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Carriage Rates of Haemophilus Influenzae in Naso-Pharyngeal Aspirates of Pre-School Children in Enugu State, Nigeria

KEYWORDS: Hemophilus influenzae, Carriage rate, Naso-pharyngeal aspirates

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ABSTRACT *Throat and nasal swabs were obtained from 158 children who were aged 6 months to 6 years. They comprised those in nursery schools (A), in their homes (B), those who attended day-care centers (C). H. influenzae was identified after isolation in culture by satelitism and biochemical tests. The organism was isolated in 50 samples giving an average carriage rate of 31.7%. Data were analyzed using SPSS version 15.0. Mean carriage rate of 30.60% was obtained from children in nursery schools, 41.08 from those at home and 22.64 from those at day care centers. The highest carriage rate was obtained from those 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. The carriage rate is significant in pre-school children in Enugu State.*

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 out patient 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 nonmotile (Yeh, 2013). H. influenzae 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 meningitis and pneumonia in children <5-yr-old, with approximately 386,000 deaths each year worldwide (WHO, 2006).

The development and widespread use of Haemophilus 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 & 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 95–100%. 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 haemophilus influenza type b, all through a single dose. With this introduction, nearly 400,000 cases of haemophilus influenza type B would be prevented with about 27,000 lives saved annually in Nigeria (NAN, 2012).

The aim of this study was to determine the carriage rates of H. influenza in nasopharyngeal aspirates of pre-school children so as to make a clarion call to mothers and all caregivers to avail their children < 5 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) collected from 158 children in Enugu metropolis 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 2 mm in diameter.

Ethical clearance to conduct the study was got 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 (twenty) 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 dis-

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ease, 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.influezae, 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 highest isolation rate (57.1%) while the lowest rate was among the three years old (15.4%). No isolation was made amongst those < 11months or those aged six years (Table 1). In group B twenty-three were positive giving a carriage rate of 41%. In group B, the highest carriage rate 100% (3 out of 3) was obtained in the 6-11 months age group. 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 (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 yr and the least carriage rate of 11.1% (1 out of 9) in the 6 years aged children. None was isolated in the 2 and 4 years aged children (Table 3).

Table 1: Nasal and Throat Carriage of H.influenzae for group A (Nursery Schools)

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

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

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 & throat)	% Isolation by age groups	Carriage Rate (%)
6-11 months	14	6	42.9	10.71
1 yr	3	1	33.3	1.79
2 yrs	3	3	100.0	5.36
3 yrs	10	0	00.0	0.00
4 yrs	9	7	77.8	12.50
5 yrs	4	1	25.0	1.79
6 yrs	13	5	38.5	8.93
Total	56	23		41.08

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

Mean Carriage rate = 30.60% approximately 31%

Table 3: Nasal and Throat Carriage of H.influenzae for group C (Day Care Centres)

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

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 & 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 naso pharyngeal 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. influenza was a dynamic process corresponding to the carriage of a single strain for several months.

In this study, the highest incidence of H. influenzae carriage was observed among children 12-23 month age group A similar finding (8.3 per 1000) was also got in Central Vietnam byYoshida 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% vs. 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). Haemophilus influen-

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zae 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 asymptomatically in the naso and oro -pharynx 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 6 months to one 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.

Conclusion

From the results, it can be concluded that the carriage rate is significant. Interventions are needed to reduce high transmission and carriage rates. 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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