Original Article

# Pattern of cerebrospinal fluid analysis in children above the neonatal age as seen at the University of Port Harcourt Teaching Hospital

Awopeju Abimbola Temitayo Oluwajenyo, Yaguo-Ide Lucy Eberechukwu[1]

Departments of Medical Microbiology and Parasitology and [1]Paediatrics, University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria

## Abstract

**Background:** Bacterial meningitis is a potent cause of morbidity and mortality in the paediatric age group. The aim of this study was to review the results of analysis the cerebrospinal fluid (CSF) collected from children above the neonatal age who were suspected to be having meningitis and to also determine the susceptibility pattern of these isolates.

**Methods:** A descriptive retrospective study of results of CSF culture reports taken from paediatric patients aged 1 month to 16 years with clinical suspicion of bacterial meningitis at the University of Port Harcourt Teaching Hospital between January 1, 2009, and December 31, 2014. The CSF samples were subjected to macroscopic examination, white blood cell count, Gram's stain and culture. Organisms isolated were characterised by standard procedure and antibiotic susceptibility testing was done according to the Clinical Laboratories Standard Institute guidelines. Data were retrieved from laboratory record books and analysed using Microsoft Excel sheet. Results are presented as tables and pie chart.

**Results:** Five hundred and seventy-four samples were received from children who were above the neonatal age (28 days). Of these, 329 were male (57.3%), 240 were female (41.8%), while in 5 (0.9%) of the samples, the sex was not indicated. Only 10 (1.7%) samples were positive for culture. Of these, Gram-positive cocci were the most common organisms, of which *Staphylococcus aureus* was the most prevalent, being 7 (70%), followed by *Escherichia coli* (20%), while the least prevalent organism was β-haemolytic streptococci (10%). The organisms all exhibited resistance to amoxicillin-clavulanic acid. Ceftazidime exhibited 100% sensitivity against *E. coli* isolates. Ceftriaxone, cefotaxime and ciprofloxacin exhibited good sensitivity among all the bacteria isolates that were cultured.

**Conclusions:** There was a low yield of bacteria in CSF culture of paediatric patients at the University of Port Harcourt. The third-generation cephalosporins are still effective in bacterial meningitis in paediatric patients.

**Keywords:** Antibiotics, cerebrospinal fluid, meningitis, paediatric

**Address for correspondence:**
Dr. Awopeju Abimbola Temitayo Oluwajenyo, Department of Medical Microbiology and Parasitology, University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria. E-mail: drawopeju@yahoo.co.uk
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## 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 areas with low socioeconomic resources.[1] The aetiologic agent can be a viral, bacterial or fungal pathogen. Bacterial meningitis is one of the most potentially serious infections occurring in infants and older children. Meningitis has a high mortality generally and causes serious complications in those who survive, especially children.[2] The nonspecific clinical presentations of meningitis in children often make it difficult for early diagnosis. Incidentally, it may present with nonspecific symptoms such as headache, fever, neck stiffness and loss of consciousness; progressing over 2–5 days before a clinical diagnosis can be made. Thus, increasing the risk of patient developing acute complications and risk of long-term morbidity.[3]

Despite recent progresses being made in the treatment of cerebrospinal meningitis, it is a major cause of morbidity and mortality in different parts of the world.[4,5] It has been previously reported that about 1.2 million cases of meningitis occur annually, causing about 170,000 deaths.[6]

Neurological defects, epilepsy, deafness and mental retardation are among the complications observed in about 20% of individuals who survive cerebrospinal meningitis.[7] Acute bacterial meningitis is among the top five leading causes of disability, especially in the middle- and low-income countries.[4] These countries have a relatively higher prevalence of bacterial meningitis compared to developed countries.

Laboratory reports are crucial in determining the aetiology of pathogens associated with meningitis and necessary for improving clinical management and prevention of meningitis. A presumptive diagnosis of meningitis and institution of empirical antibiotics can be made based on the appearance of the cerebrospinal fluid (CSF), white blood cell (WBC) count and the Gram-stain picture of the fluid while awaiting the result of microbial culture and antibiotic susceptibility results.[8] Viral meningitis is often difficult to diagnose in the laboratory because of the need for a specialised virology laboratory with facilities for cell culture or polymerase chain reaction which most patients cannot afford to pay for on routine basis.

In Nigeria, several studies[9,10] have been done to determine the aetiologic agent of meningitis; however, information on pathogens contributing to meningitis in hospital-based studies in the South–South region is limited. This study was carried out to assess the aetiological agents and antibiogram of organisms associated with meningitis among children above neonatal age in Port Harcourt, Rivers State.

## Methods

The study was carried out at the University of Port Harcourt Teaching Hospital (UPTH) Rivers State, Nigeria. The teaching hospital is located in the South–South region of Nigeria. Moreover, it provides medical care to patients residing in the state and also attends to referral cases from the entire region. A retrospective analysis of results of CSF of 574 children above the neonatal age, with clinical suspicion of bacterial meningitis who presented at the various entry points in the UPTH, within a 5-year period from January 1, 2009, to December 31, 2014, was carried out.

Data were retrieved from the laboratory record of the Department of Medical Microbiology, and information obtained include demographic data, clinical diagnoses, CSF appearance, WBC count, isolated organisms and antibiotic susceptibility profile of the isolates. Data were entered into Microsoft Excel sheet for descriptive analysis and results are expressed as tables, figures and texts.

The CSF samples were aseptically collected into sterile universal containers by the attending physicians and were sent to the Medical Microbiology Laboratory where they were processed, and microscopy was done for WBC counts and Gram-staining. Specimens were cultured on blood agar, chocolate agar, MacConkey agar and Sabouraud agar, and incubated aerobically at 37°C in $CO_2$-enriched environment. Specimen processing was done following standard microbiological methods described by Jorgensen *et al*. in the manual of microbiology.[11] The isolated bacteria were identified by standard techniques.[12] Antibiotic susceptibility tests were carried out on bacterial isolates using the Kirby–Bauer disk diffusion method and interpreted in accordance with the Clinical Laboratories Standard Institute guidelines.[13] The isolates were tested against gentamicin 10 μg, penicillin 10 IU, ciprofloxacin 5 μg, cefotaxime 30 μg, amoxicillin-clavulanic acid 30 μg, ceftazidime 30 μg, chloramphenicol 30 μg, erythromycin 30 μg, ceftriaxone 30 μg and cloxacillin 10 μg. Fungal growth was identified by Indian ink staining.

## Results

A total of 574 paediatric patients above neonatal age were suspected of meningitis between 2009 and 2014. Table 1 shows there were more males (57.3%, $n = 329$) than female patients (41.8%, $n = 240$) while there were no records of the age in 5 (0.9%) of the samples. Age group distribution showed that 53.8% ($n = 309$) were between 1 month and 1 year, 32.6% ($n = 187$) were within 1–5 years, 10.1% ($n = 58$) were within 6–10 years, while 3.5% ($n = 20$) were within 11–16 years. About 76.8% ($n = 441$) of the samples were from the children emergency ward, 5.6% ($n$ = 32) from the children outpatient ward, children medical ward 1 (4.9%, $n$ = 28), special care baby unit (3.1%, $n$ = 18), while 6.8% ($n$ = 39) of the samples did not have the ward of presentation indicated.

WBC count was <5 in 85.7% ($n$ = 492) of the samples; it was 5 in 1.4% ($n$ = 8) while it was >5 in 2.4% ($n$ = 14). Twenty-five (4.3%) of the samples were not suitable for cell count because of their bloody appearance. Five hundred and sixty-four (98.3%) of the samples did not yield any growth when incubated, while only 1.7% ($n$ = 10) of the samples yielded positive growth. Of these, 9 (1.5%) were Gram-positive organisms and 1 (0.2%) Gram-negative organism [Table 2].

Patients aged between 1 and 12 months ($n$ = 4, 40%) had the highest number of bacterial growth; 30% ($n$ = 3) of the bacteria isolates were from patients between 1 and 5 years. Two of the bacterial isolates (20%) were from patients between 6 and 10 years and the remaining 10% ($n$ = 1) were from a patient between the age group of 11–16 years [Table 3].

The results showed that of the 10 bacteria isolated, *Staphylococcus aureus* was the most prevalent 7 (64%), followed by *Escherichia coli* 2 (18%), while the organism with the least prevalence was the β-haemolytic *Streptococcus* 1 (9%). *Cryptococcus neoformans* was isolated in a child who was coinfected with human immunodeficiency virus [Figure 1].

Table 4 shows the antibiotics sensitivity pattern of the bacteria isolates. All the isolated organisms exhibited the most sensitivity to ceftriaxone (80%), followed by ciprofloxacin and gentamicin (70%, respectively). Overall sensitivity to cloxacillin was 40% and 20% to penicillin and ceftazidime, respectively, with chloramphenicol and cefotaxime exhibiting the least sensitive (10% each). All the isolates were resistant to amoxicillin-clavulanic acid (100%). *S. aureus* was 100% sensitive to ceftriaxone, followed by gentamicin and ciprofloxacin (71.4%, respectively), cloxacillin (51.4%), while the least sensitivity was against penicillin (28.6%). The β-haemolytic *Streptococcus* isolate was 100% sensitive to gentamicin, ciprofloxacin and ceftriaxone and resistant to other antibiotics. All the *E. coli* isolates exhibited 100% sensitivity to ceftazidime, 50% sensitivity to gentamicin, chloramphenicol, ciprofloxacin and cefotaxime.

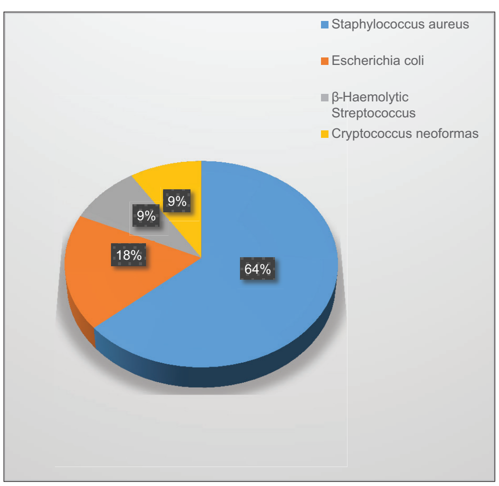


**Figure 1:** Frequency occurrence of bacterial isolates

**Table 1: Demographic and ward distribution of patients (*n*=574)**

| | Frequency, *n* (%) |
|---|---|
| Gender | |
| Male | 329 (57.3) |
| Female | 240 (41.8) |
| Undisclosed | 5 (0.9) |
| Age group | |
| 1-12 months | 309 (53.8) |
| 1-5 years | 187 (32.6) |
| 6-10 years | 58 (10.1) |
| 11-16 years | 20 (3.5) |
| Ward | |
| SCBU | 18 (3.1) |
| CHEW | 441 (76.8) |
| CHOP | 32 (5.6) |
| CHMW1 | 28 (4.9) |
| CHMW2 | 14 (2.4) |
| CHSW | 2 (0.3) |
| UD | 39 (6.8) |

SCBU: Special care baby unit, CHEW: Children emergency ward, CHOP: Children outpatient, CHMW1: Children medical ward 1, CHMW2: Children medical ward 2, CHSW: Children surgical ward, UD: Undisclosed

**Table 2: Microscopic characteristics of cerebrospinal fluid and white blood cell count of subjects (*n*=574)**

| Pattern | Frequency, *n* (%) |
|---|---|
| Culture | |
| No growth | 563 (98.1) |
| Observed growth | 11 (1.9) |
| Gram-stain | |
| None | 563 (98.1) |
| Gram-positive | 9 (1.5) |
| Gram-negative | 1 (0.2) |
| Yeast cell | 1 (0.2) |
| Cell count (WBC/mm$^3$) | |
| <5 | 492 (85.7) |
| 5 | 8 (1.4) |
| >5 | 14 (2.4) |
| Not suitable | 25 (4.4) |

WBC: White blood cell

**Table 3: Distribution of bacterial isolates according to age groups**

| Age group | Frequency of bacterial isolates, *n*=10 (%) |
|---|---|
| 1-12 months | 4 (40) |
| 1-5 years | 3 (30) |
| 6-10 years | 2 (20) |
| 11-16 years | 1 (10) |

**Table 4: Antibiotic sensitivity pattern of bacterial isolates**

| Antibiotics | *Staphylococcus aureus*, *n*=7 (%) | β-haemolytic *Streptococcus*, *n*=1 (%) | *Escherichia coli*, *n*=2 (%) | Total, *n* (%) |
|---|---|---|---|---|
| Penicillin 10 IU | 2 (28.6) | 0 | - | 2 (20) |
| Gentamicin 10 μg | 5 (71.4) | 1 (100) | 1 (50) | 7 (70) |
| Chloramphenicol 10 μg | - | - | 1 (50) | 1 (10) |
| Ciprofloxacin 5 μg | 5 (71.4) | 1 (100) | 1 (50) | 7 (70) |
| Ceftriaxone 30 μg | 7 (100) | 1 (100) | - | 8 (80) |
| Cefotaxime 30 μg | - | - | 1 (50) | 1 (10) |
| Amoxicillin-clavulanic acid 20 μg | 0 | 0 | 0 | 0 |
| Ceftazidime 30 μg | - | - | 2 (100) | 2 (20) |
| Cloxacillin 5 μg | 4 (51.4) | - | - | 4 (40) |



## Discussion

It has been well documented that CSF WBC examination serves as a guide for presumptive diagnosis of meningitis, while awaiting CSF culture outcome.[14] In this study, the prevalence of confirmed cases of meningitis was 1.9% which was slightly lower than 3.3% in Ghana has reported by Owusu *et al.*[15] WBC per high-power field had <5 in 92.69% of the patient samples reviewed which also correlates with a high percentage with negative culture. This study also shows that there were more males to females (1.3: 1) showing symptoms suspected to be meningitis and that meningitis was more common in male and in those children below the age of 5 years as observed in similar studies conducted in Nigeria and other parts of the world.[9,10,16-18] This may be due to the relatively low immune status in this age range. There was a low yield (1.9%) of bacteria isolated in the subjects. This may be attributed to technical challenges in the laboratory which may include but not limited to nonavailability of special medium for the isolation of some fastidious pathogens, delay in the transportation of specimen to the laboratory for analysis among others.[17-19] Furthermore, prior use of antibiotics may be a contributory factor because of availability of antibiotics off the counter without prescription leading to a use or abuse of antibiotics before presentation to health-care centres.[19,20] The relatively high prevalence of Gram-positive bacteria, especially *S. aureus* observed in this, is slightly different from the findings of Modi *et al.*[18] which reported a higher prevalence of *Klebsiella pneumonia,* followed by *E. coli,* while *S. aureus* was the third most prevalent bacteria isolated in that study. This finding, however, is in agreement with the findings of Johnson *et al.*[10] in Nigeria and Sudharshan *et al.*[17] in India that Gram-positive cocci was the prevalent organism above neonatal age group. Although the other bacteria isolated observed in this study are common pathogens associated with meningitis, their relative frequency varies in different geographical area.[8,10]

The antibiotic susceptibilities of the organisms isolated showed that amoxicillin-clavulanic acid is not effective in the treatment of meningitis since all the isolates were all resistant to this antibiotic. This finding is significant because this antibiotic is often used empirically in the treatment of meningitis secondary to otitis media.[15,19] *S. aureus* showed 100% sensitivity to ceftriaxone and high sensitivity to gentamicin which are part of the first line antibiotics in use in the hospital. This finding also correlates with the findings of Owusu *et al.*[15] in Ghana and shows that the antibiotics regimen being use is still effective.

This study has some limitations which include improper filling of patients' biodata by the attending clinicians, prior usage of antibiotics by most patients before presentation in the hospital, samples are usually not processed immediately they are submitted which might have led to loss of fastidious organisms, for example, *Haemophilus influenzae* which has been reported in other studies but not isolated in the present work. Lack of special culture media for isolation of fastidious organisms such as *Neisseria meningitidis* and reliance of culture method for identification of organisms instead of latex agglutination tests and other molecular assay were part of the setbacks encountered during this study.

## Conclusions

There was a low yield of CSF cultures in children suspected of meningitis at the UPTH. The study showed that children under the age of five (especially below the age of 1 year) were more susceptible to bacterial meningitis. Antibiotic susceptibility pattern of the isolates showed that the third-generation cephalosporins are still effective against common bacterial pathogens that may cause bacterial meningitis in paediatric patients.

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## Conflicts of interest
There are no conflicts of interest.

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