**Nasopharyngeal Carriage of *Streptococcus pneumoniae* among Children in an
Urban Setting in Brazil prior to PCV10 Introduction**

Run title: Pneumococcal carriage at community-level

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24 **Abstract**

25 Information on pneumococcal carriage in the pre-vaccine period is essential to predict
26 and assess the impact of PCV in settings where disease surveillance is particularly
27 difficult. Therefore, we present data on pneumococcal carriage before the introduction
28 of the 10-valent-pneumococcal conjugate vaccine (PCV10) in Brazil. We conducted a
29 prospective study on a cohort of 203 children aged < 5 years-old, randomly selected in
30 an urban community located in the periphery of the city of Salvador, Brazil and
31 followed them from January/2008 to January/2009. Nasopharyngeal swabs were
32 collected from each child at four times. In total, 721 swabs were collected, yielding a
33 pneumococcal carriage prevalence of 55% (n=398). In multivariate analyses, the
34 variables associated with carriage were having contact with three or more children <2
35 years old (OR, 2.00; 95% CI 1.33-2.89) and living in a house with an average of 3
36 residents per room (OR, 1.77; 95% CI 1.05-3.10). Also, white participants were more
37 likely to be protected from colonization (OR, 0.52; 95% CI 0.29-0.93), and prevalence
38 of carriage varied over time, with lower prevalence occurring from February to June
39 (OR, 0.53; 95% CI 0.37-0.78) compared to July to January. Contact with children under
40 two years of age and living in crowded housing also were associated with colonization
41 by highly invasive serotypes, although this relationship was not significant. The most
42 prevalent vaccine serotypes were 6A/B (25.4%), 19F (10.1%) and 14 (9.0%), while the
43 most prevalent non-vaccine serotypes were 16F (4.8%), 15B/C (4.5%) and 6C/D
44 (3.5%). Overall, 38.4% (153/398) of the isolates were non-susceptible to penicillin, and
45 of those, 73.8% (113/153) were non-susceptible to trimethoprim/sulfamethoxazole.
46 Colonization rate by PCV10 serotypes was 52.2%. Routine PCV10 vaccination can lead
47 to significant changes in pneumococcal serotypes found in NP colonization, indicating a

48 need for continued monitoring, especially in crowded settings, as occurs in Brazil's
49 slums.

50

51 **Keywords:** Children, nasopharyngeal carriage, *Streptococcus pneumoniae*, serotypes,
52 PCV10-vaccine

53

54 **Background**

55 Asymptomatic carriage of pneumococci is common in young children and has been
56 related to the development of disease and transmission of the pathogen [1-3]. The
57 prevalence of pneumococcal carriage increases in the first few years of life, peaking at
58 approximately 50% to 80% in children 2-3 years of age and decreasing thereafter until
59 stabilizing at 5% to 10% in children over 10 years of age [4]. Effective vaccines against
60 10-, or 13- of the more than 90 pneumococcal serotypes are now used in many
61 countries, resulting in a substantial decline in invasive disease and carriage of vaccine
62 serotypes [5, 6]. Despite this success, serotypes not targeted by the vaccine have
63 increased among healthy carriers and could potentially become important causes of
64 invasive diseases. As Brazil started nationwide vaccination with 10-valent
65 pneumococcal conjugate vaccine (PCV10) in 2010, it is essential to have information on
66 pneumococcal carriage in the pre-vaccine era to assess potential vaccine impact.

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

78

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

84

85 **Methods**

86 **Study site and population**

87 The study was conducted in the Pau da Lima community, which is situated in the
88 periphery of Salvador, a city of 2.7 million inhabitants [10] in Northeast Brazil. We
89 selected an area of 0.46 km² where a cohort study for leptospirosis was conducted in
90 2003. As a part of this study, a census was completed during visits to 3,689 households;
91 this identified 14,122 inhabitants, of which 8% (n=1, 131) were aged < 5 years [11]. A
92 total of 130 households were randomly selected within the census tract in order to have
93 203 children < 5 years old enrolled in the study. This sample size was based on a
94 previous study conducted in Salvador, where 65% of Children < 5 years were colonized
95 at a single time-point [9]. Eligible subjects, defined as children 1 to 59 months of age
96 who lived continuously in one of the selected households during the month prior to
97 recruitment, were enrolled into the study according to informed consent procedures
98 approved by the Oswaldo Cruz Foundation, Brazilian Ministry of Health.

99 **Data collection**

100 During house visits, a standardized questionnaire was used to document information on
101 demographics, underlying medical conditions, hospitalizations, occurrence of an upper
102 respiratory tract infection (URTI) in the previous month, antibiotic therapy in the last
103 four weeks before the visit, childcare arrangements, school attendance and household
104 inhabitants' habits such as smoking. Information for children was obtained by
105 interviewing the parent or legal guardian. Household crowding was defined as the
106 number of people divided by the number of rooms in the house, or the number of people

107 divided by the number of beds in the house. Household contact with children less than
108 two years old was defined as contact of at least 4 hours per day.

109

110 **Isolation of pneumococci**

111 Between January 2008 and January 2009, nasopharyngeal swabs were collected from
112 each child at four times, at enrollment and then again at three month intervals. Samples
113 were collected with calcium alginate swabs (Calgiswab type 1, Spectrum USA) and
114 inoculated into modified Stuart transport medium and sent to the Clinical Microbiology
115 Laboratory at the Gonçalo Moniz Research Institute. All swabs were plated within 4
116 hours onto agar plates with 5% sheep blood and 5.0 µg/ mL of gentamicin. Plates were
117 incubated at 35°C in 5% CO₂-enriched atmosphere for up to 48 hours. Three α-
118 hemolytic colonies exhibiting morphologic characteristics suggestive of *S. pneumoniae*
119 were isolated. Identification of these isolates as *S. pneumoniae* was confirmed by
120 optochin disc susceptibility (BBL Microbiology Systems, Cockeysville, USA) and the
121 bile solubility test. One *S. pneumoniae* colony per plate was then sub-cultured,
122 harvested, and kept frozen at -70 °C for further testing. When *S. pneumoniae* isolates
123 from the same primary plate exhibited a clearly different colony morphology, dissimilar
124 colonies were frozen separately.

125

126 **Serotyping**

127 The isolates were serotyped by multiplex-PCR as described elsewhere [12]. DNA
128 extraction and PCR conditions were performed as described by the US Centers for
129 Disease Control and Prevention (CDC) [12]. Isolates with negative multiplex PCR

130 results were subjected to single-plex-PCR with primer 19F variation [13] and Quellung
131 reaction testing for capsular type definition.

132

133 **Antimicrobial susceptibility testing**

134 The broth microdilution method was performed according to Clinical and Laboratory
135 Standard Institute recommendations [14] to determine susceptibility of isolates to
136 penicillin, cefotaxime, tetracycline, erythromycin, trimethoprim/sulfamethoxazole
137 (TMP/SMX) and levofloxacin (Sigma–Aldrich, Germany). Quality control was
138 performed by testing *S. pneumoniae* ATCC 49619. Isolates with a penicillin MIC value
139 ≥ 0.12 $\mu\text{g/mL}$ were defined as penicillin non-susceptible.

140

141 **Genotyping**

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

147

148 **Data management and statistical analysis**

149 Data were entered and managed by Epi Info version 3.5.1 (CDC, Atlanta, GA, USA).
150 Statistical analyses were performed in SAS v9.3. Univariate and multivariate logistic
151 regression models were constructed to identify risk factors for colonization (PROC
152 GLIMMIX). To construct confidence intervals that accounted for the non-independence
153 of samples from the same individual, we created 1000 bootstrap samples, where all

154 observations from an individual were grouped together and sampled with replacement.
155 Household crowding was analyzed as continuous variables .A variable was considered
156 to be significantly associated with colonization ($p < 0.05$) if the 95% CI did not include
157 1.

158

159 For multivariate models, variables that were significant in the univariate analyses were
160 included in different combinations, with the best-fitting model determined by Akaike
161 Information Criteria (AIC) [17]. To test for an association between the demographic
162 risk factors and the odds of being colonized with a high or low-invasiveness serotype,
163 we created three outcome categories: uncolonized, colonized with a high invasiveness
164 serotype (4, 7F, 8, 9V, 14, 18C and 19A;), or colonized by a low-invasiveness serotype
165 (3, 6A/B/C, 11A, 13, 15A, 15B/C, 16F, 17F, 19F, 20, 21, 22F, 23B, 23F ,35F and NT [
166 Not Typeable]) [18]. We then fit univariate generalized logit models to these data and
167 again used the bootstrap samples to test for significance at $p = 0.05$.

168

169 **Results**

170

171 **Demographic characteristics**

172 In January 2008, a total of 203 children were enrolled into the cohort study. Ages
173 ranged from 1 to 48 months, and the median age was 24 months (interquartile range:
174 12-36). There was a predominance of mixed race (70%), and 48% of participants were
175 males. The families of the enrolled children reported low monthly income (less than
176 USD\$ 430.00), and crowded environments were observed in the households, with a
177 median of five (range: 2 to 15) inhabitants per household. Most of the study children
178 lived in households of two rooms (81.8%), with a ratio of 3.5 residents per bed (Table
179 1).

180

181 **Prevalence of pneumococcal carriage**

182 In total, 721 swabs were collected throughout the study period, yielding 398
183 pneumococcal isolates. The prevalence of *S. pneumoniae* nasopharyngeal carriage was
184 50.5% (February), 46.3% (June), 63.2% (September) and 48.8% (December) at each
185 sampling point, respectively. Of the 203 children eligible for the study, 156 (76.8%)
186 provided nasopharyngeal samples at all four visits (Figure 1). At least one
187 pneumococcal isolate from the nasopharyngeal sample was found in 74.4% (116 of the
188 156) of all children; 9.0% (14 of the 156) were not colonized at all; 19.9% (26 of the
189 156) were only once colonized; and 12.2% (19 of the 156) were colonized in all four
190 visits.

191

192

193

194 **Risk factors for colonization**

195 Children who lived in households, where there was at least one child under two years,
196 who lived in crowded households, and had a recent URTI in the last month had greater
197 odds of being colonized in univariate analysis. Carriage prevalence varied in time, with
198 decreased prevalence from February to June (dry season) compared to July to January
199 (rainy season). Additionally, white children were less likely to be colonized than mixed
200 children (OR, 0.52; 95% CI 0.29 - 0.93) (**Table 1**). From multivariate analyses shown in
201 Table 1, prevalence of carriage varied over time, with lower prevalence occurring
202 during dry season (OR, 0.53; 95% CI 0.37 - 0.78). Also, having contact with three or
203 more children under two years old (OR, 2.00; 95% CI 1.33 - 2.89) and living in a house
204 with a greater number of persons per room (OR, 1.77; 95% CI 1.05 - 3.10) were each
205 independently and positively associated with pneumococcal carriage.

206

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

218

219 Serotype Distribution and Antibiotic susceptibility

220 Table 3 shows the distribution of serotypes recovered throughout the period of the
221 study. The most prevalent serotypes were 6A/B (25.4%), 19F (10.1%) and, 14 (9.0%).
222 The serotypes included in PCV10 and PCV13 accounted for 52.2% and 55.5%,
223 respectively. The most frequent non-vaccine serotypes were 16F (4.8%), 15B/C (4.5%),
224 6C/D (3.5%), 34 (3%) and not typeable (7.3%); 15.3% (61/398) of the isolates of *S.*
225 *pneumoniae* did not have the capsular type determined by multiplex-PCR. We did not
226 find any fluctuation in the distribution of serotypes during the study period.

227

228 Overall, 38.4% (153/398) of the pneumococci were non-susceptible to penicillin, with
229 MICs ranging from 0.12 to 8.0µg/ml. The percentage of capsular types included in the
230 PCV10 vaccine among penicillin non-susceptible accounted for 73.2% (112/153) as
231 follows: 6A/B (45/112; 40%), 19F (29/112; 25.9%), 14 (20/112; 18%), 23F (12/112;
232 11%) and others (6/112; 5%). The non-vaccine serotypes commonly associated with
233 penicillin non-susceptibility (41/153; 22%) were: NT (6/41; 14.6%), 16F (4/41; 9.8%),
234 13 (3/41; 7.3%), 21 (3/41; 7.3%), 34 (1/41; 2.4%). In addition, 58% (231/398) were
235 non-susceptible to TMP/SMX, 18.6% (74/398) to tetracycline, 3% (12/398) to
236 erythromycin, and 2% (8/398) to cefotaxime. Of the 153 penicillin non-susceptible
237 isolates, 113 (73.8%) were also non susceptible to TMP/SMX. The drugs involved in
238 the most frequently identified patterns of multidrug nonsusceptibility, defined as being
239 intermediate or resistant to three or more classes of antibiotics, were penicillin,
240 TMP/SMX and tetracycline (14 of 398 isolates), penicillin, TMP/SMX and
241 erythromycin (7 of 398 isolates), penicillin, TMP/SMX, cefotaxime (3 of 398 isolates)
242 and penicillin, TMP/SMX, cefotaxime, and erythromycin (3 of 398 isolates).

243

244 **Genotyping**

245 PFGE analysis confirmed that 24 of the 156 (15.4%) children were highly likely to have
246 been colonized by the same pneumococcal PFGE type at multiple time points: 6A/B
247 (n=9/24; 37.5%), 14 (n=5/24; 20.8%), 19F (n=4/24; 16.7%), 34 (n=2/24; 8.3%), 23F
248 (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
249 most commonly identified sequence types were ST156 (14;[n=5]), ST 66 (14;[n=10]),
250 ST177 (19F; [n=7]), ST 338 (23F;[n=6]), ST 3930 (6C; [n=3]) and ST 771 (34; [n=4]).
251 Strains belonging to the ST 66, ST 156 and ST 177 were isolated more than once in the
252 same child, indicating persistence in the environment for up to six months.

253

254

255 **Discussion**

256

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

266

267 In Brazil, as in many developing countries, a significant proportion of the population
268 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 towards 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

294 (3and 19A) represented only 3% of carriage isolates. In Brazil, PCV13 is only available
295 in private clinics at a high cost (about US\$ 100/dose). The most common non-vaccine
296 serotypes found in this study (16F, 15B/C, 6C and 34) are rarely associated with
297 invasive disease in Salvador [30]. However, some of these non-vaccine serotypes (34
298 and 15B / C) are successful in carriage, with persistent carriage in the same children for
299 up to six months. Other studies of colonization identified a high prevalence of those
300 serotypes [21, 31], and these findings might indicate the possibility of serotype
301 replacement, as observed in others places after PCV7 introduction [32, 33].

302

303 The rates of antimicrobial resistance observed in this study population were higher for
304 both penicillin non-susceptible and SXT than previously shown in another colonization
305 study conducted in Salvador [9]. Likewise, increasing resistance has already been
306 documented in invasive disease in Salvador, with rates growing from 15% (1999) to
307 22.2% (2007) [30, 34]. Geographical variations in the frequency of antibiotic resistance
308 have been observed in different regions of Brazil and others countries [7, 23, 35, 36],
309 and these differences may reflect, in part, true geographical differences in antibiotic
310 resistance rate, but most likely reflect differences due to investigation methodology and
311 populations sampled.

312

313 We also identified carriage of internationally spread clones of pneumococci with
314 penicillin non-susceptibility as the ST66, 156, 177. All of these clones have been
315 associated with carriage and invasive disease in Salvador and others places [6, 32]. In
316 this community, these clones also account for persistent carriage, having been identified
317 in the same child at intervals up to six months. Swabbing every 3 months is unlikely to
318 detect the same *S. pneumoniae* carriage episode, as a recent Kenyan study described the

319 mean duration of carriage to be 30-days [37]. A study conducted in Gambia showed that
320 serotype 14 had longer duration of carriage [38]. In this study community, the serotypes
321 6A/B, 14 and 19F were isolated in the same child in more than one visit during the year.

322

323 There are some limitations to the study. Firstly, nasopharyngeal swabs were not taken in
324 monthly intervals; the monthly intervals between nasopharyngeal swabs improves
325 detection of serotypes carried for short durations and assessment of persistence of
326 carriage. Secondly, we used the World Health Organization culture protocol that
327 underestimates the prevalence of multiple serotype carriage. Thus, we must have
328 identified the predominant serotype, missing the minor carried ones. Also, we did not
329 discriminate between serotypes 6A from 6B, considering both as a PCV-10 serotype. In
330 addition, the serotypes identified as highly invasive were chosen based upon a single
331 study from the UK and that that invasive serotypes 1 and 5 which are often associated
332 with IPD in children were not detected in this study. However, invasiveness patterns
333 among serotypes are generally consistent worldwide [39]. Finally, the loss of follow-up,
334 which is a major problem in cohort studies, did not affect the analysis, since the risk of
335 been colonized was considered for all children.

336

337 This study provides baseline information on pneumococcal carriage that may be
338 particularly relevant for monitoring and evaluation of the PCV-10 vaccine, which was
339 introduced in the Brazilian Immunization Program in March 2010. This vaccine would
340 have a considerably impact on asymptomatic carriage among children throughout the
341 community (52.2%). Our study's findings indicate that conditions of high density, as
342 happens in houses of slum settlements in Brazil, could have a relevant role in
343 community transmission of pneumococcus. Serotype shift and replacement, together

344 with clonal expansion of pneumococci with non-vaccine serotypes, have been noted in
345 other countries following the introduction of pneumococcal conjugate vaccine and may
346 become major concerns. Thus the contribution of these crowded communities in
347 keeping non-vaccine serotypes circulating, and their ability to cause invasive disease
348 should be monitored after introduction of conjugate vaccines.

349

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360

361 **Author Contributions**

362 Conceived and designed the experiments: JNR, AK, MGC. Performed the experiments:
363 APOM, MGC. Collect the samples: APOM, JA, LCC, MCL, JNR. Analyzed the data:
364 APOM, MC,DW, GR. Contributed reagents/materials/analysis tools: JNR, MR, MGC.
365 Wrote the paper: JNR, APOM, GR, DW. Reviewed and revised the final version of the
366 manuscript: All authors.

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483

484 **Legend Figure 1.** Flowchart of participants enrolled in the study.

485

486 Table 1. Risk Factors for pneumococcal carriage among children < 5 years old at
 487 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 $\leq$ 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 ($\pm$ 1.7)	1.90 (1.19-3.13)	1.77 (1.05 - 3.10)
No. of residents/No. of bed^c	3.5 ($\pm$ 1.8)	1.53 (1.10-2.23)	
Season (Feb/June vs July/January)	-	0.60 (0.42-0.82)	0.53 (0.37 - 0.78)

488 a- Number of swabs for each variable.

489 b-REF – reference for analysis

490

491

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

Characteristics	N (%)	Pneumococcal Invasive property	
		OR (95% CI)	
		Low Invasiveness ^a	High Invasiveness ^b
		(n = 263)	(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 (Feb/June vs July/January)		0.57 (0.39 - 0.84)	0.68 (0.30 - 1.28)

494 a- High invasiveness serotype: 4, 7F, 8, 9V, 14, 18C and 19A;

495 b- Low invasiveness serotype: 3, 6A/B/C, 11A, 13, 15A, 15B/C, 16F, 17F, 19F, 20, 21, 22F,
 496 23B, 23F, 35F and NT.

497 c- REF – reference for analysis.

498

499 Table 3. Serogroup/type distribution of all pneumococcal carriage isolates (N= 398)
500 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

501 ^a ND= Not Determined by Multiplex PCR.

502 ^b NT=Not typeable by both methods (Multiplex PCR and Quellung Reaction)

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506

507 Figure 1. Flowchart of participants enrolled in the study.