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Prevalence and risk factors for nasopharyngeal carriage of *Streptococcus pneumoniae* among adolescents

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Data on the prevalence of pneumooccal nasopharyngeal carriage and its risk factors among adolescents are scarce. The aim of this study was to provide such information. A cross-sectional, population-based prospective study was conducted. Participants were 1013 adolescents (age range 10–19 years) randomly recruited in 22 public schools. Those schools were randomly chosen among 307 public schools from 11 Sanitary Districts of Salvador, Brazil. Nasopharyngeal samples were assessed by standard procedures to recover and identify *Streptococcus pneumoniae*. Data on potential risk factors were gathered by confidential interview based on a standardized questionnaire. Pneumococci were recovered from 8.2 % [83/1013, 95 % confidence interval (CI) 6.6–10.0]. By stepwise logistic regression, pneumococcal colonization was independently associated with younger age [odds ratio (OR) 0.85, 95 % CI 0.77–0.94, $P=0.001$], being male (OR 1.78, 95 % CI 1.11–2.85, $P=0.02$), exposure to passive smoke in the household (OR 1.76, 95 % CI 1.10–2.79, $P=0.02$), having an upper respiratory infection during recruitment (OR 2.67, 95 % CI 1.67–4.28, $P<0.001$) and having a history involving an episode of acute asthma during the last year (OR 2.89, 95 % CI 1.18–7.08, $P=0.03$). The estimated probability of pneumococcal colonization decreased with age (χ^2 for trend=8.52, $P=0.003$). These findings provide tools for increasing the use of prevention strategies for pneumococcal diseases, such as pneumococcal vaccination among asthmatic patients and public health measures to stop smoking.

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INTRODUCTION

The incidence of invasive pneumococcal disease is highest among children and the elderly (CDC, 1997). However, respiratory infections are an important cause of morbidity and mortality among adolescents (Benguigui, 1992). Among children, *Streptococcus pneumoniae* is a major causative agent of such infections (Heiskanen-Kosma *et al.*, 1998). The nasopharynx is known to be the main ecological reservoir of *S. pneumoniae*, from where it can give rise to disease after extending to other areas of the respiratory tract or penetrating normally sterile body fluids (Austrian, 1986). Although nasopharyngeal isolates are not useful for predicting the causative agent of invasive disease in individuals, they reflect epidemiological aspects of pneumococcal disease in the community (Brueggemann *et al.*, 2003).

Studies conducted over the last decades have gradually revealed the connection between pneumococcal carriage, and mucosal and invasive infections caused by the

Abbreviations: CDC, Centers for Disease Control and Prevention; CI, confidence interval; OR, odds ratio.

organism (Bogaert *et al.*, 2004a), and identified young age (Austrian, 1986), viral respiratory infections (Avadhanula *et al.*, 2006) and day-care centre attendance (Bogaert *et al.*, 2004b) as risk factors for both nasopharyngeal colonization and occurrence of disease in children. Because the burden of pneumococcal disease is lower in adolescents, the carriage of the organism in this section of the population has not been explored in depth. Basic knowledge of this is important for understanding the transmission patterns of pneumococci in families and communities. The aims of this study were to provide data on the prevalence of nasopharyngeal carriage and on conditions predisposing pneumococcal colonization among adolescents.

METHODS

Sampling. This cross-sectional survey was conducted in Salvador, North-East Brazil, from November 2002 to July 2003. A single nasopharyngeal specimen was collected from adolescents (between 10 and 19 years of age) (PAHO, 1998) from public schools. The city has a total of 307 public schools distributed among 11 Sanitary Districts. The list of all the public schools by Sanitary District was obtained from the Secretariat of Education of Salvador. A two-sample stage sampling was applied to select participant adolescents. Two schools from each District were randomly chosen. The number of adolescents registered at each studied school ranged from 1200 to 7530, and the number of adolescents recruited by school varied between 88 and 104. The sample size was estimated taking into account the expected pneumococcal carriage for adults (Inostroza *et al.*, 1998). Thus, we estimated that 900 participants would be sufficient to detect a prevalence and the respective 95 % confidence interval (CI) of nasopharyngeal colonization ranging from 8–12 % (point prevalence=10 %, 2 % error). We assumed a 12 % refusal to participate, so the sample size was established as 1000 participants. Demographic, clinical and epidemiological data were collected by a standardized questionnaire.

Bacteriological data. Two trained biochemists collected nasopharyngeal specimens by rotating cotton-tipped flexible swabs (Transwab pernasal charcoal medium; Medical Wire & Equipment)) during the smooth insertion through the nostrils of each individual, whose head was tipped backward; the swabs were left in the posterior nasopharynx for 5 s to saturate the tip and then they were transported in Amies medium within 4 h to the Central Laboratory of Bahia where they were inoculated onto trypticase soy agar with added 5 % sheep blood and 5 μg gentamicin ml^{-1}. Plates were incubated at 37 °C in 5 % CO_2 for 18 to 24 h. Micro-organisms whose colonies displayed pneumococcus morphology (Gram stain) and that were susceptible to a 5 μg optochin disc (inhibition zone $\geqslant$14 mm) and had bile solubility were defined as pneumococcus. All strains were frozen in skim milk and transported in dry ice by airplane to the Bacteriology Branch, Adolfo Lutz Institute, where the identification was confirmed by the same procedures. Collection of samples and laboratory procedures were performed in accordance with World Health Organization guidelines (WHO, 1994).

Data analysis. All data were entered into a database using Epi Info 6.04. Descriptive analysis was performed by SPSS package (version 9.0) and STATA software (version 8.0) was applied for logistic regression analysis. The rainy season was defined as the period between April and September, and the dry season as the period between October and March. Rain is the main difference between seasons in the study area. Prevalence rates and the respective 95 % CI were calculated. Association between categorical variables was initially tested by univariate analysis with the Pearson chi-square or Fisher's exact test, when appropriate. Means of continuous variables were compared by Student *t*-test or Mann–Whitney U test, as appropriate. A P value <0.05 was considered significant and all tests were two-tailed tests. Potential variables associated with pneumococcal carriage were assessed by odds ratio (OR) with the respective 95 % CI. Independent variables that gave significant or borderline results in the univariate analysis were used in a four stepwise multiple logistic regression analysis to determine their independent effect on pneumococcal nasopharyngeal carriage. This multivariate analysis allowed the examination of the effects of all exposure variables reciprocally and simultaneously adjusting for all the other variables in the model. All possible interactions were assessed. After the written consent of the director of the school, each adolescent was exposed to a brief explanation about this investigation and he/she was requested to give written informed consent before recruitment. This procedure was based on the assumption of the low risk of this investigation and on the capacity of an adolescent to provide informed consent in such a situation (Santelli *et al.*, 2003). The ethics committees of the participating institutions approved the study.

RESULTS AND DISCUSSION

We collected nasopharyngeal swabs from 1013 adolescents (age range 10–19 years, mean 14.6 ± 2.3 years). Twenty adolescents refused to consent to take part in the study and were replaced using the same procedure of random selection. Pneumococcus was recovered from 83 samples (8.2 %, 95 % CI 6.6–10.0) and the estimated probability of pneumococcal colonization decreased with age (Table 1). Data on antimicrobial resistance and serotype distribution have been presented elsewhere (Cardozo *et al.*, 2006b). Most studies evaluating pneumococcal nasopharyngeal carriage enrolled only children (O'Brien *et al.*, 2003). In Brazil, pneumococcal colonization rates in children aged 8–71 months have ranged from 21.2–55.0 % (Cardozo *et al.*, 2006a). A few studies have analysed adolescents along with children (Bogaert *et al.*, 2004b; Inostroza *et al.*, 1998; Ciftçi *et al.*, 2000; Muhlemann *et al.*, 2003; Hussain *et al.*, 2005)

Table 1. Prevalence of nasopharyngeal pneumococcal colonization among Brazilian adolescents by age in years

The χ^2 value for the trend is 8.52, P value=0.003.

Age (years)	Pneumococcal carrier	
	n/total	Percentage (95 % CI)
10	5/43	11.6 (3.9–25.1)
11	7/62	11.3 (4.7–21.9)
12	11/94	11.7 (6.0–20.0)
13	12/117	10.3 (5.4–17.2)
14	13/138	9.4 (5.1–15.6)
15	14/154	9.1 (5.1–14.8)
16	10/179	5.6 (2.7–10.0)
17	7/116	6.0 (2.5–12.0)
18	3/72	4.2 (0.9–11.7)
19	1/38	2.6 (0.06–13.8)
Total	83/1013	8.2 (6.6–10.0)

or the whole population (Hennessy *et al.*, 2002), reporting prevalence of pneumococcal carriage from 19 to 43 %. These studies do not allow proper evaluation of adolescents (PAHO, 1998), and thus are lacking the identification of risk factors. The pneumococcal carriage rate reported herein was similar to the rate reported by Hussain *et al.* (2005) for people aged ⩾18 years (8 %), but an independent association of age with pneumococcal colonization was found, that is, the younger the adolescent the greater the risk of pneumococcal colonization (Table 1). A similar finding was observed by Bogaert *et al.* (2004b), although those authors have not analysed statistically the trend of colonization rate through adolescence. Colonization of the nasopharynx may be detected in early infancy, with a peak incidence during the first 3 years of life (Bogaert *et al.*, 2004a). It has been demonstrated that infants <2 months of age and adults aged 18 to 50 years are the age groups with the lowest nasopharyngeal pneumococcal carriage rates (Dagan *et al.*, 1998). Therefore, one can infer that age is an important risk factor for pneumococcal colonization and its influence varies with the phase of growth throughout life.

The frequency of the baseline characteristics of the study group and the significant differences in relation to pneumococcal isolation are presented in Table 2. The maximum number of children aged less than 5 years in the same sleeping room was two. No significant differences were found in all other searched variables when compared with pneumococcal carriage, including underlying diseases (immunodeficiency 2.4 vs 2.1 %, sickle cell disease 1.3 vs 1.8 %, diabetes mellitus 0 vs 0.9 %, chronic lung disease 1.2 vs 0.8 % or liver disease 0 vs 0.5 %). Two, one and no individuals reported cardiac disease, renal disease and human immunodeficiency virus infection, respectively. The frequency of nasal medicine use during the previous 24 h was the same (1.2 %) among pneumococcal colonized and non-colonized adolescents. The isolation rates were statistically similar in the rainy (8.6 %) and the dry (7.5 %) season. Being of the male sex has been described as an independent risk factor for more frequent and more severe lower respiratory infections (Falagas *et al.*, 2007). In this investigation, males were significantly more likely to be pneumococcal carriers than females (Table 2). The higher probability to have respiratory

Table 2. Baseline characteristics for 1013 Brazilian adolescents and potential factors associated with pneumococcal carriage

Characteristic	Pneumococcal colonization		OR (95 % CI)	
	Yes=83	No=930	Unadjusted	Adjusted
Age (years)*	14±2.2 (median 14)	14.7±2.3 (median 15)	0.87 (0.78–0.95)	0.85 (0.77–0.94)†
Males‡	46 (55.4)	427 (45.9)	1.47 (0.93–2.32)	1.78 (1.11–2.85)†
Passive exposure to cigarette smoke‡	40 (48.2)	307 (33.0)	1.89 (1.20–2.97)	1.76 (1.10–2.79)†
Presence of children aged <5 years‡				
at home	12 (14.5)	187 (20.2)	0.67 (0.34–1.31)§	–
in the sleeping room	3 (3.6)	67 (7.2)	0.48 (0.12–1.64)§	–
URI during recruitment‡	39 (47.0)	227 (24.4)	2.74 (1.74–4.33)	2.67 (1.67–4.28)‖
URI in the household contacts‡	15 (18.1)	183 (19.7)	0.90 (0.48–1.66)§	–
Occurrence during the last year of:‡				
URI	71 (85.5)	766 (82.6)	1.27 (0.65–2.52)§	-
Otitis	17 (20.5)	165 (17.8)	1.19 (0.66–2.15)§	-
Rhinitis	12 (14.6)	84 (9.1)	1.70 (0.84–3.39)§	-
Sinusitis	6 (7.3)	85 (9.2)	0.77 (0.29–1.91)§	-
Episode of acute asthma	7 (8.4)	30 (3.2)	2.76 (1.17–6.49)	2.89 (1.18–7.08)†
Pneumonia	2 (2.4)	14 (1.5)	-	–
Hospitalization	3 (3.6)	50 (5.4)	0.66 (0.13–2.12)§	–
Antimicrobial use during previous 3 months‡	5 (6.0)	76 (8.17)	0.72 (0.22–1.83)§	–
No. of individuals in the house*	5±1 (median 4, range 2–9)	5±2 (median 5, range 2–22)	–	–
No. of individuals in the same sleeping room*	2±1 (median 2, range 1–5)	2±1 (median 2, range 1–9)	–	–
Age (months) of children aged <5years in the same sleeping room*	20±7 (median 24, range 12–24)	29±15 (median 24, range 1–59)	–	–

URI, Upper respiratory infection.
*Results as continuous variables.
†*P* value <0.05.
‡Results are reported as *n* (%).
§*P* value >0.05; log likelihood ratio test.
‖*P* value <0.001.

disease may be partly secondary to the higher probability to be colonized.

Among non-smoking adolescents, exposure to tobacco smoke in the household was associated with pneumococcal carriage (47.6 vs 32.8 %, $P<0.01$); among 13 active smoking adolescents, pneumococcus was recovered from one person who also reported exposure to passive smoke in the household (100 %, 1/1); among 12 smokers non-colonized by pneumococcus, exposure to passive smoke was reported by 6 (50 %). Our findings present evidence that asthmatic individuals are independently more prone to be pneumococcal carriers (Table 2). In a recent study, Talbot *et al.* (2005) identified asthma as an independent risk factor for invasive pneumococcal disease in people aged 2–49 years. Nonetheless, exposure to tobacco was not measured in that study. In our study, the stratified evaluation included asthma and passive exposure to tobacco (Table 2), and each of them was identified as an independent risk factor for pneumococcal colonization. The association of exposure to tobacco and pneumococcal carriage in children and their mothers has been demonstrated (Greenberg *et al.*, 2006). Cigarette smoking has been identified as the strongest factor for invasive pneumococcal disease among immunocompetent, non-elderly adults (Nuorti *et al.*, 2000). Contact with smokers has also been identified as a risk factor for meningococcal disease in adolescents (Coen *et al.*, 2006). In this last study, the authors differentiated the exposure to smoke from exposure to smokers, and the first variable was not associated with meningococcal disease. It is supposed that smokers may be more infectious than non-smokers (Nuorti *et al.*, 2000).

The preceding data show evidence that asthma and exposure to tobacco are independent risk factors for pneumococcal carriage. The conjugate pneumococcal vaccine decreases pneumococcal carriage (O'Brien & Dagan, 2003) and it has proven to be effective in preventing invasive disease (Black *et al.*, 2006). Our findings emphasize the inclusion of asthma as a condition for the use of pneumococcal vaccine, in addition to the evidence that asthmatic patients have a higher risk of invasive pneumococcal disease (Talbot *et al.*, 2005). Measures to prevent cigarette smoking should include information regarding the risk of pneumococcal carriage and invasive disease.

According to the Advisory Committee on Immunization Practices (ACIP, 2000), conjugate pneumococcal vaccine has not been studied sufficiently among older children or adults to make recommendations for its use among persons aged $\geqslant 5$ years. The results observed in the present investigation are aligned with previous studies that found asthma as an important risk factor for invasive pneumococcal disease (Talbot *et al.*, 2005). Thus, adolescents should be considered a potential group that deserves consideration regarding the investigation of pneumococcal morbidity and conjugate vaccine efficacy. Further studies conducted in other regions should be instigated in order to confirm our findings.

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