Prevalence of Pneumococcal Nasopharyngeal Carriage Among Children 2–18 Months of Age

Baseline Study Pre Introduction of Pneumococcal Vaccination in Cuba

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Background: A new vaccine candidate against pneumococcus is being developed in Cuba, and it is a priority of the national health system. There is limited information on nasopharyngeal colonization burden, though it is essential for monitoring the impact of the vaccine. The study aims to estimate the prevalence of nasopharyngeal colonization in children 2–18 months of age and identify circulating serotypes, antimicrobial resistance and its association with selected risk factors.

Methods: A cross-sectional study was conducted between October and December 2013 in Cienfuegos municipality. Inclusion criteria were evaluated, and informed consent was obtained from the parents. Clinical and epidemiologic data were collected through a semistructured questionnaire. Nasopharyngeal swabs according to established protocols were taken. Data analysis included frequency distributions and comparison of proportions. The association between colonization and selected risk factors was assessed by multivariate analysis.

Results: A total of 984 children (87.2% living in urban areas) were included. The overall prevalence of colonization was 21.6%. The most frequent serotypes isolated were 6A (23.1%), 23F (10.8%), 6B (10.3%), 19F (8.5%) and 14 (3.3%). We found no resistance to β-lactamases in circulating serotypes. Living with sibling younger than 5 years, previous respiratory infections, previous hospitalization and day-care attendance were determinants of nasopharyngeal carriage.

Conclusions: The findings suggest that the burden of pneumococcal disease and colonization in Cuba could be significantly affected after vaccine introduction.

Key Words: *Streptococcus pneumoniae*, nasopharyngeal carriage, serotypes, pneumococcal conjugate vaccines, Cuba

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Streptococcus pneumoniae is a common cause of invasive disease and respiratory tract infections worldwide.[1] It is associated with high morbidity and mortality especially in young children, seniors older than 65 years and immune-compromised individuals. Each year 1 million children under 5 years die from pneumonia or invasive pneumococcal disease (IPD). Nasopharyngeal (NP) carriage is recognized as a risk factor for invasive disease.[2]

Colonization by pneumococcus mainly occurs in the first year of life. The prevalence of NP carriage in children younger than 2 years varies from 30% to 62%. NP carriage is also believed to be an important source of horizontal spread of this pathogen within the community.[3–6] Crowding, as in day-care centers, schools or hospitals, increases horizontal spread of pneumococcal strains.[7,8] The combination of factors such as high prevalence and high levels of crowding present mainly among young children makes this group the highest risk of horizontal dissemination of the infection.[9] Therefore, part of the strategy to prevent pneumococcal disease focuses on prevention of NP colonization.

Because vaccination is the most effective public health tool to prevent IPD, pneumococcal conjugate vaccines (PCVs) represent an important step to preventing and controlling invasive and noninvasive diseases. The new generation of conjugate vaccines is highly immunogenic in children younger than 2 years.[10–14] They have been introduced in several countries where a substantial decrease in incidence of IPD has been reported in the target population of less than 5 years of age.[13] Also it has been reported as herd immunity effect among nonvaccinated populations by reducing NP colonization and transmission of vaccine-type (VT) pneumococci from vaccinated children.[14]

In Cuba, PCV is not included in the national immunization program, and no studies that explore the pneumococcal NP colonization rates in children have been found.

A new conjugate heptavalent pneumococcal vaccine (PVC7-TT) is under development in Cuba. It contains 2 μg of serotypes 1, 5, 14, 18C, 19F and 23F and 4 μg of 6B, each one conjugated to tetanus toxoid (TT). This vaccine was designed with the serotypes that cause most IPDs around the world.[15] Preliminary results of clinical evaluation in adults, children and toddler 7–11 months of age are showing that it is safe and immunogenic.[16–18] Clinical evaluation for effectiveness and impact of the new vaccine includes exploring changes in the distribution of *S. pneumoniae* serotypes in invasive disease and NP carriage after its introduction in Cuba. This report explores the prevaccination prevalence of pneumococcal NP colonization among children 2–18 months of age. *S. pneumoniae* serotypes are identified by age group and their association with individual risk factors and household.

MATERIALS AND METHODS

Study Design and Subjects

A cross-sectional study was conducted between October and December 2013 in Cienfuegos municipality, located in the central zone of Cuba. This selection was based on annual series of incidence of pneumococcal meningitis, laboratory capacity at provincial level for sampling and culture of *S. pneumoniae* and previous experience in performing clinical trials.

A pediatrician in each health area (primary health organization in Cuba) was responsible for informing parents and obtaining parental consent for clinical evaluation of eligible children. All children 2–18 months of age from 8 urban and peri-urban health areas who met inclusion criteria were considered eligible and were randomly selected to participate. Subjects were excluded if they had (1) anatomic defects that limit NP samples, (2) blood disorders referred by parents, (3) antibiotic treatment 7 days before samples were collected or (4) previous history of pneumococcal vaccination.

Individual and household risk factors were determined at baseline. Also, pneumococcal vaccination history, records of antibiotics use, hospitalizations and outpatient disease were collected using a semistructured questionnaire. Regional differences were recorded.

NP Specimen Collection

NP samples were collected by laboratory technician previously trained using flexible sterile nylon flocked swabs (COPAN, Brescia, Italy). The swabs were inserted into the posterior NP and rotated around 5 seconds, removed and directly placed into 1.0-ml skim-milk–tryptone–glucose–glycerol (STGG) broth transport medium. NP samples were transported to the laboratory in dry ice and vortexed for 10 seconds and frozen at −70°C.

Culture of Samples and Serotyping

Specimen testing, detection of *S. pneumoniae*, serotyping and antibiotic susceptibility testing were conducted by National Reference Laboratory of Tropical Medicine Institute "Pedro Kouri" (IPK).

Identification of *S. pneumoniae* was based on the presence of α-hemolysis and inhibition by optochin.[19] After fully thawing the NP-STGG specimen at room temperature (25°C) and vortex for approximately 10–20 seconds, each specimen was inoculated onto a trypticase soy agar supplemented with 5% sheep blood plus gentamicin 5 mg/L plate and incubated for 18–24 hours at 37°C in CO_2 incubator. The suspected pneumococcal isolates were passed to a fresh blood agar plate and processed by a conventional diagnostic approach: isolation of a single α-hemolytic colony susceptible to optochin and soluble in deoxycholate. When more than 1 pneumococcal colony morphology was evident, all different morphologies were tested.

A fresh culture (overnight/24 h) of each isolated specimen confirmed as *S. pneumoniae* was stored at –70°C in 1.0 mL of STGG medium until serotyping and antibiotic susceptibility test.

Pneumococcal capsular polysaccharide serotype was determined by capsular swelling reaction (Quellung reaction) analysis using group- and type-specific antisera obtained from Statens Serum Institute, Copenhagen, Denmark.[18] Isolates for which a capsule could not be assigned were reported as nontypeable. Serotypes were classified as vaccine type (VT; 1, 5, 6B, 14, 18C, 19F and 23F), vaccine-related type or cross-reaction (VRT; 6A and 19A), nonvaccine type (NVT) and nontypeable (NT).

Susceptibility testing was conducted by minimal inhibitory concentration determination by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI)-recommended procedures.[20] All isolates were tested against penicillin, ceftriaxone, trimethoprim sulfamethoxazole (SXT), erythromycin and chloramphenicol.

Data Collection and Statistical Analysis

Interviews were conducted using a nonstructured questionnaire to explore demographic variables (age, sex, geographic area and health area) and individual risk factors (type of daycare, breastfeeding exclusive, previous hospitalization and respiratory infections) and household conditions (sharing bed, exposure to cigarette smoke and sibling).

The demographic information of each child was recorded in a data file using Microsoft Access version 2007 (Redmond, WA). The NP colonization prevalence rates were estimated for VT, VRT and NVT serotypes per age specific and per strata of age. The antibiotic resistance rate was calculated. The bivariate analysis and multivariate logistic regression were conducted to evaluate determinants of NP colonization using prevalence ratio as risk estimator. The statistical significance differences were assessed using χ^2 test with a significance level of 0.05. The statistical analysis was carried out using R software (Vienna, Austria).[21]

Ethics Statements

Ethics committees of IPK and Medicine University in Cienfuegos approved the study protocol. Informed consent was obtained from parents or guardians of all children before enrollment.

RESULTS

General Characteristics of Study Population

Out of 1050 children between 2 and 18 months of age enrolled, 984 (93.71%) met the inclusion criteria and entered the study, as shown in Figure 1. A total of 980 (99.59%) NP samples were obtained, and 21.6% (212/980) were found positive to *S. pneumoniae.*

Table 1 describes sociodemographic characteristics of study population distributed in NP carriage and not carriage. A total of 87.2 % of children enrolled are living in urban areas. Children living in peri-urban and rural zones are mainly linked to health areas V and VIII. There was no significant difference in age ($P = 0.560$), sex ($P = 0.379$) and geographic distribution ($P = 0.2714$) among both groups.

Carriage NP Rates by Age Group

Overall carriage of *S. pneumoniae* was 22% (95% confidence interval [CI]: 19.1–23.3). The lower prevalence rate was found at 6 months of age (16%; 95% CI: 9.5–22.2) and the higher rate at 10 month of age (28%; 95% CI: 14.3–47.5). However, in children younger than 6 months the prevalence rate was more than 22% (Fig. 2). There was no significant difference in carriage NP prevalence distributed by strata of age: <6 months (24.62%; 95% CI: 18.87–31.39), 6–12 months (20.55%; 95% CI: 14.38–21.93)

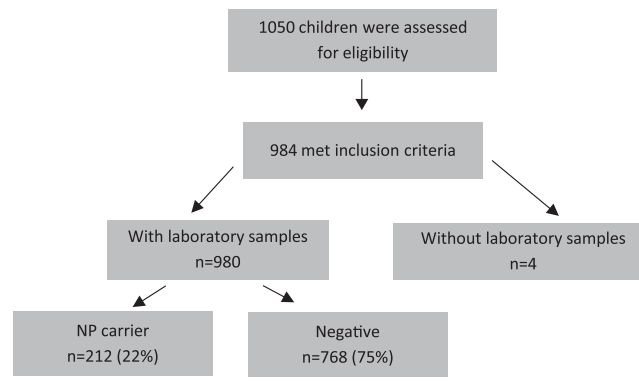

FIGURE 1. Flowchart of subjects and sampling cultures.

and >12 months (21.19%: 95% CI: 20.13–29.18). None child carried more than 1 pneumococcal serotype in their nasopharynx.

Serotype Distribution by Age and Geographic Area

For pneumococcal capsular detection, overall 212 isolates were serotyped by the Quellung reaction and 30 were identified of more than 90 serotypes known. Figure 3 depicts the specific serotype distribution by strata of age. Of the 212 carriage pneumococcal isolate, 49 (23.1%) were 6A, 23 (10.8%) were 23F, 22 (10.3%) were 6B, 18 (8.5%) were 19F and 7 (3.3%) were 14. Among VT serotype, 23F was the most prevalent in infants (19, 15.45%), whilst 6B (10, 11.2%) and 19F (7, 7.9%) were predominant in children older than 12 months. Serogroups 14 and 18 were more frequently detected in infants younger than 6 months. NVT serotypes represent 28.3% of total isolations. Less frequently, 11A (3.30 %), 15B (2.83%), 23B (2.83%), 15C (1.89%), 11D (1.42%), a.o, were detected. Serotype 6A—considered VRT because preliminary cross protection observed in nonclinical studies and Phase I clinical studies[16]—was the most prevalent in all group: <6 months (19%), 6–12 months (28%) and >12 months (21%). None of the serotyped isolates was serotype 1 and 5. By Quellung reaction, 25 isolates (11.8%) were NT. None child carried more than 1 pneumococcal serotype in their nasopharynx.

Significant differences ($P = 0.01$) were found among health areas in the proportion of VT plus VRT and NVT serotypes (Fig. 4). In general, VT plus VRT serotypes are predominant in the majority of health areas ranging from 50% to 81%. In suburban and rural areas pertain to health areas V and VIII, the proportion carrying *S. pneumoniae* nonvaccine type was higher, representing 42% and 40%, respectively.

TABLE 1. Sociodemographic Characteristics of Children Enrolled

	NF Carriage	Not NF Carriage
	N = 212	N = 768
Variables	n (%)	n (%)
Age		
2–3	19 (8.96)	62 (8.07)
4–5	29 (13.68)	85 (11.07)
6–7	20 (9.43)	106 (13.80)
8–9	32 (15.09)	101 (13.15)
10–11	7 (3.30)	18 (2.34)
12	16 (7.55)	65 (8.46)
13–18	89 (41.98)	331 (43.10)
Gender (male)	120 (56.60)	406 (52.86)
Geographic area		
Urban	181 (85.38)	674 (87.76)
Suburban	13 (6.13)	28 (3.65)
Rural	18 (8.49)	61 (7.94)
Health area		
I	45 (21.23)	116 (15.10)
II	14 (6.60)	84 (10.94)
III	32 (15.09)	111 (14.45)
IV	15 (7.08)	55 (7.16)
V	43 (20.28)	137 (17.84)
VI	19 (8.96)	69 (8.98)
VII	14 (6.60)	103 (13.41)
VIII	30 (14.15)	93 (12.11)
Type of day care		
At home	171 (80.66)	672 (87.50)
Private household care	22 (10.38)	65 (8.46)
Daycare attendance	19 (8.96)	31 (4.04)
Sibling		
Presence of older sibling	34 (16.04)	193 (25.13)
Sibling younger than 5 years	96 (45.28)	193 (25.13)

Antimicrobial Susceptibility

Antimicrobial susceptibility was determined in 194 (91.04%) isolates. All of them were susceptible to penicillin and ceftriaxone. Erythromycin, SXT and chloramphenicol resistance was observed in 91 (46.9%), 83 (42.7%) and 12 (6.1%) isolates, respectively. Forty-four isolates (22.6%) and a wide range of serotypes (particularly 6B, 6A, 19F and 23F) showed resistance to erythromycin and SXT. The recent use of antibiotics was reported in 22.3% of children; however, there was no significant difference among carriage and not carriage ($P = 0.34$).

Individual and Household Risk Factors

Bivariate analysis (Table 2) showed that (1) previous hospitalization, (2) previous episodes of respiratory infections and (3) day-care attendance were significantly associated with NP colonization ($P = 0.000$, $P = 0.007$ and $P = 0.006$, respectively).

At household level, sharing bed with parents ($P < 0.001$) and cohabiting with senior older than 60 years ($P = 0.007$) and children younger than 5 years ($P < 0.001$) were risk factors significantly associated with colonization.

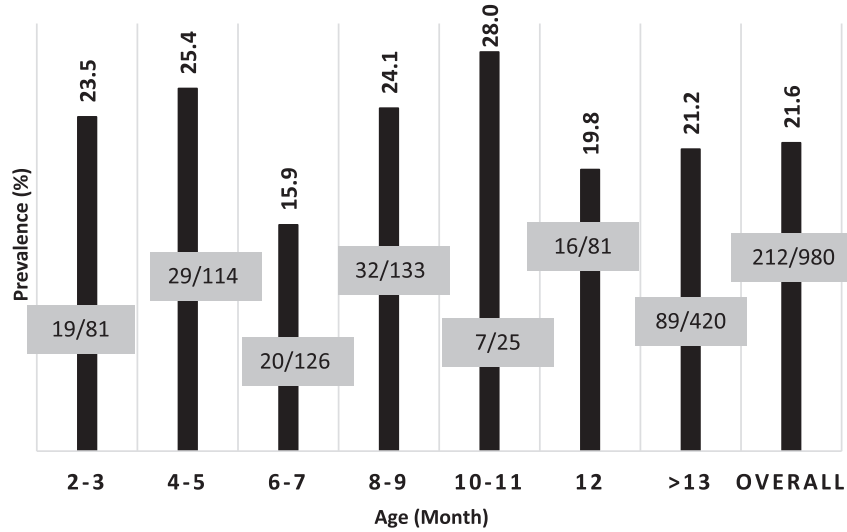

FIGURE 2. Carriage pneumococcal NP prevalence by age.

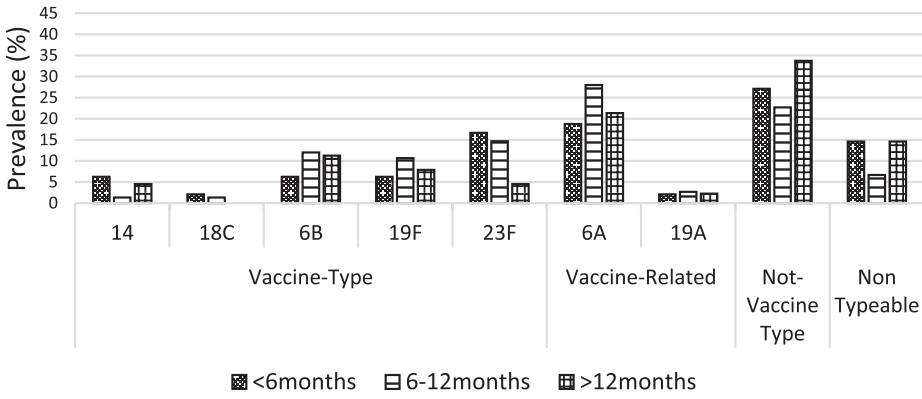

FIGURE 3. Serotype-specific distribution by strata of age.

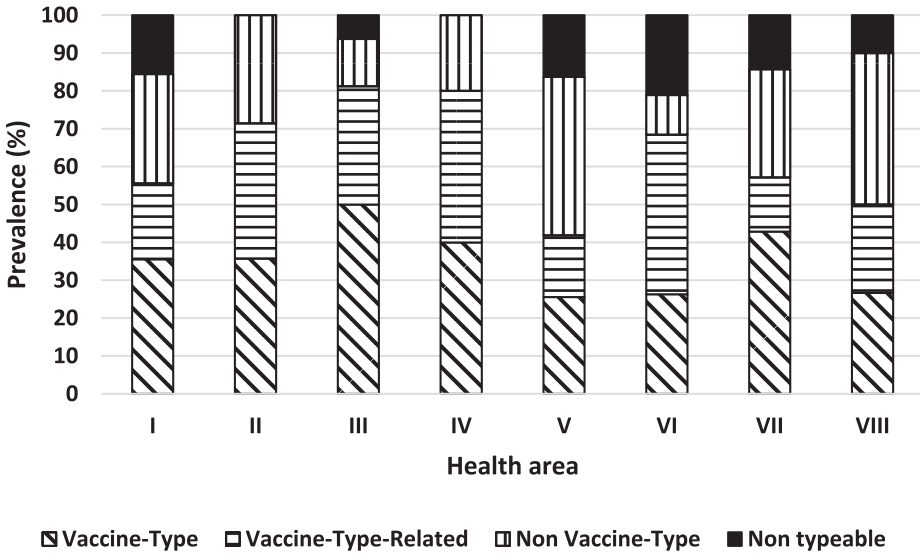

FIGURE 4. Distribution of type of serotype by health area.

Multivariate analysis (Table 3) showed that sibling children younger than 5 years (odds ratio [OR] 3.48; 95% CI: 1.82–2.51), previous respiratory infections (OR 2.45; 95% CI: 1.03–1.57), previous hospitalization (OR 2.48; 95% CI: 1.05–1.62) and day-care attendance (OR 4.33; 95% CI: 1.26–2.36) were determinants of NP colonization.

DISCUSSION

This article explores the burden of pneumococcal colonization at community level in the first months of life to provide scientific evidence for decision making about pneumococcal vaccination in Cuban population.

The main limitation of the study is regarding the retrospective data collection on individual risk factors as hospitalization, respiratory infection and antibiotic use in the last 2 months.

Our finding regarding of 25 NT pneumococcal isolate (11.8%) is in harmony with the prevalence of NP colonization with NT pneumococci to different regions, ranging from 0% to 11%.[22] Among nonsterile-site isolates, sputum, oral pharyngeal isolates or NP isolates, it is common to find that around 10% of the isolates fail to react with pneumococcus-typing antisera.[23] Conversely, serotyping based on the Quellung reaction with anticapsular sera, besides expensive and labor intensive, is error-prone due to factors like differences in the quality of the antisera, lack of capsular production as a result of passage of isolates on agar plates and human error.[24]

Data of baseline pneumococcal carriage and invasive disease rates are crucial for assessing the impact of vaccination and for monitoring serotype changes and antimicrobial resistance patterns. There are over 90 serotypes of pneumococci, but worldwide 6–11 serotypes are responsible for 70% of IPD in children younger than 5 years.[25] The distribution of pneumococcal serotypes in NP carriage and IPD vary by age and geographic areas.[2,26,27] Pneumococcal carriage is highly prevalent in developing countries particularly among children younger than 5 years.

The NP niche becomes colonized during the first year of life, and the scientific literature also has reported an increase before the age of 2 years.[28,29] We did not find significant differences among strata of age divided in the first 6 months, between 6 and 12 months and toddler. Despite the peak prevalence of pneumococcal colonization was found at the age of 10–11 months, we did not detect significant differences among prevalence rates from 2 months of age. The overall prevalence in children between 2 and 18 months before vaccine introduction in Cuba is in range of international reports from Asia, Europe and North America where have found carriage prevalence of 11%–100%.[30–32]

The most frequent serotypes in all strata of age (67.5% in urban areas and 45.2% in rural areas) will be covered with Cuban vaccine (VT or VRT), despite the variation among health areas and the difference detected between urban and rural. Also among cases with IPD, the coverage will be more than 65% of isolates serotypes.[33] In these analysis, we are assuming the expected cross

TABLE 2. Individual and Household Risk Factors in Nasopharyngeal Carriage: Bivariate Analysis

Risk Factor	Not Carriage N = 768 n (%)	NF Carriage N = 212 n (%)	Total N = 980 n (%)	*P*
Individual risk factors				
Breastfeed exclusive until 6 months	652 (84.9)	188 (88.7)	840 (85.7)	0.200
Previous hospitalization	83 (10.8)	42 (19.8)	125 (12.7)	0.001
Previous antibiotic use	166 (21.6)	53 (25.0)	219 (22.3)	0.340
Previous respiratory infections*	554 (78.2)	174 (82.1)	728 (74.7)	0.007
Daycare attendance	31 (4.0)	19 (9.0)	50 (5.1)	0.007
Household risk factors				
Sharing bedroom with more than 2 persons	761 (99.1)	212 (100)	973 (99.3)	0.350
Sharing bed with parents†	61 (8.0)	27 (12.7)	88 (9.0)	<0.001
Exposure to cigarettesmoke‡	328 (43.7)	99 (46.9)	427 (44.4)	0.433
Older sibling > 60	193 (25.1)	34 (16.0)	227 (23.2)	0.007
Sibling younger than 5 years	193 (25.1)	96 (45.3)	289 (29.5)	<0.001

*Six volunteers (not carriage) without data.
†Seven not carriage without data.
‡Seventeen not carriage and 1 carriage without data.

TABLE 3. Determinant of Nasopharyngeal Colonization

NP Colonization Determinants	OR (95% CI)	*P*
Intercept	0.13 (0.08–0.19)	<0.001*
Previous Hospitalizations	1.62 (1.05–2.48)	0.028†
Previous respiratory infections	1.57 (1.03–2.45)	0.039†
Daycare attendance	2.36 (1.26–4.33)	0.006‡
Sharing bed with parents	1.60 (0.96–2.62)	0.067§
Older sibling >60 years of age	0.58 (0.38–0.87)	0.010†
Sibling younger than 5 years	2.51 (1.82–3.48)	<0.001*

Values are significant at *P <0.001, ‡P < 0.01, †P = 0.05, §P = 0.01.

protection between 6B/6A and 19F/19A, because there are evidences in the phase I clinical trials in old children[17] and infants (unpublished data). Studies conducted in several countries report comparisons among rural and urban areas for NP colonization and antibiotic resistance and have found that rural residence is a protector factor.[30,32,34] However, we did not find studies reporting difference among VT and NVT serotype detection in rural and urban areas.

Further studies should be conducted to explore the main determinants of differences among areas, but had been well documented by the literature that serotype distribution among NP carriage isolates varies by country, age group and type of cohort, but our results were similar of much of them. For instance, Europe and the United States show similar serotype distributions with minor differences in several serotypes.[35] In Gambia, serotypes 19F (19%), 6B (16%), 6A (13%), 9V (7%) and 23F (7%) were most frequently found among children under 3 years of age. In Finland, serotypes 6B (16%), 23F (14%), 19F (14%) and 6A (9%) were most prevalent.[2] In the United States, serotypes 6B, 14, 19F and 23F were also common.[35]

In Asia, similar serotypes and serogroups have been found among NP isolates in healthy children. For example, in India, the most common serogroups are 6, 14, 19 and 15,[36,37] and in Vietnam, the most common serogroups are 19, 23, 14, 6 and 18.[38] The serogroup distribution in Indonesia is slightly different, with the most common being 6 (25%) and 23 (21%) followed by 15 (8%), 33 (8%), 19 (6%), 12 (5%) and 3 (4%).[34] In Kenya, serotype 13 was with 15, 14, 6B and 19F most commonly identified. In South Africa, a similar distribution was found with the exception of serotype 13, which was not found at all.[39]

Despite the prevalence serotype specific of NP colonization is useful to monitoring changes associated with vaccine introduction, it does not represent exactly the contribution of each serotype to the burden of pneumococcal disease. It has been demonstrated for serotype 1 and 5 that frequently produce IPD but are uncommon detected in carriage. They are poor colonizer because short time colonizing NP, but have a high invasive capacity. In contrast, serotypes 6A, 6B, 14, 19A and 19F are frequently isolated causing IPD and also are prevalent nasopharynx colonizing.[40] A study in The Gambia showed that despite pneumococcal serotypes 1 and 5 were rarely isolated from the nasopharynx of children, they were nevertheless responsible for about one-third of all cases of IPD.[41]

In Cuba, although the data limitations on serotypes isolations in IPD, serotypes 14, 19A, 19F, 6B, 23F and 6A were frequently detected in IPD in children younger than 5 years.[42] Our findings in carriage and the available information on prevalent serotypes in IPD[43]suggest that introduction of PCV7-TT Cuban vaccine will reduce morbidity and mortality associated with pneumococcus in short and long term.

Currently, NP colonization in preschool children between 1 and 5 years of age is explored as part of efficacy clinical trial of the new vaccine candidate in Cuba.[44] Preliminary results showed that in institutionalized children the prevalence was 31% and more than 48% of all isolations were VT serotypes (ongoing studies in Cuba).

Carriage in this group of age varies from 44% to 90% in Papua New Guinea, Fiji, Indonesia, Venezuela and The Gambia.[34,45–47] In a Finnish study, the frequency of NP carriage in children 2–24 months of age increased from 13% in children younger than 6 months to 43% in children older than 19 months.[48]

Rates of carriage with serotypes penicillin resistance varied greatly within regions and continents.[49] There is no preceding information regarding antimicrobial resistance patterns of NP *S. pneumonia* between Cuban healthy children, but the prevalence of invasive pneumococcal penicillin resistant isolates has been reported as 44.3% in a study performed between 2007 and 2012.[42] However, this figure was supported by the revision of meningeal isolates of *S. pneumoniae*, predominantly.

In the nasopharynx, antimicrobial use selects for antibiotic-resistant pneumococcal, mainly of vaccine-related serotypes, whereas it may promote an increase in the frequency of colonization with nonvaccine serotypes. Although the children enrolled in the current study not received any antibiotic therapy 7 day before samples collection to explain the high degree of susceptibility for penicillin will be necessary to know the history of the use of this drug during the 60-day period before the time of sampling.

The high resistance by erythromycin and SXT detected in our study population is similar to reports worldwide regarding invasive pneumococcal isolates.[39] Since the early 1990s, SXT and macrolide antibiotics have been widely prescribed for the empiric treatment of community-acquired respiratory tract infections. In 1993, concerns over the increasing prevalence of pneumococcal resistance to β-lactam antibiotics, coupled with an increased awareness of the role of atypical pathogens in community-acquired pneumonia (CAP). Subsequently, the macrolides

have been included as a first-line therapeutic option for outpatients with community-acquired respiratory tract infections in treatment guidelines worldwide, resulting in the widespread use of these agents. However, high levels of pneumococcal resistance to macrolide antibiotics have led to concerns over the continued clinical efficacy of these agents.[50]

The association of previous respiratory infections and hospitalization with the prevalence of colonization NP, it supports the theory of increased risk during viral infections.[51–55] In the healthy population, risk factors also seem to determine the frequency of pneumococcal carriage. We found as main determinants at individual and household level the daycare attendance and sibling with children younger than 5 years.[2,41,56] Crowding is a major factor in colonization and in spread of pneumococcal strains. In young children, especially, day-care visits are associated with significantly increased colonization rates.[29,56–61] In a study from the Netherlands, the relative risk of NP colonization by pneumococci in children who attended day-care centers was 1.6 compared with children who were cared for at home.[2] Pneumococcal colonization, especially with antibiotic-resistant bacteria, is also increased as a result of recent antibiotic treatment.[56,62] The selection of antibiotic-resistant pneumococci at the nasopharynx is commonly assumed to be the cause of the spread of resistant pneumococcal strains within the community.[63]

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