ANTIMICROBIAL RESISTANT PATTERN OF *Streptococcus pneumoniae* ISOLATED FROM CLINICAL SAMPLES OF CHILDREN AGED 1-12 YEARS IN SELECTED HOSPITALS FROM SAPELE DELTA STATE, NIGERIA

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ABSTRACT: The efficiency of treatment for pneumonia infections has been hampered by *Streptococcus pneumoniae* resistance to antimicrobial agents and the spread of resistant strains is also significantly influenced by the underuse of antibiotics. The current study reveals the antimicrobial resistant pattern of *Streptococcus pneumoniae* isolated from clinical samples of children in selected hospitals of Sapele, Delta state Nigeria. One hundred blood and nasal swab samples of children showing different symptoms of pneumonia like chest pain, cough, fever, chills, difficulty in breathing were collected at different intervals from five hospitals in Sapele, Delta state Nigeria for isolation of *S. pneumoniae*. The children were separated into four age brackets. The age brackets included patients within the ages of 1-3years, 4-6 years, 7-9years and finally 10-12 years. Samples were aseptically plated on nutrient agar and blood agar and the isolates identified macroscopically and microscopically using standard microbiological methods. The age group 4-6years had highest percentage positive of *S. pneumoniae* 32%, followed by 1-3 years the percentage of *S.pneumoniae* isolated was 27%, 7-9 years had 22% *S. pneumoniae,* while 10-12 years had 19% *S. pneumoniae*. Antibiotics susceptibility was performed using disc diffusion technique on Mueller Hinton agar for all the bacterial isolates. Thirteen different antibiotics were used against identified *Streptococcus pneumoniae*. The antibiotic study revealed that the isolated organism, *Streptococcus pneumoniae* was sensitive to six antibiotics, Neomycin, Streptomycin, Erythromycin, Amikacin, Azithromycin, Bacitracin with the following zones of inhibition 18mm, 22mm, 18mm, 20mm, 15mm and 18mm respectively and resistant to Ampicillin, Methicillin, Penicillin, Clindamycin, Ciprofloxacin, Vancomycin, followed by Cefixime. Data obtained were analyzed using SPSS version 21 and a p-value less than 0.05 was statistically considered significant. Hence children below the 12years should be vaccinated against bacterial pneumonia to decrease the death rate of this age group.

Keywords: Resistance, susceptibility, age, *Streptococcus pneumoniae*.

Introduction

Lung infected disease known as pneumonia results in an accumulation of bacteria fluid and inflammatory cells in the air sacs of the affected person's lung, impairing their ability to breathe. The symptoms and signs of pneumonia infection is usually acute lower respiratory tract infection, which is confirmed by chest X-ray.*Streptococcus pneumoniae* is a gram positive bacterium that has spherical shape and belongs to the genus streptococcus; it also exhibits alpha hemolytic characteristics (Barroso *et al.*, 2012). They do not form spores and usually occur as diplococcic (in pairs) and are non-motile (Castaneda *et al.*, 2021). As an important pathogen that affects humans *Streptococcus pneumoniae* was known as the main cause of pneumonia disease towards the end of 19th century and also the point of attraction in the study of humoral immunity. *S. pneumoniae* lives without significant symptoms in a healthy carrier and settles in the upper respiratory tract, nasal cavity and sinuses. Howsoever, In individuals with weak immune system such as older people and children who are likely to be infected, the organism may cause disease, damage tissues and be transmitted to other parts of the body.

According to Albiger *et al.*, 2007, it is transmitted directly between individuals through respiratory droplets and by autoinoculation in those who have the organism in their upper respiratory tracts. It might be the source of infections in newborns. The primary cause of sepsis in HIV-positive individuals as well as community-acquired pneumonia and meningitis particularly in elderly people and children, is *Streptococcus pneumoniae*. The organism is also aetiologic agent of other forms of illnesses caused by pneumococci. Bronchitis, rhinitis, acute sinusitis, otitis media, conjunctivitis, meningitis, sepsis, osteomyelitis, septic, arthritis, endocarditis, peritonitis, pericarditis and cellulitis are among the invasive pneumococcal illnesses.

Streptococcus pneumoniae possesses capsule that consists of polysaccharide which contributes to the organism's virulence. There are more than a hundred distinct *S. pneumoniae* serotypes identified. They differ in prevalence, virulence and medication resistance's limits. Pneumonia is an inflammation of one or both lungs, typically due to bacteria, viral or fungal infection. 151.8 million new cases of community-acquired pneumonia are projected to occur annually among children under five years in developing and poor nations, 11-20 million of these cases necessitate hospital stays (Rudan *et al.*, 2004).

In underdeveloped nations, pneumonia occurs often and is more severe than in developed nations, and carries higher incidence and death rates. Pneumonia accounts for one- fifth of under-five deaths within the underdeveloped world (Bryce *et al.*, 2005). Regarding the susceptibility of *S. pneumoniae* to antibiotics the minimum inhibitory concentration (MIC) breakpoints for the antibiotic drug were initially established in response to the necessity for making certain roaring treatment of diplococcus infectious disease. More than three decades past, increasing MICs to penicillin have emerged in pneumococci, and the proportion of penicillin-intermediate and penicillin-resistant strains have risen (Berezin et al., 1996; Whitney *et al.*, 2000).

Materials and methods

Study area

This study was conducted in Sapele metropolis, in Delta state, Nigeria. Sapele which is today one of the most important industrial port town in the western location of the Niger Delta Nigeria was a small village belonging to the people of Okpe in Urhobo. it is situated at the left bank of the Ethiope River. The population of people in Sapele metropolis according to 2006 population census was 142,052. Sapele is located within longititude 050 41'27" E to 05042'05" E and Latitude 05051'55" N to 05052'03" N on the western side of the Niger Delta. (Owhoudue and Agbini, 2021). The people's occupation is majorly fishing and farming. The religious beliefs of the Sapele indigenes are majorly traditional religion though some belong to Christian religion. The local government of Sapele has its headquarters at Sapele a cosmopolitan town and an important seaport for trade in timber, palm oil and rubber related products. It is one of the oil producing areas of the state. Major communities Amukpe, Sapele, Elume, Oghedi, Ikeresian and Ughorhen

Isolation of *Streptococcus pneumoniae*

A total of 100 blood and nasal swab samples of children with pneumonia were collected at different intervals from five hospitals in Sapele, for isolation of *Streptococcus pneumoniae*. Out of 100 samples, 40 samples were collected from patients attending Central Hospital Sapele, 20 samples were from Graceville children Hospital Sapele, 20 samples from Obule Medical Diagnostic centre, 10 samples from Jetcare clinic Sapele and 10 from Biomed diagnostic. The patients were divided by age into four groups. The first group included children within the age1-3yrs, followed by 4-6yrs then 7-9 yrs and finally 10-12yrs. Samples were plated on were on nutrient agar and blood agar then incubated at 37^0C for 24hours for microbiological growth formation. Pure culture of the isolate was obtained by subculturing in blood agar and hemolytic reactions observed in the blood agar. *Streptococcus pneumoniae* gives alpha hemolysis.

Characterization of Bacteria Isolate

The bacterium isolated was characterized on the basis of its macroscopic, microscopic and biochemical characteristics based on Cheesbrough, 2006. The following tests were carried out for the identification of the isolate; Gram reaction, catalase, methyl red test, voges Proskauer, urease, oxidase, Indole, coagulase test

Antibiotics Susceptibility Test

The standard Kirby-Bauer disk diffusion method was used to examined the antimicrobial susceptibility pattern of the organism isolated based on the recommendations of National Committee for Clinical Laboratory Standards Institute (Weinstein and Lewis 2020). Bacterial inoculum was prepared by suspending fresh bacteria growth in 4-5ml sterile nutrient broth and the turbidity was adjusted to 0.5 McFarland standards. The testing of antimicrobial susceptibility was done using Mueller Hinton medium, Antibiotic disks were applied using sterile forceps. Agar containing plates were incubated at 37^0C for 18 hours. After 24 hours incubation at 37^oC the diameter in millimeters of the inhibition zones around each of the antimicrobial discs was recorded and categorized as resistant or sensitive.

Statistical Analysis

Data were analyzed using SPSS version 21. The chi-square ($\chi 2$) test was used to assess statistical differences between the groups. A p-value below 0.05 was statistically considered significant.

Results

Out of 100 samples collected from children between the age of 1-12 years who attended five different hospitals in Sapele 68 (68%) positive cases of pneumonia were found to be present. The results of morphological, staining, cultural, biochemical and values of percentage of incidence of isolated bacteria are shown in different tables.

Figure1 reveals the occurrence of *Streptococcus pneumoniae* from the samples in relation to age range. From all the samples collected, the patients within the age of 4-6years had the highest percentage of *Streptococcus pneumoniae* 22 (32%), followed by patients within the age of 1-3yrs 18(27%), 7-9yrs had 15(22%) *S. pneumoniae*, while 10-12 years had 13(19%) *S.pneumoniae*.

Table 1 shows frequency of *S. pneumoniae* in the samples according to hospitals (study areas). The study area that showed highest prevalence was Grace ville children Hospital 19(28%), followed by central hospital 15(22%) and Obule medical diagnostic centre 15(22%) then Jetcare clinic and Biomed diagnostic centre had 10(15%) and 9 (13%) respectively. This research reveals that the area of study had no significant effect ($P > 0.05$).

Figure 2 shows the Occurrence of *S. pneumoniae* in positive samples of respondents. Out of 68% *S.pneumoniae* isolated, 58 (85%) were recovered from nasal swabs while 10(15%) were from blood. The blood culture was carried out by filling the sterile bottles containing brain heart infusion with 3ml of collected blood samples and incubated for 24hrs at 37^0C. Table 2 summarizes the phenotypic and biochemical properties of the isolated organism. The outcomes of microscopic appearance, cultural characteristics and biochemical tests were used for the identification of the organisms. Samples of nasal swab and blood were plated on nutrient agar and blood agar at 37^0C for 24 hours and finally observed Convex, smooth &gray-white colonies in nutrient agar, the alpha-hemolytic colony on blood agar. Then bacterial colonies were identified using Gram's stain which showed spherical chain shape diplococci cells and purple color.

The antibiotics susceptibility was conducted by utilizing the disc diffusion technique on Mueller Hinton agar for all the isolates to the common antibiotic agents. Thirteen different antibiotics were used against identified *Streptococcus pneumoniae* (Table 3). The antibiotic study revealed that the isolate, *S. pneumoniae* was sensitive to six antibiotics, Neomycin showed zone of inhibition of 18mm, Streptomycin 22mm, Erythromycin 18mm, Amikacin 20mm, Azithromycin 15mm and Bacitracin 18mm. The isolated organism was resistant to Penicillin, Ampicillin, Methicillin, Clindamycin, Ciprofloxacin, Vancomycin, followed by Cefixime.

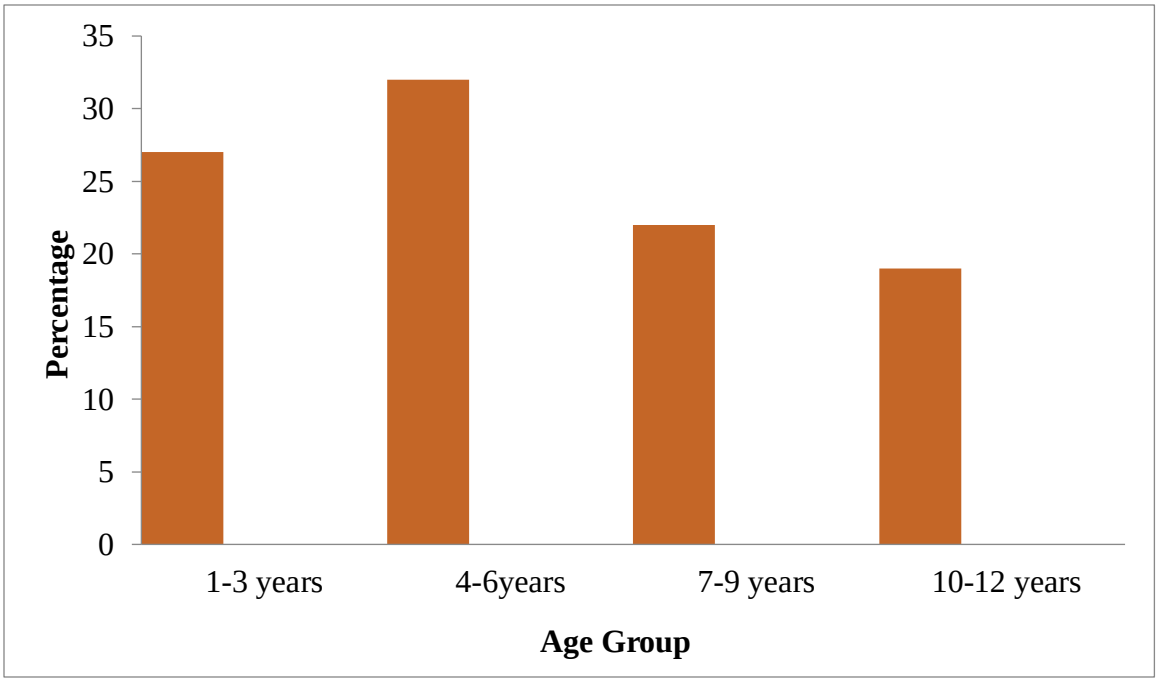

Figure1: Distribution of *Streptococcus pneumoniae* according to age group

Table 1: Frequency of the *Streptococcus pneumoniae* in the Hospitals

Hospitals	No Examined	No of *S. pneumoniae*	Percentage (%)
Central hospital	40	15	22%
Graceville children hospital	20	19	28%
Obule medical diagnostic centre	20	15	22%
Jet care clinic	10	10	15%
Biomed diagnostic centre	10	9	13%
Total	100	68	100

Table 2: Biochemical Properties of *Streptococcus pneumoniae* Isolated

Biochemical Tests	Reactions
Growth on blood agar	Alpha hemolytic
Gram Staining	Gram positive
Shape	Spherical/diplococci
Catalase	-
Oxidase	-
Methyl red	+
Voges Prausker	-
Indole	-

KEY += positive reaction; - = negative reaction

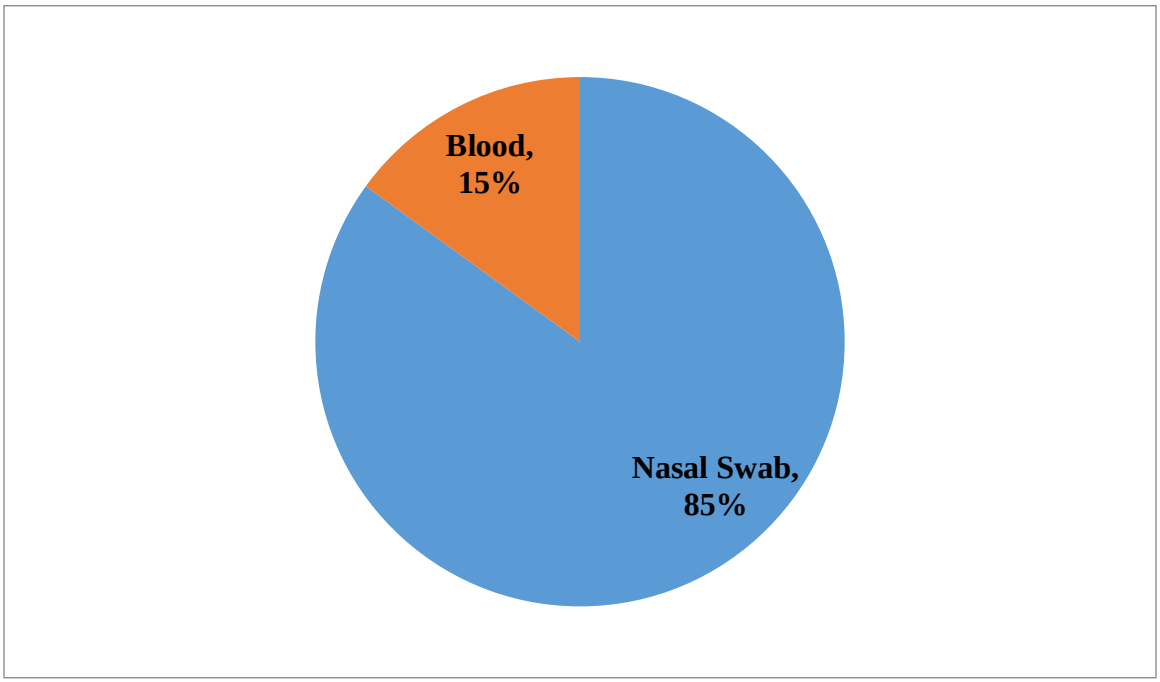

Figure 2: Distribution of *Streptococcus pneumoniae* in Samples

Table 3: Antibiotic Susceptibility Patterns of *S. pneumoniae*

Names of Antibiotics	Interpretation
Methicillin (30µm)	R
Penicillin (30µm)	R
Clindamycin (30µm)	R
Streptomycin (30µm)	S
Bacitracin (30µm)	S
Erythromycin (30µm)	S
Ampicillin (25µm)	R
Ciprofloxacin (10µm)	R
Vancomycin (30µm)	R
Cefixime (25µm)	R
Azithromycin (30µ)	S
Neomycin (30µ)	S
Amikacin (30µ)	S

Discussion

S. pneumoniae infection is among the most extensively disseminated illnesses causing high children death and morbidity rates. The present study observed higher prevalence of *S. pneumoniae* 68% in children which is greater than those of Onipede *et al.*, 2009 from Ile-Ife Nigeria. Differences in methodology may account for this. Also high isolation of Streptococcus *pneumoniae* in this research is a pointer to the magnitude of invasive *S. pneumoniae* infections in the area of study, which suggests the importance of preventive strategies like immunization, adequate nutrition and addressing environmental factors such as indoor air pollution aimed at curtailing the immediate and late untoward effects of invasive Streptococcal infections.

The present study showed an increased prevalence of pneumonia in children within age of 4-6yrs to be 8(36%) followed by children who were between the age bracket 1-3 years, this might be as a result of immature immune system, young children have underdeveloped immune systems, especially in the first few years of life. They rely more on maternal antibodies (which decline after birth) and have lower levels of specific antibodies against *S. pneumoniae*, reducing their ability to clear the bacteria.

Table 3 shows the occurrence of *S. pneuminiae* in positive samples of children. The nasal swab sample showed higher percentage of *S. pneumoniae* 85% than in blood samples 15%. This occurs as a result of the fact that *S. pneumoniae* can induce ultimate illness in the normally sterile area of children's nose mucous membranes by colonizing them in an asymptomatic manner. This organism lives in the nasopharyngeal mucosa of healthy people; surface adhension protein facilitate the attachment of the organism to the epithelial cells. The microorganisms in the nasopharngeal retains small amount of pneumococcus but was not allowed to exhibit its pathogenic capacity and be the cause of disease. As soon as the typical microflora's dominance is compromised, following adhesion and local replication, pneumococcus can be transmitted into the sinuses, middle ear or lungs. The low proportion of the infectious agent *S. pneumoniae* in blood indicates that few numbers of these children had bacteremia, these can be related to type of treatment given to them and socio economic background of the participants.

The antibiotics study revealed that the isolate was susceptible to streptomycin, erythromycin, azthramycin and backtracking which are related to Nnanna *et al.,* 2023 in a research carried out at Warri, Delta State Nigeria. The organism isolated was not susceptible to penicillin, amoxicillin which is related to Huang *et al.*, 2015. *S. pneumoniae* exhibited extreme resistance to penicillin and Amoxicillin which was comparable to research of Kandakai-Olukemi and Dido, 2009 who investigated the antibiotic resistant profile of *S.pneumoniae* from the nasopharynx of secondary school children in Jos, Nigeria. The following authors showed that azithromycin and erythromycin are usually prescribed for resistant antibiotics (Thummeepak, *et al.*, 2015 Sabrina *et al.*, 2012. While this research found azithromycin and erythromycin highly sensitive. Rapee *et al.*, 2015 found in their work that *Streptococcus pneumonia*e was resistant to these antibiotics. This research suggested streptomycin, erythromycin, bacitracin, azithromycin, neomycin and amikacin for the management and treatment of infections brought-on by *S. pneumoniae*. The multi drug resistance exhibited by this organism may be the consequences of misuse and underused of the antibiotics, lack of clean water and sanitation inadequate disease prevention and control promotes the transmission and spread of microorganisms, some of which may be resistant to antibiotic treatment.

Conclusion

The bacteria *S.pneumoniae* is the aetiologic agent of children pneumonia worldwide especially in developing countries. The current study discovered that bacterial pneumonia was predominant in children within 4-6 years and 3-5years age limit. The organism isolated is multi-drug resistant which may occur due to misuse and overuse of antibiotics.

Recommendations

Vaccines are recommended for:

- Children below 12 years, (it is currently a component of the standard immunization scheduled for infants and children.
- Adults and children with immune deficiencies and chronic diseases are susceptible to pneumococcal infections.
- Adults 65 years and older
- Individuals between 19 and 64 years who are at risk for certain medical conditions.
- Individuals that work in a long-term care facility or in a nursing homes.

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