Original Research Article

Streptococcus pneumoniae Colonization and Antibiogram of Isolates from Paediatric Population in Enugu, South East Nigeria

ABSTRACT

Aims: The aim of this research was to evaluate and identify *Streptococcus pneumoniae* from nasopharynx sample of children and carry out antibiotic susceptibility assay on the isolates; being that pneumococci are well noted as a significant cause of invasive pneumonia in infants with challenge of antibiotic resistance, which may worsen due to the emergence of such strains among children population

Study Design: This was a descriptive, cross sectional studies

Place and Duration: Research was conducted between March and September 2023 in Nsukka area of Enugu.

Methodology: Samples were collected from 100 childrenusing sterile swab sticks. These were inoculated unto blood and chocolate agar then incubated at 37^{o} Cfor 24 hours. Morphological and biochemical characteristics were used forthe identification. Antimicrobial susceptibility test using disk diffusion method was performed on isolates. The multi-discs used were pefloxacin 10mcg, gentamicin 10mcg, ampiclox 30mg, cefuroxime 20mg, amoxacillin 30mcg, ceftriazone 25mcg, ciprofloxacin 10mcg, and streptomycin 30mcg, co-tirmoxazole 30mcg, and, erythromycin 10mcg.

Results: The results identified 8.0% pediatrics with pneumococcal carriage. Males were 10.0% while females were 5.0%. The age distributions were as follows: 0-5 years, 4(8.7%); 6-10 years, 1(3.1%); and 11-15 years, 3(13.6%).There was 66.7% overallsensitivity rate of *S. pneumonia*. The resistant rates were 75% each to ampiclox, and amoxicillinwhilecefuroxime was 87.5% resistant.

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Conclusions: The findingsof this study indicated the emergence of multi-drug resistance pattern among *S. pneumonia* and their prevalence in the nasopharynx of childrenwhich increases the risk of resistant strains that cause pneumococcal infection.

Keywords: *Streptococcus pneumonia,* Antibiotics,Paediatrics

1. INTRODUCTION

Streptococcus pneumoniae, also known as *pneumococcus,* is a micro-organism that colonizes the human nasopharynx causing several invasive and non-invasive diseases. The organism characteristically has 90 different serotypes [1]. It is one of the major bacteria responsible for causing various diseases such as community acquired pneumonia (CAP), bacteremia, meningitis and sepsis [2]. It can cause mastoiditis, cellulitis, and arthritis in infants and early childhood [3]. Pneumococcus is often implicated in acute otitis media (AOM) and pneumonia [4], which is the main cause for antibiotics uses in children in so many countries [5].

Nasopharyngeal colonization of *S.pneumonia*is a predisposing factorforpneumococcal infection [6]. The bacteria are often transmitted through respiratory droplets, making children to be at risk, especially those in daycare or school settings. It can also be transmitted through direct contact [7]. Transmission of pneumococcus from children to household contacts or adults is also the principal cause of nasopharyngeal carriage and the spread of antibiotic-resistant clones. Predisposing factors to Nasopharyngeal carriage include younger age, attendance to nursery or school, number of siblings, and unfavorable social conditions [8, 9]. Pneumococcal acquisition is usually seen in adults, who are in close contacts with children. In a similar study, approximately 20% of adults were intermittent carriers and 10% were carriers for >4 months (median duration seven weeks) [10]. High pneumococcal

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carriage rates (up to 90 %) have been described in resource-limited countries with low vaccination coverage [11]. Co-infection can also be a major concern since *S. pneumonia* frequently co-inhabits nasopharynx with other bacteria such as *H. influenzae*. *S. pneumoniae* and *H. influenzae* migrate to other niches within the body to cause various diseases both in children and adults [12].

The global prevalence of *Streptococcus pneumoniae* infections in children varies significantly across different regions, age groups, and socioeconomic factors. This variation is influenced by factors such as access to healthcare, vaccination coverage, living conditions, and population density. *S. pneumoniae* infections are more prevalent in developing countries, particularly in sub-Saharan Africa and parts of Asia. In high-income countries, the incidence of invasive pneumococcal infections is 8 to 75 cases per 100 000 children under 5 years of age every year, with a case fatality rate of 6.5%, while in low-income countries, this rate increases from 100 to 500 cases per 100 000 children every year, with a case fatality rate of 8% [13].Thelow-incomeregions often experience higher rates of pneumococcal diseases due to limited access to medical care, poor sanitation, and crowded living conditions.

Based on research, invasive pneumococcal diseases (IPDs) are the leading cause of death in children under 5 years of age [11] and a prominent cause of antibiotic consumption. The high morbidity and mortality burden of *S. pneumoniae* disease is mostly found in children less than 5 years of age [3]. According to the Global Burden of Disease (GBD), in 2016, *S. pneumoniae* was the cause of more than 341,000 deaths in children below 5 years in 195 countries[14]. Infection can also be treated with low-cost medications and care. There are effective antibiotics against pneumococci, but management of the infection can be complicated by antimicrobial resistance.

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The World Health Organization recommended for countries to introduce safe pneumococcal conjugal vaccine (PCV) to protect children against *S. pneumococcus* infection [15].The incidence of*S. pneumoniae* pediatric infections has remarkably decreased, following introduction of pneumococcal conjugate vaccine (PCV). However, antibiotic pressure and the introduction of new conjugate vaccines can lead to changes in serotypes and rate of nasopharyngeal colonization [16]. Antibiogram assay of *S. pneumonia* is usually carried outto test the activities of selected antibiotics against isolates of *S. pneumococi* in accordance with the guidelines provided by the Clinical and Laboratory Standards Institute [17]. The emergence of drug resistant pneumococci has been reported around the world including 13 Asian countries, Africa and the Middle East [18] and Ethiopia [19]. Interestingly, *S. pneumoniae* strains with increased resistance to penicillin were found to frequently show cross-resistant to other antibiotics. For that reason, resistance to penicillin is reported to be a surrogate marker for the presence of a multi drug-resistant phenotype. These conditions inevitably lead to limitation of treatment options [20].

Some mechanisms may account for the increased rate of pneumococcal resistance against antibiotics. *S. pneumonia* can undergo genetic transformation and acquire DNA from other *streptococci* during asymptomatic nasopharyngeal carriage stage. This selection of antibiotic resistant pneumococcus occurs especially in children, because children are more often colonized and for longer periods. They are also more frequently exposed to antibiotics. Pneumococci were found to pick up a specific genome from bacteria that often colonize the nasopharynx such as *Streptococcus mitis*.The transferred genes were incorporated into their own DNA resulting in all β-lactam antimicrobials such as penicillin having a decreased binding affinity by the penicillin-binding proteins (PBPs). Several other mechanisms for antibiotics resistance have been identified such as increased drug efflux, target mutations, decreased bacterial permeability, and reduced penetration of the antibiotics to the target sites [21].

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Pneumococcal strains revealing resistance to more than three separate classes of antibiotics is considered to be multi drug resistant [22]. Multiple factors are potentially involved in emerging and spreading multi drug-resistant *S. pneumoniae* (MDR-SP), but prior excessive and improper uses of antibiotics, day care attendance, young age as well as horizontal gene transfer are the most frequently identified risk features [23]. Data about the resistance rates in young infants (especially six month or less) are relatively rare, therefore, knowledge is essential for determining the proper antibiotics against pneumococci isolates. Indeed, numerous physicians prescribe the antibiotics empirically before the results of microbiological culture are obtained particularly in infants. It is therefore imperative to study the antibiogram pattern to enable the detection of changes in resistance over time, allowing for timely adjustments in treatment guidelines and interventions especially among the very young children. Therefore, the present studies will contribute to the ongoing monitoring of *S. pneumoniae* characteristics in the nasopharynx and also provide valuable epidemiological information based on the antibiogram of isolates obtained from the pediatric populations in order to offer a valuable insight and assist in clinical decision-making when choosing empirical antibiotic therapies.

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2. MATERIALS AND METHODS

2.1 Population and Sampling

Nasopharyngeal swabs were collected from 100 children aged 0–15 years randomly selected from two nursery and primary schools, which represented up to 12.7% of the population of children. Infants six months of age or less from both genders were also enrolled in the study. The subjects were mobilized by public awareness of the dangers of pneumonia caused *Streptococcus pneumoniae* and ways to prevent it. The population was randomly selected from nursery and primary school pupils within Enugu in Nigeria where the head teachers of the school gave consent. Prior to the sampling, informed consents forms

were filled by the parents for participation in the study. Participation forms were also provided and completed by the parents, which provided useful personal information of the children. Samples from nasopharynx were collected by sterile swab sticks under aseptic precautions and carried to the laboratory.

2.2 Inclusion and Exclusion Criteria

Inclusion criterion was infants/children at age 0 – 15 years from both genders. While exclusion criteria were those above 15 years and those who had known immunologic disorders. Also, those who had received antibiotic treatment within past four weeks during the research were excluded.

2.3. Culture and Identification of Isolates

The media used were blood and chocolate agar (supplemented with 5% sheep blood prepared from nutrient agar, Oxoid, UK). The content of the swab was aseptically inoculated unto the agar surface and streaked to obtain discrete colonies for easy identification. The plates were incubated overnight at 37°C with added 5% CO_2.

All agar plates were incubatedfor 18-24 hours. Plates showing no growth after 48 hours were recorded as negative cultures. The microbial colony growths were identified according to colonies morphology. Gram's stain reaction and standard biochemical tests were carried out. Biochemical tests carried out include catalase enzyme test, bile solubility test and optochin sensitivity tests. Bile solubility reagent was prepared using 2 grams of sodium deoxycholate bile salt put into 100 ml of sterile water, making it 2% bile salt. Then, the solution containing saline and bacterial suspension was parted into two sterile test tubes and labeled as control and test culture. The turbidity was adjusted to that of 0.5-1 McFarland standard. In a test culture tube, two drops of bile salt reagent were added and two drops of

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saline were added in the control tube. The test tubes were shaken vigorously to ensure uniform mixing of the cell suspension with the reagent. After that, the tubes were incubated for 18-24 hours at 35 -37 ^{o}C. At last, the tubes were observed for the turbidity or clearance.

2.4 Antibiotic Susceptibility Testing

Antibiotic Susceptibility test was carried out using Kirby-Bauer disk diffusion method following the recommended standard of Clinical and Laboratory Standard Institute [17]. A broth suspension with sterile normal saline was made from a 24 hours growth of the test organisms and adjusted to match the0.5 McFarland turbidity standard. The 0.5 McFarland standards was equivalent to a bacterial suspension containing between 1 x 10^{8} and 2 x 10^{8} CFU/ml of *S. pneumonia*. The suspension was seeded on the entire surface of solidified Mueller-Hinton agar (Sigma-Aldrich, U.S. A) supplemented with 5% sheep blood. Antibiotics disks (Oxoid, Hamshire, UK) were carefully placed centrally on the seeded plate.. The antibiotics used were pefloxacin (10mcg), gentamicin (10mcg), ampiclox (30mcg), cefuroxime(20mcg), amoxicillin (30mcg), ceftriaxone (25mcg), ciprofloxacin (10mcg), streptomycin (30mcg), co-trimoxazole (30mcg), and, erythromycin (10mcg). The disk was not moved once it has contacted the agar surface even if the disk was not in the proper location, because some of the drugs start diffusing as soon as it comes in contact with the agar. The incubation of the agar plates was at 35 -37 ^{o}C for 24 hours. The antimicrobial agent diffused from the disc to the medium and the growth of the organism was inhibited at a distance from the disc that was equivalent to the sensitivity of the organism whereas resistant strains had little or no inhibition zones. The zones were measured by placing the metric ruler on the plate across the zone of inhibition, measuring from one edge of the zone to the other edge. The diameter of the disk was also included in the measurement. The plate was viewed using a direct, vertical line of sight to avoid any parallax that may result to misreading.

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3. RESULTS

Colonies growth suspected of pneumococci displayed typical morphological characteristic of mucoid or smooth colonies of 0.5-2mm in size. The gram stain microscopic morphology showed typical lancet–shaped Gram -positive cells. Colonies appeared white or mostly yellow with yellowish clear zones of haemolysis on the chocolate agar media but partial heamolytic clear zones on the blood agars. Those isolates from the 100 subjects subsequently confirmed by bile solubility and optochin disk sensitivity and labeled as *S. pneumonia* isolates were 8(8.0%). The 6(10.0%) were from males while 2(5.0%) were positive among the females. The age distributions were as follows: 0-5 years, 4(8.7%), 6-10 years, 1(3.1%), 11-15 years, 3(13.6%); (Table 1).

There was 66.7% overallsensitivity rate of *S. pneumoniae*. The resistant rates were 75% each to ampiclox & amoxacillin while cefuroxime was 87.5% resistant.

Streptomycin, Ceftriazone, Cotirmoxazole, Erythromycin & Gentamicin was 100% sensitive each, while ciprofloxacin was 75.0% sensitive. Pefloxacin showed intermediate sensitivity.

Table 1

Prevalence of Pneumococcal carriage in children by gender and age range

Variable	Number of positive result (%)	Total Number (%)
Gender:		
Male	6(10.0)	60(60.0)
Female	2(5.0)	40(40.0)

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Age:		
0 -5 years	4 (8.7)	46 (46)
6 -10 years	1(3.1)	32(32)
11 -15 years	3(13.6)	22(22)
Total	8(8.0)	100 (100)

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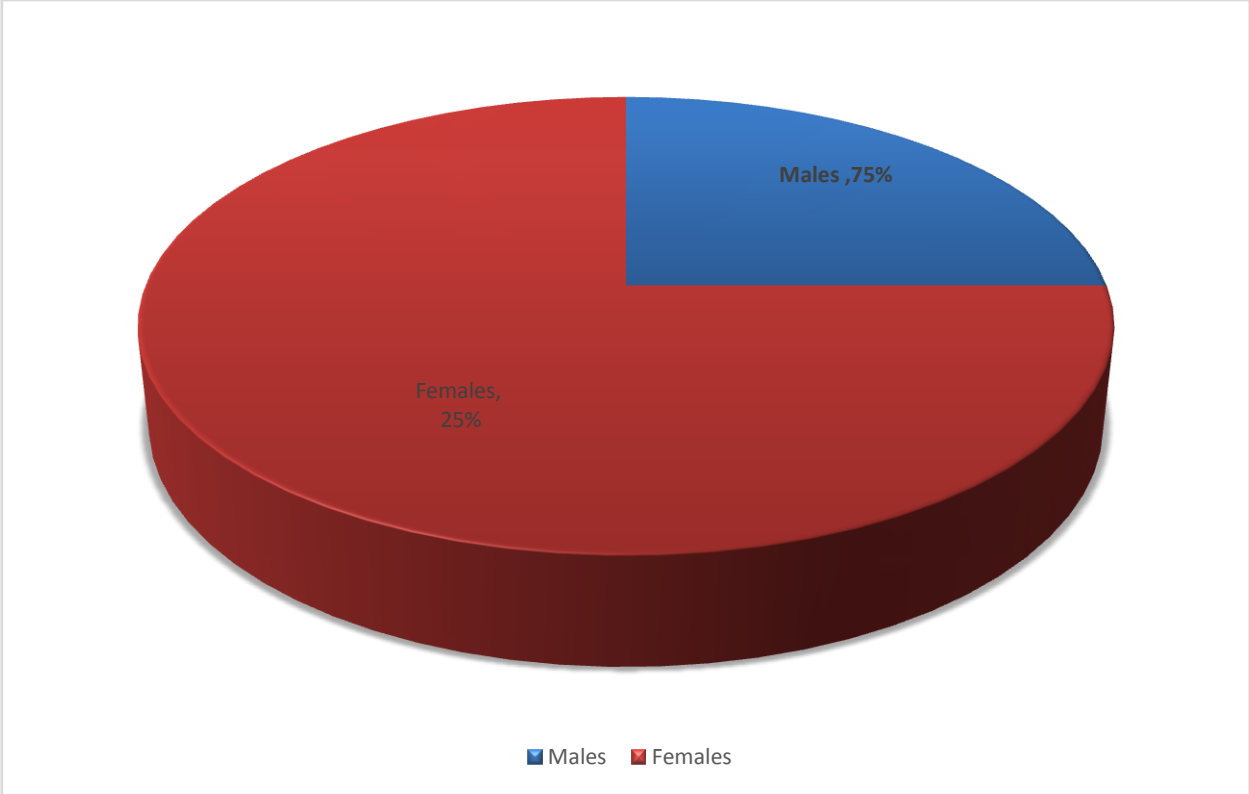

Figure 1. Male- Female Ratio Prevalence of *Pneumococci* Carriage

Table 2

Susceptibility pattern of the Antibiotics tested against the *Streptococcus pneumonia* isolates

Antibiotics(Interpretation guide)	**Sensitivity Rates**	**Resistant rates**
Ciprofloxacin(S>21; R<15)	**6(75)**	**2(25)**
Streptomycin(S>15; R<11)	**8(100)**	**0(0.0)**
Cotirmoxazole (S>15; R<11)	**8(100)**	**0(0.0)**

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Erythromycin(S>23; R<13)	**8(100)**	**0(0.0)**
Pefloxacin(S>23; R<14)	**7(87.5)****	**1(12.5)**
Gentamicin(S>15; R<12)	**8(100)**	**0(0.0)**
Ampiclox(S>19; R<8)	**2(25)**	**6(75)**
cefuroxime(S>20; R<7	**1(12.5)**	**7(87.5)**
Amoxacillin(S>18; R<13)	**2(25)**	**6(75)**
Ceftriazone(S>12; R<9)	**8(100)**	**0(0.0)**

**** = Intermediate Sensitivity**

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4. DISCUSSION

The pediatric subjects in study identified with pneumococcal carriage were mostly within the range of 0-5 years old. It is well documented that children less than 5 years of age are more susceptible to pneumonia than older children [13], and tend to have worse prognosis of the disease [24, 25]. The prevalence of *S. pneumoniae* infections varies by age. The overall prevalence rate of colonization obtained in this study was low in contrast to that obtained from China[26] and Northern Tanzania[27]. Koliou *et al* [28] also reported that about one-third (25.0%) of children but 3–4% of adults were asymptomatically carriers of pneumonia. In Nigeria also, during a pre-vaccination study,the prevalence of carriage, the rate was higher in children (67.4%) compared to adults (26.0%)[29]. Similarly, Dananché *et al*., [30] reported, the incidence of pneumococcal conjugate vaccine (PCV13) serotypes to be as high as 67.1%, in nasopharyngeal carriagecases among children aged < 5 years. Higher pneumococcal carriage rates (up to 90%) were reported in many resource-limited countries with low vaccination coverage [11]. Streptococcal carriage in this study was, however, comparable to some in mid-income countries against higher rates (65%-48%) in low income

nations [31]. Many past studies in India observed high nasopharyngeal (NP) colonization with SP [32].In Nigeria by 2016 carriage prevalence had reduced from 21 to 12% as childhood (<5 years) vaccine coverage rose from 7 to 84% [33]. This downward slide of prevalence was also noted in this present study with a prevalence of 8.0% indicating wide vaccination coverage in the country. Apart from socioeconomic and environmental factors, seasonal variation may also affect the prevalence carriage rates being that the incidence of the pneumococci in higher in colder months.

Among the tested antibiotics in the current study, Streptomycin, and gentamicin showed an excellent activity against all *S. pneumoniae* isolates, which was in line with the study conducted in Nigeria [34], while three out of four (75.0%) sample of *S. pneumoniae* isolates were resistance to b-lactams(cefuroxime, ampiclox, and amoxicillin). It is known that amoxicillin and ampicillin/ampiclox is most frequently used in treating pneumonia in children. The resistances noticed may have evoked as a result of abuses of this antibiotic. Unlike the cephalosporin (cefuroxime), that is rarely prescribed or used. The prevalence rate of resistance to b-lactams among *S. pneumonia* strains has also been increasing and is becoming one of the most important antimicrobial resistant threats world-wide [35]. In this study, the isolates were found to be resistant to more than one class of drug and more than 30% resistance prevalence, which is a pointer to multi-drug resistance trait. In a recent study among US children the majority (56.8%) of isolates were resistant to at least 1 drug class during and 30.7% of isolates were resistant to ≥2 drug classes [36].

The antibiogram result in this present study also indicated the emergence of a multi-drug resistance (i.e., non-susceptibility to three or more antibiotic classes) pattern among *S. pneumoniae* isolates. It is often noted that increase of multi-drug resistance in *S. pneumoniae* isolates makes the treatment of these infections more difficult. It is highly recommended that further studies will be conducted among children with CAP to carry out antibiotic susceptibility test against such isolates within this same region.

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5. CONCLUSION

The antibiogram pattern obtained in this study indicates the emergence of multi-drug resistance among *S. pneumonia* and the prevalence of antibiotics-resistant especially the beta-lactam. The low prevalence of pneumococcal carriage highly commends good vaccination coverage in this region. However, campaigns against self-medication and antibiotic abuses should be boosted in the country.

CONSENT (WHEREEVER APPLICABLE)

"All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

"All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

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