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**Nasopharyngeal Carriage of Streptococcus Pneumoniae Among Apparently Healthy Children Attending Clinics in Ikara and Makarfi Local Government Areas of Kaduna State, Nigeria**

Bitet, E.D. 1\*, Fyaktu, F.J. 2, Mohammed A.B. 3

General hospital Kagarko, Ministry of Health and Human Services, Kaduna State, Nigeria 1, Department of Medical Microbiology, College of Medical Science, University of Jos, Plateau State, Nigeria 2, Department of Medical Laboratory Science, Faculty of Allied Health Science, Collage of Health Science, Bayero University Kano, Kano State Nigeria 3.

Author for Correspondence: [ezekiidogobetet@gmail.com](mailto:ezekiidogobetet@gmail.com)/ +234-803-652-3901

**Abstract**

The aim of this study was to investigate the nasopharyngeal carriage of *Streptococcus Pneumoniae* among apparently healthy children < 3 years attending 2 health facilities in Ikara and Makarfi Local Government Areas of Kaduna State. Nasopharyngeal Swab samples were collected from three hundred and seventy (370) children comprising of 188 (50.80%) males and 182 (49.20%) females. The samples were cultured and the isolates were identified using standard biochemical and serological methods. All optochin resistant isolates were also identified using polymerase chain reaction (PCR). A total of 110 *S. pneumoniae* isolates were recovered giving a nasopharyngeal carriage of 29.70%. The carriage rate was found to be slightly higher among females 57(31.30%) than males 53 (28.20%). In relation to age, the highest 63(57.30%) was recorded for children 0 – 5 months and the lowest 3(2.70%) for children 21 – 25 months. Overall carriage rate was found to decrease with increasing age. However, these findings were not statistically significant ( $p > 0.05$ ). Six serotypes were distinguished, these included the following according to decreasing rate of occurrence; serotype 1, 43(39.10%), 3, 33(30.00%), 5, 5(4.50%), 12, 5(4.50%), serotype 4, 3(2.70%) and 6, 3(2.70%) respectively. However, 18(16.40%) of the isolates were non-typeable. Three isolates were observed to be optochin resistant and were further identified using polymerase chain reaction (PCR). Based on this study, there may be a relationship between optochin resistance to prior chloroquine treatment. Introduction of pneumococcal conjugate vaccination (PCV)

among high risk age groups may help in reducing nasopharyngeal carriage as well as the risk of both invasive and non-invasive pneumococcal diseases in the study area.

**Keywords:** *Streptococcus pneumoniae*, optochin resistance, under three, serotypes, nasopharyngeal carriage.

**Introduction**

*Streptococcus pneumonia* is a gram-positive, alpha – haemolytic, rod – oval to lancet shape, aero-tolerant member of the genus *Streptococcus* (Ruyan *et al.*, 2004), *S. pneumoniae* forms part of the normal flora in 40-70% of humans (Henrichsen, 1999). *S. pneumoniae*, causes respiratory infection in children younger than five years.

The organism was first named *Diplococcus pneumoniae* (Winslow *et al.*, 1920) because of its appearance in gram stained sputum; it was renamed *S. pneumoniae* in 1974 because of its growth in chains in liquid media (Far *et al.*, 1994). *S. pneumoniae* normally colonizes the human nasopharynx (throat and nose) often asymptotically. The pathogen remains a major cause of childhood morbidity and mortality worldwide (Henrichsen, 1999). Pneumococcal disease has been a major public health problem worldwide. In 2005, the World Health Organization (WHO) estimated that 3-5 million deaths occur annually in children younger than five years of age due to pneumococcal disease (Adetifa *et al.*, 2012).

The pathogen can cause several diseases such as:

bacteremia, pneumonia, meningitis, and septicemia in persons infected with Human Immune Deficient Virus (HIV) (Adetifa *et al.*, 2012). Other infections include; otitis media, acute sinusitis, bronchitis, osteomyelitis, conjunctivitis, peritonitis, endocarditis and arthritis (Ifedayo *et al.*, 2012).

Based on structural differences in its capsular polysaccharides, more than 93 pneumococcal types have been identified (Nahm *et al.*, 2011). Some serotypes are more frequently associated with invasive disease while others are related to nasopharyngeal carriage (Hautsдорff *et al.*, 2000).

The increase incidence of pneumococcal disease in children and the occurrence of antimicrobial resistance have been correlated to the levels of carriage (Onyemelekwé *et al.*, 1999).

Factors influencing carriage include; immune status, seasonal or geographical variation, socio economic, crowding and other demographic factors. It is highest in infants and decreases with age (Sorensen *et al.*, 1993).

Optochin resistant *S. pneumoniae* isolates have been reported, hence, indicating the potential for misidentification of this important pathogen (Tatiana *et al.*, 2013).

Therefore, correct detection, identification and serotyping have opened way for the production of new generation of pneumococcal conjugate vaccines to prevent invasive pneumococcal diseases (IPD) (Sorensen *et al.*, 1993) and for proper treatment protocol (Arai *et al.*, 2001). Variation of serotype, prevalence and the emergence of antibiotic resistance in different geographical areas, necessitate the typing and characterization of strains from these areas for effective vaccine protection (Arai *et al.*, 2001). Thus, study on the molecular epidemiology of *S. pneumoniae* for children in one region is beneficial to monitor pneumococcal resistant clones (Lin *et al.*, 2010). Therefore, there is the need to update data information based on the increasing resistance to recommended antibiotic from different geographical areas of the world for better management.

## Materials and methods

### Study area

This study was carried out in Ikara and Makarfi Local Government Areas (LGAs) of Kaduna State, in North-West Nigeria. Kaduna State has a population of 2,603,084 inhabitants according to the 2006 census (Kaduna encyclopedia). It has a land area of 3,080 square kilometers. Kaduna State was created by the Federal Government of Nigeria in 1976 from Northern region. It is bounded on the North by Kano and Katsina States, in the East by Abuja FCT and Nasarawa State, in the West by Niger State and in the South by Plateau and Bauchi States. Kaduna has experienced a huge population growth at an annual rate of 3%. The influx of people mostly to the urban centre is believed to be an important factor in this growth. The rainy season begins from April and ends in October with a brief interlude of harmattan occasioned by the north east tides with a main feature of dryness. The annual total rainfall is 1,200- 1,800mmHg. There are diverse ethnic groups in Kaduna State, these include; Hausa, Mwanghavul, Fulani, Bagyi, Atyep, Gwandara, Jaba, Marwa, Bajura, among others. The major occupations are; farming, cattle rearing, and business. A minority of the population are civil servants. The two LGAs have a number of primary health centers and a secondary health institution offering pediatric clinics making it suitable for the study.

Laboratory analysis was carried out at the Microbiology Department of General Hospital Makarfi, established in 1986. The hospital laboratory department was supported and equipped by Center for Integrated Health Program (CIHP).

### Study population

The study population consisted of apparently healthy children < 3 years. The following individuals were included as subjects in the study; age between < 36 months, attendance to the immunization programme in Primary Health Care center (PHC) in Ikara and General Hospital (GH) Makarfi in Ikara and Makarfi local government areas of Kaduna State. Parents and guardians gave verbal informed consent before their participation in the study.

**Sample size**

A total of three hundred and seventy (370) samples were used for this research. This was obtained using the standard formula as provided by Bonnet *et al.* (2006).

Following the assumption that the local prevalence rate of *S. pneumoniae* carriage among children in developing countries is 52.50 % (Adetifaet *al.*, 2008), and a precision of 5 % (with 15% confidence).

Formula:  $n = \frac{Z^2 P(1-P)}{d^2}$

Where:

n = Sampling size

P = Prevalence rate

Z = Standard normal distribution of 95% confidence limit = 1.96

d = Absolute desired precision at 5% = 0.05

Hence;  $1.962 \times 0.5(1-0.525) = 364.$   
0.052

However, in order to obtain equal samples from each sampling point, 370 samples were used for the study.

**Inclusion criteria**

Apparently healthy children < 3 years of both gender attending immunization program whose parents and guardians gave verbal informed consent were eligible for enrollment in the study.

**Exclusion criteria**

The following individuals were excluded from this study; adults, children  $\geq$  3 years, children whose parents/ guardian refused to offer a verbal consent for their children/ward to participate in the study and children who exhibited overt symptoms of respiratory tract infections.

**Ethical consideration**

The study protocol was approved by the ethics committee of the State Ministry of Health, Kaduna, Kaduna State, Nigeria. Also, the Medical Director and The Head of Laboratory Department (HOD) of General Hospital Makarfi in Kaduna State approved the use of their Microbiology Laboratory for processing and analysis of samples.

**Sampling method**

Nasopharyngeal samples were obtained from three hundred and seventy (370) apparently healthy children < 3 years from one (1) primary health care centre (PHC Ikara) and one (1) General hospital (GH Makarfi). The opt in option was adopted on several clinics days to obtain the required sample size.

**Data and sample collection**

A questionnaire was administered to the parent/ guardian of each child and the health personnel on duty to help obtained demographic, personal, simple clinical and age information.

Nasopharyngeal samples were obtained by a trained Paediatrician and a Specialist Nurse of the Ear, Nose and throat (ENT) Unit of General Hospital, Makarfi and Ikara. Using sterile flexible swabs, manufactured by Evepon Industries Limited. The swab was inserted into the nostril of each participant until meeting slight resistance and was rotated twice to obtain the sample.

**Transportation of swabs**

After collection of samples, the swabs were placed in a cold box containing ice pack of temperature between 2 – 8<sup>o</sup>c and was transported to the laboratory, according to Ontario Association of Medical Laboratories (OAML), community laboratory practice guidelines (OAML 2004). All samples were processed within 45minutes – 1hr after collection, at the Microbiology Laboratory of General Hospital Makarfi of Kaduna State.

**Culture and incubation of samples**

Samples were inoculated on 5% sheep blood agar (SBA) prepared according to the manufacturer's instructions and incubated at 37<sup>o</sup>c for 24 hours in 5% carbon dioxide (candle extinction jar).

**Identification of isolates**

After the period of incubation, the inoculated agar plates were inspected for bacterial growth. Isolates were identified as *S. pneumoniae* using the following criteria.



### Physical examination (macroscopy)

After the period of incubation, each plate was observed macroscopically. The suspected *S. pneumoniae* colonies were large (others small), grey, mucoid and others non- mucoid and about 1 – 2mm in diameter. The colonies had raised edges and exhibited alpha haemolysis (green - discoloration) after incubation (typical of *S. pneumoniae*). After 48 hours of incubation, colonies became flattened.

### Gram's staining

Isolates were tested for Gram Stain. Gram staining is a method of differentiating bacteria species into two large groups; gram- positive and gram-negative. Gram staining differentiate bacteria based on the chemical and physical properties of their cell walls by detecting peptidoglycan which is present in a thick layer in gram –positive bacteria (Tankeshwar, 2013). *S. pneumoniae* has a positive gram reaction.

### Catalase test

Isolates were tested for catalase. Catalase is the enzyme that breaks down hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) into water (H<sub>2</sub>O) and Oxygen (O<sub>2</sub>). The Oxygen is given off as bubbles in liquid. The catalase test is primarily used to differentiate between gram–positive cocci. Members of the genus *Staphylococcus* are catalase positive, and members of the genera *Streptococcus* and *Enterococcus* are catalase –negative.

### Optochin sensitivity test

Isolates were tested for Optochin sensitivity. *Streptococcus pneumoniae* strains are sensitive to the chemical optochin (Ethylhydrocupreine hydrochloride). Optochin sensitivity allows for the presumptive identification of alpha – hemolytic Streptococci as *S. pneumoniae*, although some pneumococcal strains are optochin – resistant. Zone size of  $\geq 14$ mm indicated susceptibility which was diagnostic of *S. pneumoniae* while zone size < 14mm indicates resistance.

### Bile solubility test

Isolates were tested for bile solubility. The bile salt (sodium deoxycholate) solubility test distinguishes *S. pneumoniae* from all other alpha

– haemolytic streptococci. *S. pneumoniae* is bile salt soluble whereas all other alpha – haemolytic streptococci are bile salt resistant. Sodium deoxycholate (2% in water) will lyses the pneumococcal cell wall. Clearance of the tube or loss of turbidity indicated a positive result while persistence turbidity indicates negative result.

### Serotyping

The Quelling reaction has been the “gold standard” method for typing of *S. pneumoniae*. The conventional method of serotyping *S. pneumoniae* uses pneumococcal typing antisera. The Quelling reaction (swelling reaction) forms the basis of serotyping and relies on the swelling of the capsule upon binding of anticapsular antibody (Korir, 2004). Serotyping is the gold standard of distinguishing between *S. pneumoniae* strains based on their capsular type. Serotyping of the isolates was conducted using antisera to polysaccharide capsules representing polyvalent 1, 2, 3 and monovalent 1, 3, 4, 5, 6 and 12 (Denka Seiken Co., Ltd, Japan). The rapid slide agglutination method of serotyping *S. pneumoniae* using pneumococcal antisera was used. A positive reaction is indicated by agglutination within 5 – 10 seconds. A negative reaction is indicated by no agglutination within 5 – 10 seconds.

### DNA Extraction

Before beginning, set a heating block at 60C. Isolate of *S. pneumoniae* were scraped the using a plastic loop and suspended in a 1.5ml tube containing 400ul of lysis buffer to which 25ul of protenase K was added. The tube was placed on heat block at 60C for minimum 1hours. Four hundred microliters (400ul) of phenol chloroform (1:1) was added to the lysate and vortexed briefly. The tube was centrifuged at 10000rpm for 10 minutes to separate the phases. The upper layer was carefully removed with a pipette and added to a new 1.5ml tube. Avoid extracting the white interphase. Four hundred microliters (400ul) of chloroform was added to the recovered clear layer and vortexed briefly at 10000rpm for 10 minutes to separate the phases. The upper layer was carefully removed with a pipette and added to a new 1.5ml tube. Avoid extracting the white interphase. Equal volumes of 100% ethanol and 20ul of 3M sodium acetate



were added and mixed by inverting the tube several times. The tube was incubated at -20C overnight. Subsequently, the tube was centrifuged at a maximum speed for 10-30 minutes (preferably in a refrigerated centrifuge) in the same orientation. The ethanol was removed and 400ul 70% ethanol added. Centrifuge the tube at a maximum speed for 5 minutes at 4oC. Repeat again. This is just to make sure that the salt is all gone. This procedure may need to be repeated one more time. As much ethanol as possible was removed and the tube was centrifuged for 30 seconds at high speed to remove all traces of ethanol. There may be need to spin again to remove the remaining traces. The DNA was dried out by leaving the tube open for 3-10 minutes. The pellet was resuspended in 20-50ul sterile water or in appropriate volume of water.

### **Polymerase chain reaction (PCR) of pneumococcal autolysin (Lyt A)**

The LytA gene was detected by PCR using primer described by Masaki *et al.* (2012). Using a Biometra T Gradient Thermocycler (Biometra GmbH, Goettingen, Germany). Thirty cycles of DNA amplification were performed as follows: denaturation at 94 oC for 15 seconds, annealing at 50 oC for 30 seconds, and extension at 72oC for 30 seconds. The presence of an amplified 273 – bp sequence of autolysin gene indicated the presence of the LytA gene. The DNA size marker was 100 bp ladder DNA (Pharmacia Biotech Co., Ltd, Tokyo, Japan); forward primer 5' – CAA CCG TAC AGA ATG AAG CGG – 3' and reverse primer 5' – TTA TTC GTG CAA TAC TCG TGC G – 3'. Green PCR master mix 2x (Pharmacia Biotech Co., Ltd, Tokyo, Japan) was used in 50µl reaction; (25µl Taq PCR master mix 2x, 2.5µl forward primer, 2.5µl reverse primer, 5µl DNA template and nuclease free water up to 50µl). Bands were suspected to be seen at 300bp.

Agarose gel electrophoresis of amplified products; 10µL reaction mixture and 5µL of 1000bp ladder water separated on 2% agarose gel with 0.3ug/ ml ethidium bromide. The bands were visualized using UV trans illuminator captured and analyzed by computer software.

### **Statistical Analysis**

SPSS software version 22.0 was use for the statistical analysis of data collected. Chi-square test was used. A p-value of  $\leq 0.05$  was considered statistically significant in all statistical analysis.

### **Result**

A total number of 370 apparently healthy children <3 years attending two health facilities in two local government areas of Kaduna State participated in this study. The participating health facilities were made up of one primary health care center (PHC Ikara) and one secondary health facility (General Hospital Makarfi) running immunization programs. Equal numbers of subjects (185 subjects) were recruited from each facility for the study. The total number consisted of 188(50.8%) male's subject and 182 (49.2%) female subjects giving an approximate male: female ratio of 1:1. Sample collection from the subjects lasted between June to August 2015.

Apparently healthy children between the ages of 0-5 months formed the bulk of the study population with 201 (54.32%) Subjects, followed by children between the ages of 6-10 months with 118(31.90%). Children between the ages of 11-15 months and 16-20 months formed 29(7.83%) and 12(3.24%) of the study subjects respectively. The least number 10(2.70%) among the study population comprise of children between 21 – 25 months.

Data derived from the administered questionnaire about the subjects and laboratory results were entered into a computer and analyzed by the statistical program SPSS software to obtained p-value. For all statistical analysis, a p-value  $\leq 0.05$  were considered significant and p - values  $> 0.05$  were considered not significant. Results were expressed in simple frequencies with 95% confidence interval.

Out of the total number of subjects sampled, *S. pneumoniae* was isolated from 110 of them, accounting for 29.70%. The occurrence of *S. pneumoniae* carriage varied between the different age groups with highest frequency of 63 (57.30%) among children between 6-10 months.

The lowest observed frequency of *S. pneumoniae* carriage was 3(2.70%) found among children age 21-25 months ( $p < 0.05$ )

Isolation of *S. pneumoniae* among the study population also varied slightly between subjects

of different genders. *S. pneumoniae* was isolated from 53 (28.20%) of male subjects and 57 (31.30%) of female subjects. This distribution however, had no statistically significant difference ( $p > 0.05$ ).

**Table 1: Carriage rate of *S. pneumoniae* among apparently healthy children (0 – 25 months) attending health facilities in Ikara and Makarfi L.G.A of Kaduna state in relation to Gender and Age.**

Age (Months)	Number of subjects		Number (%) positive		Total (%)
	Male	Female	Male	Female	
0 – 5	105	96	29(27.60)	34(35.40)	<b>63(57.30)</b>
6-10	61	57	14(23.00)	16(28.10)	<b>30(27.30)</b>
11-15	11	18	5(45.50)	5(27.80)	<b>10(9.10)</b>
16-20	6	6	3(50.00)	1(6.70)	<b>4(3.60)</b>
21-25	5	5	2(40.00)	1(20.00)	<b>3(2.70)</b>
<b>Total</b>	<b>188</b>	<b>182</b>	<b>53(28.20)</b>	<b>57(31.30)</b>	<b>110(29.70)</b>

Age:  $p = 0.004$  Gender:  $p = 0.231$

**Table 2: Distribution of *S. pneumoniae* serotypes among apparently healthy children (0 – 25 months) in Ikara and Makarfi LGAs of Kaduna State in relation to age.**

Age (Months)	Serotypes No. (%) Isolated							Total
	1	3	4	5	6	12	NT	
0-5	21(33.30)	23(36.50)	2(3.20)	3(4.80)	1(1.60)	1(1.60)	12(19.00)	<b>63(57.30)</b>
6-10	14(46.70)	8(26.70)	1(3.30)	1(3.30)	0(0.00)	2(6.70)	4(13.30)	<b>30(27.30)</b>
11-15	3(30.00)	2(20.00)	0(0.00)	1(10.00)	2(20.00)	1(10.00)	1(10.00)	<b>10(9.00)</b>
16-20	3(75.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(25.00)	<b>4(3.60)</b>
21-25	2(66.70)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(33.30)	0(0.00)	<b>3(2.70)</b>
<b>Total</b>	<b>43(39.10)</b>	<b>33(30.00)</b>	<b>3(2.70)</b>	<b>5(4.50)</b>	<b>3(2.70)</b>	<b>5(4.50)</b>	<b>18(16.40)</b>	<b>110(29.70)</b>

Age:  $p$ -value = 0.004

Key: NT = Non-typeable

**Table 3: Serotypes distribution of *S. pneumoniae* among apparently healthy children (0 – 25 months) among the health facilities.**

Serotypes	Health Facilities		N =370 Total No (%) isolated
	GH Makarfi No (%) isolated	PHC Ikara No (%) isolated	
1	20 (38.50)	23(39.70)	43(39.10)
3	17(25.00)	16(27.50)	33(30.00)
4	0(0.00)	3(5.20)	3(2.70)
5	3(5.77)	2(3.40)	5(4.50)
6	2(3.80)	1(1.70)	3(2.70)
12	2(3.80)	3(5.20)	5(4.50)
NT	8(15.40)	10(17.20)	18(16.40)
<b>Total</b>	<b>52(47.30)</b>	<b>58(52.70)</b>	<b>110(29.70)</b>

**p-value = 0.0132**

**Key:**

*G.H.* = General Hospital

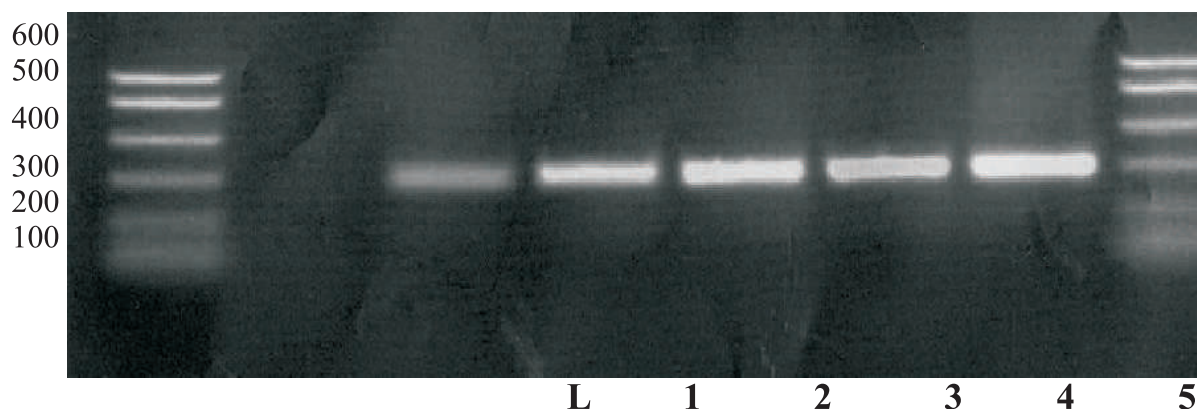
*PHC* = Primary Health Center

*NT* = Non- typeable

**Table 4: Optochin-resistant *S. pneumoniae* serotypes, isolated from apparently healthy children (0 – 25 months) in relation to prior chloroquine treatment.**

Serotypes	No. isolated	No (%) isolates	Children with prior Chloroquine treatment No (%)
<b>Optochin Resistant</b>			
1	43	2(4.70)	8(13.80)
3	33	1(4.30)	3(13.00)
4	3	0(0.00)	0(0.00)
5	5	0(0.00)	0(0.00)
6	3	0(0.00)	0(0.00)
12	5	0(0.00)	0(0.00)
NT	18	0(0.00)	0(0.00)
<b>Total</b>	<b>110</b>	<b>3(2.73)</b>	<b>12(10.90)</b>

Key: No. = Number %=Percentage (p-value= 0.386)



**Figure 1: Gel electrophoresis; pneumococcal lytA gene amplified products (bands appeared at 300bp) DNA ladder in lanes L. Lanes 1 and 6 are negative and positive controls respectively, lanes 2, 3, and 4 are Optochin resistant. Lane 5, Optochin sensitive.**



## Discussion

*Streptococcus pneumoniae* is a major pathogen that causes fatal infectious diseases such as pneumonia, septicemia and meningitis, particularly in children and elderly individuals.

In this study, 110 isolates of *S. pneumoniae* from 370 cultured nasopharyngeal swab samples were recovered, giving a carriage rate of 29.70%. Our finding is consistent with previous reports from Accra, Ghana (Holliman *et al.*, 2007) and from Bangalore, India Kumar *et al.* (2014) which indicated carriage rates of 35% and 27.90% respectively. It is, however, lower than carriage rate of 52.50% reported by Adetifa *et al.* (2008) in the South Western, Nigeria. A rate of 79.00% was reported from Gambia (Phillip *et al.*, 2011) while rates of 70% and 68% have been reported in young children in Zambia (Philips *et al.*, 2007) and in Guinea (Munoz *et al.*, 2006) respectively. Similar studies showed that young children in Nigeria experience averagely high prevalence of *S. pneumoniae* carriage rates like those in Zambia, Guinea and Ghana (Adetifa *et al.*, 2012). A possible reason for the differences in carriage rate is that pneumococcal carriage varies within countries and settings from less than 10% in some developed countries like Italy, France and United State of America (Antonio *et al.*, 1999) and 20 – 93.4% in developing countries (Philips *et al.*, 2008).

There was no significant difference in relation to carriage rate based on gender in this study, which agrees with many studies on *S. pneumoniae* carriage (Iliyasu *et al.*, 2014; Adetifa *et al.*, 2012; Kim *et al.*, 2010).

The observed higher isolation rate of *S. pneumoniae* among apparently healthy children younger than five years agrees with previous findings in Nigeria and other endemic countries of the world (Adetifa *et al.*, 2012; Kim *et al.*, 2010). This may be as a result of children being colonized with *S. pneumoniae* at an early age, usually five weeks old (Antonio *et al.*, 2008). Generally, the carriage rate of *S. pneumoniae* is consistently higher in developing countries than in developed countries which could be attributed to availability of pneumococcal pentavalent conjugate vaccine and awareness in the developed countries compared to their

counterparts in the developing countries.

Carriage was highest in lower age group children 0 – 5 months with 63(57%) followed by 6 – 10 months with 30 (27.3%). Prevalence rates of 9.10%, 3.60% and 2.70% were observed among children aged 11 – 15, 16 – 20 and 21 – 25 months respectively. Suggesting that age could be a determining factor to carriage rate of the organism in the nasopharynx (Cheng, 2004). Carriage rate decreases with increasing age (Table 1). This result agrees with the findings of Adetifa *et al.* (2003) from their studies on the nasopharyngeal carriage of *S. pneumoniae* in a Nigerian community. They reported that nasopharyngeal carriage reduced significantly with increasing age ( $p < 0.001$ ). Several reasons have been given for decrease in the carriage rate with increasing age. These include; personal hygiene, environmental sanitation, reduction in risk factors and maturation of immune system. However, the relationship between carriage and the development of natural immunity is poorly understood (CDC, 2010).

The serological result showed that 43(39.1%) isolates were of serotype 1, 33(30.0%) of serotype 3, 3(2.7%), 5(4.5%), 3(2.7%) 5(4.5%) were of serotype 4, 5, 6, and 12 respectively while 18(16.4%) were non-typeable.

Globally identified serotypes associated with carriage and invasive disease are; 1, 3, 4, 6a, 6b, 8, 9v, 7f, 14, 18c, 19f, 23f, 46, and 48 (Iliyasu *et al.*, 2014). The predominant serotypes in West African countries are types; 1- 10, 12, 14, 18, 19, 23, 25, 45, 46, and 48 (Adetifa *et al.*, 2012).

The serotypes identified in this study (Table 3) were similar to that identified by Falade *et al.* (2009) in Ibadan, Nigeria and Iliyasu *et al.* (2011) in Kano (North Western Nigeria).

Interestingly the type distribution pattern in developing countries is often similar to that seen in the USA and Europe in the beginning of the 20th century, with few types predominating, particularly types 1 and 5. Types 1, 2, 3 and 5 compared to other types which are more immunogenic have been less often isolated from countries and seems to have a more pronounced tendency to spread epidemiologically (Masaki *et*

*al.*, 2012). It may be suggested that with poor living conditions with poverty and overcrowding prevalent in Nigeria, exposure to active cases of pneumococcal disease may be prevalent, whereas in Western countries small children that are asymptomatic carriers may be the predominate source from which spread of pneumococci occurs.

Remarkable difference in the distribution of prevalent types may also exist between different geographical areas and there may be substantial changes over time within specific area (Masaki *et al.*, 2012).

In USA, Brazil and United Kingdom, only a few pneumococcal serotypes have been reported to be involved in nasopharyngeal carriage and internally displaced people (IDP). In contrast to Asia and Africa, a wide variety of serotype has been related to nasopharyngeal carriage and invasive disease (Lalitha *et al.*, 1999).

Serotypes 1 and 3 were found to be predominant among carrier isolates found in the 2 local Government areas (Table 3). The serotypes in this study are often the types implicated in carriage and invasive diseases.

A striking finding in this study was the isolation of serotype 4, which is rarely isolated by most authors although isolated by Iliyasu *et al.* (2014) in their study in Kano, North-Western Nigeria.

In this study 3 isolates were resistant to Optochin which is widely used for identification of *S. pneumoniae*. The 3 isolates were further confirmed using PCR and were found to be *S. pneumoniae* (Figure 1). Optochin resistance in *pneumococcus* was first reported in Finland in 1987 and since then has been widely reported (Masaki *et al.*, 2012; Aguiar *et al.*, 2006).

Optochin (ethylhydrocupreine hydrochloride) is a quinine analog. There is speculation of a possible association between exposure to antimalarial drugs and evolution of Optochin resistance.

The findings of this study have important implications for practicing Physicians and

Laboratory Scientist with regard to diagnosis, for stakeholders involved in hospital formulary decisions, and in the development of policies regarding laboratory diagnosis, infection control and public healthcare towards children. Therefore, it is significant for hospital and laboratories to improve the process of diagnosis by ensuring strict adherence to standard operating procedures to avoid misdiagnosis.

### Conclusion

This study reported the occurrence of a moderate carriage rate of 29.70% of *S. pneumoniae* in apparently healthy children younger than three years in Ikara and Makarfi LGA's of Kaduna State. Young age was the major risk factor for carriage and transmission within the 2 communities. Data from this study can be used for future reference. However, there is the need for extensive regional and national surveillance of pneumococcal carriage which is imperative to the burden of invasive pneumococcal diseases (IPD) in Nigeria. The isolation of Optochin resistant strains is a major setback in the diagnosis of pneumococcal diseases and there is need for enlightenment of Clinicians and Laboratory Scientist.

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