

## Rare Bacterial Meningitis among Hospitalized Children in a Teaching Hospital

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A study on the incidence of bacterial meningitis among hospitalized children in Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife was carried out using standard microbiological techniques. A sum of one hundred and thirty-five patients with the age range from 1 hour to 8 years was involved in the study and was placed in classes; the highest study class being 41.5 % ranged from 1 hour to 6 days, followed by age range 1 year to 4 years (25.9 %). Three of them (2.22%) showed positive presence of bacterial pathogens in their cerebral fluid (statistically non-significant ( $p \geq 0.05$ ) but medically important). These three positive patients were between the 1 < 12 months (33.3%) and <4 years to 8 years (66.7%). Two isolates of recovered were resistant to ampicillin, chloramphenicol, streptomycin and gentamycin, cotrimoxazole but sensitive to ceftriazone, augmentin, cefixime and ofloxacin. At least one of the isolates was resistant to cloxacillin, cephalixin and ceftazidime. The only *Haemophilus influenzae* recovered was resistant to ampicillin, cloxacillin, chloramphenicol, streptomycin, cotrimoxazole, cefixime and erythromycin but sensitive to gentamycin, cephalixin, ceftazidime, ceftriaxone, augmentin and ofloxacin. Multiple antibiotic resistance index (MARI) of *S. pneumoniae* (n=2) were 0.57 and 0.43 while *H. influenzae* (n=1) had MARI of 0.50. These showed the isolates to be of high risk sources and suggestive of prior antibiotic abuse. The incidence of Meningitis is unusual in the study area and suggests the need for proactive health control measures against rare infections.

**Key words:** meningitis, *Streptococcus pneumoniae*, *Haemophilus Influenzae*, children, antibiogram.

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Diseases from virulent bacteria have been adduced as the main cause of childhood mortality of about half the total number of children born in sub-Saharan African countries <sup>1</sup>. Meningitis, for instance, is a microbial infection that is commoner in children than adults and it is the inflammation of the meninges (protective layer that shields the central nervous system) <sup>2</sup>. The inflammation may

be caused by infection with viruses, bacteria, or other microorganisms, and less commonly by certain drugs <sup>3</sup>. Sáez-Llorens and McCracken <sup>4</sup> had observed that the mortality rate for meningitis can be low even to 2% in infants and high up to 20–30% in neonates and adults. It also leaves quite terrible physiological deformity in about 33.3% of the survivor <sup>5</sup>. So, this clinical condition is handled as a medical emergency <sup>2, 6</sup> as its widespread emergence sense great danger to public health. This situation however seemed appalling round the world, as decline in prevalence have been reported <sup>7-9</sup> in many countries.

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Group B Streptococcus remains the prevalent aetiology of meningitis among the new born and the premature babies<sup>10</sup>, though *Escherichia coli* have also been implicated<sup>11,12</sup>. An epidemic proportion of meningitis due to *Listeria monocytogenes* have also been earlier reported<sup>13</sup>. This Listerial meningitis is also of concern to public health scientist. This has been of peculiar concern to many researchers. For instance, Amaya-Villar, *et al.*<sup>14</sup> propelled by this venture to evaluate the clinical features, management, and outcome of Listerial meningitis. The occurrence, affected age-group, the aetiology and outcome are important factors in meningitis study. Chang Chien *et al.*<sup>15</sup> reported 60% of their study populations were < 7 days old age-group, and nosocomial spread in such conditions have been reported<sup>16,17</sup>. For children above  $\geq 7$ , the prevalent bacterial aetiologies are commonly some serotypes of *Streptococcus pneumoniae*, *Neisseria meningitidis* but those patients < 5 years are usually affected by *Haemophilus influenzae* type B<sup>18</sup>. A combination of Gram negative *N. meningitidis* and the Gram positive *S. pneumoniae* were responsible for meningitis in adults but *Listeria monocytogenes* meningitis affect mainly the elderly people with ages of  $\geq 60$  years<sup>19</sup>. Goetghebuer *et al.*<sup>20</sup> also observed that 52.1 % of the childhood meningitis in Gambia was caused by *S. pneumoniae*. Other meningitis are encountered due to the prevalence of such aetiologies known for other clinical manifestations, for example *Mycobacterium tuberculosis* meningitis is rampant in low-income countries already being ravaged by tuberculosis<sup>21</sup> or by immune system related diseases like HIV/AIDS<sup>22</sup>.

*H. influenzae* type b (Hib) is recognized for causing over 95% of invasive diseases including childhood meningitis before the administration of routine infant immunization<sup>23</sup>. The advent of this vaccine has however led to the reduction of infant meningitis (age range 0-4 years) to about 1 in 2,000 individuals in United Kingdom, United States and Canada<sup>24,25</sup>. In developing countries especially in Sub-Saharan African countries, the incidence is several multiples of those in developed countries<sup>26</sup>. These may be due to prevailing socio-economic differences. Even at that, *H. influenzae* type b (Hib) still showed similar trend in some developing countries compared to

the developed ones, though with higher incidence in the former<sup>26,27</sup>. An incidence rate of 47.9% childhood meningitis was caused by *H. influenzae* as reported by Goetghebuer *et al.*<sup>20</sup>. This explains the need to continuously monitor the incidence of meningitis and other such rare deadly infection especially in developing countries, in order to proffer prompt solution.

Meningitis prognosis is a threat to the susceptible children. Fatality has been reported in both developed and developing countries<sup>28,29</sup>. A study conducted by Trotman *et al.*<sup>30</sup> showed a 100% mortality rate for the entire infant below the age 1 year and a general of 36% poor outcome for all the patients. The post therapy outcome even showed 35% hearing loss among the convalescent. Goetghebuer *et al.*<sup>20</sup> reported that 50% of the patients with meningitis had various abnormalities after treatment. The major physical abnormalities they found include hearing loss, mental retardation, motor abnormalities and seizures.

The appropriate therapy depends on aetiology of the disease and of course, the child's age.  $\beta$ -Lactam antibiotics including the cephalosporins are relatively safe for children and therefore constitute the most appropriate initial regimen. The high *in-vitro* activity of monotherapy with the third generation cephalosporin has been reported<sup>31</sup> and it may be administered. However, the administration of vancomycin along with the cephalosporin may be necessary when *S. pneumoniae* (Pneumococcal) is detected as the pneumococcal strains are known for vancomycin tolerant when administered alone<sup>32,33</sup>. Another multicenter study reported and justified the administration of carbapenem plus cephalosporin therapy as the starting regimen in about 61.9% of the cases studied<sup>34</sup>. This study examines the incidence of childhood bacterial meningitis in Obafemi Awolowo University Teaching Hospitals complex, Ile-Ife, Nigeria and their antibiotic susceptibility profile.

## MATERIALS AND METHODS

### Study Design

Bacterial meningitis is a rare occurrence in the study location. A retrospective report of the incidence observed in May-July, 2011 is hereby presented. A verbal consent was obtained from all

the patients' parents before the collection of samples. The research was carried in accordance with the ethical guideline of the Teaching Hospitals.

#### Study Locations

This study was conducted at Obafemi Awolowo University Teaching Hospitals Complex. The samples were collected from the children ward while the microbiological analysis was carried out in Bacteriology Laboratory, Microbiology Department. Obafemi Awolowo University Teaching Hospitals is a referral centre for about 5 millions difficult cases within and outside Osun State, Nigeria<sup>35</sup>.

#### Sample Collection and Analysis

Sample collection, isolation and identification were carried out using the scheme of Perilla *et al.*<sup>36</sup> with very minor adjustment. Cerebrospinal fluid (CSF) samples from one hundred and thirty-five patients were collected aseptically and transported immediately to the microbiology laboratory for immediate analysis. In the laboratory the CSF was centrifuged and the sediment from a centrifuged specimen was cultured on blood agar (BA), MacConkey agar (MA) and vitox-enriched chocolate agar (CA) plates. The culture plates were incubated at  $35\pm 2^{\circ}\text{C}$  for 1-2 days in both aerobic (blood agar and macConkey agar) and anaerobic (chocolate agar) condition. The suspected *Streptococcus* species from the blood agar and chocolate agar were subjected to Neufeld's Quellung reactions (Pneumococcal test). In all the identification steps, *S. pneumoniae* NCTC 12977 was used as positive control. The Gram negative thread-like coco-bacillus suspected to be *Haemophilus influenzae* was confirmed by its positive reaction to the production of satellitism when grown with *Staphylococcus aureus*, factors x and v, and iridescence on slide. *H. influenzae* ATCC 49247 and *H. influenzae* ATCC 10211 were used as control for some of the analysis.

#### Antibiotic Susceptibility Testing (AST)

The diffusion technique was used to determine the profile of the following antibiotics on the isolates: ampicillin, cloxacillin, streptomycin, chloramphenicol, cotrimoxazole, erythromycin, gentamycin, cephalexin, augmentin, cefixime and ceftriaxone. The multi-antibiotic discs were commercially prepared by Abtek. The antibiogram was performed in accordance with standards

described by the National Committee for Clinical Laboratory Standards, NCCLS and Cheesebrough<sup>37-39</sup>.

#### Standardization of inoculum, plates preparation and interpretation of results

The method earlier described by Komolafe and Adegoke<sup>40</sup> was used with slight modifications. Few isolates on a 24 h plate culture were randomly selected for the preparation of uniform dilution equivalent to McFarland standard. These isolates were inoculated into 2 mL of sterile peptone water broth in bijou bottles. This was incubated at  $37^{\circ}\text{C}$  for 6 h and the turbidity was adjusted by serial dilution in phosphate buffer saline (pH 7.2) to match an opacity tube containing 0.5 mL of 1% barium chloride in 1% sulphuric acid (a McFarland's 0.5 barium sulphate standard containing  $10^5$  cfu/mL of the inoculums). One millilitre of the bacteria suspension was transferred into a well dried surface of diagnostic sensitivity test agar (DST) medium and tilted to spread evenly over the entire surface of the tilted plate. The excess fluid was drained off and dried within 5 min multi-antibiotic discs were then placed on the surface of the inoculated plate and incubated aerobically at  $37^{\circ}\text{C}$  for 18 to 24 h. The diameter of the zone of inhibition was measured in millimetre which was interpreted as either resistance (R) or sensitive (S) using the approved manufacturer's standard manuals.

#### Statistical analysis

Descriptive analyses were performed using the SPSS statistical programme, version<sup>19</sup>. A *p*-value,  $p\geq 0.05$  was regarded as being statistically significant (Dahiru)<sup>41</sup>.

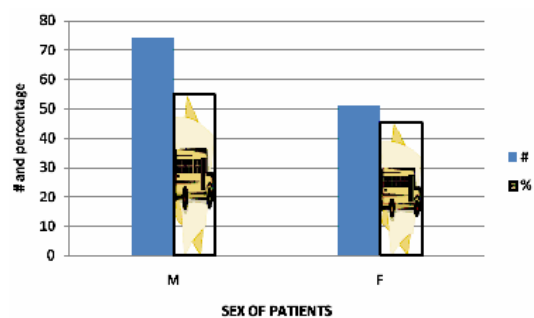


Fig. 1. Sex distribution (Number and Percentage) of the studied patients

## RESULTS

The study population includes the children with clinical manifestation like headache and neck stiffness. Most of these children also exhibit multiple symptoms like fever, vomiting, unfocused consciousness and photophobia all of which suggest meningitis, the children were then recommended for CSF bacteriological examinations and the number includes 74 males (54.8%) and 51 female children (Fig 1). Forty-one and a half percentage of these patients fall in the age range of 1 hour to 6 days, followed by age range 1 year to 4 years (25.9%), 4 years to 8 years (17%), 1 week to < 1 month (8.1%) and 1 month to < 12 months (7.4%) (Table 1). The observation of few positive cerebro spinal fluid samples was made in this study. Out of one hundred and thirty five patients studied, three of them (2.22%) showed positive presence of bacterial pathogen in their cerebral fluid. This number of the positive patients were statistically non-significant ( $p \geq 0.05$ ) but medically important. These three positive patients were between the 1 month to < 12 months (33.3%) and <4 years to 8 years (66.7%) Table 1.

Following the biochemical and serological analysis to identify the organisms isolated, two of the bacterial species were *S. pneumoniae* (Pneumococcus) and one was *H. influenzae*. The summary of the biochemical and serological characteristics of the isolates are as shown in Table 2.

Table 3 summarizes the antibiotic susceptibility profile of the isolates. Both *S. pneumoniae* isolates were resistant to ampicillin, chloramphenicol, streptomycin, gentamycin and cotrimoxazole while both were sensitive to ceftriazone, augmentin, cefixime and ofloxacin. One isolate was resistant while the other was sensitive to cloxacillin, cephalixin and ceftazidime. For *H. influenzae*, the isolate was resistant to ampicillin, cloxacillin, chloramphenicol, streptomycin, cotrimoxazole, cefixime and erythromycin, while sensitivity to gentamycin, cephalixin, ceftazidime, ceftriaxone, augmentin and ofloxacin were observed in this organism. Multiple antibiotic resistance index (MARI) of *S. pneumoniae* (n=2) were 0.57 and 0.43 while *H. influenzae* (n=1) had MARI of 0.50.

**Table 1.** Age Range and the positive samples per age range

Age range	No and Percentage Age Range of the Sampled Patients		No of culture with positive results	
	No	%	No	%
1 h-6days	56	41.5	0	0
1 w- <1 m	11	8.1	0	0
1m-<12m	10	7.4	1	33.3
1y-d"4 y	35	25.9	0	0
>4y-8y	23	17	2	66.7
	135	100		

**Note:** The number of positive sample is non-significant ( $p$ -values  $\geq 0.05$ ).

**Table 2.** Biochemical and Serological Characteristics of the isolates.

Haemolysis	Gram Reaction	Catalase	Optochin Susceptibility	Bile Solubility	Quellung reactions Test	Identification
Á	+	-	+	+	+	<i>S. pneumoniae</i>
Satellitism (+ <i>S. aureus</i> )	Gram Reaction	Indole	Urease	Factors X V	Iridescence	Identification
+	-	+	+	+	+	<i>H. influenzae</i>

**Table 3.** Antibiotic Susceptibility profile of *S. pneumoniae* and *H. influenzae*.

Antibiotics	% Resistance (R) or Sensitive (S)	
	<i>S. pneumoniae</i> (n=2)	<i>H. Influenza</i> (n=1)
Ampicillin	2R	1R
Cloxacillin	1R, 1S	1R
Chloramphenicol	2R	1R
Streptomycin	2R	1R
Gentamycin	2R	1S
Cotrimoxazole	2R	1R
Cephalexin	1R, 1S	1S
Ceftazidime	1R, 1S	1S
Ceftriaxone	2S	1S
Augmentin	2S	1S
Cefixime	2S	1R
Ofloxacin	2S	1S
Ciporex	1S,	1S
Erythromycin	1R, 1S	1R

**DISCUSSION**

The results of this study involving 135 children showed low profile incidence of 2.22% in this area known to be free of such cases compare to the hyper-endemic regions like Chad, Niger and Northern Nigeria<sup>42</sup>. Yet, the presence of this case at all calls for high level preparedness, considering it all an alert signal reminding all the stakeholders the deadly impact of meningitis on many African children as previously reported elsewhere<sup>43</sup>. Following the use of culture and immunological assays for effective detection<sup>44</sup>, the *S. pneumoniae* (Pneumococcus) detected in grown up children (8 years and 7 years) and *H. influenzae* detected in much younger child (4 months) were in tandem with earlier report by Schuchat and Wenger<sup>25</sup>, which emphasized the prevalence of *H. influenzae* b (Hib) meningitis in younger children. As observed in this study, *S. pneumoniae* (Pneumococcus) has higher frequency of occurrence than *H. influenzae* in meningitis cases and this is similar to published studies<sup>45,46</sup>. For this pneumococcal meningitis, poor nutritional status and less competent immune system might be contributory factors<sup>47,48</sup> as the older children have better immune competence than neonates<sup>49,50</sup>. Monitoring meningitis caused by *S. pneumoniae* accompanied by non-meningitis infection is important due to usual underlining illnesses that

sometime facilitate the pathogenesis of meningitis which ultimately affect the therapeutic outcome<sup>51</sup>, thus, making the infection difficult to manage and recurrent<sup>3</sup>. Lower survival was reported in cases with *S. pneumoniae* meningitis than with *H. influenzae* (Hib) meningitis by Goetghebuer *et al.*<sup>20</sup>.

A study showed that *S. pneumoniae*, *H. influenzae* type B, and *Meningococcal meningitidis* infections depend on the age, sex and immune status of the patients<sup>52</sup>, two of these incidences were also observed in this study. More interestingly, the two bacteria isolated in this study (*H. influenzae* and *S. pneumoniae*) have been reported as the predominant aetiologies of meningitis among young black children in many parts of Africa<sup>43</sup>. This makes these organisms important in meningitis related studies in Africa.

However, due to general low incidence, our observations cannot be confirmed to be sex-based but the 2 older children with *S. pneumoniae* meningitis were incidentally males while the 4 month old baby with *H. influenzae* was a female. Yet, it agrees with earlier reports that pneumococcal disease is usually with a percentage of 60% for male while female has 40% frequency of occurrence<sup>53-54</sup>.

The observation of three cases report in this research shows the need for preparedness against the old infectious diseases<sup>55</sup>. The observed cephalosporin-resistant *S. pneumoniae* and cephalosporin-susceptible *S. pneumoniae* makes the prescription of such a wide spectrum antibiotic unreliable as there lies 50% therapeutic failure as other reported cases<sup>56,57</sup> except a prior antibiotic susceptibility testing confirms the potential for effective therapy with the antibiotic. When such failure occurs, complications may set in before further action is taken. This further explains why meningitis should be addressed as an emergency which cannot be underestimated even at low incidence rate<sup>58</sup>. Resistance to ampicillin, chloramphenicol, streptomycin and cotrimoxazole by the *S. pneumoniae* and *H. influenzae* observed in this study corroborated the reports elsewhere<sup>59,60</sup>. Also, the observation that at least one of the two *S. pneumoniae* and the only *H. influenzae* were resistant to erythromycin and cloxacillin in this study support the much advocated need to shift from monotherapy to combination therapy for efficacy<sup>61</sup> though combination therapy is



sometimes accompanied with attending challenges<sup>62</sup>. Prior antibiotic susceptibility testing is important on whichever regimen is to be adopted.

Resistance to ampicillin, cloxacillin, chloramphenicol, streptomycin, cotrimoxazole, cefixime and erythromycin by the *H. influenzae* showed the need to recourse to vaccine administration, especially in meningitis endemic area. While supporting the use of Hib conjugate vaccine immunogenic in young infants, WHO<sup>63</sup> reported that it is safe, and offer long-term protection. The level of preparedness in infection control in Sub-Saharan African countries should be reviewed and strengthened intermittently to prevent the emergence of such infections like meningitis in epidemic proportion even in areas where it was never or rarely reported.

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