

Nasopharyngeal carriage of *Streptococcus pneumoniae* among children in an urban setting in Brazil prior to PCV10 introduction



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ABSTRACT

Information on pneumococcal carriage in the pre-vaccine period is essential to predict and assess the impact of PCV in settings where disease surveillance is particularly difficult. Therefore, we present data on pneumococcal carriage before the introduction of the 10-valent-pneumococcal conjugate vaccine (PCV10) in Brazil. We conducted a prospective study on a cohort of 203 children aged <5 years old, randomly selected in an urban community located in the periphery of the city of Salvador, Brazil and followed them from January/2008 to January/2009. Nasopharyngeal swabs were collected from each child at four times. In total, 721 swabs were collected, yielding a pneumococcal carriage prevalence of 55% ($n=398$). In multivariate analyses, the variables associated with carriage were having contact with three or more children <2 years old (OR, 2.00; 95% CI 1.33–2.89) and living in a house with an average of 3 residents per room (OR, 1.77; 95% CI 1.05–3.10). Also, white participants were more likely to be protected from colonization (OR, 0.52; 95% CI 0.29–0.93), and prevalence of carriage varied over time, with lower prevalence occurring from February to June (OR, 0.53; 95% CI 0.37–0.78) compared to July to January. Contact with children under 2 years of age and living in crowded housing also were associated with colonization by highly invasive serotypes, although this relationship was not significant. The most prevalent vaccine serotypes were 6A/B (25.4%), 19F (10.1%) and 14 (9.0%), while the most prevalent non-vaccine serotypes were 16F (4.8%), 15B/C (4.5%) and 6C/D (3.5%). Overall, 38.4% (153/398) of the isolates were non-susceptible to penicillin, and of those, 73.8% (113/153) were non-susceptible to trimethoprim/sulfamethoxazole. Colonization rate by PCV10 serotypes was 52.2%. Routine PCV10 vaccination can lead to significant changes in pneumococcal serotypes found in NP colonization, indicating a need for continued monitoring, especially in crowded settings, as occurs in Brazil's slums.

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1. Background

Asymptomatic carriage of pneumococci is common in young children and has been related to the development of disease and transmission of the pathogen [1–3]. The prevalence of pneumococcal carriage increases in the first few years of life, peaking at

approximately 50–80% in children 2–3 years of age and decreasing thereafter until stabilizing at 5% to 10% in children over 10 years of age [4]. Effective vaccines against 10-, or 13- of the more than 90 pneumococcal serotypes are now used in many countries, resulting in a substantial decline in invasive disease and carriage of vaccine serotypes [5,6]. Despite this success, serotypes not targeted by the vaccine have increased among healthy carriers and could potentially become important causes of invasive diseases. As Brazil started nationwide vaccination with 10-valent pneumococcal conjugate vaccine (PCV10) in 2010, it is essential to have information on pneumococcal carriage in the pre-vaccine era to assess potential vaccine impact.

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Many studies have been conducted in Brazil to investigate the distribution of pneumococcal serotypes from invasive disease and nasopharyngeal colonization. However, there are no reports of prospective studies conducted in communities. The previous studies were undertaken in schools and daycare settings, which represent important risk factors for the transmission and circulation of pneumococcus [7,8]. In addition, a previous cross-sectional study that we conducted in a slum community in Salvador did not observe an association between prevalence of carriage with the size of the household or numbers of household contacts. It was hypothesized that study design and sample size could have affected the ability to adequately evaluate the effect of risk factors related to population or household density [9].

Thus, we carried out a cohort study of pneumococcal carriage in a slum community in Salvador to describe the risk factors for carrier status of *Streptococcus pneumoniae* in children under 5 years old. We also determined the distribution of serotypes, characterized antimicrobial susceptibility, and defined the possible coverage provided by the 10 or 13 valent pneumococcal conjugate vaccine (PCV10 or PCV13).

2. Methods

2.1. Study site and population

The study was conducted in the Pau da Lima community, which is situated in the periphery of Salvador, a city of 2.7 million inhabitants [10] in Northeast Brazil. We selected an area of 0.46 km² where a cohort study for leptospirosis was conducted in 2003. As a part of this study, a census was completed during visits to 3689 households; this identified 14,122 inhabitants, of which 8% ($n = 1131$) were aged <5 years [11]. A total of 130 households were randomly selected within the census tract in order to have 203 children <5 years old enrolled in the study. This sample size was based on a previous study conducted in Salvador, where 65% of children <5 years were colonized at a single time-point [9]. Eligible subjects, defined as children 1–59 months of age who lived continuously in one of the selected households during the month prior to recruitment, were enrolled into the study according to informed consent procedures approved by the Oswaldo Cruz Foundation, Brazilian Ministry of Health.

2.2. Data collection

During house visits, a standardized questionnaire was used to document information on demographics, underlying medical conditions, hospitalizations, occurrence of an upper respiratory tract infection (URTI) in the previous month, antibiotic therapy in the last four weeks before the visit, childcare arrangements, school attendance and household inhabitants' habits such as smoking. Information for children was obtained by interviewing the parent or legal guardian. Household crowding was defined as the number of people divided by the number of rooms in the house, or the number of people divided by the number of beds in the house. Household contact with children <2 years old was defined as contact of at least 4 h/day.

2.3. Isolation of pneumococci

Between January 2008 and January 2009, nasopharyngeal swabs were collected from each child at four times, at enrollment and then again at 3-month intervals. Samples were collected with calcium alginate swabs (Calgiswab type 1, Spectrum, USA) and inoculated into modified Stuart transport medium and sent to the Clinical Microbiology Laboratory at the Gonçalo Moniz Research Institute. All swabs were plated within 4 h onto agar plates with

5% sheep blood and 5.0 µg/mL of gentamicin. Plates were incubated at 35 °C in 5% CO₂-enriched atmosphere for up to 48 h. Three α-hemolytic colonies exhibiting morphologic characteristics suggestive of *S. pneumoniae* were isolated. Identification of these isolates as *S. pneumoniae* was confirmed by optochin disc susceptibility (BBL Microbiology Systems, Cockeysville, USA) and the bile solubility test. One *S. pneumoniae* colony per plate was then subcultured, harvested, and kept frozen at –70 °C for further testing. When *S. pneumoniae* isolates from the same primary plate exhibited a clearly different colony morphology, dissimilar colonies were frozen separately.

2.4. Serotyping

The isolates were serotyped by multiplex-PCR as described elsewhere [12]. DNA extraction and PCR conditions were performed as described by the US Centers for Disease Control and Prevention (CDC) [12]. Isolates with negative multiplex PCR results were subjected to single-plex-PCR with primer 19F variation [13] and Quellung reaction testing for capsular type definition.

2.5. Antimicrobial susceptibility testing

The broth microdilution method was performed according to Clinical and Laboratory Standard Institute recommendations [14] to determine susceptibility of isolates to penicillin, cefotaxime, tetracycline, erythromycin, trimethoprim/sulfamethoxazole (TMP/SMX) and levofloxacin (Sigma–Aldrich, Germany). Quality control was performed by testing *S. pneumoniae* ATCC 49619. Isolates with a penicillin MIC value ≥ 0.12 µg/mL were defined as penicillin non-susceptible.

2.6. Genotyping

Pulse field gel electrophoresis (PFGE) analysis was performed to define the molecular profile of the isolates. Chromosomal digests generated by *Sma*I were prepared and analyzed as described elsewhere [15]. A CHEF DRII apparatus (Bio–Rad, Hercules, CA) was used for running the gels. The bacterial strains were also analyzed by multilocus sequence typing (MLST), as described elsewhere [16].

2.7. Data management and statistical analysis

Data were entered and managed by Epi Info version 3.5.1 (CDC, Atlanta, GA, USA). Statistical analyses were performed in SAS v9.3. Univariate and multivariate logistic regression models were constructed to identify risk factors for colonization (PROC GLIMMIX). To construct confidence intervals that accounted for the non-independence of samples from the same individual, we created 1000 bootstrap samples, where all observations from an individual were grouped together and sampled with replacement. Household crowding was analyzed as continuous variables. A variable was considered to be significantly associated with colonization ($p < 0.05$) if the 95% CI did not include 1.

For multivariate models, variables that were significant in the univariate analyses were included in different combinations, with the best-fitting model determined by Akaike Information Criteria (AIC) [17]. To test for an association between the demographic risk factors and the odds of being colonized with a high or low-invasiveness serotype, we created three outcome categories: uncolonized, colonized with a high invasiveness serotype (4, 7F, 8, 9V, 14, 18C and 19A), or colonized by a low-invasiveness serotype (3, 6A/B/C, 11A, 13, 15A, 15B/C, 16F, 17F, 19F, 20, 21, 22F, 23B, 23F, 35F and NT [not typeable]) [18]. We then fit univariate generalized logit models to these data and again used the bootstrap samples to test for significance at $p = 0.05$.

Table 1

Risk factors for pneumococcal carriage among children <5 years old at community-level prior to PCV10 introduction in Brazil.

Demographic characteristics	N (%) ^a	Pneumococcal carriage – OR (95% CI)	
		Univariate analysis	Multivariate analysis
Age (months)			
<6	50 (7%)	REF ^b	
6–11	93 (13%)	0.62 (0.27–1.41)	
12–23	145 (20%)	1.35 (0.64–2.91)	
24–35	132 (18%)	0.82 (0.38–1.70)	
36–47	173 (24%)	0.71 (0.35–1.42)	
48–59	128 (18%)	0.70 (0.32–1.49)	
Gender			
Male	347 (48%)	1.03 (0.73–1.44)	
Race ^a			
Mixed	505 (70%)	REF ^b	REF ^b
White	59 (8%)	0.52 (0.30–0.90)	0.52 (0.29–0.93)
Black	153 (21%)	0.77 (0.50–1.20)	
Day care attendance			
No	685 (96%)		
Yes	28 (4%)	1.40 (0.67–3.50)	
URTI in the last month			
No	287 (40.5%)	REF ^b	REF ^b
Yes	421 (59.5%)	1.42 (1.06–1.94)	1.38 (1.00–1.89)
Exposure to cigarette smoking			
No	490 (68%)	REF ^b	
Yes	231 (32%)	1.11 (0.75–1.67)	
Antibiotic use in the last month			
No	653 (92%)	REF ^b	
Yes	58 (8%)	0.90 (0.51–1.60)	
Number of children ≤2 years living in the same household			
None	288 (40%)	REF ^b	REF ^b
One contact	358 (50%)	1.60 (1.10–2.31)	1.44 (0.99–2.20)
Two contacts	67 (9%)	1.90 (1.10–3.50)	1.38 (0.73–2.53)
Three contacts	4 (1%)	3.20 (2.4–4.31)	2.00 (1.33–2.89)
No. of residents/no. of rooms	3.3 (±1.7)	1.90 (1.19–3.13)	1.77 (1.05–3.10)
No. of residents/no. of bed	3.5 (±1.8)	1.53 (1.10–2.23)	
Season (February/June vs July/January)	–	0.60 (0.42–0.82)	0.53 (0.37–0.78)

^a Number of swabs for each variable.^b REF, reference for analysis.

Bold means statistically significant results.

3. Results

3.1. Demographic characteristics

In January 2008, a total of 203 children were enrolled into the cohort study. Ages ranged from 1 to 48 months, and the median age was 24 months (interquartile range: 12–36). There was a predominance of mixed race (70%), and 48% of participants were males. The families of the enrolled children reported low monthly income (less than USD\$ 430.00), and crowded environments were observed in the households, with a median of five (range: 2–15) inhabitants per household. Most of the study children lived in households of two rooms (81.8%), with a ratio of 3.5 residents per bed (Table 1).

3.2. Prevalence of pneumococcal carriage

In total, 721 swabs were collected throughout the study period, yielding 398 pneumococcal isolates. The prevalence of *S. pneumoniae* nasopharyngeal carriage was 50.5% (February), 46.3% (June), 63.2% (September) and 48.8% (December) at each sampling point, respectively. Of the 203 children eligible for the study, 156 (76.8%) provided nasopharyngeal samples at all four visits (Fig. 1). At least one pneumococcal isolate from the nasopharyngeal sample was found in 74.4% (116 of the 156) of all children; 9.0% (14 of the 156) were not colonized at all; 19.9% (26 of the 156) were only once colonized; and 12.2% (19 of the 156) were colonized in all four visits.

3.3. Risk factors for colonization

Children who lived in households, where there was at least one child under 2 years, who lived in crowded households, and had

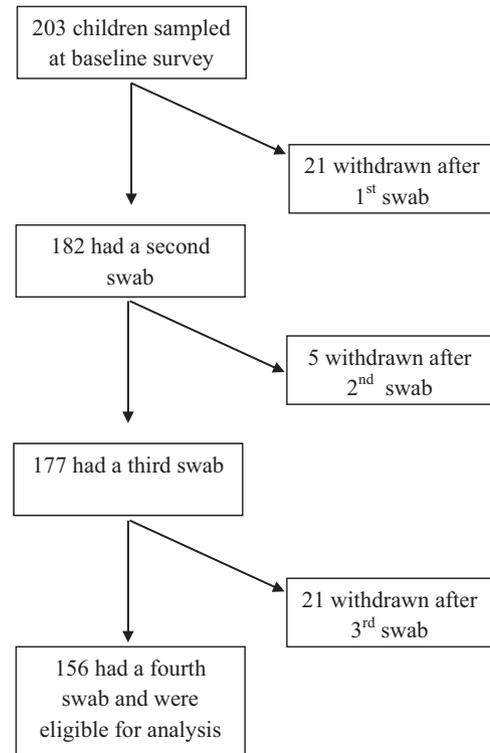
**Fig. 1.** Flowchart of participants enrolled in the study.

Table 2
Risk factors for pneumococcal carriage among children <5 years old, stratified by invasiveness property of serotypes prior to PCV10 introduction in Brazil.

Characteristics	N (%)	Pneumococcal invasive property – OR (95% CI)	
		Low invasiveness ^a (n = 263)	High invasiveness ^b (n = 60)
Age (months)			
<12	119 (18%)	0.74 (0.26–1.99)	0.18 (0.00–0.90)
13–23	133 (21%)	1.54 (0.67–3.80)	0.57 (0.16–2.01)
25–35	126 (20%)	1.09 (0.47–2.50)	0.48 (0.15–1.81)
36–47	155 (24%)	0.76 (0.34–1.69)	0.61 (0.20–1.95)
48–59	113 (18%)	0.82 (0.35–2.06)	0.40 (0.10–1.42)
Gender			
Male	312 (48%)	1.07 (0.73–1.55)	1.18 (0.62–2.25)
Race			
Mixed	452 (70%)	REF ^c	REF ^c
White	52 (8%)	0.48 (0.25–0.89)	0.45 (0.00–1.28)
Black	138 (22%)	0.70 (0.42–1.15)	1.31 (0.58–2.62)
Day-care attendance			
No	613 (96%)	REF ^c	REF ^c
Yes	25 (4%)	1.05 (0.37–2.80)	2.11 (0.00–6.34)
URTI in the last month			
No	259 (41%)	REF ^c	REF ^c
Yes	375 (59%)	1.53 (1.04–2.24)	1.01 (0.61–1.70)
Exposure to cigarette smoking			
No	441 (68%)	REF ^c	REF ^c
Yes	205 (32%)	0.97 (0.63–1.53)	1.21 (0.54–2.27)
Antibiotic use in the last month			
No	586 (92%)	REF ^c	REF ^c
Yes	51 (8%)	0.62 (0.28–1.23)	1.16 (0.41–2.61)
Number of children ≤2 years living in the same household			
None	264 (41%)	REF ^c	REF ^c
One contact	315 (49%)	1.38 (0.94–2.12)	1.95 (1.02–3.99)
Two contacts	62 (9.7%)	1.92 (1.05–3.60)	1.60 (0.47–3.75)
Three contacts	1 (0.2%)	0.00 (0.00–0.00)	0.00 (0.00–0.00)
No. of residents/no. of room	3.3 (±1.7)	1.65 (1.00–2.82)	2.49 (1.15–5.38)
No. of residents/no. of bed	3.5 (±1.8)	1.38 (0.98–2.07)	2.25 (1.28–3.89)
Season (February/June vs July/January)		0.57 (0.39–0.84)	0.68 (0.30–1.28)

^a High invasiveness serotype: 4, 7F, 8, 9V, 14, 18C and 19A.

^b Low invasiveness serotype: 3, 6A/B/C, 11A, 13, 15A, 15B/C, 16F, 17F, 19F, 20, 21, 22F, 23B, 23F, 35F and NT.

^c REF, reference for analysis.

Bold means statistically significant results.

a recent URTI in the last month had greater odds of being colonized in univariate analysis. Carriage prevalence varied in time, with decreased prevalence from February to June (dry season) compared to July to January (rainy season). Additionally, white children were less likely to be colonized than mixed children (OR, 0.52; 95% CI 0.29–0.93) (Table 1). From multivariate analyses shown in Table 1, prevalence of carriage varied over time, with lower prevalence occurring during dry season (OR, 0.53; 95% CI 0.37–0.78). Also, having contact with three or more children under 2 years old (OR, 2.00; 95% CI 1.33–2.89) and living in a house with a greater number of persons per room (OR, 1.77; 95% CI 1.05–3.10) were each independently and positively associated with pneumococcal carriage.

We also considered whether specific demographic risk factors were associated with having higher odds of being colonized with a highly invasive serotype or being colonized with a lower invasive serotype. Children who lived in crowded households (persons per room, persons per bed) had greater odds of being colonized by high-invasiveness serotypes. On the other hand, having had an episode of URTI in the last month increased the odds of colonization with a low-invasive serotypes. Carriage of low-invasiveness serotypes also varied over time, with a lower prevalence in the period from February to June (Table 2). Being white was associated with a lower odds of colonization with a low invasiveness serotype compared with mixed race children. However, these differences in effect sizes for the high and low invasiveness serotypes were not statistically significant.

3.4. Serotype distribution and antibiotic susceptibility

Table 3 shows the distribution of serotypes recovered throughout the period of the study. The most prevalent serotypes were 6A/B

(25.4%), 19F (10.1%) and, 14 (9.0%). The serotypes included in PCV10 and PCV13 accounted for 52.2% and 55.5%, respectively. The most frequent non-vaccine serotypes were 16F (4.8%), 15B/C (4.5%), 6C/D (3.5%), 34 (3%) and not typeable (7.3%); 15.3% (61/398) of the isolates of *S. pneumoniae* did not have the capsular type determined by multiplex-PCR. We did not find any fluctuation in the distribution of serotypes during the study period.

Overall, 38.4% (153/398) of the pneumococci were non-susceptible to penicillin, with MICs ranging from 0.12 to 8.0 µg/mL. The percentage of capsular types included in the PCV10 vaccine among penicillin non-susceptible accounted for 73.2% (112/153) as follows: 6A/B (45/112; 40%), 19F (29/112; 25.9%), 14 (20/112; 18%), 23F (12/112; 11%) and others (6/112; 5%). The non-vaccine serotypes commonly associated with penicillin non-susceptibility (41/153; 22%) were: NT (6/41; 14.6%), 16F (4/41; 9.8%), 13 (3/41; 7.3%), 21 (3/41; 7.3%), 34 (1/41; 2.4%). In addition, 58% (231/398) were non-susceptible to TMP/SMX, 18.6% (74/398) to tetracycline, 3% (12/398) to erythromycin, and 2% (8/398) to cefotaxime. Of the 153 penicillin non-susceptible isolates, 113 (73.8%) were also non-susceptible to TMP/SMX. The drugs involved in the most frequently identified patterns of multidrug nonsusceptibility, defined as being intermediate or resistant to three or more classes of antibiotics, were penicillin, TMP/SMX and tetracycline (14 of 398 isolates), penicillin, TMP/SMX and erythromycin (7 of 398 isolates), penicillin, TMP/SMX, cefotaxime (3 of 398 isolates) and penicillin, TMP/SMX, cefotaxime, and erythromycin (3 of 398 isolates).

3.5. Genotyping

PFGE analysis confirmed that 24 of the 156 (15.4%) children were highly likely to have been colonized by the same pneumococcal

Table 3

Serogroup/type distribution of all pneumococcal carriage isolates ($N=398$) recovered from children <5 years old prior to PCV10 introduction in Brazil.

Serotype	No. of isolates	(%)
PCV10	209	52.2
4	5	1.3
6A/B	101	25.4
7F/A	1	0.3
9V/A	2	0.5
14	36	9.0
18	10	2.5
19F	40	10.1
23F	14	3.5
PCV13	221	55.5
3	7	1.8
19A	5	1.3
Non-vaccine	177	44.5
6C/D	14	3.5
8	1	0.3
11A	4	1.0
13	5	1.0
15A	2	0.6
15B/C	18	4.5
16F	19	4.8
17F	2	0.6
20	2	0.6
21	3	0.8
22F/A	2	0.6
23B	1	0.3
34	12	3.0
35B	1	0.3
35F	1	0.3
ND ^a	61	15.3
NT ^b	29	7.3

^a ND, not determined by multiplex PCR.

^b NT, not typeable by both methods (multiplex PCR and Quellung reaction).

PFGE type at multiple time points: 6A/B ($n=9/24$; 37.5%), 14 ($n=5/24$; 20.8%), 19F ($n=4/24$; 16.7%), 34 ($n=2/24$; 8.3%), 23F ($n=1/24$; 4.2%), 3 ($n=1/24$; 4.2%), 6C ($n=1/24$; 4.2%) and 15B/C ($n=1/24$; 4.2%). The most commonly identified sequence types were ST156 (14; [$n=5$]), ST 66 (14; [$n=10$]), ST177 (19F; [$n=7$]), ST 338 (23F; [$n=6$]), ST 3930 (6C; [$n=3$]) and ST 771 (34; [$n=4$]). Strains belonging to the ST 66, ST 156 and ST 177 were isolated more than once in the same child, indicating persistence in the environment for up to 6 months.

4. Discussion

We found carriage prevalence of 55% with temporal fluctuations, with lower prevalence of carriage occurring in the period from February to June. Temporal variation was not observed in a previous study conducted in England in a period of 10 months [19] but was observed among Navajo children [20]. As has been reported [19,21], higher carriage rates are observed in children less than 5 years of age. The younger the patient, the greater the chance of pneumococcal colonization [22]. Although, variability in carriage rates were not observed in different age groups from this study population, in general, the observed carriage rate is in agreement with previous studies conducted in others Brazilian cities [7,23].

In Brazil, as in many developing countries, a significant proportion of the population lives in slum communities [24]. These urban informal settlement, characterized by crowded households and lower incomes, have been identified as factors associated with increased pneumococcal carriage in children [25]. The household density phenomenon of pneumococcal disease and carriage has been discussed in previous studies [22,25,26]. In this cohort study, conducted in an urban community, the prevalence of pneumococcal carriage increased with increasing household density. Furthermore, we have identified that in this community, living in

a crowded home (as defined by the number of household contacts with other children, the number of people per bed, or number of people per room) is associated with an increased risk for being colonized with pneumococcus. Households in Brazilian slums are very small in size and can be overcrowded with as many as 5 persons per room [11].

This study suggests that having a URTI in the last month increases the odds of being colonized, although not statistically significant. In addition, URTI also trended toward increasing the odds of carriage pneumococcal serotypes of lower invasiveness potential. Another study showed that influenza co-infection was associated with the greatest increases in the incidence of URTI caused by pneumococcal serotypes of lower invasiveness potential [27]. Unfortunately, we have not done laboratory diagnosis of influenza in this population to further explore this association.

The serotype distribution among nasopharyngeal isolates in the present study was similar to that found in previous studies in Brazil [7–9,23] and other countries in Latin America and Europe [28,29]. Overall, the serotypes isolated from the nasopharynx included the most common serotypes causing invasive disease [30] and represented in the PCV-10 vaccine (~52%). No significant additional protection against carriage was provided by the PCV-13 formulation of the vaccine, as the two additional serotypes (3 and 19A) represented only 3% of carriage isolates. In Brazil, PCV13 is only available in private clinics at a high cost (about US\$ 100/dose). The most common non-vaccine serotypes found in this study (16F, 15B/C, 6C and 34) are rarely associated with invasive disease in Salvador [30]. However, some of these non-vaccine serotypes (34 and 15B/C) are successful in carriage, with persistent carriage in the same children for up to 6 months. Other studies of colonization identified a high prevalence of those serotypes [21,31], and these findings might indicate the possibility of serotype replacement, as observed in others places after PCV7 introduction [32,33].

The rates of antimicrobial resistance observed in this study population were higher for both penicillin non-susceptible and SXT than previously shown in another colonization study conducted in Salvador [9]. Likewise, increasing resistance has already been documented in invasive disease in Salvador, with rates growing from 15% (1999) to 22.2% (2007) [30,34]. Geographical variations in the frequency of antibiotic resistance have been observed in different regions of Brazil and others countries [7,23,35,36], and these differences may reflect, in part, true geographical differences in antibiotic resistance rate, but most likely reflect differences due to investigation methodology and populations sampled.

We also identified carriage of internationally spread clones of pneumococci with penicillin non-susceptibility as the ST66, 156, 177. All of these clones have been associated with carriage and invasive disease in Salvador and others places [6,32]. In this community, these clones also account for persistent carriage, having been identified in the same child at intervals up to 6 months. Swabbing every 3 months is unlikely to detect the same *S. pneumoniae* carriage episode, as a recent Kenyan study described the mean duration of carriage to be 30 days [37]. A study conducted in Gambia showed that serotype 14 had longer duration of carriage [38]. In this study community, the serotypes 6A/B, 14 and 19F were isolated in the same child in more than one visit during the year.

There are some limitations to the study. Firstly, nasopharyngeal swabs were not taken in monthly intervals; the monthly intervals between nasopharyngeal swabs improve detection of serotypes carried for short durations and assessment of persistence of carriage. Secondly, we used the World Health Organization culture protocol that underestimates the prevalence of multiple serotype carriage. Thus, we must have identified the predominant serotype, missing the minor carried ones. Also, we did not discriminate between serotypes 6A from 6B, considering both as a PCV-10 serotype. In addition, the serotypes identified as highly invasive

were chosen based upon a single study from the UK and that that invasive serotypes 1 and 5 which are often associated with IPD in children were not detected in this study. However, invasiveness patterns among serotypes are generally consistent worldwide [39]. Finally, the loss of follow-up, which is a major problem in cohort studies, did not affect the analysis, since the risk of been colonized was considered for all children.

This study provides baseline information on pneumococcal carriage that may be particularly relevant for monitoring and evaluation of the PCV-10 vaccine, which was introduced in the Brazilian Immunization Program in March 2010. This vaccine would have a considerably impact on asymptomatic carriage among children throughout the community (52.2%). Our study's findings indicate that conditions of high density, as happens in houses of slum settlements in Brazil, could have a relevant role in community transmission of pneumococcus. Serotype shift and replacement, together with clonal expansion of pneumococci with non-vaccine serotypes, have been noted in other countries following the introduction of pneumococcal conjugate vaccine and may become major concerns. Thus the contribution of these crowded communities in keeping non-vaccine serotypes circulating, and their ability to cause invasive disease should be monitored after introduction of conjugate vaccines.

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