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Long-term effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of *Streptococcus pneumoniae* in children in Brazil

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ABSTRACT

Brazil introduced the 10-valent pneumococcal vaccine (PCV10) to the routine national immunization program (NIP) in March 2010. In 2017, we investigated the effects of PCV10 on nasopharyngeal carriage of vaccine-types (VT) and non-vaccine-types (NVT) of *Streptococcus pneumoniae* (Spn) among children living in São Paulo city. We also compared the prevalence of VT and NVT with previous carriage surveys performed in 2010 (baseline) and 2013.

Method: The carriage survey was conducted among 531 children, aged 12 months to <24 months, recruited from public Primary Health Units during the immunization campaign, using previous surveys methodology, except for qPCR, which was performed in the 2017 survey only.

Results: No statistical difference was found in the prevalence of Spn either by culture (59.7%) or by qPCR (61.2%). Spn carriage increased from 40.3% (baseline) to 59.7% (2017 survey) ($p < 0.001$). Colonization by VT isolates significantly decreased by 90.9% (19.8–1.8%) and 95.5% (19.8–0.9%) in the 2013 and 2017 surveys, respectively, compared to that at baseline. NVT isolates increased significantly by 128% (19.6–44.8%) and 185% (19.6–55.9%) in the respective post-PCV10 surveys, most led to high prevalence of serotypes 6C (27%), 15B (9.8%), 19A (9.2%), 15A (6.0%), and 16F (5.7%). In 2017, reduction in serotype 6A (4.2–0.6%, $p < 0.001$) and increase in serotype 19A (1.8–6.0%, $p = 0.001$) were found; serotype 3 isolate was not detected in the present survey. We identified the emergence of 19A isolates CC320, associated with high penicillin (MIC $\geq$ 2.0 mg/L) and cefotaxime (MIC $\geq$ 1.0 mg/L) values.

Conclusion: After 7 years of PCV10 introduction in the NIP, colonization by VT among toddlers decreased substantially to a residual level, along with substantial serotype replacement by novel serotypes not present in any current conjugated pneumococcal vaccine and serotype 19A. The present findings can assist policy decisions in Brazil.

1. Introduction

Streptococcus pneumoniae (Spn) is the cause of high morbidity and mortality worldwide mainly among infants and the elderly [1,2]. Spn colonize the mucosa of the upper respiratory tract soon after birth, peaking in the first two years of life [3]. Nasopharynx [the nasopharyngeal colonization (NPC)] is the reservoir of Spn, a precondition for pneumococcal disease development, and spreads to the community [3]. The pneumococcal conjugate vaccines (PCVs) are highly effective against Spn invasive and mucosal diseases and in the prevention of new acquisition of vaccine-type

Abbreviations: CC, clonal complex; CLSI, Clinical and Laboratory Standards Institute; Ctx, ceftriaxone; DCC, day-care center; IAL, Adolfo Lutz Institute; IPD, invasive pneumococcal disease; MIC, minimum inhibitory concentration; MLST, Multilocus sequence typing; NIP, national immunization program; NPC, nasopharyngeal colonization; NVT, non-vaccine-types; PCV10, 10-valent pneumococcal vaccine; Pen, penicillin; PHU, Primary Health Units; Spn, *Streptococcus pneumoniae*; STGG, skim milk-tryptone-glucose-glycerol; VT, vaccine-types.

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serotypes in the nasopharynx [4–7]. Consequently, the inclusion of PCVs in the routine immunization program of various countries has modified the epidemiological setting for pneumococcal serotypes worldwide [8]. Studies on prevalence of colonizing serotypes carried out before and after PCVs introduction have provided valuable information to assess the effect of vaccination on vaccine serotype colonization [9,10].

Brazil introduced the 10-valent pneumococcal conjugate vaccine (PCV10) into the childhood immunization program in March 2010, with a schedule of three primary doses at ages 2, 4, and 6 months, and a booster dose for children aged 12–18 months (3 + 1 doses) [11]. In addition, a catch-up campaign offered two primary doses at 2 and 4 months plus a booster dose at 7–11 months of age, and a single dose for children aged 12–23 months at vaccine introduction [11]. In 2016, the vaccine schedule reduced to two primary doses at 2 and 4 months, and a booster dose for children aged 12–18 months (2 + 1 doses) [12].

In Brazil, studies on the PCV10 effectiveness and impact reported great reduction in invasive pneumococcal diseases (IPD) caused by vaccine-types among children shortly after PCV10 introduction [11,13]. Data obtained from the national laboratory-based surveillance for Spn from IPD conducted before and 5 years after the introduction of PCV10 revealed that IPD cases caused by PCV10-serotypes declined by 85.6% among children from 2 months to 4 years of age, whereas non-vaccine types increased from 20.7% to 88.7%, driven mainly by the increase in serotypes 3, 6C and 19A [14].

Few studies on PCV10 impact on NPC have been conducted in Brazil. In a large carriage survey among children living in the city of São Paulo conducted in 2013, after 3 years of PCV10 use, a 90.9% reduction in NPC by vaccine types (VT) was reported along a significant increase in non-vaccine PCV10 types (NVT), specially by the increase in serotype 6C [15].

We investigated the impact of PCV10 on the pneumococcal colonization and the distribution of serotypes over a long-term period after the introduction of PCV10 in the national immunization program in Brazil. We also compared our findings with those from previous carriage surveys among unvaccinated children (2010) and those observed after a short period of PCV10 vaccination (2013). Because of the expansion of the specific clonal complex (CC320) among 19A isolates from IPD cases in Brazil, we also investigated the genetic lineages of all serotype 19A isolates recovered from the three nasopharyngeal surveys [16].

2. Materials and methods

2.1. Study area, population and study design

This was a cross-sectional study conducted in 2017 among healthy children residing in the urban region of the municipality of São Paulo, the biggest metropolitan area in Brazil, capital of the state of São Paulo, Southeast Brazil.

Study participants were children aged 12 to <24 months of age recruited during an immunization campaign in 20 public Primary Health Units (PHUs) that included five geographic regions in São Paulo city. Nasopharyngeal (NP) samples were collected between August 16 and 19, 2017, as required by children at the PHUs. Children vaccinated with any PCV10 dose were included in the survey. Children with fever, acute illness, or reported antibiotic used during the previous seven days, or those vaccinated with any dose of other PCV than PCV10 [7-valent (PCV7) or 13-valent (PCV13), or the 23-valent polysaccharide vaccine (PPV23)] were excluded from the study. Only one child per household was enrolled. Vaccination status was assessed by reviewing the child's immunization card.

In the pre-PCV10 survey conducted in 2010 (baseline data) we only included unvaccinated children with no history of previous vaccination with any conjugated or unconjugated pneumococcal vaccine. For the surveys performed between 2013 and 2017 we included a group of children unvaccinated with any pneumococcal vaccine and children vaccinated with only PCV10. Children vaccinated with PCV7, PCV13 or PPV23 were excluded from all the study period [15]. Prevalence of PCV10-types (VT, serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F), and the non-PCV10-types (not included in the PCV10 formulation and excluding non-typeable) were compared over the three surveys. We also analyzed the additional serotypes included in the PCV13 (serotypes 3, 6A, and 19A) and serotype 6C because of its high prevalence as a carrier and IPD case after PCV10 introduction.

2.2. Sampling and sample size

For the current survey (2017), 545 children were recruited, 531 met the inclusion criteria while 14 children were excluded: for living in another municipality (n = 3), belonging to another age group (n = 3), incoherence on vaccination data (n = 6), and the informed consent not signed (n = 2). The sample size for this survey was estimated taking into account the baseline prevalence of PCV10 serotypes (20%) [15], considering an additional 5% of children who would not attend the eligibility criteria.

The distribution of the number of study participants per geographic regions for the 3 surveys is shown in Table S1 [15].

2.3. Ethical considerations

The study was approved by the Ethics Committees of the Federal University of Goiás, the Adolfo Lutz Institute and the Municipal Health Secretary of the São Paulo City. Written informed consent was obtained from each child participant's parent(s)/legal guardian(s) before enrollment.

2.4. Data and specimen collection

Socio-demographic data of participants enrolled in the 2017 survey was obtained through a standardized questionnaire applied to the children's parents/legal guardians before the collection of nasopharyngeal specimen. The potential factors investigated for pneumococcal colonization were gender, age at time of sampling, day-care attendance, mother's education level, household income, and smoker in the household. Vaccination status for PCV10 and other pneumococcal vaccine was assessed by checking the child's vaccination card.

NP specimen was collected by a single transnasal nasopharyngeal sample per child by trained nurses according to the World Health Organization (WHO) working group standard methods using a flexible sterile swab (FLOQ Swabs, Copan) [17]. NP specimens were immediately inoculated into 1 mL of skim milk-tryptone-glucose-glycerol transport medium (STGG) [18] placed in a cold-box and delivered to the laboratory at Adolfo Lutz Institute (IAL) within 4–5 h after collection. At IAL, NP-STGG vials were vortexed for 20–30 s and then stored at −70 °C.

2.5. Microbiology methods

For culture, we followed the same procedures previously described. In brief, NP-STGG vials were thawed at room temperature and then vortexed for 20–30 s; 120 μL aliquots were inoculated into 3 mL of TYS broth (Todd-Hewitt broth with 0.5% yeast extract and 600 μL of rabbit serum), as an enrichment culture [19]. Identification of *S. pneumoniae* was based on the presence of α-hemolysis in sheep blood agar plate, and optochin susceptibility and bile solubility tests. About 3–4 suspected colonies by plate were investigated [20]. Pneumococcal isolates were serotyped by

Quellung reactions with antisera from Statens Seruminstitut (Copenhagen, Denmark). Non-typeable (NT) pneumococcus by Quellung was confirmed by 8 sequential PCR-multiplex comprising 70 serotypes [19,21–23].

The molecular detection of Spn by quantitative real-time PCR (qPCR) was performed only in the 2017 survey. An NF-STGG aliquot of 200 μL was incubated with lysozyme (0.015 g/mL) and mutanolysin (25 U/mL) (Sigma Chemicals) [24]. DNA was extracted and purified through the automated extraction system MagNAPure LC2.0 (Roche MagNAPure LC DNA isolation Kit I™) and stored at −20 °C. DNA from each NP sample was tested for the presence of the *lytA* gene (*S. pneumoniae*)] and for the human *RNase* P gene [25,26]. The positive samples for Spn were serotyped in 7 sequential qPCR comprising 21 serotypes [19,22,23].

Multilocus sequence typing (MLST) was performed for all 19A isolates found in the three surveys according to Enright and Spratt [27]. The sequence types (ST) were assigned using the pneumococcal MLST webpage [28], while the clonal complexes (CC) were defined using eBURST software [29].

Penicillin (Pen) and ceftriaxone (Ctx) susceptibility was performed by screening for oxacillin (Oxa) susceptibility by disk diffusion (OXOID, Basingstoke, England), following the Clinical and Laboratory Standards Institute (CLSI) procedure [30]. Spn with diminished susceptibility to Pen (Oxa halo ≤19 mm) were analyzed for minimum inhibitory concentration (MIC) to Pen and Ctx by strip test (Liofilchem, Italy). Spn showing Oxa susceptibility (halo >19 mm) and MIC thresholds conferring resistance to Pen and Ctx were considered as ≥0.12 mg/L and ≥1.0 mg/L, respectively.

2.6. Data analysis

For the current survey (2017), the primary outcome included the pneumococcal colonization/detection obtained by culture and qPCR. Cocolonization was defined when a child was simultaneously colonized by more than one serotype or serotype plus NT isolate – herein, namely serotype/NT. Children colonized with more than one serotype were classified as being colonized with vaccine serotype as long as one of the serotypes was VT, and as additional PCV13-types as long as one of the serotypes belonged to serotypes 3, 6A or 19A. Chi-square test was used to investigate the potential socio-demographic factors of Spn colonization at univariate analysis. P-values less than 0.05 were considered statistically significant.

The comparative analysis of participant characteristics, pneumococcal colonization, and serotypes obtained from three surveys (2010, 2013 and 2017) included outcomes obtained exclusively by culture, since in the previous surveys (2010 and 2013) the qPCR was not performed.

Changes in the prevalence of the serotypes between the post-PCV10 surveys and pre-PCV10 survey were calculated by the formulae: [(percentage of serotypes in the post-PCV10 period – percentage of serotypes in the pre-PCV10 period/percentage of serotypes in the pre-PCV10 period) * 100]. A positive percentage change expresses an increase in the serotype in the post-PCV10 period, whereas a negative percentage change expresses a reduction in the serotype in the post-vaccination period. We used chi-square test to compare prevalences of nasopharyngeal colonization by *Streptococcus pneumoniae* serotypes between the baseline survey (2010) and post-PCV10 introduction (2017) survey. Data analysis was conducted using the IBM SPSS Statistics software version 25.0 (IBM Corp. Released 2017. Armonk, NY, USA).

3. Results

In the current survey (2017), 531 participants were included (male, 47.6%); with higher proportion of males in the pre-PCV10 (55.9%) and in the early-post-PCV10 (50.5%) surveys ($p < 0.029$); similar mean age (17.2 months (sd = 3.4), $p = 0.807$); 97.9% of the children received 2 or more PCV10 doses. The prevalence of Spn carriage detected by culture was 59.7% (n = 317) and by qPCR was 61.2% (n = 325), with an overall Spn prevalence of 62.3% (n = 331) ($p > 0.05$) (Table 1). qPCR detected 8 additional Spn carrier in relation to the culture although no statistical significant difference was found for the number of pneumococcal isolates (n = 348) and pneumococcal detection by qPCR (n = 347) ($p > 0.05$). Overall, 51 children were colonized by more than one serotype or serotype plus NT: 30 and 21 cocolonizations identified by culture and qPCR, respectively. qPCR added 31 serotypes among 26 children: 6C/6D (n = 10), 19A (n = 5), 15F/15A (n = 4), 6A/6B, 16F and 22F/22A (n = 2, each), and 3, 9A/9V, 11A/11D, 19F, 23A and 12F/12A/12B/44/46 (n = 1, each). The social-demographic factors associated with Spn colonization were being aged 12–17 months and attendance at day-care center (DCC) ($p < 0.05$). Colonization by the additional PCV13-types, non-PCV10-types, serotype 6C, and serotype 19A were significantly associated with DCC ($p < 0.05$); 6C colonization was also associated with having received the PCV10 booster dose ($p < 0.05$) (Table 2).

Pneumococcal colonization increased from 40.3% in the pre-PCV10 to 59.7% in the late-post-PCV10 surveys ($p < 0.001$) (Table 3). Colonization by VT significantly decreased by 90.9% and 95.5% in the 2013 and 2017 surveys, respectively, compared to that at baseline (19.8%), while NVT carriage increased significantly by 128% and 185% in the respective post-PCV10 surveys. Similar additional PCV13-types carrier was observed among the three surveys ($p > 0.05$), however 85.7% reduction in 6A (4.2–0.6%, $p < 0.001$) and 233.3% increase in 19A (1.8–6.0%, $p = 0.001$) was observed between the baseline and the late-post-PCV10 survey. A few serotype 3 isolates were found in the 2010 (n = 3) and 2013 (n = 2) surveys, and none in the 2017 survey. Colonization by non-PCV types, serotype 6C, and NT isolates increased by 273.9% (13.4–50.1%), 883.3% (1.8–17.7%, $p < 0.001$), and 501% (1.6–6.4%, $p < 0.001$), respectively, in the late-post-PCV10 survey versus baseline (Table 3).

Spn cocolonization by culture was 5.6% (n = 30/531) in the late-post-PCV10 survey, including 11 children with 2 serotypes, 1 child with 3 serotypes and 18 children with one serotype and one NT isolate. In the baseline and 2013 survey, 5 and 3 participants, respectively, were colonized by 2 serotypes.

Table 4 shows the distribution of serotypes per survey. A total of 33, 29, and 37 Spn serotypes were identified among the 207, 198, and 348 isolates at the baseline, in the 2013 and 2017 surveys, respectively. The high prevalence of PCV10 types (47.8%) before vaccination decreased into few isolates, by 2017 survey (1.4%), being represented by serotypes 6B, 9V, 14, 18C, and 23F (n = 1, each). In 2017, the most prevalent non-PCV10 types were 6C

Table 1
Prevalence, number of isolates and nasopharyngeal colonization by *Streptococcus pneumoniae* among children according to microbiologic method in the late-post-PCV10 survey (2017), in São Paulo, Brazil.

Colonization (N = 531)	Microbiologic method		
	Culture	qPCR	Culture or qPCR
Number of carriers (%; 95%CI)	317 (59.7; 55.5–63.8)	325 (61.2; 57.0–65.3)	331 (62.3; 58.1–66.4)
Number of *S. pneumoniae*	348	347	387
Children with cocolonization*	30	21	51

* Children colonized with one serotype and non-typeable isolate, or with multiple serotypes.

Table 2
Potential factors associated with *Streptococcus pneumoniae* colonization, vaccine types and non-vaccine types by culture among children in the late-post-PCV10 survey (2017), São Paulo, Brazil.

	S. pneumoniae			PCV10 types			Additional PCV13 types			Non-PCV types			Serotype 6A			Serotype 6C			Serotype 19A			Non-typeable		
	N	%	P-value	N	%	P-value	N	%	P-value	N	%	P-value	N	%	P-value	N	%	P-value	N	%	P-value	N	%	P-value
Gender																								
Male	166	59.7	0.995	2	0.8	1.000	13	5.1	0.198	129	51.0	0.694	0	0.0	–	45	17.8	0.961	13	5.1	0.412	15	5.9	0.670
Female	151	59.7		3	1.1		22	7.9		137	49.3		3	1.1		49	17.6		19	6.8		19	6.8	
Age group																								
12–17 months	185	63.8	**0.035**	3	1.0	1.000	22	7.6	0.311	156	53.8	0.061	2	0.7	1.000	61	21.0	**0.027**	20	6.9	0.355	16	5.5	0.360
18–23 months	132	54.8		2	0.8		13	5.4		110	45.6		1	0.4		33	13.7		12	5.0		18	7.5	
Day care attendance																								
Yes	179	77.8	**<0.001**	0	0.0	–	24	10.4	**0.002**	151	65.7	**0.000**	1	0.4	1.000	70	30.4	**<0.001**	23	10.0	**0.001**	**26**	**11.3**	**0.000**
No	138	45.8		5	1.7		11	3.7		115	38.2		2	0.7		24	8.0		9	3.0		8	2.7	
PCV10 booster dose																								
Yes	208	57.1	0.076	3	0.8	0.652	20	5.5	0.133	175	48.1	0.170	1	0.3	0.234	56	15.4	**0.039**	19	5.2	0.249	23	6.3	0.907
No	109	65.3		2	1.2		15	9.0		91	54.5		2	1.2		38	22.8		13	7.8		11	6.6	
Mother education																								
≤8 years	84	65.1	0.146	1	0.8	1.000	11	8.5	0.319	72	55.8	0.129	2	1.6	0.149	21	16.3	0.603	9	7.0	0.616	5	3.9	0.172
>8 years	231	57.9		4	1.0		24	6.0		192	48.1		1	0.3		73	18.3		23	5.8		29	7.3	
Household income ≤3 minimum wage per month																								
Yes	255	61.2	0.130	4	1.0	1.000	28	6.7	0.761	214	51.3	0.260	2	0.5	–	77	18.5	0.672	26	6.2	0.894	31	7.4	**0.042**
No	54	52.9		1	1.0		6	5.9		46	45.1		0	0.0		17	16.7		6	5.9		2	2.0	
Smoker in the household																								
Yes	83	62.9	0.412	2	1.5	0.603	8	6.1	0.762	73	55.3	0.175	1	0.8	1.000	23	17.4	0.895	7	5.3	0.674	6	4.5	0.306
No	233	58.8		3	0.8		27	6.8		192	48.5		2	0.5		71	17.9		25	6.3		28	7.1	
Region																								
North	149	61.3	0.176	3	1.2	0.542	15	6.2	0.132	128	52.7	0.325	2	0.8	–	46	18.9	0.673	13	5.3	0.189	15	6.2	0.471
South	49	58.3		1	1.2		5	6.0		42	50.0		0	0.0		14	16.7		5	6.0		3	3.6	
East	7	77.8		0	0.0		2	22.2		5	55.6		0	0.0		3	33.3		2	22.2		1	11.1	
Central West	68	52.3		0	0.0		5	3.8		55	42.3		0	0.0		22	16.9		5	3.8		12	9.2	
South East	44	67.7		1	1.5		8	12.3		36	55.4		1	1.5		9	13.8		7	10.8		3	4.6	

P-values less than 0.05 were considered statistically significant.

Table 3
Numbers, prevalence and percentage changes of nasopharyngeal colonization by *Streptococcus pneumoniae* serotype at baseline (2010) and post-PCV10 introduction (2013 and 2017) in São Paulo, Brazil.

Colonization[¥]	Year of survey									
	2010		2013				2017			
	N	%	N	%	% change	p-value	N	%	% change	p-value
Number of enrolled children	501		400				531			
S. pneumoniae carrier	202	40.3	195	48.8	+21.1	0.011	317	59.7	+48.1	**<0.001**
PCV10 types[¥]	99	19.8	7	1.8	−90.9	0.000	5	0.9	−95.5	**<0.001**
Non-PCV10-types[¥]	98	19.6	179	44.8	+128.6	**<0.001**	297	55.9	+185.2	**<0.001**
Additional PCV13 types[¥]	32	6.4	28	7.0	+9.4	0.714	35	6.6	+3.1	0.894
Serotype 6 A	21	4.2	16	4.0	−4.8	0.885	3	0.6	−85.7	**<0.001**
Serotype 19 A	9	1.8	10	2.5	+38.9	0.465	32	6.0	+233.3	**0.001**
Serotype 3	3	0.6	2	0.5	−16.7	1.000	0	0.0	−100.0	–
Non-PCV types[¥]	67	13.4	152	38.0	+183.6	**<0.001**	266	50.1	+273.9	**<0.001**
Serotype 6C	9	1.8	45	11.3	+527.8	**<0.001**	94	17.7	+883.3	**<0.001**
Non-typeable[¥]	8	1.6	10	2.5	+56.6	0.336	34	6.4	+501.0	**<0.001**

P-values less than 0.05 were considered statistically significant.
[¥] Pneumococcal isolates by culture; children colonized with more than one serotype were classified as being colonized with vaccine serotype as long as one of the serotypes was VT, and as additional PCV13-types as long as one of the serotypes belonged to serotypes 3, 6A or 19A.

(27%), 15B (9.8%), 19A (9.2%), 15A (6.0%), and 16F (5.7%); NT pneumococcus was found in 9.8% of the isolates.

Among the 348 pneumococcal isolates from the late-post-PCV10 survey, 216 (62.0%) showed MIC to Pen ≥0.12 mg/L, and belonged to serotypes 6C (n = 82), 19A (n = 30), 15B (n = 21), 16F (n = 15), 15A (n = 12), 23A (n = 7), 35F (n = 6), 35B (n = 5) 6A, 11A, and 15C (n = 3, each), 9V, 14, 23F, 7C, 15F, 17F, and 34 (n = 1, each), and NT isolates (n = 22); 24 (6.9%) isolates showed Ctx ≥1.0 mg/L, belonged to serotypes 19A (n = 22) and 11A (n = 1), and to NT isolates (n = 1). MIC_{90} to Pen and Ctx were 1.0 mg/L, and 0.5 mg/L, respectively, and MIC_{50} to Pen and Ctx were 0.12 mg/L and 0.06 mg/L, respectively.

Overall, 51 isolates belonging to serotype 19A were identified. The molecular typing identified the CC320 in 11.1% (n = 1/9), 20% (n = 2/10), and 75% (n = 24/32) of the 19A isolates from the pre-PCV10, early-post-PCV10, and late-post-PCV10 surveys, respectively (Fig. 1). Among the CC320 strains, 97.7% displayed MIC values to Pen ≥2.0 mg/L, and 74% showed MIC to Ctx ≥1.0 mg/L.

Table 4
Numbers and proportions of isolates of *Streptococcus pneumoniae* serotype at baseline (2010) and post-PCV10 introduction (2013 and 2017) in São Paulo, Brazil.

Serotype	2010 N = 207		2013 N = 198		2017 N = 348	
	n	%	n	%	n	%
PCV10-types	99	47.8	7	3.5	5	1.4
4	1	0.5	0	0.0	0	0.0
6B	30	14.5	2	1.0	1	0.3
7F	1	0.5	0	0.0	0	0.0
9 V	0	0.0	0	0.0	1	0.3
14	20	9.7	0	0.0	1	0.3
18C	4	1.9	0	0.0	1	0.3
19F	27	13.0	1	0.5	0	0.0
23F	16	7.7	4	2.0	1	0.3
Additional PCV13-types	33	15.9	28	14.1	35	10.1
3	3	1.4	2	1.0	0	0.0
6A	21	10.1	16	8.1	3	0.9
19A	9	4.3	10	5.1	32	9.2
Non-PCV types	67	32.4	153	77.3	274	78.7
6C	9	4.3	45	22.7	94	27.0
7B	1	0.5	0	0.0	0	0.0
7C	3	1.4	0	0.0	1	0.3
8	0	0.0	2	1.0	2	0.6
9N	4	1.9	0	0.0	0	0.0
10A	1	0.5	1	0.5	2	0.6
10F	1	0.5	0	0.0	0	0.0
11A	6	2.9	14	7.1	14	4.0
11C	1	0.5	0	0.0	0	0.0
12F	0	0.0	1	0.5	1	0.3
13	5	2.4	6	3.0	3	0.9
15A	3	1.4	7	3.5	21	6.0
15B	3	1.4	11	5.6	34	9.8
15C	4	1.9	11	5.6	3	0.9
15F	0	0.0	0	0.0	1	0.3
16F	4	1.9	9	4.5	20	5.7
17F	0	0.0	2	1.0	3	0.9
18A	0	0.0	1	0.5	0	0.0
18B	1	0.5	0	0.0	0	0.0
19B	0	0.0	0	0.0	5	1.4
19C	0	0.0	0	0.0	1	0.3
20	0	0.0	3	1.5	1	0.3
21	0	0.0	0	0.0	7	2.0
22F	0	0.0	3	1.5	4	1.1
23A	1	0.5	8	4.0	13	3.7
23B	8	3.9	4	2.0	11	3.2
24F	1	0.5	1	0.5	3	0.9
28A	3	1.4	4	2.0	0	0.0
28F	1	0.5	0	0.0	0	0.0
29	3	1.4	7	3.5	1	0.3
31	0	0.0	3	1.5	1	0.3
33A	0	0.0	0	0.0	1	0.3
34	1	0.5	0	0.0	5	1.4
35A	1	0.5	1	0.5	1	0.3
35B	0	0.0	2	1.0	8	2.3
35C	0	0.0	0	0.0	1	0.3
35F	2	1.0	7	3.5	6	1.7
38	0	0.0	0	0.0	6	1.7
Non-typeable	8	3.9	10	5.1	34	9.8

4. Discussion

This survey shows on a long-term period after PCV10 introduction, the prevalence of colonization by VT remained very low (<1%) showing a robust vaccine impact on VT carriage. This has already been observed in a survey conducted after three years of PCV10 use that demonstrated a 1.8% of VT prevalence. Only 5 isolates belonging to 5 different VT were identified in the present survey, showing 95% reduction of VT versus the baseline. This residual colonization by VT found in the post-PCV10 surveys is probably a consequence of the high vaccine uptake with at least two vaccine doses by the study participants.

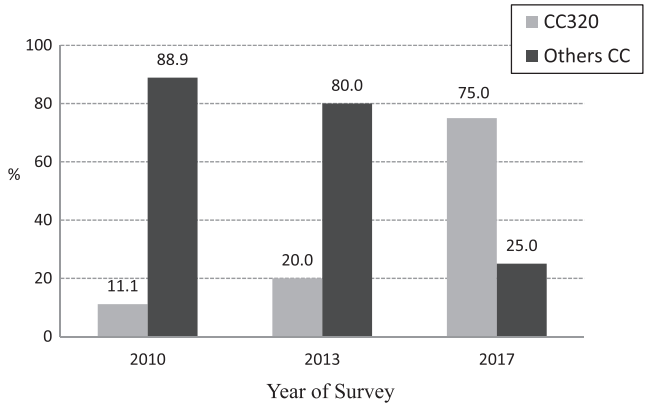

Fig 1. Distribution of complex clonal 320 among *Streptococcus pneumoniae* serotype 19A by colonization survey. CC, clonal complex; Others CCs: 2878–2880, Singletons (STs 733, 276, 9801, 13837).

We also observed an increase in pneumococcal carriage led by the increase in NVT. High diversity of colonizing serotypes (n = 37/348) was identified compared with the number of serotypes identified in the 2013 survey (n = 29/198), suggesting that serotype replacement was still in process after 3 years of PCV10 use. In 2017, 57.7% of Spn isolates belonged to only five NVTs (6C, 15B, 19A, 15A, and 16F) and 9.7% were NT isolates. We identified a significant increase in 19A carriage, a reduction in the number of children carrying 6A, and serotype 3 was not detected by culture. Importantly, in the 2013 survey, there was no significant difference in the prevalence of serotypes 19A and 6A compared with baseline, supporting the hypothesis that a longer period after vaccination is crucial to evaluate the contribution of a specific NVT on carriage. In a carriage study conducted shortly after PCV10 introduction in Kilifi, Kenya, VT was rapidly reduced, and no effect of PCV10 was observed against vaccine-related serotypes 6A and 19A [31]. The few serotype 3 isolates identified in the 2010 and 2013 carriage surveys contrast with the high prevalence of this serotype for IPD which we found in Brazil mainly in patients with meningitis 5 years after the PCV10 introduction [14]. This difference may be consequence of source of the pneumococcal isolates since carriage data came from cross-sectional surveys and invasive serotypes from the Brazilian national passive surveillance systems [14]. We also draw attention to the substantial serotype 6C increase observed earlier in the 2013 survey, indicating that this serotype was the most prevalent colonizer after 7 years of PCV10 introduction. All these findings except for serotype 3 are in line with the results obtained from the laboratory-based surveillance of IPD conducted after 4 years of PCV10 introduction in Brazil (2014–2015) that showed the huge reduction in VT-IPD and serotype 6A-IPD, along with the increase in IPD, caused by serotypes 19A and 6C, as well as for the other NVTs [14]. Thus, the present study does not support the potential cross-protection of the VT 6B and 19F, respectively against 6C and 19A, as suggested by other studies [11,32]. In a recent study conducted on IPD in Finland, no reduction in 19A-IPD was observed in the vaccine-eligible cohort 6 years after the PCV10 introduction to the routine immunization [33].

Resistance to beta-lactamic antibiotics was most associated with serotypes 6C, 15B, 19A, 15A, and 16F, and NT isolates which may contribute to the serotype replacement by these serotypes. The genetic characterization of all 19A isolates, recovered from the three surveys, identified the expansion of the antibiotic-resistant CC320 in the post-PCV10 surveys. The presence of CC320 among carriage toddlers is worrying because of a great chance of its spread. Currently, the CC320 has been the most

prevalent genetic lineage among 19A-IPD isolates in Brazil [16]. Similarly, it has been reported in other settings, after PCV7 or PCV10 introduction [34–39]. Thus, antibiotic and vaccine pressures, beside the differences on the potential of invasiveness of NVT, might explain in part the high prevalence of 19A carriage on the long term, after PCV10 introduction [40,41].

On the other hand, the early increase in serotype 6C after vaccination might also be associated with antimicrobial resistance and vaccine pressure as the VT 6B does not induce cross-protection for 6C [42–44].

Among the potential factors of Spn colonization, we found DCC attendance strongly associated with colonization by the NVT 10A and 6C, and NT isolates, possibly because these serotypes are associated with resistance to beta-lactams and children are the a population with higher consumption of antibiotics [16].

In this survey, the qPCR did not improve the detection of Spn colonization compared with culturing, as we identified only eight NP specimens with positive qPCR and negative cultures (Table 1). Although molecular tests are highly sensitive for detection of multiple serotypes, the qPCR can identify only 21 serotypes. In the present study, we did not test the positive samples in the qPCR by the conventional PCR-multiplex that identify 70 serotypes; therefore our rate of cocolonization, may be underestimated as some serotypes may have been missed. Notwithstanding, the qPCR added 31 serotypes over the culture.

This study was conducted in the biggest city of Brazil, and the participants were recruited by a convenience sampling (a non-probability sampling), which could limit the generalization of the results for the whole population of the city. Also, seven years after the 2010 survey, some of the socio-demographic characteristics of the population may have changed. We observed an improvement in DCC attendance, which may lead to the increase of Spn colonization over the three surveys. However, the results of this survey are consistent with the findings from a survey carried out in 2013, which suggested the need for a longer follow up of the results of repeated carriage studies after vaccination, to understand the sustainability of NVT.

In conclusion, we evidenced a tremendous impact of PCV10 on vaccine type-Spn carriage, changing the epidemiological picture of serotype distribution after 7 years of PCV10 introduction in Brazil, with increased non-PCV10 types, mainly serotypes 6C and 19A. The increase in 19A is worrisome, was associated with the expansion of the antibiotic-resistant CC320 in the post-PCV10 surveys and require close follow-up. These findings can corroborate policy decisions and the development of new PCVs.

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Contributors

All authors attest they meet the ICMJE criteria for authorship. The idea and contents of the article emerged from discussions among the authors. RM and ALSA performed the analyses, MCCB wrote the first draft. All authors contributed to the subsequent revisions and approved the final version.

Declaration of Competing Interest

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Disclaimer

All authors have approved the final version of the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.07.043.

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