

Full Length Research Paper

Carriage rates of *Haemophilus influenzae* in Nasopharyngeal aspirates of pre-school children in Enugu State, Nigeria

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This study involved the investigation of carriage rates of *Haemophilus influenzae* in nasopharyngeal aspirates of pre-school children in Enugu State, Nigeria. Throat and nasal swabs were obtained from 158 children who were aged 6 months to 6 years. The children comprised those in nursery schools (A), those in their homes (B), and those who attended day-care centers (C). *H. influenzae* was identified after isolation in culture by satellitism and biochemical tests. Of the samples studied, the organism was isolated in 50 giving an average carriage rate of 31.7%. Descriptive statistics was used for general description of study participants and to evaluate the distribution. Data obtained were analyzed using SPSS version 15.0 software. Mean carriage rate of 30.60% was obtained from children in nursery schools, 41.08% from those at home and 22.64% from those attending day care centers. The highest carriage rate was obtained from children who were in their homes and had never attended nursery schools or day-care centers. In each of the 3 groups of children with the exception of those at home, children aged 1 year had the highest isolation rate. It can be concluded from this work that the carriage rate of *H. influenzae* is significant in pre-school children in Enugu State, Nigeria.

Key words: *Haemophilus influenzae*, carriage rate, nasopharyngeal aspirates.

INTRODUCTION

Respiratory Tract Infections (RTIs) have been identified as one of the leading causes of child mortalities that can be easily prevented. RTIs account for 40-50% of outpatient clinic visits by children, 20-40% of pediatric hospitalizations, and 18-20% of child deaths (NAS, 2009). The nasopharynx is densely colonized by a broad variety of microorganisms, including commensal bacteria as well as potential pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae* (essentially non-typeable strains) and *Moraxella catarrhalis*. In most cases, these organisms are carried without causing clinical symptoms. When the condition of the host is altered, micro-organisms may invade adjacent sites and/or invade the bloodstream, causing disease.

H. influenzae are small, pleomorphic gram-negative rods that are oxidase-positive, facultatively anaerobic, and non-motile (Yeh, 2013). They are important human-restricted gram-negative bacterial pathogen, which can cause severe invasive disease, such as meningitis, sepsis, and bacteremic pneumonia in susceptible individuals (Ulanova, 2013). *H. influenzae* cause predominantly mucosal infections. It is estimated that the bacilli cause >3 million cases of serious disease, mainly

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meningitis and pneumonia in children <5 year old, with approximately 386,000 deaths each year worldwide (WHO, 2006).

H. influenzae type b (Hib) still causes a substantial number of deaths among children in developing countries, despite the availability of effective conjugate vaccines. Haemophilus influenzae serotype b (Hib) can cause severe life threatening disease in healthy individuals and is a major global cause of childhood meningitis, pneumonia, epiglottitis, septicaemia, cellulitis, osteomyelitis and septic arthritis. It is estimated that Hib causes three million cases of serious disease and 700,000 deaths annually worldwide, with case fatality rate of around 5% in developed countries and up to 2% in developing countries (Ramsay and Noakes, 2006; Peltola, 2000). The organism can be carried asymptotically in the naso and oro-pharynx and acquisition most commonly results from asymptomatic carriers, rather than from cases. The development and widespread use of *H. influenzae* type b (Hib) conjugate vaccines have nearly eradicated invasive Hib disease in children in countries where the vaccines have been included in the national immunization programs. Hib conjugate vaccines induce protective humoral immune responses and also reduce circulating strains of Hib in the population by reducing nasopharyngeal carriage of Hib (Agrawal and Murphy, 2011). The widespread use of Hib conjugate vaccines in infancy has led to a dramatic decline in the incidence of invasive Hib disease in children. However, the disease remains common in countries not using the vaccine. Hib conjugate vaccines have been shown to be universally effective against all manifestations of Hib disease, with a clinical efficacy among fully vaccinated children estimated to be between 95 and 100%. The vaccine has also been shown to be immunogenic in patients at high risk of invasive disease (CDC, 2008).

With a view to improving child health, the Nigerian government has introduced pentavalent vaccine into her routine immunization schedule. Pentavalent vaccine is a combination of five vaccines-in-one that prevents diphtheria, tetanus, whooping cough, hepatitis b and *H. influenzae* type b, all through a single dose. With this introduction, nearly 400,000 cases of *H. influenzae* type B would be prevented with about 27,000 lives saved annually in Nigeria. It is estimated to achieve 82% coverage of all the local government areas of the federation by 2014. The vaccination would be carried out in three phases at yearly intervals over a three-year period from May 2012 to May 2014 (NAN, 2012).

The aim of this study was to determine the carriage rates of *H. influenzae* in nasopharyngeal aspirates of pre-school children so as to make a clarion call to mothers and all care-givers to avail their children below 5 years old of the opportunity to be immunized with this pentavalent vaccine to greatly reduce the risk of these invasive diseases.

MATERIALS AND METHODS

A total of 316 specimens (nasal and throat swabs) were collected from 158 children in Enugu metropolis and divided into 3 different groups (groups A, B and C). Anterior nasal swab specimens were obtained from a depth of 1 cm in the nostril with a cotton-tipped wooden swab of 2 mm in diameter.

Ethical clearance to conduct the study was obtained from the University of Nigeria Teaching Hospital, Ituku Ozalla, Enugu State and informed written consent was obtained from the parents of the subjects. The ages of this study population were from 6 months to 6 years.

Group A consisted of 49 children chosen randomly from nursery schools. Group B were 56 children selected from 20 different families. Group C were 53 children who attended day-care centers regularly. The study population did not show or manifest any clinical symptoms of respiratory tract infections caused by *H. influenzae*, so they were all assumed to be healthy control group (added). Information about the children were collected from their mothers and their teachers. The information included: age, sex, if there had ever been an outbreak of respiratory disease recently in the school, home or day-care center, if ever the subjects had received previous treatment of any respiratory tract disease, and if the subjects had ever received immunization against *H. influenzae*.

Swabs were simultaneously collected from the throat and nose of each subject using sterile commercially prepared swabs and sterile tongue depressors for the throat swabs. The swabs were cultured unto oxalated horse blood agar and chocolate agar and were incubated at 35-37°C over-night for "satelitism" growth colonies characteristic of *H. influenzae*. These were followed up with biochemical tests to confirm identification of the isolates.

Data analysis

Descriptive statistics was used for general description of study participants and to evaluate the distribution, while Fisher's exact test was used to investigate association between the identified isolates. Odds ratios, their 95% confidence intervals and p-values were obtained. Level of significance was set at $p < 0.05$. Data generated were analyzed using SPSS version 15.0 software.

RESULTS

The results of this study showed that of the 158 samples collected, 50 throat/swab specimens were positive for *H. influenzae*, thus a carriage rate of 31.7%. Cumulatively the highest carriage rate of 61.1% was in children aged one year. The least carriage rate of 14.8% was obtained in those aged 3 years. In group A, fifteen were positive with a carriage rate of 31%. Those aged one year had the

Table 1. Nasal and throat carriage of *H. influenzae* for group A (Nursery schools).

Age	No. of children	No. Isolated (nasal and throat)	% Isolation by age groups	Carriage rate (%)
1 year	7	4	57.1	6.86
2 years	10	4	40.0	8.16
3 years	13	2	15.4	4.08
4 years	8	3	37.5	6.12
5 years	11	2	18.1	4.08
Total	49	15		30.60

$$\text{Carriage rate} = \left[\frac{\text{No. isolated}}{\text{No. collected}} \right] \%$$

Mean carriage rate = 30.60% approximately 31%.

Table 2. Nasal and throat carriage of *H. influenzae* for group B (Children at home).

Age	No. of children	No. Isolated (nasal and throat)	% Isolation by age groups	Carriage rate (%)
6-11 months	14	6	42.9	10.71
1 year	3	1	33.3	1.79
2 years	3	3	100.0	5.36
3 years	10	0	00.0	0.00
4 years	9	7	77.8	12.50
5 years	4	1	25.0	1.79
6 years	13	5	38.5	8.93
Total	56	23		41.08

$$\text{Carriage rate} = \left[\frac{\text{No. isolated}}{\text{No. collected}} \right] \%$$

Mean carriage rate = 30.60% approximately 31%.

highest isolation rate (57.1%), while the lowest rate was among the three years old (15.4%). No isolation was made amongst those under eleven months nor those aged six years (Table 1). In group B, twenty-three were positive giving a carriage rate of 41%. Also in group B, the highest carriage rate of 100% (3 out of 3) was obtained in the 6-11 months age group, whereas the least carriage rate of 25% (1 out of 4) was obtained among the 5 years old group. No isolate was made in the 10 samples that were collected from the 3 years old children (Table 2). In group C, twelve were positive giving a rate of 23%. The highest carriage rate of 75% (6 out of 8) was recorded in children aged 1 year and the least carriage rate of 11.1% (1 out of 9) was recorded in the 6 years aged children. However, none was isolated in the 2 and 4 years aged children (Table 3).

DISCUSSION

The carriage rate of *H. influenzae* in the noses and

throats of pre-school children reported in this work is on the average (31.7%). This is similar to that observed (36.5%) in the study by Mackinze et al. (2010). High carriage rate was also reported in North Indian primary school children (Kumar and Awasthi, 2005), and also 41% on Aboriginal children in Australia (Watson et al., 2006). The average carrier rate of *H. influenzae* in children attending French Day Care Centers in France was 40.9% (Dabernat et al., 2003). This nasopharyngeal carriage can be explained by a prospective study carried out with three children attending a day care center (Spinola et al., 1986). It was shown that colonization of the nasopharynx by *H. influenzae* was a dynamic process corresponding to the carriage of a single strain for several months. In the United Kingdom, a concerning increase in invasive disease caused by *H. influenzae* type b (Hib) over the last 4 years was observed pre-dominantly in fully immunized children (McVernon et al., 2004; Yoshida et al., 2013). Many factors have been implicated in this rise including loss of an initial 'catch-up' immunization

Table 3. Nasal and throat carriage of *H. influenzae* for group C (Day care centres).

Age	No. of children	No. Isolated (nasal and throat)	% Isolation by age groups	Carriage rate (%)
6-11 months	6	1	16.7	1.89
1 year	8	6	75.0	11.32
2 years	9	0	00.00	00.00
3 years	4	2	50.0	3.77
4 years	8	0	00.0	00.00
5 years	9	2	22.2	3.77
6 years	9	12	11.1	1.89
Total	53	23		22.64

campaign's effect, and the use of less immunogenic Hib vaccines.

In this study, the highest incidence of *H. influenzae* carriage was observed among children in the 12-23 months age group. A similar finding (8.3 per 1000) was also obtained in Central Vietnam by Yoshida et al. (2013) and dissimilar with findings of study on infants and young children in Santo Domingo, Dominican Republic. Hib carriage was 51% lower among currently breast-fed 6 to 11 month olds than among those not currently breast-fed (18.2 versus 9.0%) (Gómez et al., 1998). The result of this study is also in contrast to that found in Kenya, where the prevalence of *H. influenzae* type b in children <5 years was 1.7% (Abdullahi et al., 2008). *H. influenzae* type b was also isolated from 8% of the children in Gaborone and from 3% of the children in Francistown in Botswana (Huebner et al., 1998).

The highest carriage rate (15%) in this study was obtained from children who were in their homes and had never attended nursery schools or day-care centers. This finding can be explained by the fact that the organism can be carried asymptotically in the naso and oropharynx and acquisition most commonly results from asymptomatic carriers, rather than from cases. Individuals may transfer the organism to close contacts though airborne or droplet spread by coughing and sneezing (Millar et al., 2000; Sauver et al., 2000). The reported rates of bacterial acquisition and carriage vary extensively between different studies and geographical sites. These differences have been related to genetic background variables and socio-economic conditions including housing, access to health care, poor hygiene, family size, overcrowded living conditions, day-care contact, number of siblings, etc (Rodríguez and Martínez, 2002). Results from studies such as Millar et al. (2000) and Fontanals et al. (2000) have suggested that both acquisition and carriage status in newborns and infants are influenced in great measure by the socio-economic status of the study population. Host factors associated with variation in the risk of nasopharyngeal colonization in children include use of pacifiers, hospitalization,

conjunctivitis and xylitol consumption (Rodríguez and Martínez, 2002; Ladhani et al., 2013; Millar et al., 2000; Uhari et al., 2000).

The 6 months to 1 year olds showed the highest carriage rate of 6.96%. This may have been as a result of lower immunity in this age group because of weaning which goes on at this time and gives them passive immunity from their mothers.

With a view to improving child health, the Nigerian government has introduced pentavalent vaccine into her routine immunization schedule. Pentavalent vaccine is a combination of five vaccines-in-one that prevents diphtheria, tetanus, whooping cough, hepatitis b and *H. influenzae* type b, all through a single dose. With this introduction, nearly 400,000 cases of *H. influenzae* type B would be prevented with about 27,000 lives saved annually in Nigeria. It is estimated to achieve 82% coverage of all the local government areas of the federation by 2014. The vaccination would be carried out in three phases at yearly intervals over a three-year period from May 2012 to May 2014 (NAN, 2012).

Conclusion

From the results obtained in this study, it can be concluded that the carriage rate is significant. Interventions are needed to reduce high transmission and carriage rates. The ability of Hib vaccines to diminish carriage to levels that will effectively reduce transmission and lead to herd immunity in this setting needs to be determined. Surveillance of transmission and immunity across all ages of the population is essential to monitor the evolution of Hib epidemiology. Continued efforts must concentrate on parental education and enforcement of recommendations for management of pediatric upper respiratory tract infections. Families should also be encouraged to ensure that their children are up-to-date with all their immunizations. With good standard of living, personal hygiene, good environmental sanitation and immunization of the population at risk, this high carriage rate may be checked.

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