any colonies observed were further characterized by Gram staining. The pathogens were identified by standard microbiological techniques adapted from the WHO laboratory manual for diagnosing bacterial meningitis [5].

Gram negative bacilli were further identified using the Microbact 24E identification system [6].

Antimicrobial susceptibility testing was done on the bacterial isolates using the Kirby-Bauer disk diffusion method and following the CLSI guideline the result were interpreted [7]. The isolates were tested against ceftriaxone 30µg, gentamicin 10µg, penicillin 10IU, cefotaxime 30µg, Amoxicillin-clavulanic acid 30µg, ceftazidime 30µg, ciprofloxacin 5µg, chloramphenicol 30µg, erythromycin 30µg, cloxacillin 10µg.

RESULTS

During the period of this study, a total of 657 Cerebrospinal fluid samples were submitted to confirm the diagnosis of meningitis in children that were aged 0day to 17 years who had features suggestive of meningitis. Samples from male patients were 381(57.99%), those from female patients were 267(40.64%) while the remaining 9(1.37%) samples had forms which did not indicate their sexes. A hundred and thirteen (17.20%) of the CSF were from children that were within the neonatal age group of 0 to 28 days while the remaining 544 (82.80%) were aged between 29 days and 17 years. Seventy-five percent of the samples were from the children emergency room, 12.1% were from the Special Care Baby Unit (SCBU) while the remaining samples were from patients on admission in children wards. The demographic data is shown in table 1.

Table 1- Demographic characteristics of Paediatrics patient suspected of having Bacterial Meningitis at University of Port Harcourt Teaching Hospital between 2009 and 2014

Gender	Number	Percentage (%)
Male	381	57.99
Female	267	40.64
Not indicated	9	1.37
Age		
0-28 days	113	17.20
Under 5 years	548	83.41
5 years and above	94	14.31
Not indicated	15	2.28
Ward		
SCBU	74	12.05
CHEW	459	74.76
CHOP	32	5.21
CHMW1	30	4.89
CHMW2	14	2.28

Key:

SCBU- Special Care Baby Unit

CHEW- Children Emergency Ward

CHOP- Children Outpatient

CHMW1- Children Medical Ward 1

CHMW2-Children Medical Ward2

White Blood Cell count (WBC) in 609(92.69%) of the sample were less than 5WBC per ml, 9(1.37%) had about 5 WBC per ml, 14(2.13%) were more than 5 WBC per ml while 25 samples were not suitable for cell count because of their appearance.

Direct Gram staining done on 642(97.72%) of the samples did not show any organism, 11(1.68%) showed Gram positive cocci in clusters, 1(0.15%) showed Gram negative bacilli, 1 stained positive for *Cryptococcus neoformans* with Indian ink stain while 2(0.30%) were considered unsuitable for staining. table 2.

The correlation between the presence of organism seen during direct Gram staining and subsequent growth when the samples were cultured is also highlighted on table 2. The total sample that had a positive bacterial growth were lower than what was demonstrated by direct gram staining.

Table 2- Correlations between WBC count, Gram stain appearance and the presence of microbial growth.

Cell count (WBC/mm ³)	Number	Percentage (%)
<5	609	92.69
5	8	1.37
>5	14	2.13
Not suitable	25	3.81
Gram Strain Appearance		
No organism	642	97.72
Gram Positive	11	1.68
Gram Negative	1	0.15
Not suitable	2	0.30
Yeast	1	0.15
Any growth on culture?		
No bacterial growth	645	98.17
Bacterial growth	11	1.67
Fungi growth	1	0.15

Only 12(1.83%) of the total samples had a positive growth, of which 9(75%) were from male patients while the remaining 3(25%) were female with male to female ratio of 3:1.

The organism that were isolated were *Staphylococcus aureus* 8(66.67%), *Escherichia coli* 2(16.67%), group B beta haemolytic *Streptococcus* 1(8.33%) and one case of *Cryptococcus neoformans* was isolated from a child with HIV infection table 3.

Table 3- Organisms Isolated From Paediatric Patients With Acute Meningitis In University Of Port Harcourt Between Jan 2009 And Dec 2014

Organism cultured	Number	Percentage %
<i>Staphylococcus aureus</i>	8	66.67
<i>Escherichia coli</i>	2	16.67
Group B-Haemolytic <i>Streptococcus</i>	1	8.33
<i>Cryptococcus neoformans</i>	1	8.33
Sex		
Male	9	75
Female	3	25

Staphylococcus aureus was susceptible to Ceftriaxone 100%, ciprofloxacin 63%, gentamicin 75%, penicillin 25%; Group B hemolytic *Streptococcus* susceptibility was gentamicin 100%, ceftriaxone 100%, ciprofloxacin 100%. *Escherichia coli* susceptibility pattern was chloramphenicol 100%, ceftazidime 100%, gentamicin 50%, ciprofloxacin 50% and cefotaxime 50%. The organisms were all 100% resistance to amoxicillin-clavulanic acid as shown on Table 4.

Table 4- Antibigram Of Organisms Isolated In Children Suspected Of Having Bacterial Meningitis In UPTH Between Jan 2009 And Dec 2014.

Antibiotics	<i>Staphylococcus aureus</i> n=8 %	<i>Group B Streptococcus</i> n=1 %	<i>Escherichia coli</i> n=2 %	Total N%
Penicillin 10 IU	2 (25)	0 (0)	-	2 (16.7)
Gentamicin 10µg	6 (75)	1 (100)	1 (50)	8 (66.6)
Chloramphenicol 10µg	-	-	1 (100)	1 (8.3)
Ciprofloxacin 5µg	5(63)	1(100)	1(50)	7 (58.3)
Ceftriaxone 30µg	8 (100)	1(100)	-	9 (75)
Cefotaxime 30µg	-	-	1 (50)	
Amoxicillin-Clavulanic acid20µg	0(0)	0 (0)	0 (0)	0 (0))
Ceftazidime 30µg	-	-	2(100)	2 (16.7)
Cloxacillin 5µg	4 (50)	-	-	4 (33.3)

Key:

N= total number of isolates

n= number of individual organisms isolated

DISCUSSION

Meningitis is a medical emergence that carries a high mortality with serious sequel for those who survive especially children [8].

Diagnosis of meningitis in children is usually difficult in the early stage because clinical presentations are often non-specific. It may present insidiously with specific symptoms that progress over 2 to 5 days before the diagnosis can be made. It may also present with symptom and signs of the infection which rapidly progress over 1 to 2 days. The third mode of presentation may be such in which there is rapid deterioration and shock early in the course of illness [9].

In our study clinicians had the suspicion of meningitis more in children aged 1-5 years from which 84.41% of the samples came and 381(58%) of which were male patients. The diagnosis of meningitis was confirmed in male patient in ratio 1.4 to 1 and this finding is lower than male to female ratio of 3.2 to 1 that was reported by Singhi et al but it still shows that meningitis is commoner in male than females. [10]

Examination of CSF White blood cell has been documented as serving as a guide to the presumptive diagnosis of meningitis while awaiting the CSF culture result [11]. Our finding from this study was that 92.69% of the patients had less than 5WBC per high power field.

Gram staining of CSF sediment has also been reported as serving as the most useful single test in identifying the likely pathogen causing infection when the suspicion is high. We also found that 642(92.72%) of the samples were acellular on Gram microscopy.

CSF samples of 645 (98.17%) of the patient had no positive culture with only 1.83% positivity rate. This finding is similar to 1.6% positivity rate that was reported by Ogunlesi TA et al in Ilesa, south west Nigeria and 0.69% in Nnewi, South east Nigeria [12, 13]. Gram positive bacteria were the commonest pathogen isolated in those that had a positive growth. Seventy-five percent of the isolate were Gram positive cocci and this is different from Gram negative bacilli which have been widely reported. [8, 9, 14]. Our finding is however similar to 65.9% and 57.75% prevalence of Gram positive facultative anaerobic bacteria reported in two similar studies done by Mani and Sigauque respectively [15, 16].

Madhumita P et al [17] gave some reasons for the low yield of CSF culture which included prior use of antibiotics before presentation, improper technique of lumbar puncture, delay in transportation of the specimen to the laboratory, non-availability of special culture medium that could aid the isolation of the pathogen. This work was cited by Modi Gaurav B et al 2012 [18]. These factors were also considered to have affected our finding because specimen were only accepted for processing in the laboratory after payment or when there is evidence that such patient is indigent and cannot afford to pay for the test. Also, in Nigeria, antibiotics are readily available as off counter without any prescription and this low yield might also be due to prior administration of antibiotics before presenting in the hospital [19].

Limitation of the study; we could not ascertain whether the high proportion of negative culture we had in the study was as a result of antimicrobial inhibition of the pathogen or actually absence of bacteria. Also, the diagnostic facility in the hospital still relies on the 'old culture method' whereas newer methods like latex agglutination test and polymerase chain reaction with better sensitivities are available in advance countries [20, 21, 22]. Also, clinical condition like aseptic meningitis, TB meningitis may resemble septic meningitis in their mode of presentation and these were supposed to be ruled out but this wasn't done in this study.

CONCLUSION

There is a low yield of CSF cultures in Paediatric patients as seen at the University of Port Harcourt Teaching Hospital. The third generation cephalosporins : ceftazidime, Ceftriaxone (which is a first line drug in the treatment of bacterial meningitis) are still very effective in the eradication of the common pathogens causing meningitis in the paediatrics age group at the University of Port Harcourt Teaching Hospital.

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