Prevalence of *H. Influenzae* among Under-Five Children Presenting At the Emergency Paediatric Unit (EPU) Of Two Teaching Hospitals in Jos, Plateau State, Nigeria.

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Abstract: Background: *Haemophilus influenzae* is a leading cause of bacterial meningitis in under-five children globally. It is also the leading cause of endemic bacterial meningitis in infants and young children. *H. influenzae* infection is severe where vaccine is not routinely used and one-third to one-half of the children either dies or suffers permanent disability such as deafness, paralysis or mental retardation.

Objective: Therefore, this research set out to study and to determine the prevalence of *H. influenzae* in under-five children in Jos.

Methodology: This was a descriptive cross-sectional study. One hundred and sixty consecutive under-five children who presented with signs and symptoms consistent with *H. influenzae* infection were recruited. Socio-demography data was obtained with structured questionnaire. Specimens were collected and carefully processed for isolation of *H. influenzae*.

Results: The prevalence of *H. influenzae* was low in Jos with prevalence of 6.3% among 160 under-five children studied, with mean age of 34 months and M: F ratio of 1:1. About 60% of these isolates were obtained from patients with acute pyogenic bacterial meningitis and *H. influenzae* were isolated predominantly from children whose parents were Farmers and Artisans. Most of the isolates were biotype 1 and fifty percent of the isolates were serotype b.

Conclusion: The outcome of this study can be used in the identification of *H. influenzae* and treatment of children with invasive *H. influenzae* infections in order to avoid complications.

Key words: *Haemophilus influenzae*, prevalence, under-five, EPU, Jos.

I. Introduction/ Background

*Haemophilic* bacteria are so designated because they require growth factors contained in blood. The most important human pathogen in this genus is *Haemophilus influenzae*. Other *Haemophilus* species include *Haemophilus parainfluenzae*, *Haemophilus haemolyticus*, *Haemophilus segnis*, *Haemophilus aphrophilus*, and *Haemophilus ducreyi*. *Haemophilus influenzae* is a fastidious gram negative bacillus which was initially recognized as the viral agent causing influenza. The type species (*H. influenzae*) is responsible for a variety of diseases in humans. It is a common aetiological agent of diseases such as pneumonia, meningitis, otitis media, and conjunctivitis. Meningitis caused by *H. influenzae* occurs almost exclusively in children less than five years of age. Most infections are due to serotype b, commonly abbreviated as Hib. *Haemophilus influenzae* type b causes meningitis in children between six months, when the acquired antibodies would have disappeared from the serum, and four years, when active immunity would have been acquired. In this situation, headache is followed rapidly by development of a stiff neck, with progression to coma and, in the absence of treatment, death. Type b *H. influenzae* also causes cellulitis and epiglottitis. But, prompt and adequate treatment with antibiotics would reduce mortality and complications.

Non-typable *H. influenzae* strains commonly cause infection of the middle ear (otitis media) and are the most common cause of *Haemophilus* infection in adults, such as bronchitis and pneumonia, especially in the presence of underlying disease of the bronchi and lungs. Non-typable *H. influenzae* strains also commonly cause acute or chronic sinusitis in patients of all ages.

Human immunodeficiency virus positive children were prone to invasive *H. influenzae* infection and leads to severe complications and disabilities in the analysis presented by Mulholland, 18% of children admitted with *H. influenzae* infection were infected with HIV and 28% of HIV positive children admitted to the
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hospital with invasive H. influenzae died. These children were difficult to manage which increases their morbidity and mortality.

Apart from infections by type b H. influenzae, Pittman showed that a small fraction of H. influenzae infections was caused by H. influenzae type a and by H. influenzae type f. These types may be identified by an agglutination reaction that uses antisera raised in rabbits; this result in false positive result due to cross-reactions with somatic antigens. This type of error is eliminated by using counter-immunoelectrophoresis, in which migration under an electric current removes somatic (protein) antigens from the reaction, leaving only capsular polysaccharides to react with antibody.

In a study by Mulholland, the incidence and mortality of invasive disease due to H. influenzae type b in Africa remain high when compared with findings from other continents. These were linked to the combined effect of high level of poverty and malnutrition in this continent.

Haemophilus influenzae is a leading cause of under-five bacterial meningitis and bacterial pneumonia globally. And is also the leading cause of endemic bacterial meningitis in infants and young children. One-third to one-half of the children who suffer from this infection either dies or suffers permanent disability. Although safe and effective H. influenzae vaccines have been used in developed countries for more than seventeen years, they are under-utilized in developing countries.

Haemophilus influenzae serotype b is a silent killer. Every year, an estimated 400,000 children less than five years of age die from H. influenzae serotype b disease worldwide. Africa accounts for more childhood deaths due to H. influenzae than any other continent. Haemophilus influenzae meningitis has more fatality and disability rate. In Africa, more than one in five cases of H. influenzae meningitis die and 15-35% of survivors suffer permanent disability. Haemophilus influenzae pneumonia is more common than H. influenzae meningitis. It is estimated that for every case of H. influenzae meningitis there are 4 to 10 cases of pneumonia. Haemophilus influenzae is transmitted via aerosol and spread by direct contact and there is no animal reservoir for this organism.

The annual incidence of H. influenzae infections is between 22 and 24 cases/100 000 children under-five. Type b H. influenzae caused about 8.13 million serious illnesses worldwide and caused 371,000 deaths (247,000-527,000) in children aged 1-59 months, of which 8100 (5600-10,000) were HIV-positive and 363,000 (242,000-517,000) were HIV-negative children. Therefore, the global burden of H. influenzae infections is substantial. Data on H. influenzae in Jos is scanty, but a study in south-west Nigeria, which determined the causative organisms, age, sex and seasonal incidence in pyogenic meningitis, by Babalola A.A and Coker A.O in 1982, the prevalence of H. influenzae was 28% and noted that most of the cases occurred in the dry season than during the rainy season. In a study to determine the clinical and investigative indicators of aetiology of pyogenic meningitis, by Olanrewaju and colleagues in Ilorin, the prevalence of H. influenzae was 7.1% and noted a high mortality associated with H. influenzae meningitis. In a prospective study in the Gambia where H. influenzae meningitis were studied over a two year period among children less than five years, recorded an incidence of 60 cases/100,000 children under-five and 297 cases/100,000 children less than 1 year of age. In a descriptive epidemiology study carried out in Kilifi district of Kenya to determine nasopharyngeal carriage and risk factors in children and adult in 2004, H. influenzae prevalence was 26% in the age 0-4 years.

Biotyping and serotyping have been used to investigate patterns of infection. Biotype I, serotype b, for instance, is often associated with severe meningitis in children. In contrast, non-serotypable strains of H. influenzae, particularly biotypes II and III, are frequently commensal to the upper respiratory tract.

**Objective of the study**

Aim of the study was to determine the prevalence of H. influenzae among under-five children presenting at Emergency Paediatric Unit (EPU) of JUTH and BUTH.

**II. Materials and Methods**

**Study background**

This study was carried out at the Emergency Paediatric Unit (EPU) of Jos University Teaching Hospital (JUTH) and Bingham University Teaching Hospital (BUTH). JUTH is a 600 and BUTH a 350 bedded tertiary Health Institutions serving Plateau state and majority of the states in the north central geopolitical zone of Nigeria. The temperature in the state is generally cold. The harmattan period encourages clustering and crowding of family units. The housing units, made from clay mostly are closely packed together and poorly ventilated.

**Study population**

Consecutive children between six months and five years who presented in the Emergency Paediatric Unit of Jos University Teaching Hospital and Bingham University Teaching Hospital, with symptoms and signs
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of meningitis, otitis media, conjunctivitis, sinusitis, pneumonia, and epiglottitis, and whose parents or guardians consented to participate in the study were enrolled and were informed about the need for this work.

INCLUSION CRITERIA:  
i. Children of both sexes aged six months to five years with symptoms

EXCLUSION CRITERIA:  
i. Children with known history of bleeding disorder, skin disease extending to the lumbar area and raised intracranial pressure.  
ii. Children below six months or above five years.  
iii. Children not on antibiotics two weeks prior to presentation.

Study design  
This was a descriptive cross–sectional prospective study conducted from October 2009 to March 2010.

Sample size  
Using the prevalence rate of 28% documented by Babalola and Coker, the minimum sample size was set at 160.

Sampling methods  
Diagnosis was achieved in collaboration with the Paediatrician. Children diagnosed of H. influenzae infection were recruited, for instance; meningitis (fever, neck pain, headache, nausea, vomiting, and neck stiffness), otitis media (fever irritability, difficulty in sleeping, fever, ear discharges and ear pain), conjunctivitis (red eyes and eye discharge), sinusitis (sneezing fever and breathing difficulty), pneumonia (cough fever flaring of alar nasal and tachpnoea), epiglottitis (presenting with drooling of saliva, inability to talk and mouth agape). The purpose of this work was explained to the parents before their consent to participate was sought. The consent form was filled by the investigator and the parent or guardian of each child signed the form.

Interviewer-administered, structured questionnaires were used as the study tool. The questions outlined in the data forms were explained to the parent or guardian and then completed with the required information which included the child’s bio-demographic data (such as child’s age, sex, school, type of house and number of people sleeping in a room), provisional diagnosis and laboratory processes, such that the eventual result was noted in the data forms and communicated to the physicians and the parents.

The data obtained was coded entered and analysed using Epi Info version 3.5.1 package. Confidence interval was 95% and the p value was 0.05.

III. Specimen Collection, Transportation And Processing  
The type and quality of specimens submitted to the laboratory usually determine the success of isolating H. influenzae. Each specimen received should therefore be examined for quality, in terms of amount, sterility and presence or absence of blood.

Cerebrospinal fluid (CSF) was collected in universal sterile container; Blood was collected into two aerobic blood broth; Throat swab collected with Stuart swab and returned into its container. Ear and eye swab samples collected using pen torch to illuminate the ear. Eye and Ear examined for evidence of exudates. A moistened sterile swab stick was used to swab the conjunctivitis and the middle ear to obtain the specimen, the swab was carefully removed return back to the container.

The specimen were macroscopically and microscopically examined, Gram stain was done. Using a sterile wireloop, the specimens were streaked onto a sheep chocolate agar, sheep blood agar, sheep chocolate agar plate with vancomycin and clarithromycin and Haemophilus testing media with supplement.

The inoculated plates were placed in a canister and incubated at 37°C in a moist atmosphere supplemented with 5 – 10% CO₂ for 18 to 24 hrs. The colonies were observed, those that were mucoid, shiny, convex and greyish were recorded. The colonies were Gram stained for evidence of Gram negative bacilli or coccobacilli. Pure colonies were identified by satellitism and X+V factor requirement.

Satellitism: Three Sheep blood agar plates were allow to dry. Using a wire loop, pure colonies from the primary plates were streaked on two Sheep blood agar. Haemolytic Staphylococcus aureus colony was streaked across one of the Sheep blood agar plate. Nonhaemolytic coagulate negative Staphylococcus strain was streaked across the second Sheep blood agar plate, to serve as negative control.

Quality control strain of Haemophilus influenzae was streaked on the third Sheep blood agar and a haemolytic Staphylococcus aureus streaked across the plate, to serve as positive control. The three plates were incubated at 37°C in a moist atmosphere supplemented with 5 – 10% CO₂ for 18 to 24 hrs. Growth was seen in
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the first and the third Sheep blood agar plates along the zone of haemolysis. No visible growth seen in the second Sheep blood agar plate.

Factor requirement: A nutrient agar plates was allowed to dry. Pure colonies from the primary culture plate were streaked on the nutrient agar plate. After 2mins, three growth requirement disk; X factor disk, V factor disk, and X+V factor disk, were placed on the streaked nutrient agar plate. The factor disks were well spaced apart on the plate. The plate was incubated at 37°C in a moist atmosphere supplemented with 5 – 10% CO₂ for 18 to 24 hrs. There were growth around the X+V factor disk and no growth around the X factor disk and V factor disk15.

Ethical Approval

Ethical approval for this study was obtained from the ethical committee of the Jos University Teaching Hospital and permission was sought from Bingham University Teaching Hospital.

IV. Results

This study was carried out among 160 children between the ages of six months and five years. Samples were collected from these children and examined between November 2009 and March 2010, and no parent withdrew their child after consenting to the study. There were 76 males (47.5%) and 84 females (52.5%) and the male to female ratio (M:F) was 1:1. The mean age of children studied was 34 months, with the highest proportion within the age range of 31-40 months accounting for 22.0% of the children and the lowest proportion being 0-10 months accounting for 8.2%. However, this distribution was not statistically significant (P >0.05). (Table 1).

From the 160 children, H. influenzae was isolated from 10 children, three from Jos University Teaching Hospital (JUTH) and seven from Bingham University Teaching Hospital (BUTH) (figure1) representing 6.3% of the study population (JUTH and BUTH represents 1.9% and 4.4% respectively). Three H. influenzae isolated from female children while seven H. influenzae was isolated from males representing 4.4% of the study population. (Table 2). Of the 10 isolates, 3 were isolated from the 11-20 months age group giving a prevalence of 13.6%, another 3 (9.4%) from the 41-50 months age group; 2 (6.1%) from the 21-30 months age group and 1 each from the 31-40 months and 51-60 months age groups, giving a prevalence of 2.9% and 4.0% respectively. None were isolated from the 0-10 months age groups. (Table 1).

Among the 160 children studied, 34 (21.3%) and 43 (26.9%) children presented with features of meningitis and pneumonia, while 41 (25.6%) and 15 (9.4%) children presented with features of acute suppurative otitis media and septicaemia respectively. Other clinical presentation were tonsillitis, epiglottitis, conjunctivitis and sinusitis with frequencies of 12 (7.5%), 8 (5.0%), 5 (3.1%) and 2 (1.3%) respectively. Out of the 10 isolates of H. influenzae obtained from the study population, 6 (17.6%) were isolated from children that presented with features of meningitis while 2 isolates each were obtained from children who presented with acute otitis media and bacterial pneumonia giving a prevalence of 4.9% and 4.7% respectively. No isolate was obtained from children who presented with features of bacterial conjunctivitis, epiglottitis, septicaemia, sinusitis and tonsillitis. (Table 3).

With regard to the colony characteristic of the isolates obtained from specimen of the children examined, six (6) of the isolates exhibited mucoid characteristic while four (4) of the total isolates gotten were non-mucoid. Of the total isolates obtained in this study, eight (8) of the isolates (80.0%) were biotype 1; while 2 (20.0%) were biotype 3. This was statistically significant (P<0.05) (Figure2). Five of the total isolates (50.0%) in this study were serotype b, three of the total isolates (30%) were nonserotype b although this study was unable to test for other specific serotypes (a,c,d,e,f) while the remaining two (2) isolates (20.0%) were non-typable. This was statistically significant (P<0.05). In relation to infection, five (83.3%) out of six H. influenzae meningitis were serotype b while the remaining one was nonserotype b, all the isolates of H. influenzae pneumonia were nonserotype b, while all the isolates causing H. influenzae otitis media were nontypable.

<p>| Table 1: Distribution of Haemophilus influenzae isolates among under-five children in Jos. |
|-----------------------------------------------|-----------------------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Age group (Months)</th>
<th>Total</th>
<th>Percent (%)</th>
<th>H. influenzae Percent (%)</th>
<th>isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>13</td>
<td>8.1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>11 – 20</td>
<td>22</td>
<td>13.8</td>
<td>3</td>
<td>13.6</td>
</tr>
<tr>
<td>21 – 30</td>
<td>33</td>
<td>20.6</td>
<td>2</td>
<td>6.1</td>
</tr>
<tr>
<td>31 – 40</td>
<td>35</td>
<td>21.9</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>41 – 50</td>
<td>32</td>
<td>20.6</td>
<td>3</td>
<td>9.4</td>
</tr>
<tr>
<td>51 – 60</td>
<td>25</td>
<td>15.6</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td>100.0</td>
<td>10</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>X² = 3.24</td>
<td>df = 5</td>
</tr>
</tbody>
</table>

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Table 2: Sex distribution and Haemophilus influenzae isolates among under-five children in Jos.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency</th>
<th>Percent</th>
<th>Haemophilus isolate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>76</td>
<td>47.5</td>
<td>7 (4.4)</td>
</tr>
<tr>
<td>Females</td>
<td>84</td>
<td>52.5</td>
<td>3 (1.9)</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td>100.0</td>
<td>10 (6.3)</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 2.74 \quad df= 1 \quad P>0.05 \]

Table 3: Distribution of Haemophilus influenzae isolates by infections among under-five children in Jos.

<table>
<thead>
<tr>
<th>Provisional diagnosis</th>
<th>Total</th>
<th>H. influenzae isolates</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASOM</td>
<td>41</td>
<td>2</td>
<td>4.9</td>
</tr>
<tr>
<td>B. meningitis</td>
<td>34</td>
<td>6</td>
<td>17.6</td>
</tr>
<tr>
<td>B. conjunctivitis</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>B. epiglotitis</td>
<td>8</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>B. pneumonia</td>
<td>43</td>
<td>2</td>
<td>4.7</td>
</tr>
<tr>
<td>B. septicaemia</td>
<td>15</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>B. Sinusitis</td>
<td>2</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>B. tonsillitis</td>
<td>12</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td>10</td>
<td>6.3</td>
</tr>
</tbody>
</table>

V. Discussion

The prevalence of H. influenzae among under-five children presenting at the emergency unit of JUTH and BUTH was 6.3%. This figure varies with findings in Nigeria and other parts of the world.

The finding of 6.3% prevalence in this study is higher than studies in UK\(^1\). In the UK study, the wide scale use of conjugate H. influenzae vaccine might have contributed to a reduction in incidence of H. influenzae infection. Immunization of children in the United Kingdom is very efficient and the relationship between the use of conjugate vaccines and subsequent reduction of H. influenzae infections is well established. In Nigeria the value obtained in this study is lower than a study done in south-west Nigeria, where the value of 28% was obtained by Babalola and Coker in Lagos\(^10\). Also, this value was lower than studies in East Africa. A value of
26% was documented in Kilifi district of Kenya\textsuperscript{13}. In southern America, a study by Bryant and colleagues in Brazil\textsuperscript{18} reported prevalence rate of 23%. These differences observed may be due to geographic and climatic differences\textsuperscript{10, 13}. Also, in this study the age limit was restricted to under-five, whereas in the other studies, children aged five years and above were examined\textsuperscript{10}.

The finding of 6.3% in this study is similar with the isolation rate in Cuba\textsuperscript{19} where the prevalence rate was 6.0%. This may be due to low immunization coverage despite the commencement of the conjugate \textit{H. influenzae} vaccine in Cuba. This is a poor nation with suboptimal basic health facilities. This scenario is the same in Nigeria.

From this study, more than 60% of the isolates obtained from older children the children were from 21-30, 31-40, and 41-50 months age groups and no isolate was obtained from children within the 0-10 months age group, this findings was consistent with establish knowledge that acquired maternal immunity wanes as the child grows older\textsuperscript{2} and this was statistically significant. Although there was female preponderance among those investigated, \textit{H. influenzae} was isolated more in males than in females in about 3:1 ratio, this findings was consistent with other findings\textsuperscript{10,13}.

The prevalent infection caused by \textit{H. influenzae} in this study was bacterial meningitis (17.6%) which was predominantly encapsulated; this was in line with other study. Dry harmattan favours \textit{H. influenzae} meningitis because \textit{H. influenzae} can be transmitted directly to the CNS and its transmission aided by dry harmattan\textsuperscript{10}.

In this study, eight of the isolates out of 10 (80%) were biotype 1; this is consistent to finding from other studies\textsuperscript{14}. This finding implied that \textit{H. influenzae} isolated from these children were causing invasive disease. The findings of less of biotype 1 and none of other biotypes signifies that isolates were not from normal flora or colonization.

In terms of \textit{H. influenzae} isolate reaction to antisera b, \textit{H. influenzae} serotype b and other serotypes, not determined in this study contributes to invasive \textit{H. influenzae} infections. This was contrary to findings from other studies\textsuperscript{6,14}.

VI. Conclusion

The outcome of this study can be used in the identification of \textit{H. influenzae} and treatment of children with invasive \textit{H. influenzae} infections in order to avoid complications. Improved health education and prompt referral to tertiary health institution will improve patient outcome. Although, the prevalence of \textit{H. influenzae} in this environment was low, the incorporation of vaccination against \textit{H. influenzae} into the national immunization schedule is advised because of its potential complications.

Conflict of interest

There is no conflict of interest

Acknowledgement

All my secretariat staff

Reference


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