Original Research Article

Antibiotic Susceptibility Patterns of *Streptococcus pneumoniae* Isolated from the Nasopharyngeal Mucosa of Children in Enugu Metropolis, Nigeria

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A B S T R A C T

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This study reports the prevalence and antibiotic susceptibility patterns of *Streptococcus pneumoniae* in the nasopharyngeal mucosa of sick and healthy children aged between 1-10years in Enugu metropolis. A total of 380 swabs of nasopharyngeal mucosa was collected using sterile swab sticks and plated on blood agar at 35°C for 18-24 hours. The suspected isolated *S. pneumoniae* bacteria were identified and characterized using standard microbiology techniques. Susceptibility of *S. pneumoniae* isolates to antibiotics was done on Mueller Hinton agar (Oxoid, Uk) by disk diffusion technique. Two hundred and eleven samples (55.5 %) out of the 380 swab samples collected were positive for *S. pneumoniae*. Female children had a higher prevalence rate of 115 (54.5 %) as against 96(45.5 %) for the male children. A prevalence rate of 122(57.8 %) and 89(42.2 %) were observed among sick and healthy children respectively. Antibiotic sensitivity results show that *S. pneumoniae* was highly susceptible to cefotaxime 52 (58 %), followed by chloramphenicol 58 (65 %), gentamycin 70 (78%), doxycycline 71 (79 %), clindamycin 73 (82 %), and ciprofloxacin 80 (89 %) while it was resistant to trimethoprim-sulphamethoxazole 27 (30 %), followed by amoxicillin 24 (27 %), erythromycin 21 (24 %) and clindamycin 16 (18 %). *S. pneumoniae* was also susceptible to leavofloxacin 122 (100 %), ciprofloxacin 111(91 %), doxycycline 101(83 %), gentamycin 100(82 %) and chloramphenicol 82(67 %) but was highly resistant to cefotaxime 74(60 %), followed by amoxicillin 36(29 %); trimethoprime-sulphamethoxazole 30(25 %), erythromycin 18(14 %), and clindamycin 14 (11 %) among sick children. This study recorded a high prevalence/carriage rate of antibiotic resistant *Streptococcus pneumoniae* among both hospitalized and healthy children in Enugu metropolis. However, there are extremely limited data on the healthy carriers of *S. pneumoniae* in the pediatric population in Enugu State, hence the probable under-estimation of the resulting disease burden in the area. Therefore, there is need to investigate the colonization rate of *S. pneumoniae* among hospitalized and healthy children in selected primary schools and those admitted at the Enugu State University Teaching Hospital (ESUTH), Enugu State, Nigeria, with a view to studying the susceptibility of the pneumococcal isolates to antimicrobial agents.

Introduction

Streptococcus pneumoniae (Pneumococcus) bacteria are lancet-shaped, Gram-positive, facultative anaerobic bacteria with more than 90 known serotypes. *Streptococcus pneumoniae* colonize the mucosal surfaces in the nasopharynx of human beings from the first day of life through transmission by contact with respiratory secretions, thereby making individuals especially children healthy carriers of the bacteria. After primary colonization of the nasopharynx, they can migrate to other sites, such as middle ear, sinus, lung, blood, or cerebrospinal fluid and cause damage, leading to invasive disease (Cunha, 2003). Pneumococcus is spread by airborne droplets and is a leading cause of serious illness, including bacteremia, meningitis, and pneumonia among children and adults worldwide (Nuorti and Whitney, 2010; Thigpen *et al.,* 2011). Around 14.5 million episodes of severe pneumococcal disease occur annually in the world, causing 1,612,000 deaths; 825,000 of them among children under 5 years old, representing 11% of the total number of infant deaths. In the year 2000, the estimated number of serious pneumococcal diseases to occur globally was 14.5 million, leading to about 826 000 deaths in children aged one month to five years (O'Brien *et al.,* 2009). *Streptococcus pneumoniae* is the leading cause of potentially life-threatening community-acquired diseases and is associated with an estimated global mortality rate that is in the same order of magnitude as that of tuberculosis (3-5 million deaths per year). Pneumonia is the leading cause of death in children worldwide and the most important pathogen causing the disease is the bacterium *Streptococcus Pneumoniae* (the pneumococcus). Infections caused by *S. pneumoniae* including pneumonia, meningitis, bacteremia, sinusitis and otitis are extremely common, and their associated morbidity and mortality place a tremendous financial burden on the society (Hoffman *et al.,* 2005). Pneumococcal diseases are a major public health problem all over the world. Resistance of *Streptococcus pneumoniae* to penicillin and other antibiotics is increasing worldwide (Applebaum, 2002). Many bacteria, including *Streptococcus pneumoniae* (pneumococcus) have become resistant to one or more classes of antibiotics which in turn lead to treatment failures. The last two decades of the 20th century were marked by an increasing resistance rate among several bacteria. Threat of resistance has been observed in *Staphylococcus spp*., *Enterococcus spp*., *Pseudomonas spp.* and *Enterobacteriaceae*, which are the major pathogens in nosocomial infections. Misdiagnosis and unnecessary prescription of antibiotics, as well as lack of education on bacterial antibiotic resistance are important factors in the emergence of resistance with attendant public health and economic loss consequences.

Since it has been scientifically proven that *S. pneumoniae* is a leading cause of morbidity and mortality especially in children, the elderly and the immunocompromised, therefore, knowledge of its antimicrobial resistance is of major concern. The antimicrobial resistance profile of *S. pneumoniae* isolated from nasopharynx can be used to predict antimicrobial resistance that may arise in clinically significant isolates since the nasopharynx is the primary source of pneumococi. This study is therefore designed to determine the antimicrobial susceptibility pattern and also to evaluate the colonization rate of *Streptococcus pneumoniae* in the nasopharyngeal mucosa of healthy children from selected primary schools and those admitted at the Enugu State University

Teaching Hospital (ESUTH), Enugu state.

Material and Methods

Sample collection: Sterile swab stick was inserted into the anterior nares (nostril) of each participant and swept upwards the top of the nares. The procedure was repeated with the same swab stick in the other nares and the swab was used to inoculate the surface of a blood agar medium forty-five minutes after collection. The nasal swabs were promptly refrigerated in cases where immediate inoculations were not possible.

Characterization of isolates: All the bacterial isolates were identified using standard microbiology techniques.

Optochin sensitivity test: Using inoculating wire loop, 3-4 pure colonies of *S. pneumoniae* were selected on the blood agar plate. One-half of newly prepared culture plate was streaked in an area around 3cm square. Optochin disc was placed in the upper third of the streaked area and the disc pressed with flamed forceps so that disc adheres to the agar surface. The plate was then incubated at 35ºC for 18–24 hours. Alpha hemolysis showing a zone of inhibition of 14mm around a 6mm disc presumptively indicates the presence of *S. pneumoniae*.

Bile solubility test: Several colonies of the test organism were emulsified in a test tube containing 2ml of sterile physiological saline to give a turbid suspension. The suspension of the organism was divided into two test tubes. To the first test tube, two drops of sodium deoxycholate reagent was added and mixed while two drops of sterile distilled water was added to the second test tube. The two test tubes were left on a bench at 37ºC for 10–15 minutes and the observation of a gradual clearing of turbidity

in the test tube containing sodium deoxycholate shows a positive test (Cheesbrough, 2006).

Disc diffusion susceptibility testing

Twenty milliliter each of molten Mueller Hinton agar was poured aseptically into sterile Petri dishes and then allowed to gel and labeled. A sterilized wire loop was used to inoculate the pure culture of the organism on the plate of Mueller Hinton agar medium. The surface of the medium was streaked in four directions while the plate was being rotated approximately 60 degree to ensure even distribution. With the Petri dish lid in place, the surface of the Mueller Hinton agar medium was allowed to dry for 25 minutes. A sterilized forceps was used to place the antibiotic discs evenly distributed on the inoculated Mueller Hinton agar so that the disc should be about 15mm from the edge of the plate and not close than 25mm from disc to disc. After 30 minutes, the plates were inverted and incubated for at least 18–24 hours. A ruler was used to measure the diameter of each zone of inhibition in mm on the underside of the plate. The interpretation as 'Sensitive' or 'Resistant' was done on the basis of diameters of zones of inhibition of bacterial growth as recommended by Clinical and Laboratory Standards Institute (CLSI, 2005). The following standard antibiotic discs were used against the isolates: Leavofloxacin, Gentamycin, Trimethoprim-sulpha-methoxazole, Ciprofloxacin, Chloram-phenicol, Cefotaxime, Doxycycline, amoxicillin, erythromycin and Clindamycin. The minimum inhibitory concentrations of the antibiotics against *S. pneumoniae* were determined by plotting a line graph of the squares of the inhibition zone diameters against the log of the concentrations of antibiotics used. The antilog of the intercept of the graph at the horizontal axis was

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obtained and recorded as the Minimum Inhibitory Concentrations of the drugs against the organism.

The colonization rate of *S. pneumoniae* isolated from the nasopharyngeal mucosa of both sick and healthy children in Enugu metropolis was calculated using:

Rate = n/N X 100/1

Where:

R= Carriage rate for the organism
Where n = number of participants that tested positive for the organism
N= total number of people from which sample was collected

Results and Discussion

The widespread use of broad-spectrum antibiotics has led to the emergence of nosocomial infections caused by drug resistant microbes (Courvalin and Weber, 2005; Chikere *et al.,* 2008).

In this study, the carriage rate of *Streptococcus pneumoniae* among children according to the gender of the participants was higher among the females with a percentage of 115(54.5 %) than males 96 (45.5 %). This result corroborates the findings of Lin *et al.* (2009) who observed a carriage rate of 43.5 % among healthy children and in contrast with the findings of El-Mahmood, (2010) and Cheng, (2003) who reported the carriage rate of *S. pneumoniae* to be 22(19.3); 28(23.7) and 20(6.9) and 27(9.2) in males and females respectively. The carriage rate of *S. pneumoniae* was higher among the participants who were sick (57.8 %) whereas the rate in healthy children who participated in the study was observed and recorded as 42.8 % (Table 3). This was in agreement

with the findings of Kandakai-Olukemi and Dido (2009) who recorded a prevalence of 42.04 % among healthy carriers and in contrast with the findings of Christian *et al.* (2009) who recorded a high prevalence rate of 82.0 % and 76.7 % among healthy and sick carriers respectively.

The highest prevalence rate of 70.8 % was recorded among children aged 1-2 years while the lowest prevalence rate of 44.1 % was observed among children aged 9-10 suggesting age could be a determining factor to carriage rate of the organism in the nasopharynx (Table 4). Prevalence rates of 60.9 %, 54.1 % and 49.5 % were observed among children aged 3-4, 5-6 and 7-8 years respectively (Table 4). This is in agreement with the findings of Cheng (2004).

S. pneumoniae isolated from sick children were susceptible (97.8 %) and resistant (2.2 %) to leavofloxacin while the organism was susceptible (58.4 %) and resistant (41.6 %) to cefotaxime (Table 5). *S. pneumoniae* was sensitive (60.7 %) and resistant (39.3 %) to cefotaxime in sick children. This is in agreement with the findings of Joseph and George, (2010) who recorded susceptibility of 98.0 % of *S. pneumoniae* to leavofloxacin.

S. pneumoniae isolated from the healthy children showed 89.9 % susceptibility and 10.1 % resistance values to Ciprofloxacin while *S. pneumoniae* isolated from the sick children showed 91.0 % susceptibility and 9.0 % resistance values to ciprofloxacin. This is in agreement with the findings of Kandakai-Olukemi and Dido (2009) and that of Joseph and George (2008) who observed resistance and sensitive scores of 10.81 % and 7.9 % respectively but contradicts El-Mahmood *et al.* (2009) with observations of 10.6 % susceptibility by *S. pneumoniae* to ciprofloxacin. *S. pneumoniae* isolated from

healthy children was resistant (18.0 %) and susceptible (82.0 %) to clindamycin while *S. pneumoniae* isolated from sick children were resistant (11.5 %) and susceptible (88.5 %) to clindamycin.

The susceptibility and resistance patterns of *S. pneumoniae* to the range of antibiotics tested in this study were higher among the sick children than among healthy children.

In the test of the organism against Chloramphenicol, it was observed that *S. pneumoniae* showed susceptibility and resistance of 65.2 % and 34.8 % respectively in the swab samples from healthy children and susceptibility and resistance of 67.2% and 32.7 % respectively for the sick children. This is not in concord with the findings of El-Mahmood *et al.* (2009) that observed a susceptibility of 4.2 % and Kandakai-Olukemi and Dido (2009) who observed a resistance of 21.6 % by *S. pneumoniae* to chloramphenicol. *S. pneumoniae* showed susceptibility and resistance of 79.8 % and 20.2 % respectively to doxycycline among healthy children and susceptibility and resistance values of 82.8 % and 17.2 % respectively among sick children.

S. pneumoniae isolated from healthy children was susceptible (76.4 %) and resistant (23.6 %) to erythromycin while *S. pneumoniae* isolated from sick children was susceptible (85.5 %) and resistant (14.8 %). This is in contrast with the findings of Kandakai-Olukemi and Dido (2009) who recorded a resistance and susceptibility of 32.43 % and 10.1 % respectively by *S. pneumoniae* to erythromycin.

S. pneumoniae showed susceptibility and resistance of 73.0 % and 27.0 % respectively to amoxicillin in healthy children but recorded a susceptibility and resistance of

70.5 % and 29.5 % to amoxicillin in sick children. *S. pneumoniae* isolated from healthy children was susceptibility (78.7 %) and resistant (21.3 %) to gentamycin but *S. pneumoniae* isolated from sick children was susceptible (82.0 %) and resistant (18.0 %) to gentamycin. This corroborated the findings of Kandakai-Olukemi and Dido (2009) who recorded a resistance of (21.62 %) by *S. pneumoniae* to gentamycin and highly in contrast to the findings of El-Mahmood (2009) who recorded a susceptibility of 7.6 % by *S. pneumoniae* to gentamycin. *S. pneumoniae* isolated from sick children was resistant (24.6 %) and susceptible (75.4 %) to Trimethoprim-sulphmethoxazole while *S. pneumoniae* isolated from healthy children was susceptible (69.7 %) and resistant (30.3 %) to trimethoprime-sulphamethoxazole.

Multidrug resistance and the presence of several virulence factors in strains of many pathogens responsible for different diseases pose an increasing threat to the successful management of disease scourge. Also, the rising prevalence of drug resistance such as penicillin-resistant *pneumococci* worldwide mandates selective susceptibility testing and epidemiological investigations during outbreaks (Okonko *et al.,* 2008). Infections caused by resistant pathogens result in significant morbidity and mortality, and contribute to escalating healthcare costs worldwide. Despite the availability of newer antibiotics, emerging antimicrobial resistance has become an increasing problem in many pathogens throughout the world (Keith and John, 2005).

High rates of drug resistance of isolates of *S. pneumoniae* were recorded in most of the antibiotics that were used in this study. In developing countries like Nigeria, self medication is a common practice and this might probably be a major cause of

antibiotic resistance in clinical isolates since many infected people only think of going to the hospitals or taking their children to the hospital when self-medication fails. Inappropriate practices like misuse and abuse of antibiotics and unskilled practitioners can also lead to emergence of antibiotic resistant bacteria especially *S. pneumoniae* which is a normal flora of humans.

Table.1 The carriage rate of *Streptococcus pneumoniae* among male and female children in Enugu metropolis

S/N	Sex of participants	Number of participants	Number positive	Percentage carriage rate (%)
1.	Females	196	115	54.5
2.	Males	184	96	45.5
	Total	380	211	100

Table.2 Carriage rate of *Streptococcus pneumoniae* among Sick and Healthy Children in Enugu Metropolis

S/N	Status of Participants	Number of Participants	Number Positive	Percentage (%)
1.	Sick children	200	122	57.8
2.	Healthy children	180	89	42.2
	Total	380	211	100

Table.3 Carriage rate of *Streptococcus pneumoniae* among Children in Enugu Metropolis According to Age Groups

Age Group of Participants	Number of Participants	Number Positive	Percentage (%)
1-2	72	51	70.8
3-4	69	42	60.9
5-6	74	40	54.1
7-8	97	48	49.5
9-10	68	30	44.1
Total	380	211	55.5

Table.4 Antibiotic Resistance and Susceptibility Patterns to *S. pneumoniae* of Sick and Healthy Children

	Healthy Children		Sick Children	
	Susceptible (%)	Resistance (%)	Susceptible (%)	Resistance (%)
Antibiotics Used				
Ciprofloxacin	80(89.9)	9(10.1)	111(91.0)	11(9.0)
Cefotaxime	52(58.4)	37(41.6)	74 (60.7)	48(39.3)
Clindamycin	73(82.0)	16(18.0)	108(88.5)	14(11.5)
Chloramphenicol	58(65.2)	31(34.8)	82 (67.2)	40(32.7)
Doxycycline	71(79.8)	18(20.2)	101(82.8)	21(17.2)
Erythromycin	68(76.4)	21(23.6)	104(85.5)	18(14.8)
Leavofloxacin	87(97.8)	2(2.2)	122(100)	0(0.0)
Amoxicillin	65(73.0)	24(27.0)	86(70.5)	36(29.5)
Gentamycin	70(78.7)	9(21.3)	100(82.0)	22(18.0)
Trimethoprim-sulphmethoxazole	62(69.7)	27(30.3)	92(75.4)	30(24.6)

Expired antibiotics, self-medication, counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates (Prescott *et al.,* 2005). According to Suchitra and Lakshmidevi (2009), intensive medical therapies and frequent use of antimicrobial drugs are capable of selection of resistant microbial flora.

Nosocomial infections due to resistant organisms have been a problem with an increase in the incidence of methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus* (VRE) *P. aeruginosa* (Suchitra and Lakshmidevi, 2009) and *S. pneumoniae*. These results suggest that multi-drug resistance among clinical pathogens is common and significant in Nigeria and call for nationwide surveillance program to monitor microbial trends and antimicrobial resistance patterns in Nigeria. One of the explanations for these high resistance rates could be antibiotic usage in the respective health institutions in Nigeria (Doughari *et al.,* 2005). Determining the antimicrobial patterns of the disease causing organism will enable health institutions to restrict the use of antimicrobials and take active measures in preventing the spread of drug resistance at home and most especially in hospitals.

In line with the assertions by Doughari *et al.* (2005), the findings of this study have important implications for practicing physicians with regard to empirical antibiotic selection, for authorities involved in hospital formulary decisions, and in the development of policies regarding antibiotic

utilization, infection control and public healthcare towards children. Therefore, it is important for hospitals to improve the processes of care known to impact nosocomial infection rates by ensuring adequate adherence to standard operating procedures to avoid the spread of the pathogenic organism especially *S. pneumoniae* since it has been observed that resistance to drugs is worse with hospital-related pathogens. There is also a high need of sensitization among the general public and most especially mothers on the need for avoidance of drug abuse and misuse especially towards children.

In conclusion, however, the judicious use of antibiotics by health workers and efforts to control procurement and use of antibiotics officially in all localities in Nigeria will probably help to limit the increasing rates of drug resistance to pathogens especially *S. pneumoniae*. Therefore, it is of prime importance that all sectors (medicine, veterinary, horticulture, etc.) using antibiotics cooperate to minimize the proliferation of resistant *S. pneumoniae* bacteria, which may more generally have important consequences for virulence evolution and disease control especially among children.

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