ORIGINAL RESEARCH

OROPHARYNGEAL COLONIZATION BY *HAEMOPHILUS INFLUENZAE* IN HEALTHY CHILDREN FROM TAUBATÉ (SÃO PAULO), PRIOR TO THE *HAEMOPHILUS INFLUENZAE* TYPE B VACCINATION PROGRAM IN BRAZIL

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Haemophilus influenzae is one of the most important bacterial agents of otitis and sinusitis. *H. influenzae* type b (Hib) is one of the main causes of meningitis, pneumonia, and septicemia in nonvaccinated children under 6 years of age.

The aims of this study were to determine the prevalence of *H. influenzae* and Hib oropharyngeal colonization prior to the onset of the Hib vaccination program in Brazil in previously healthy children and to assess the susceptibility profile of this microorganism to a selected group of antimicrobials that are used to treat acute respiratory infections.

METHOD: Cultures of *Haemophilus influenzae* were made from oropharynx swabs from 987 children under 6 years of age who were enrolled in 29 day-care centers in Taubaté (a city of São Paulo state, Brazil) between July and December 1998.

RESULTS: The prevalence of *H. influenzae* carriers was 17.4%, and only 5.5% of the strains were beta-lactamase producers. The prevalence of Hib carriers was high, 7.3% on average (range, 0.0 – 33.3%).

CONCLUSIONS: The low prevalence of colonization by penicillin-resistant strains indicates that it is not necessary to substitute ampicilin or amoxicilin to effectively treat otitis and sinusitis caused by *H. influenzae* in Taubaté.

KEY WORDS: ***Haemophilus influenzae. Haemophilus influenzae* type b. Children. Antimicrobial resistance. Oropharynx colonization.**

Haemophilus influenzae may cause many events, which vary from an asymptomatic infection to a severe invasive disease with high mortality rates. The colonization of the upper respiratory tract by nonidentified strains and encapsulated strains of *H. influenzae* (serotypes a through f) can be influenced by personal and environmental factors.[1-6]

Upper respiratory tract colonization occurs earlier in children who go to day-care centers than in those who stay home. Consequently, the exposure and risk of these children for acquiring invasive disease is higher for children attending day-care centers.[1-6]

During the past few decades, antibiotic-resistant *H. influenzae* strains have appeared, and the major resistance mechanism has been production of beta-lactamase. The patterns of antibiotic resistance vary in different parts of the world and have been growing, hampering the therapy for diseases caused by *H. influenzae*.[7-12]

The increasing prevalence of beta-lactamase–producing strains is one of the main reasons for the prescription of wide-spectrum antibiotics to treat children with acute respiratory infection; on the other hand, the use of antibiotics is considered one of the main factors associated with the dissemination of antibiotic-resistant strains, because of alterations induced in oropharyngeal bacterial flora.[13-17]

Knowing the resistance patterns of

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microorganisms through prevalence studies of resistant strains in the oropharynx or nasopharynx of previously healthy children may be an important instrument in establishing a reference guide for acute respiratory infection therapy.[7,13-18]

In nonvaccinated populations, most invasive diseases caused by *H. influenzae* are associated with serotype b, particularly among children under 5 who attend day-care centers.[1-6]

The relationship between being a *H. influenzae* type b (Hib) carrier and the risk of disease has not yet been established, because there is disease dissemination in populations with low colonization rates and absence of disease in populations with high colonization rates by Hib. However, asymptomatic carriers play an important role in microorganism dissemination. Although few subjects colonized by Hib develop the disease, airway colonization by this microorganism is the first step to the development of invasive disease.[1,3,18,19]

In developing countries, there have been few studies concerning oropharyngeal colonization in children and the resistance profile of *H. influenzae* in nonhospitalized children.[6,20,24]

In Brazil, vaccination against Hib was only introduced at the end of 1999, and until that year, no study of Hib carriers had been published.[23] The choice to perform this study in children who attend day-care centers is due to the higher Hib colonization rates and antibiotic exposure in this population.

The purposes of this study were to identify the prevalence of *H. influenzae* colonization and the prevalence of strains resistant to antibiotics in the oropharynx of healthy children prior to the onset of the Hib vaccination program in Brazil; to analyze risk factors for the acquisition of antibiotic-resistant strains; to identify the prevalence of asymptomatic Hib carriers; and to verify the main risk factors associated to Hib oropharynx colonization.

SUBJECTS AND METHOD

Study Population

Children under 6 years of age who were attending municipal day-care centers in 1998 in Taubaté (SP, Brazil) were included in the study. The city has approximately 244,000 inhabitants and 31 day-care centers that accept children under 7. Two day-care centers are specifically for children between 6 and 7; the other 29 are distributed, with 1 in each district.

The exclusion criteria were: age above 6, children whose parents or legally responsible guardians did not authorize participation in the study, those who did not attend the day-care center at the collection date or who had not been fasting on the collection occasion.

Method

After the approval of the project by the Ethical Committee of the involved Institutions, the legally responsible person for each child was informed about the study objectives and signed an Informed Consent Form. A standardized questionnaire containing information about the child, sociodemographic characteristics, environmental conditions, previous and current morbidity, and antimicrobial usage was filled out.

The following variables were analyzed: gender, age, race, period of time attending the day-care center, how much time the child spends at the day-care center, number of people who live in the same house, number of siblings who share the same room and who are under 5 years of age, parent's education, and number of smokers in the domicile. There were questions about respiratory symptoms and antimicrobial use on the collection date, within the last 4 weeks, and within the 3 months prior to the collection.

Sample collection and microbiological analysis

Oropharynx sample collection was carried out weekly from July through December 1998 (winter and spring) by only 1 investigator, who also interviewed the parents and filled in the forms.

A sample from each child was collected using a sterile swab that was introduced through the mouth to the oropharynx. Immediately after sample collection, the swab was placed in an adequate transportation media (*Amies* with charcoal, *StarSwab*™). The samples received a code number and were sent to the microbiology laboratory within 4 hours after sample collection. The sample was plated in the same day of collection in the following culture media: blood agar (BA), blood agar containing gentamycin (BA-G), and chocolate agar (CHOC). All the media were incubated at 35°C in a 5% CO_2 atmosphere for at least 48 hours. After incubation, plates were analyzed, and microorganisms were isolated and identified through a standard methodology.[25-27]

Haemophilus isolation was done from characteristic colonies growing on chocolate agar. These colonies were identified using X and V growth factor discs (Cefar).[25-27]

Identified *H. influenzae* colonies were preserved in lamb blood and frozen at -70°C. Subsequently, they were referred to the bacteriological section, where serotyping was performed by conventional slide agglutination method using polyclonal sera against the 6 serotypes (Difco).[27]

All samples were tested to detect the production of beta-lactamase (chromogenic-cefinase cephalosporin,

nitrocefin, BBL) and susceptibility to some antimicrobials, using disc diffusion methodology in HTM (*Haemophilus* Test Medium) agar plates.[25,26]

The tested antibiotics were ampicillin, amoxicillin/clavulanic acid, azithromycin, ceftriaxone, cefuroxime, chloramphenicol, and levofloxacine.

Statistical analysis

Data from a standard questionnaire containing information about the child and laboratory results were entered in a computer and analyzed by the statistical program, Epi Info 6.04. Analysis of variables was performed by using the χ^2 or Fisher exact test, whenever appropriate. To compare means, the Student *t* test was used; a *P* value < . 05 was considered significant.

Results were expressed in simple frequencies, and the coefficients for Hib colonization risk factors were expressed as odds ratios (OR) with a 95% confidence interval.

RESULTS

Of the total of 1200 children under 6 years of age that attended the 29 day-care centers in Taubaté, 213 did not participate in the study.

Nine hundred eighty-seven children, aged 8 to 71 months (median = 53 mo) participated in the study. Twenty children (2.0%) were under 24 months of age.

The children's main sociodemographic characteristics are listed in Table 1. Children attending day-care centers in Taubaté are, in general, from low-income families, and more than 65.0% of parents were poorly educated.

The environmental conditions are summarized in Table 2. The period of time that children had been attending the day-care center ranged from 0 to 48 months (median = 8 months), and the majority of children (68.0%) stayed at the day-care center for 8 hours per day.

The number of people living in the house ranged from 2 to 15 (median = 4), and the number of people sleeping in the same child's room ranged from 0 to 7 (median = 2). Children's exposure to tobacco smoke at home was high (51.5%); 24.3% of the mothers and 39.1% of the fathers were smokers. Both parents of 131 children were smokers (13.2%). No child presented meningitis, epiglottitis, or contact with people presenting those diseases during the previous year.

Two hundred forty-six children (24.9%) presented respiratory symptoms at the collection date. The most common problems were cough or wheezing (14.3%), common cold, influenza, or watery nose (10.0%).

Only 32 children (3.2%) were receiving antibiotics on the day of swab collection; 18.9% (n = 187) had received antibiotics during the previous month, and 30.1% (n = 297) had received antibiotics during the previous 3 months. The most commonly used antibiotics are listed in Table 3.

Table 1 - Sociodemographic characteristics of the study population: number and percentage of children, according to age, gender, race, and parents' education.

Characteristics	Number	%
Age (months)		
≤24	20	2.0
25 –71	967	98.0
Gender		
Male	498	50.5
Female	489	49.5
Race		
Caucasian	812	82.3
African	71	7.2
Brown	98	9.9
Asian	6	0.6
Maternal education		
Illiterate	57	5.8
1 to 8 years of study	640	65.1
≥9 years of study	287	29.3
Paternal education		
Illiterate	66	7.1
1 to 8 years of study	639	68.7
≥9 years of study	199	24.1

H. influenzae colonization and susceptibility profile to antibiotics

Of the 987 children who were swabbed, 172 (17.4%) had a positive culture for *H. influenzae*; 91 of these were tested for antimicrobial susceptibility. Five of these tested isolates (5.5%) were beta-lactamase producers (4 were type b). Resistance to other

Table 2 - Distribution of number and percentage of studied children according environmental conditions.

Environmental conditions	Number	%
Period of time attending the day-care center (months)		
≤6	381	38.6
>6	606	61.3
Time at the day-care center		
Part time (4 hours/day)	316	32.0
Full time (8 hours/day)	671	68.0
Presence of smokers at domicile	509	51.5
Number of people who live with the child		
<4	252	25.5
≥4	735	74.5
Children with siblings ≤5 years	382	38.7
Number of people sharing the same room with the child		
<2	309	31.3
2 – 3	569	57.7
≥4	109	11.0

tested antimicrobials was nil (Table 4).

There was no relationship between microorganism resistance and the analyzed variables. The *P* value was greater than .05 for gender, age, antibiotics usage, number of siblings, presence of smokers, history of respiratory diseases, and vaccine status.

Table 3 - Most common antibiotics used by children who attended day-care centers in Taubaté on the day of swab collection and 30 and 90 days prior to the sample collection.

Antibiotics	At collection date	Last month	Previous 90 days
Amoxicillin	47.0%	49.0%	49.5%
Penicillin benzathine	12.5%	16.6%	15.3%
TMP-SMZ*	12.5%	9.7%	10.5%

*TMP-SMZ: trimethoprim-sulphamethoxazole

Table 4 - Prevalence of *H. influenzae* strains, according to the pattern of susceptibility to different antibiotics.

Antibiotics	Sensitive (%)	Intermediate resistance (%)	Complete resistance (%)	Number of tested strains
Ampicillin *	94.4	0.0	**5.5**	91
Amoxicillin/clavulanate	100.0	0.0	0.0	91
Azithromycin	100.0	0.0	0.0	91
Cefuroxime sodium	100.0	0.0	0.0	91
Ceftriaxone	100.0	0.0	0.0	91
Chloramphenicol	100.0	0.0	0.0	91
Levofloxacin	100.0	0.0	0.0	67

* Total of beta-lactamase–producing strains = 5

Table 5 - Number of children who participated in study, number swabbed (samples), number of *Haemophilus influenzae* type b (Hib) carriers, and percentage of Hib carriers by day-care center.

Day-care center	Samples N	Hib carriers N	Hib carriers %
Alto de São Pedro	57	7	12.3
Amador Bueno	47	0	0.0
Baroneza	35	4	11.4
Belém	44	3	6.8
Canuto Borges	40	0	0.0
Cidade Jardim	46	0	0.0
Estiva	16	0	0.0
Gurilândia	74	5	6.7
Imaculada	44	0	0.0
Independência	40	6	15.0
Jaraguá	34	3	8.8
Jardim América	50	0	0.0
Mãe Maria	25	4	16.0
Marlene Miranda	13	0	0.0
Paduan	33	4	12.1
Piratininga	40	0	0.0
Parque Aeroporto	12	4	33.3
Sabará	39	10	25.6
São Gonçalo	38	3	7.8
Shalon	29	7	24.1
Santo Antonio	24	6	25.0
Santa Tereza	17	1	5.8
Santa Fé	31	0	0.0
Três Marias	35	4	11.4
Santa Isabel	17	0	0.0
Chácaras Reunidas	24	0	0.0
Chácara Silvestre	44	2	4.5
Parque Ipanema	14	0	0.0
Parque Planalto	25	0	0.0
Total	987	73	7.3

Hib colonization

From the 172 isolated cultures of *H. influenzae*, 73 (collected from 7.3% of the test population) were of type b.

Hib colonization ranged from 0.0% to 33.3% in the different day-care centers (Table 5), and except for the fact that a child attended a particular day-care center, none of the analyzed variables was significantly associated with the risk of Hib colonization (Table 6).

In order to assess whether exposure to larger numbers of children was related to the number of Hib carriers at a particular center, we made crossings between the total number of children in each day-care center (including children older than 6) and the number of Hib carriers under 5; the *P* value was not significant (OR = 0.69 [95% CI: 0.42-1.16), *P* = 0.13).

DISCUSSION

Oropharynx colonization by encapsulated strains of *H. influenzae* is very common and varies between 6.0% and 90.0%, in different populations.[2,4,6,7,13,14,16,18] In this study, the prevalence of children colonized by *H. influenzae* was 17%, similar to rates found by other authors.[20,23,28]

It is possible that some strains may not have survived the transport to the lab; however, the elevated rate of Hib colonization found (7.3%) suggests that this biasing factor did not occur.

Longitudinal studies conducted in healthy children and in children with

Table 6 - Possible risk factors for *Haemophilus influenzae* type b (Hib) oropharynx colonization.

Risk factors	Hib Carriers	Odds ratio (95% IC)	*P* value
Gender			
Female	39	1.18 (0.73 – 1.97)	0.57
Male	34		
Siblings under age of 5			
Yes	43	0.9 (0.54 – 1.51)	0.75
No	30		
Smokers at domicile			
Yes	37	0.91 (0.55 – 1.51)	0.78
No	36		
Hib Vaccination			
Yes	11	1.26 (0.60 – 2.57)	0.65
No	62		

recurrent otitis media have demonstrated that the status of a nasopharynx carrier of some pathogens like *H. influenzae, S. pneumoniae,* and *M. catarrhalis* is maintained in an almost constant proportion (21.0% to 37.0%) during the first 3 years of life; however, during high respiratory airway infections, there is an increase in those proportions.[20] In the present study, *H. influenzae* colonization was not associated with the presence of acute respiratory infections.

The profile of microorganism resistance varies from region to region; therefore, it is essential to know the local susceptibility patterns to antibiotics to appropriately guide the best therapeutic option. Most of the studies concerning microorganism resistance are related to strains isolated from body fluids of children with invasive disease, and their data are not always useful for evaluating the microorganism-resistance profile in the community.[7-17]

In many countries, the proportion of *H. influenzae* beta-lactamase–producing strains in children with invasive disease varies between 6.0% and 60.0% and has been increasing yearly.[9-11]

In Brazil between 1990 and 1999, from a total of 3204 strains of *Haemophilus influenzae* isolated from patients with invasive disease (91.0% under 4 years of age), it was noted that 18.1% of strains were resistant to ampicillin, 19.1% to chloramphenicol, and 13.9% to ampicillin and chloramphenicol, a very high prevalence compared to the results of this study. In 1996, 15.0% of the strains were beta-lactamase producers, increasing to 21.0% in 1999; during the same period, a significant increase in chloramphenicol resistance was not detected (18.8% vs. 19.9%). All strains were sensitive to rifampicin (used for chemoprophylaxis) and cephalosporin.[10]

In the present study, we found that only 5.5% of the isolated strains were beta-lactamase producers, results that are very similar to the rates found in healthy children who attended day-care centers in United Kingdom in the 1980s (5.4%)[8] and in Italian children with serous otitis.[16] In other regions of Europe, the United States, and Israel, the prevalence of strains causing serous otitis that are resistant to ampicillin and other antibiotics is much higher.[12-15]

Acute respiratory infections, particularly otitis media, are the main reason for antibiotics usage in children.[12-17] There are controversies about the value of oropharyngeal cultures for predicting the etiology of acute respiratory infections. Some studies have shown that the positive predictive value is low (50.0% for *H. influenzae*, 40.0% for *S. pneumoniae,* and 20.0% for *M. catarrhalis*)[12]; however, the negative predictive value is excellent (95%).[12,14]

The low prevalence of beta-lactamase–producing strains found in the study suggests that in Taubaté, ampicillin should be the antibiotic of choice for the therapy of otitis and sinusitis that is presumably caused by *Haemophilus influenzae.*

Although the previous use of antibiotics is related to the colonization of respiratory airways by resistant strains,[14,17] in the present study, previous usage of antibiotics was not associated with an increase in the prevalence of beta-lactamase–producing strains.

In the present study, Hib colonization rate was 7.3%, and in 11 day-care centers was greater than 10.0% (Table 5).

Of the many factors associated with Hib colonization, young age, contact with people who had invasive diseases caused by Hib, attending day-care centers, and vaccination status have been found to be the most important. Results concerning other risk factors such as gender, number of siblings under 5 years of age, and prior respiratory diseases are controversial.[1-7,20-24]

In the Dominican Republic, a higher colonization rate was found among children who had siblings under the age of 5[22]; in the present study, as in others,[2,23] there were no significant differences among Hib-colonized children regarding the different analyzed variables.

Although a quarter of the children were presenting respiratory symptoms at the time of the swab collection, this variable was not associated with an increase in Hib colonization rates. Since acute respiratory infections are risk factors for Hib invasive disease, high rates of colonization by Hib are worrisome.[1]

In 1998, the vaccine against Hib had not yet been incorporated into the pediatric vaccination calendar in Brazil. The rates of Hib carriers were similar to those found in preschool children living in developing countries such as the Dominican Republic (7.7%)[22] and Turkey (9.0%)[6] as well as Alaska (5.0% to 7.0%),[24] and they were much higher than in children living in industrialized nations in the pre-vaccine era.[1]

In populations vaccinated against Hib, the prevalence of airway carriers has significantly decreased[19,23,28-32]; however, the use of conjugated vaccines protects only partially against the carrier status and does not completely eliminate the risk of infection by this microorganism.[1,24,31,32]

In Brazil, vaccination against Hib was introduced into the routine pediatric vaccination calendar by the end of 1999. In Curitiba (PR), vaccination was introduced in 1996, and the following year the prevalence of Hib colonization in 657 vaccinated children was 1.2%, lower than that found in 643 nonvaccinated children (4.8%) living in Porto Alegre (RS).[23]

In the present study, we did not find significant differences in Hib colonization rates in vaccinated and nonvaccinated children; it is worthwhile to mention that few children had been vaccinated against Hib and that the vaccine is effective in preventing colonization in the community only when the vaccine coverage is high.[1]

In Alaska, after the introduction of conjugated vaccines against Hib, invasive disease caused by this microorganism has drastically decreased; however, a significant increase in cases of invasive disease caused by Hib has been recently detected.[24,32] It is believed that high rates of colonization by Hib in the Alaska population played an important role in the re-emergence of Hib invasive disease.[24,31,32]

In different regions in Alaska, the rates of Hib carriers were variable. In Barrow, 4.4% of the children were colonized by Hib, with higher prevalence between 6 and 7 years of age (7.8%), dropping to 0.0% in adolescents between 14 and 16 years of age. In Delta YK, they found a 6.6% rate of carriers among 365 children under the age of 4, this rate being much greater than that found in 383 children less than 4 years of age living in Anchorage (1.0%). The persistence of a great number of children who were carriers of Hib in some communities in Alaska after the introduction of conjugated vaccine against Hib suggests that children of school age (who have not been vaccinated) may constitute an important reservoir of Hib. It seems that the elevated rate of carriers among nonvaccinated children was responsible, at least in part, to the re-emergence of invasive disease in Alaska.[24,31]

These are very troubling data because in Taubaté the rates of colonization by Hib in children under 6 were as high as those found in Alaska in the prevaccine era. Unfortunately, we did not evaluate the prevalence of carriers among children older than 6 years in this city.

Preschool and school children colonized by Hib constitute the main reservoir of this microorganism, and the colonization may persist for prolonged periods of time—1 or more years. Since the status of Hib carrier, in general, does not produce an increase in the titres of protecting antibodies against Hib, children carrying this microorganism may develop invasive disease caused by this agent.[1,19,24]

The incubation period of invasive and noninvasive disease caused by *Haemophilus influenzae* is unknown; however, high rates of colonization of the respiratory airways are associated with a higher risk of disease that attacks mucous tissue as well as invasive disease. Without a doubt, vaccination against Hib is the most effective measure for reducing the impact of Hib invasive disease, although it does not play a significant role in reducing mucous tissue disease.[1,30] After this study was completed, all children between 2 and 60 months of age who attended day-care centers in Taubaté were vaccinated against Hib.

CONCLUSION

The prevalence of strains of beta-lactamase–producing *Haemophilus influenzae* (5.5%) was low among children from Taubaté, indicating that ampicillin or amoxicillin can be used as the antibiotic of choice to treat otitis and sinusitis presumably caused by this microorganism.

The prevalence of Hib carriers was high (7.3%) in this study. Since the human being is the only reservoir of Hib and colonization has a relevant role in the transmission cycle of invasive disease caused by this microorganism, it is essential to vaccinate children under 5 years of age against Hib and to monitor the impact of such a measure.

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RESUMO

BRICK LF e col. Colonização da orofaringe de crianças saudáveis de Taubaté (São Paulo) por *Haemophilus influenzae*, antes da introdução da vacina contra *Haemophilus influenzae* do tipo b no Brasil. **Rev. Hosp. Clin. Fac. Med. S. Paulo 59**(5):236-243, 2004.

Haemophilus influenzae é um dos mais importantes agentes bacterianos de otites e sinusites. Em crianças menores de seis anos de idade não vacinadas contra o *H. influenzae* do tipo b (Hib), essa bactéria é uma das principais causadoras de meningite, pneumonia e sepse.

O objetivo deste estudo foi determinar a prevalência da colonização da orofaringe de crianças previamente saudáveis por *H. influenzae* e Hib e avaliar o perfil de suscetibilidade desses microorganismos a um grupo seleto de antimicrobianos, que habitualmente são utilizados para tratar as infecções respiratórias agudas.

MÉTODO: Foram colhidos swabs da orofaringe de 987 crianças menores de seis anos de idade que freqüentavam 29 creches da cidade de Taubaté (São Paulo, Brasil), entre julho e dezembro de 1998, para realização de culturas de *H. influenzae* e antibiograma.

RESULTADOS: A prevalência de portadores do *H. influenzae* foi de 17,4% e somente 5,5% das cepas isoladas eram produtoras de beta-lactamase. A prevalência de portadores do Hib foi alta, com média de 7,3% (variando entre 0.0 e 33,3%).

CONCLUSÕES: A baixa prevalência da colonização por cepas resistentes às penicilinas indica que não é necessário substituir esses antibióticos para tratar empiricamente as otites e sinusites causadas por *H. influenzae* em Taubaté.

UNITERMOS: ***Haemophilus influenzae*. *Haemophilus influenzae* type b (Hib). Crianças. Resistência antimicrobiana. Colonização da orofaringe.**

REFERENCES

1. Barbour ML. Conjugate vaccines and the carriage of *Haemophilus influenzae* type b. Emerg Infect Dis 1996;2:1-9.

2. Michaels RH, Poziviak CS, Stonebraker FE, Norden CW. Factors affecting pharyngeal *Haemophilus influenzae* type b colonization rates in children. J Clin Microbiol 1976;4:413-7.

3. Granoff DM, Daum RS. Spread of *Haemophilus influenzae* type b: recent epidemiologic and therapeutic considerations. Pediatrics 1980;97:854-860.

4. Murphy TV, Pastor P, Medley F, Osterholm MT, Granoff DM. Pharyngeal colonization with *Haemophilus influenzae* type b in children in a day care center without invasive disease. J Pediatr 1985;106:712-6.

5. Li KI, Dashefsky B, Wald ER. *Haemophilus influenzae* type b colonization in household contacts of infected and colonized children enrolled in day care. Pediatrics 1986;78:15-20.

6. Bakir M, Yagci A, Ulger N, Akbenlioglu C, Ilki A, Soyletir G, et al. Pharyngeal colonization with *Haemophilus influenzae* type b among healthy Turkish infants and children. Pediatr Intern 2002;44:381-6.

7. Lerman SJ, Kucera JC, Brunken JM. Nasopharyngeal carriage of antibiotic resistant *Haemophilus influenzae* in health children. Pediatrics 1979;64:287-90.

8. Howard AF, Dunkin KT, Millar GW. Nasopharyngeal carriage and antibiotic resistance of *Haemophilus influenzae* in healthy children. Epidemiol Infect 1988;100:193-203.

9. Thornsberry C, Ogilvie PT, Holley HP Jr, Sahm DF. Survey of susceptibilities of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. Isolates to 26 antimicrobial agents: a prospective US Study, Antim Agent Chem 1999;43:2612-23.

10. Zanella RC, Casagrande ST, Bokermann S, Almeida SC, Brandileone MC. Characterization of *Haemophilus influenzae* isolated from invasive disease in Brazil from 1990 to 1999. Microb Drug Resist 2002;8;67-72.

11. Blosser-Middleton R, Sahm DF, Thornsberry C, Jones ME, Hogan PA, Critchley IA, et al. Antimicrobial susceptibility of 840 clinical isolates of *Haemophilus influenzae* collected in four European countries in 2000-2001. Clin Microbiol Infect 2003; 9:431-6.

12. Gehanno P, Lenoir G, Barry B, Bons J, Boucot I, Berche P. Evaluation of nasopharyngeal cultures for bacteriologic assessment of acute otitis media in children. Pediatr Infect Dis 1996;15:329-32.

13. Klein J. Role of nontypeable *Haemopilus influenzae* in pediatric respiratory tract infections. Pediatric Infect Dis J 1997;16:S5-8.

14. Harper M. Nasopharyngeal colonization with pathogens causing otitis media: how does this information help us? Pediatr Infect Dis 1999;181:1120-24.

15. Leibovitz E, Satran R, Piglansky L, Raiz S, Press J, Leiberman A, et al. Can acute otitis media caused by *Haemophilus influenzae* be distinguished from that caused by *Streptococcus pneumoniae*? Pediatr Infect Dis 2003;22:509-14.

16. Marchisio P, Claut L, Rognoni A, Esposito S, Passali D, Bellussi L, et al. Differences in nasopharyngeal bacterial flora in children with nonsevere recurrent acute otitis media and chronic otitis media with effusion: implications for management. Pediatr Infect Dis 2003;22:262-8.

17. Pichichero M. Understanding antibiotic overuse for respiratory tract infection in children. Pediatrics1999;104:1384-8.

18. Rapola S, Salo E, Leinonen M et al. Comparison of four different methods for detecting pharyngeal carriage of *Streptococcus pneumoniae* and *Haemohilus influenzae* in children. J Clin Microbiol 1997; 35:1077-9.

19. CDC. Progress toward elimination of *Haemophilus influenzae* type b invasive disease among infants and children—United States, 1998-2000. MMWR 2002;51:234-7.

20. Villaseñor-Sierra A, Herrera-Basto E, Vázquez-Salazar P et al. Prevalencia de estado de portador de *Haemophilus influenzae* in niños de Ciudad Nezahualcóyotl, estado de México, México. Salud Publica Mex 1996;38:87-93.

21. Vives M, Garcia ME, Saenz P, Mora MA, Mata L, Sabharwal H, et al. Nasopharyngeal colonization in Costa Rican children during the first year of life.. Pediatr Infect Dis 1997;16:852-8.

22. Gomez E, Moore A, Sanchez J, Kool J, Castellanos PL, Feris JM, et al. The epidemiology of *Haemophilus influenzae* type b carriage among infants and young children in Santo Domingo, Dominican Republic. Pediatr Infect Dis 1998;17:782-6.

23. Forleo-Neto E, de Oliveira CF, Maluf EM, Bataglin C, Araujo JM, Kunz LF Jr, et al. Decreased point prevalence of *Haemophilus influenzae* type b (Hib) oropharyngeal colonization by mass immunization of Brazilian children less then 5 years old with Hib polyribosylribitol phosphate polysaccharide-tetanus toxoid conjugate vaccine in combination with diphtheria-tetanus toxoid-pertussis vaccine. J Infec Dis 1999;180:1153-8.

24. Galil K, Singleton R, Levine OS, Fitzgerald MA, Bulkow L, Getty M, et al. Reemergence of invasive *Haemophilus influenzae* type b disease in a well-vaccinated population in remote Alaska. J Infect Dis 1999;179:101-6.

25. Isenberg HD. Clinical Microbiology Procedures Handbook. ASM, Washington, v1, 1992.

26. National Committee for Clinical Laboratory Standards. 1998, Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A5. Wayne, Pa.

27. Campos JM. *Haemophilus*. In: Murray PR, Baron EJ, Pfaller MA, et al. Manual of Clinical Microbiology, 7th ed. American Society for Microbiology, Washington, 1999, p.605-13.

28. Takala AK, Eskola J, Leinonen M, Kayhty H, Nissinen A, Pekkanen E, et al. Reduction of oropharyngeal carriage of *Haemophilus influenzae* type b (Hib) in children immunized with a Hib conjugate vaccine. J Infect Dis 1991;164:982-6.

29. Mohle-Boetani JC, Ajello G, Breneman E, Deaver KA, Harvey C, Plikaytis BD, et al. Carriage of *Haemophilus influenzae* type b in children after widespread vaccination with conjugate *Haemophilus influenzae* type b vaccines. Pediatr Infect Dis 1993;12:589-93.

30. Peltola H. Worldwide *Haemophilus influenzae* type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of polysaccharide vaccine and a decade after the advent of conjugates. Clin Micro Rev 2001;13:302-17.

31. Singleton R, Bulkow LR, Levine OS, Butler JC, Hennessy TW, Parkinson A. Experience with the prevention of invasive *Haemophilus influenzae* type b disease vaccination in Alaska: impact of persistent oropharyngeal carriage. J Pediatr 2000;137:313-32.

32. Trotter CL, Ramsay ME, Slack MPE. The epidemiology of *Haemophilus influenzae* type b disease in England and Wales, 1990-2002. Commun Dis Public Health 2003;6:55-8.