Cotrimoxazole Resistance in *Streptococcus pneumoniae* Isolated from Sputum of HIV-positive Patients
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**ABSTRACT**
The prevalence and cotrimoxazole susceptibility of *Streptococcus pneumoniae* isolated from sputum of 100 HIV-positive patients attending the Nigeria Institute of Medical Research clinic was investigated using standard microbiological methods. Eleven of the sputum specimens grew *Streptococcus pneumoniae*. Antimicrobial susceptibility test showed that all the isolates were sensitive to amoxicillin, augmentin, erythromycin and chloramphenicol but were resistant to cotrimoxazole. Continuous surveillance of *S pneumoniae* in sputum samples of HIV-positive subjects in this environment is necessary in order to regulate treatment regimen, considering that cotrimoxazole is the drug recommended by WHO for respiratory infections in HIV patients.

**RESUMEN**
Usando métodos microbiológicos convencionales, se investigó la prevalencia y la susceptibilidad al cotrimoxazol, del neumococo *Streptococcus pneumoniae* aislado a partir del esputo de 100 pacientes VIH-positivos que asistían a la clínica del Instituto Nigeriano de Investigaciones Médicas, Once de las muestras de esputo desarrollaron *Streptococcus pneumoniae*. La prueba de susceptibilidad antimicrobiana mostró que todos los aislados eran sensibles a la amoxicilina, la augmentina, la eritromicina, y el cloranfenicol, pero resistentes al cotrimoxazol. La vigilancia continua de *S pneumoniae* en las muestras de esputo de sujetos VIH positivos en este ambiente, es necesaria para regular el régimen del tratamiento, tomando en consideración que el cotrimoxazol es el medicamento recomendado por la OMS para las infecciones respiratorias en los pacientes de VIH.

Rate of pneumococcal bacteria in AIDS patients was 9.4 cases per 100 persons (2) and relative risk of pneumococcal bacteremia among persons with HIV-infection in Franklin county, Ohio, was 42 times that of county residence within the age of 18 to 64 years (3). The association of HIV and *S pneumoniae* in the developing world is similarly well defined (4). Respiratory disease is highly prevalent in HIV-infected subjects and *S pneumoniae* and *Mycobacterium tuberculosis* are the leading causes. Among female sex-workers in Nairobi, Kenya, *S pneumoniae* caused more diseases than *M tuberculosis* or non-typhoid salmonellae (5).

Rates of bacterial pneumonia in HIV-infected adults are increased 5–10 fold compared with age-matched controls and the mortality recorded among patients from Adis-Ababa was 11% (4). Similarly, data presented at a 2003 World Health Organization (WHO) conference indicated that pneumonia in HIV-infected children was the leading cause of...
Cotrimoxazole Resistance in HIV

hospital admission and most frequent cause of death in the six participating African countries (6).

Cotrimoxazole (CTX) is a broad spectrum antimicrobial agent strongly recommended by WHO as a primary or secondary prophylaxis for treatment of HIV bacterial infections in Africa (7). Studies conducted in Cote d’voire had indicated that daily cotrimoxazole will significantly control S pneumoniae infections (4). Alternative antibiotics would include ampicillin, first generation cephalosporins, macrolides and clindamycin. In patients with higher levels of penicillin resistance, vancomycin or imipenem/cilastatin has been recommended (8).

There is a paucity of information about the prevalence rate of Streptococcus pneumoniae in HIV infections in most parts of West Africa and particularly Nigeria. This study was carried out to provided baseline information on the prevalence of S pneumoniae in sputum samples of some HIV subjects in Lagos as well as the antibiotic susceptibility patterns of such isolates to cotrimoxazole and other antibiotics used in treating such ailment in this environment.

SUBJECTS AND METHODS

One hundred HIV-positive patients who were attending three Antiretroviral (ARV) clinics in Lagos, Nigeria, between the months of February and May 2006 were used for this study. The patients were those provisionally diagnosed by clinicians with symptoms of respiratory tract infections. Informed consent was obtained from each patient before the study was carried out. Biodata of each patient was also collected.

Early morning sputum samples were self-collected in sterile plastic containers and submitted to the laboratory and processed within two hours of collection.

A loopful of saliva free sputum was inoculated unto chocolate agar plates and incubated microaerophilically at 37°C for 24 hours. To ensure minimal saliva contamination, patients were instructed to pre-rinse their mouths prior to collection. For children, a cough plate was held before the child’s mouth as he/she coughed after pre-rinsing the mouth. Colonies with alpha-haemolytic properties were further sub-cultured for purification and later subjected to optochin sensitivity and bile solubility tests to confirm their identities (9).

Antibiotic susceptibility testing was performed according to standard procedures by the “Kirby-Bauer” method (10). The antibiotic discs used included the following: cotrimoxazole (25 mg), augmentin (25 mg), amoxicillin (25 mg), erythromycin (15 mg), tetracycline (10 mg), cloxacillin (10 mg), gentamycin (25 mg) and chloramphenicol (15 mg). A test control was set-up for comparison. Plates with antibiotic discs were incubated microaerophilically at 37°C.

RESULTS

A total of 100 subjects comprising 61 females and 39 males were used for this study. The majority (48%) were aged between 21 and 30 years while those belonging to the extreme age groups accounted for 9% (Table 1).

Table 1: Age and sex distribution of 100 patients used in study

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>No of Male Patients</th>
<th>No of Female Patients</th>
<th>Percentage (%) of population</th>
<th>No/percentage S pneumoniae positive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–10</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>11–20</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>21–30</td>
<td>18</td>
<td>30</td>
<td>48</td>
<td>4</td>
</tr>
<tr>
<td>31–40</td>
<td>8</td>
<td>11</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>41–50</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>51–60</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>–</td>
</tr>
</tbody>
</table>

There was bacterial growth on all the chocolate agar plates cultured and 58 alpha-haemolytic isolates appeared as Gram-positive cocci in chains or pairs when viewed under the microscope. However, 11 pure isolates were identified as Streptococcus pneumoniae being sensitive to optochin and bile soluble. Other bacteria encountered included Staphylococcus aureus and Moraxella catarrhalis.

Table 2: Antibiotic susceptibility patterns of S pneumoniae isolated from sputum of HIV patients

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No (%) Sensitive</th>
<th>No (%) Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin (Amx)</td>
<td>11 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Augmentin (Aug)</td>
<td>11 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Erythromycin (Ery)</td>
<td>11 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Gentamycin (Gen)</td>
<td>9 (81.9)</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>Tetracycline (Tet)</td>
<td>8 (72.7)</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>Chloramphenicol (Chl)</td>
<td>11 (100)</td>
<td>0 (100)</td>
</tr>
<tr>
<td>Cotrimoxazole (Cot)</td>
<td>0 (0.0)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Cloxacillin (Cxc)</td>
<td>8 (72.7)</td>
<td>3 (27.3)</td>
</tr>
</tbody>
</table>

Total No of isolates tested = 11

Table 2 shows the antibiotic susceptibility profile of the Streptococcus pneumoniae. All were sensitive to amoxicillin, augmentin, erythromycin and chloramphenicol. However, they were all resistant to cotrimoxazole. Some were equally resistant to cloxacillin, tetracycline and gentamycin.

DISCUSSION

It was observed in this study that the majority (88%) of HIV-positive patients were between the age of 21 and 40 years. Similar observations were made by Akinsete et al (II) among HIV-patients attending the Lagos University Teaching Hospital, Lagos, Nigeria. It has been established worldwide that the HIV scourge is mostly felt among the sexually active age group.

The prevalence of Streptococcus pneumoniae in HIV subjects with upper respiratory tract infection was observed to be 11.0% in this study. In a similar study conducted by Idika et al (12), the prevalence rate was 7.7%.

Elsewhere, Agwu et al (13) recorded a 6.4% frequency of occurrence of S pneumoniae among patients attending
tuberculosis clinics in Ekpoma, Edo State (Southern Nigeria). In contrast, rates of bacterial pneumoniae in HIV-infected adults are known to increase 5–10 fold compared with age-matched controls and figures ranging between 23% and 30% had been observed in some African countries (4). Low figures obtained in this study might be due to the limited sample size.

Antimicrobial susceptibility test showed that amoxicillin and clavoxacillin showed high activities against the S pneumoniae isolates. In contrast, Meynard et al (14) observed that 14 of the 45 hospitalized HIV-positive patients with pneumococcal disease had isolates resistant to penicillin. Similarly, we observed that erythromycin was active against all S pneumoniae isolates. In contrast, Johnson et al (15) reported that 15% of S pneumoniae isolated from HIV-patients in Atlanta (USA) were resistant to erythromycin while 27.5% resistance to this drug had been reported in Spain (16). Our study revealed that all the S pneumoniae encountered were resistant to cotrimoxazole. This antibiotic had been recommended by WHO for the treatment of pneumococcal disease in HIV/AIDS patients (7). Farrel et al (17) and Feikin (18) had similarly observed a high non-susceptibility rate in S pneumoniae isolated in paediatric patients. The resistance was due to the widespread use of trimethoprim-sulphamethoxazole (TMP/SMX) for prophylaxis and treatment of bacterial infections and chloroquine resistant malaria infections. Malaria is rampant in this environment and hence similar reason may be adduced for the non-susceptibility of all our isolates to cotrimoxazole.

This study, although limited, highlights the resistance pattern of S pneumoniae isolates from HIV-patients to cotrimoxazole, a drug of choice in the treatment of bacterial pneumoaniae. Therefore, a continuous surveillance of this pathogen and intervention strategies involving the use of other antibiotics including amoxicillin, augmentin and erythromycin which were found to be efficacious in this study, should be put in place. This is in view of the fact that vaccines against S pneumoniae are not available in Nigeria.

REFERENCES