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Prevalence, capsular types, antimicrobial resistance and risk factors associated with pneumococcal carriage among children after long-term 10-valent pneumococcal conjugate vaccine use in Brazil

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ABSTRACT

Background: The 10-valent pneumococcal conjugate vaccine (PCV10) was introduced for childhood vaccination in Brazil's National Immunization Program in 2010. After nine years of PCV10 use, we investigated the carriage prevalence, capsular types, antimicrobial resistance and risk factors among children living in Niterói city, RJ, Brazil.
Methods: Between September and December 2019, we conducted a cross-sectional study and recruited children under 6 years of age. Antimicrobial susceptibility was evaluated by the disk-diffusion method and MICs to beta-lactams and macrolides were determined by E-test®. Capsular types were deduced by multiplex PCR. Logistic regression was used to predict risk factors for pneumococcal carriage.
Results: Seventy-five (17.4%) of the 430 children were pneumococcal carriers. The most frequent capsular types were 6C/D (14.7%), 11A/D (13.3%), and 23B (9.3%). PCV10 serotypes represented 5.3%. All isolates were susceptible to levofloxacin, linezolid, rifampicin, and vancomycin. Penicillin non-susceptible pneumococci (PNSP) made up 37.3%, with penicillin and ceftriaxone MICs ranging from 0.12 to 4.0 μg/ml and 0.064–4.0 μg/ml, respectively. Of the 19 (25.3%) erythromycin-resistant (ERY-R) isolates (macrolide MICs of 6 to >256 μg/ml), most had the $cMLS_B$ phenotype (84.2%) and carried the *erm*(B) gene (73.7%). We detected 17 (22.6%) multidrug-resistant (MDR) isolates, strongly associated with serotype 6C/D. Presence of any symptoms, chronic diseases, childcare center attendance, living with young siblings, slum residence, and unstable income were predictors of pneumococcal carriage.
Conclusions: Long-term universal childhood use of PCV10 has nearly eliminated carriage with PCV10 serotypes, but the high frequency of MDR isolates, especially associated with serotype 6C/D, remains a concern. Replacing PCV10 with PCV13 should reduce the proportion of ERY-R isolates and PNSP by at least 14% and 18%, respectively.

1. Introduction

Pneumococcal diseases, including pneumonia, bacteremia and meningitis, are responsible for significant morbidity and mortality worldwide. In 2019, *Streptococcus pneumoniae* was the third most deadly bacterial pathogen in all age groups, causing >800,000 deaths, mostly due to lower respiratory infections. In addition, it was the pathogen most frequently associated with deaths among children aged <5 years [1].

In Brazil, pneumonia, mostly caused by *S. pneumoniae*, is one of the main causes of hospitalization. From 2010 to 2019, pneumonia

Abbreviations: CC, clonal complex; CLSI, Clinical and Laboratory Standards Institute; $cMLS_B$, constitutive macrolide, lincosamide and streptogramin B resistance phenotype; ERY-R, erythromycin-resistant; IPD, invasive pneumococcal disease; M, macrolide resistance phenotype; MDR, multidrug resistant; MIC, minimum inhibitory concentration; NT, non-typeable; PCV, pneumococcal conjugate vaccine; PCR, polymerase chain reaction; PNSP, penicillin non-susceptible pneumococci; ST, sequence type; STGG, skim milk-tryptone-glucose-glycerin transport medium; UOR, unadjusted odds ratios; WHO, World Health Organization.

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was responsible for over 600,000 hospitalizations per year [2], and, in the same period, the annual incidence of pneumococcal meningitis was on average 1,050 cases [3].

Since 2010, the 10-valent pneumococcal conjugate vaccine (PCV10; Synflorix®), which targets serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F, is included in Brazil's National Immunization Program for routine childhood vaccination and it is offered free of charge to any child under 5 years old. The 13-valent PCV (PCV13; Prevnar13®) contains three additional serotypes (3, 6A, and 19A) compared to PCV10 and has been available in private immunization clinics since 2010, but it started to be freely offered by the government to individuals aged 5 years or older at increased risk for pneumococcal diseases in 2019 [4]. As a result, PCV10 has been widely used by Brazilian children, whereas PCV13 has a very low (<10%) coverage [5,6].

In countries where PCVs have been universally adopted, the "serotype replacement" phenomenon has occurred, resulting in the emergence of non-vaccine serotypes associated with both colonization and disease [7,8]. Children are considered the main reservoir of pneumococci and colonization of the upper respiratory tract plays an important role in the development of pneumococcal diseases and the spread of the microorganism, including those variants associated with drug resistance [9,10].

Four years after the introduction of PCV10 for pediatric routine use in Brazil, we observed a virtual eradication of PCV10 serotypes and the emergence of multidrug resistant (MDR) serotypes associated with carriage in young children in a large metropolitan area in southeastern Brazil [5]. Although different MDR serotypes and clones have been found, we detected a clonal expansion of the CC386 associated with serotype 6C [9]. Now, we report the long-term effects of PCV10 use on pneumococcal carriage among children living in the same setting, including the capsular type distribution and the antimicrobial resistance of the pneumococcal isolates. We also created a statistical model to predict risk factors for pneumococcal carriage.

2. Material and methods

2.1. Population and study design

Between September 2 and December 17, 2019, we conducted a cross-sectional study among children <6 years old who attended two private and two public pediatric clinics for routine check-up or sick visits in Niterói, a city in the metropolitan area of Rio de Janeiro, Brazil. In line with WHO recommendations for detecting upper respiratory carriage with S. pneumoniae [11], a single nasopharyngeal specimen was collected from each child with nylon fiber mini-tip flocked swabs (Copan, Brescia, Italy). Swabs were, then, placed into a cryotube containing 1.0 mL of skim milk-tryptone-glucose-glycerin (STGG) transport medium, kept on ice until stored at −80 °C on the same day.

We also obtained clinical and sociodemographic information of the participants from their legal guardians and retrieved data on pneumococcal vaccination history from participants' vaccination cards (Table 1). Children who did not live in the city of Niterói and/or who were immunized with PCVs other than PCV10 were not included in the study.

2.2. Isolation and identification of pneumococcal strains

The broth enrichment culture method was used to isolate pneumococcal strains [12] with a few modifications [13]. After thawing at room temperature, nasopharyngeal specimens were subjected to an enrichment step, by transferring 200 μL of homogenized STGG medium into 2.0 mL of Todd Hewitt broth (Difco Laboratories, Detroit, MI, USA) supplemented with 0.5 % yeast extract (Difco Labs.) and 0.4 mL of rabbit serum. Following incubation for 6 h at 37 °C in 5% CO_2-enriched atmosphere, a 10 μL loop of broth culture was streaked onto blood agar plates (Plast Labor, RJ, Brazil). After overnight incubation at 37 °C in 5% CO_2, identification of α-haemolytic colonies suspected to be *S. pneumoniae* was based on the results of optochin susceptibility and bile-solubility tests.

2.3. Capsular type determination

Capsular types were deduced by sequential multiplex PCR [12].

2.4. Antimicrobial susceptibility testing

We determined the antimicrobial susceptibility profiles of all the isolates by the disk-diffusion method according to the Clinical and Laboratory Standards Institute guidelines [14]. The following 10 antimicrobial agents were tested: chloramphenicol (30 μg), clindamycin (2 μg), erythromycin (15 μg), levofloxacin (5 μg), linezolid (30 μg), oxacillin (1 μg), rifampicin (5 μg), sulfamethoxazole/trimethoprim (1.25 μg/23.75 μg), tetracycline (30 μg), and vancomycin (30 μg) (Cecon, São Paulo, SP, Brazil). The E-test® (BioMérieux, Marcy l'Etoile, France) was used to determine the minimum inhibitory concentration (MIC) to penicillin and ceftriaxone for isolates presenting zones of inhibition with diameter <20 mm around the oxacillin disk. Erythromycin and azithromycin MICs were also determined by E-test® (BioMérieux) for isolates that were intermediate or resistant to erythromycin in the disk-diffusion test (<21 mm diameter zone of inhibition). Macrolide resistance phenotypes were investigated by the double-disk test [14]. Isolates that were intermediate or resistant to at least one agent from three or more different classes of antimicrobial agents were classified as multidrug resistant (MDR) [15].

2.5. Investigation of macrolide resistance genes

The presence of the *erm*(A), *erm*(B) and *mef*(A/E) genes was investigated among erythromycin resistant (ERY-R) isolates by PCR [16].

2.6. Statistical analyses

We built a database using the R Studio software, version 1.3.1056, and Microsoft Excel for Mac, version 16.39. We estimated unadjusted odds ratios (UOR) for pneumococcal colonization using bivariate analysis. We used the Fisher's exact test to determine factors associated with carriage and statistical significance was considered when p-value was <0.05. We also developed multivariate generalized linear models to estimate the adjusted risk of pneumococcal colonization in a forward stepwise process. The parameters of the model were estimated by the Maximum Likelihood method. A confidence interval (CI) was then constructed for the odds ratio with 5% significance (95% CI). In addition, we used the Akaike information criterion (AIC) to evaluate how well the model provides the best determinants of pneumococcal colonization based on the addressed variables. To generate a more robust outcome, children missing any information were not included in these analyses.

2.7. Ethical considerations

This study was approved by the Ethics Committee at the School of Medicine of the *Universidade Federal Fluminense* (CAAE number 79099117.2.00005243).

Table 1
Demographic and clinical characteristics of 430 children who attended primary care clinics in Niterói city, Brazil, 2019.

Characteristic (n)	*S. pneumoniae* carriage		UOR	95% CI	*p*-value
	Yes (n = 75)	No (n = 355)			
Sex					
Male (220)	38	182	0.98	0.59–1.61	1
Female (210)	37	173			
Age					
0–<1 years (241)	25	216	0.32	0.19–0.54	<0.01
1–<6 years (189)	50	139			
Type of primary care clinic					
Public (318)	63	255	2.05	1.06–3.98	0.03
Private (112)	12	100			
Self-reported ethnicity					
White (200)	26	174	0.55	0.32–0.92	0.03
Non-white: *parda*[a], black, Asian or mestizo (219)	47	172			
Not reported (11)	2	9			
Symptomatology at time of interview [b]					
Yes (171)	42	129	2.23	1.35–3.69	<0.01
No (259)	33	226			
Has any chronic disease[c]					
Yes (56)	16	40	2.14	1.12–4.06	0.02
No (374)	59	315			
Has pneumonia at the time of interview					
Yes (6)	0	6	–	–	0.38
No (424)	75	349			
Previous pneumonia					
Yes (59)	16	43	1.97	1.04–3.72	0.04
No (371)	59	312			
Any antibiotic use in previous two weeks					
Yes (32)	4	28	0.66	0.22–1.93	0.49
No (398)	71	327			
Hospitalization (>48 h) in the past year					
Yes (57)	7	50	0.63	0.27–1.44	0.35
No (372)	68	304			
Not reported (1)	0	1			
Attends childcare center					
Yes (96)	26	70	2.16	1.25–3.71	<0.01
No (334)	49	285			
Location of primary residence					
Inside of a slum (201)	48	153	2.32	1.39–3.89	<0.01
Outside of a slum (227)	27	200			
Not reported (2)	0	2			
Number of people living in their home					
2 or 3[d] (141)	25	116	1.03	0.61–1.75	1
4+ (289)	50	239			
Has at least one sibling <6 years old					
Yes (127)	30	97	1.77	1.06–2.97	0.04
No (303)	45	258			
Lives with smokers					
Yes (141)	31	110	1.55	0.93–2.60	0.1
No (280)	43	237			
Not reported (9)	1	8			
Has stable income					
Yes (298)	41	257	0.47	0.28–0.79	< 0.01
No (131)	33	98			
Not reported (1)	1	0			

[a] The closest translation for *parda* is 'mixed' in English; CI, confidence interval; UOR, unadjusted odds ratio;
[b] Fever, coryza/sneezing, cough/expectoration, fatigue/breathlessness, hypoactivity, vomit, and/or diarrhea;
[c] Rhinitis, asthma, bronchitis, severe allergy, congenital syphilis, Aids, and/or cardiovascular diseases;
[d] Only nine children (0.5%) had two people living at home (i.e. the child and one other person). This stratum was combined with those that had three people living at home.

3. Results

We recruited 430 children who attended two public and two private clinics in Niterói city. Most (74%; 318/430) participants attended the public clinics. In total, 325 (75.6%) children were age-eligible for PCV vaccination (older than 2 months). Thirteen (4%) children aged ≥2 months did not receive any dose of the PCV10, and 312 (96%) of the 325 eligible children were vaccinated with PCV10.

The mean age of the 430 children was 1.42 year, and the median age was 0.75 year (IQR: 0.17 and 2.21 years old). Two hundred and twenty (51.2%) children were male. Seventy-two (96%) of the 75 colonized individuals had received at least 1 dose of the PCV10; the other three individuals were younger than 2 months old and, therefore, not eligible for PCV vaccination.

The prevalence of pneumococcal carriage was 17.4% (75/430). Stratifying by age, 25 (10.4%) of 241 children aged <1 year, but 50 (26.5%) of 189 children aged 1 year or older were pneumococcal carriers (p < 0.01). Table 1 shows the clinical and sociodemographic information of the study participants and the results of the bivariate analysis of risk factors for colonization.

Through multivariate analysis, attending a childcare center, having any symptoms, having any chronic diseases, living in an urban slum, co-habitation with at least one sibling <6 years old,

and not having stable income increased the odds of pneumococcal colonization (Table 2). The logistic model selected by the forward stepwise process had an accuracy of 74.2% in detecting a child colonized with *S. pneumoniae* (Fig. 1).

Twenty-one different capsular types were detected among 68 (90.7%) of the 75 pneumococcal isolates. Thirteen (17.3%) isolates were identified as serogroup 6 by sequential multiplex PCR, of which 11 (84.6%) isolates amplified the 6C/D-specific PCR product. The 68 isolates had the *cpsA* gene, but five (7.4%) isolates were not resolved by PCR. These five isolates were classified as not determined (ND). Seven (9.3%) isolates did not amplify the *cpsA* gene and were classified as non-typeable (NT).

The most frequent capsular types associated with carriage in the population investigated were 6C/D (11, 14.7%), 11A/D (10, 13.3%), 23B (7, 9.3%), and 15B/C (5, 6.7%). The distribution of pneumococcal serogroups/serotypes is shown in Fig. 2.

All the 75 isolates were susceptible to linezolid, levofloxacin, rifampicin, and vancomycin. Only one (1.7%) chloramphenicol-resistant isolate (serotype 11A/D) was found, also presenting concomitant resistance to sulfamethoxazole/trimethoprim.

The highest proportion of non-susceptible isolates was observed for sulfamethoxazole/trimethoprim (41.3%; 31/75), including 22 (29.3%) resistant and nine (12%) intermediate isolates. Tetracycline non-susceptibility was detected in 19 (25.3%) isolates, being four (5.3%) intermediate and 15 (20%) resistant isolates.

Twenty-eight (37.3%) isolates were non-susceptible to penicillin. Penicillin non-susceptible pneumococci (PNSP) were associated with 10 different capsular types and with NT isolates.

MICs to penicillin and ceftriaxone among the 28 PNSP ranged from 0.12 to 4.0 μg/ml and 0.064–4.0 μg/ml, respectively. The highest penicillin MIC (4.0 μg/ml) was detected in two isolates belonging to serotypes 15B/C and 19A. The highest ceftriaxone MIC (4.0 μg/ml) was detected in one serotype 5 and one NT isolate. Table 3 shows the interpretations for beta-lactam MIC, according to the CLSI criteria.

All the 19 (25.3%) isolates that were non-susceptible to erythromycin by the disk-diffusion method (two intermediate and 17 resistant isolates) had erythromycin and azithromycin MICs ranging from 6.0 to >256 μg/ml, indicative of resistance. For each isolate, MICs to erythromycin and azithromycin were always identical. Erythromycin-resistant (ERY-R) isolates were associated with seven different capsular types, as well as NT isolates.

The $cMLS_B$ phenotype (84.2%; 16/19) was the most frequent macrolide resistance phenotype among the ERY-R isolates, and all but one isolate had macrolide MICs >256 μg/ml. Fourteen (87.5%) of the 16 isolates with the $cMLS_B$ phenotype had the *erm* (B) gene alone (10 isolates) or in association with the *mef*(A/E) gene (four isolates). The single isolate that displayed the $cMLS_B$ phenotype with different MICs of erythromycin and azithromycin (8.0 μg/ml to both drugs) carried the *mef*(A/E) gene and had been classified as intermediate to erythromycin by the disk-diffusion method. We did not detect any of the macrolide resistance gene investigated in only one isolate with the $cMLS_B$ phenotype. Three (15.8%) of the 19 ERY-R isolates had the M phenotype, with macrolide MICs of 6.0, 8.0, and 48 μg/ml, and harbored only the *mef*(A/E) gene. Clindamycin resistance was found in 18 (24%) isolates, and 16 of them had the $cMLS_B$ phenotype.

Seventeen (22.7%) isolates were MDR, being associated with seven distinct serotypes and one NT isolate, but mostly with serotype 6C/D. Table 4 shows the characteristics of the isolates according to the multidrug resistance profiles.

Table 2
Multivariate generalized linear models predicting risk of colonization with *Streptococcus pneumoniae* among children who attended primary care clinics in Niterói city, RJ, Brazil in 2019.

	Odds Ratio	95% Confidence Interval
Childcare center attendance	2.23	1.17–4.21
Having any symptoms[a]	2.27	1.30–4.01
Having any chronic disease[b]	1.78	0.84–3.64
Living inside an urban slum	2.38	1.33–4.36
Co-residence with at least one sibling <6 years old	1.77	1.00–3.13
Having stable income	0.53	0.30–0.95

[a] Fever, coryza/sneezing, cough/expectoration, fatigue/breathlessness, hypoactivity, vomit, and/or diarrhea.
[b] Rhinitis, asthma, bronchitis, severe allergy, congenital syphilis, Aids, and/or cardiovascular diseases.

4. Discussion

Nine years after the introduction of the PCV10 for childhood immunization in Brazil, we observed a very high vaccination coverage (96%) among the age-eligible children living in the city of Niterói. Multidrug resistance was associated with several capsular types. The MDR serotype 6C/D remained the most frequent non-PCV10 vaccine serotype associated with colonization in children, but it was closely followed by the serotype 11A/D, which was, in general, susceptible to major antimicrobial agents used to treat pneumococcal diseases.

The overall prevalence of pneumococcal carriage was 17.4%. A previous study conducted in the same city after four years of PCV10 introduction had found a slightly higher proportion of colonization with *S. pneumoniae* (22.6%) among 522 children aged <6 years [5]. This small difference is largely due to the higher proportion of children younger than 1 year old recruited in 2019, since children at this age range were shown to be less colonized with *S. pneumoniae* in our setting. In São Paulo, another city in southeastern Brazil, the prevalence of pneumococcal carriage has been increasing in children aged between 12 than 24 months, since the proportion of children colonized was 40.3%, 48.8% and 59.7% in the pre-PCV10 era, after three years, and after seven years of the PCV10 introduction, respectively [17]. Beyond the age range, this divergence may be partially explained by their exclusion criteria, especially of children who had recently received antibiotics.

The four most frequent capsular types associated with carriage in the population investigated, which included 6C/D, 11A/D, 23B, and 15B/C, were already commonly found in our setting in 2014 [5]. In 2019, serotypes 6C/D, 11A/D, and 23B made up almost 40% of the isolates. In São Paulo, serotypes 6C (27%), 15B (9.8%), and 19A (9.2%) accounted for 46% of all isolates associated with carriage in children in 2017 [17]. Even when accounting for the differences in study design, the large disparity in the overall prevalence of pneumococcal carriage and the distribution of pneumococcal serotypes show how the dynamics of pneumococcal behavior can vary within the same country, even in cities located in the same geographical region.

Serotype 6C/D represented nearly 15% of the carriage isolates obtained in the present study, and, in 2019, it corresponded to the third most frequent (8.8%; 74/840) invasive serotype identified among patients of all age groups in Brazil [18]. We had already warned about the increasing incidence of serotype 6C in invasive pneumococcal diseases (IPD) in 2014, when 6C had emerged as the major serotype associated with carriage in young children in Brazil [5,19]. As stated before, the emergence of MDR isolates of the serotype 6C was largely explained by the expansion of the CC386 [9], which is likely still predominating in our setting.

In Morocco, where PCV10 replaced PCV13 for childhood immunization in 2012, serotype 6C/D was also the most frequent serotype (approximately 13%) associated with carriage in children

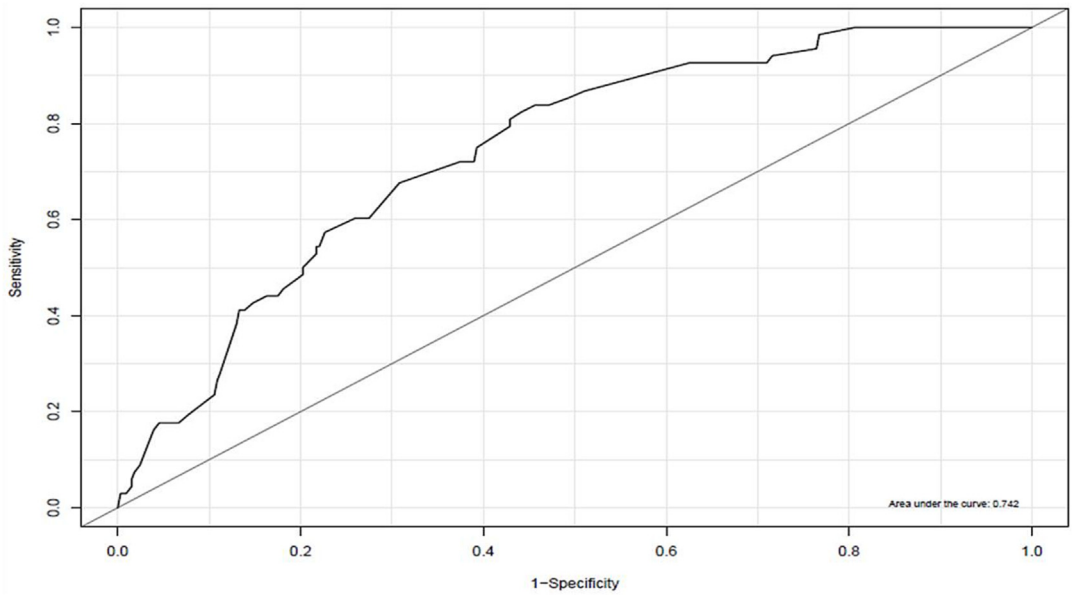

Fig. 1. ROC (receiver operating characteristic) curve showing the performance of the logistic model in predicting risk of colonization with *Streptococcus pneumoniae* among children who attended primary care clinics in Niterói city, RJ, Brazil in 2019.

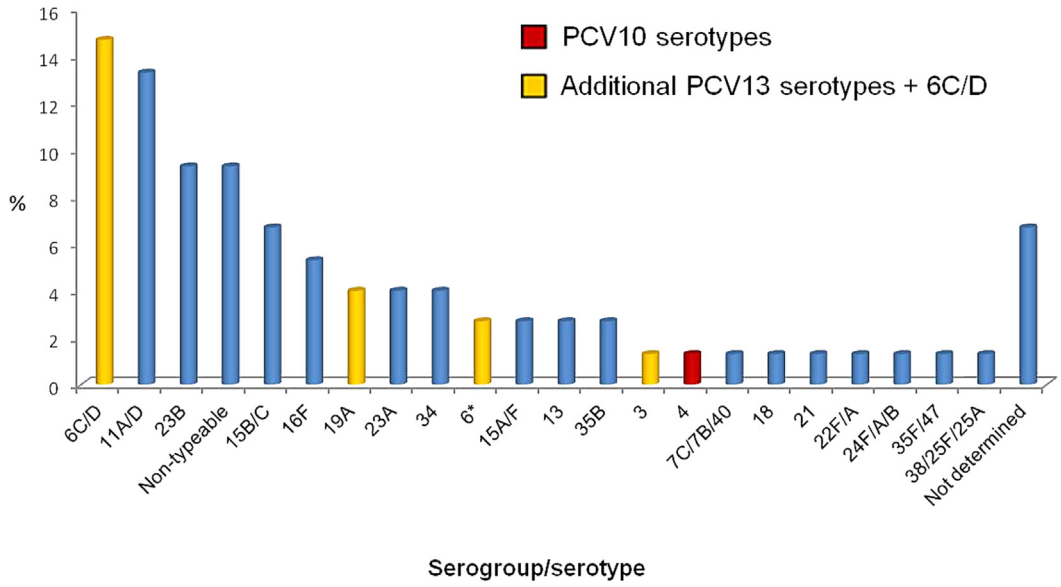

Fig. 2. Distribution of capsular types among 75 Streptococcus pneumoniae isolates obtained from children's nasopharynx in Niterói city, RJ, Brazil in 2019. *6, presumably capsular type 6A due to *cpsB* sequencing (data not shown); PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; Not determined, includes isolates that amplified the *cpsA* gene, but could not be typed by multiplex PCR.

Table 3
Susceptibility to beta-lactams among 28 penicillin non-susceptible pneumococci according to CLSI breakpoints (2022).

Beta-lactam (criterion)	N (% in relation to the total of 75 isolates)		
	Sensitive	Intermediate	Resistant
Penicillin parenteral (nonmeningitis)	23 (30.7%)	5 (6.7%)	–
Penicillin parenteral (meningitis)	–	–	28 (37.3%)
Penicillin (oral)	–	18 (24%)	10 (13.3%)
Ceftriaxone (nonmeningitis)	24 (32%)	4 (5.3%)	–
Ceftriaxone (meningitis)	19 (25.3%)	5 (6.7%)	4 (5.3%)

aged 6 to 36 months with acute otitis media who visited pediatric centers in Marrakesh in 2018 [20]. In Belgium, a significant increase in the prevalence of serotype 6C associated with carriage in children was observed following PCV13 replacement with PCV10 between 2016 and 2019 [21]. This might suggest that the emergence of the serotype 6C after national immunization with PCV10 is not a local phenomenon.

A few years after PCV10 introduction in Brazil, serotype 11A/D was already among the most common serotypes associated with carriage in children in Niterói [5], although its association with IPD in Brazil was very low at that time [22]. However, in 2019, serotype 11A was identified as the fifth most frequent invasive serotype in adults aged ≥50 years, reaching almost 4% (13/339) of the invasive isolates, although its incidence remained low among

Table 4
Characteristics of 17 multidrug-resistant pneumococci isolated from childreńs nasopharynx in Niterói city, RJ, Brazil in 2019.

Multidrug resistance (R + I) profile (n)	Capsular type (n/total)	MIC μg/ml			ERY Resistance	
		PEN	CRO	ERY/AZI	Phenotype	Genotype
CLI + ERY + PEN + TET (7)	6C/D (6/11)	0.12–1.0	0.064–1.0	>256	$cMLS_B$	*erm*(B)
	23A (1/3)	0.25	0.125			
CLI + ERY + PEN + SXT + TET (4)	19A (2/3)	4.0	0.075–2.0	>256	$cMLS_B$	*erm*(B) + *mef*(A/E)
	24A/B/F (1/1)	1.0	0.38			*erm*(B)
	NT (1/7)	2.0	4.0			*erm*(B) + *mef*(A/E)
CLI + ERY + PEN + SXT (2)	6 (1/2)	1	1	>256	$cMLS_B$	*erm*(B)
	15A/F (1/2)	4	1			none
ERY + PEN + TET (1)	6C/D (1/11)	0.25	0.19	6	M	*mef*(A/E)
CLI + ERY + PEN (1)	6C/D (1/11)	0.12	0.094	>256	$cMLS_B$	*erm*(B)
CLI + ERY + TET (1)	6C/D (1/11)	–	–	>256	$cMLS_B$	*erm*(B)
CLI + PEN + SXT (1)	11A/D (1/10)	2	1	–	–	–

AZI, azithromycin; CLI, clindamycin; $cMLS_B$, constitutive macrolide, lincosamide, and streptogramin B resistance phenotype; CRO, ceftriaxone; ERY, erythromycin; I, intermediate; M, macrolide resistance phenotype; MIC, minimum inhibitory concentration; PEN, penicillin; R, resistant; SXT, sulfamethoxazole/trimethoprim; TET, tetracycline.

young children [18]. Only one of the 10 serotype 11A/D isolates was MDR, while the others were sensitive to beta-lactams and macrolides. Of note, this serotype was also detected colonizing adults with systemic lupus erythematosus in our region [23]. CC62 is the major clonal complex associated with serotype 11A/D circulating in Niterói city since before PCV10 introduction [9,24], and this well adapted genetic lineage may have expanded even more in recent years.

Serotype 23B, on the other hand, has been associated with different genetic lineages in our setting, including ST727, ST945, ST947, ST6605, and also ST338, known as the MDR international clone Colombia^{23F}-26 [9]. The seven serotype 23B pneumococci isolated in the current study, however, were susceptible to almost all drugs tested. The incidence of this serotype as a cause of IPD in Brazil increased from 2% [22] to 3.2% [18] from 2014 to 2019, reaching the seventh position in all age groups.

In 2019, 19A (20.2%) and 3 (12.1%) were by far the serotypes most commonly associated with IPD in Brazil [18], but they do not seem to be much associated with carriage in children in our setting, since their frequencies remain ≥4% [5,24].

Other South American countries, such as Colombia and Ecuador, have also introduced the PCV10 into their national immunization programs and a major reduction in IPD due to PCV10 serotypes has also been observed. Similarly to scenario in Brazil, serotypes 19A, followed by 3 and 6C, have emerged as major causes of IPD in all age groups in these countries [25,26]. In Colombia, serotypes 15B/C and 23B have also been reported as important agents of meningitis in individuals aged <5 years and ≥60 years in 2019 [27].

Capsular type 4 was the only PCV10 serotype found among the children analyzed in the present study. It was detected in one children fully immunized with PCV10 in the scheme 2p + 1 (two primary doses plus one booster). Although we cannot assume this is a failure of the PCV10, since the primary goal of the vaccination is to prevent pneumococcal diseases, conjugate vaccines are supposed to eliminate the carrier status. Considering that children can transmit the microorganism to individuals from different age groups, this might help explain the proportion of serotype 4 associated with IPD in individuals aged 5 years or older in Brazil in 2019, when serotype 4 corresponded to 5% (34/660) of the invasive isolates [18].

In recent years, new pneumococcal conjugate vaccines have been licensed for use in children and/or adults. Pneumosil® is a different 10-valent PCV that targets serotypes 1, 5, 6A, 6B, 7F, 9 V, 14, 19A, 19F, and 23F, and was made available for children in India [28]. The 15-valent PCV (PCV15; Vaxneuvance®) targets the same serotypes included in the PCV13 plus serotypes 22F and 33F and it is now approved for individuals aged 6 weeks and older in the USA. In addition, the 20-valent PCV (PCV20; US Prevnar20® or EU Apexxnar®) contains all the PCV15 serotypes plus 8, 10A, 11A, 12F, and 15B, and, so far, it has been approved for adults aged 18 years or older [29,30].

Considering all the conjugate vaccines currently available and assuming that serotypes 6A and 15B elicit cross protection against serotypes 6C and 15C, respectively [31,32], the theoretical impact of Pneumosil®, PCV13, PCV15, and PCV20 over the isolates detected in present work would be 21%, 24%, 25%, and 45%, respectively. Additionally, these vaccines would have the potential to eliminate >60% of the PNSP, ERY-R, and MDR isolates found in the present study. Pneumosil®, PCV13, and PCV15 would eliminate 12 (63.2%) of the 19 ERY-R isolates and 17 (60.7%) of the 28 PNSP, while PCV20 would eliminate 13 (68.4%) ERY-R isolates and 19 (67.9%) PNSP. Among the 17 MDR isolates, Pneumosil® would eliminate 70.6% and PCV13, PCV15, and PCV20 would eliminate 76.5% of the isolates.

After long-term universal childhood vaccination with PCV10 in the city of Niterói, age ≥1 year, being non-white, previous pneumonia, presence of any symptoms, chronic diseases, childcare center attendance, cohabitation with young (<6 years old) siblings, public clinic attendance, slum residence, and unstable income were associated with pneumococcal carriage in the bivariate analysis. In the multivariate analysis, six of these factors were confirmed to be predictors of pneumococcal carriage.

As described in previous studies in Brazil, close contact with other children, including attending a childcare center and/or living with siblings younger than 6 years old, was also a factor that increased the likelihood of being colonized with pneumococcus [5,19] and of developing community-acquired pneumonia [33].

Some socioeconomic factors were also predictors of pneumococcal colonization. Living inside an urban slum increased approximately 2.4 times the odds of being colonized. The prevalence of pneumococcal carriage in children who lived in a slum was twice higher (around 24%) than in those who lived outside a slum (nearly 12%).

In addition, the odds of pneumococcal carriage increased by almost 50% in children whose families did not have stable household income. Although income itself is not directly associated with pneumococcal acquisition and colonization, it does have an effect on other important factors, such as access to health care, housing conditions and healthy food consumption. Since some of this information is harder to ascertain than income, it becomes a useful tool when creating prediction models. As expected, the proportion of children who lived in slums was higher among those from families with unstable income (64%; 84/131) compared to those from families with stable income (39%; 117/298).

Clinical factors also contributed to pneumococcal carriage. Having any symptoms at the time of enrollment or having chronic diseases were also associated with colonization with *S. pneumoniae*. Almost 90% of the 171 children presenting with any symptomatology had respiratory symptoms, mostly coryza/sneezing and/or cough/expectoration. Similarly, the majority (~86%; 48/56) of children with chronic diseases had rhinitis, asthma, or bronchitis. The presence of respiratory symptoms has been consistently associated with pneumococcal carriage in children from Niterói, before [24] and after [5] the introduction of the PCV10.

It is noteworthy that specimens and data collection was done immediately before the beginning of the COVID-19 pandemic. Several antimicrobial agents, especially azithromycin, have been widely used in the management of COVID-19 [34]. This intense use may help accelerate the selection of pneumococci presenting resistance to major antimicrobial drugs that are used to treat pneumococcal diseases.

The main limitation of the study concerns the exclusive use of a genotypic method to deduce pneumococcal capsular types; using the Quellung reaction would be important to confirm if the isolates express the capsule. Also, MIC values should be confirmed by broth microdilution method. In addition, we have recruited children who attended two private and two public pediatric clinics for routine check-up or sick visits in a large metropolitan area, represented by Niterói city, but the number of children recruited at public clinics was almost three times higher. Balancing the number of children from different types of clinic, expanding the number of clinics and/or recruiting participants outside primary care institutions would provide a more robust analysis.

5. Conclusions

Long-term universal childhood use of PCV10 has nearly eliminated PCV10 serotypes from carriage in the population investigated, but the high frequency of MDR isolates, especially associated with serotype 6C/D, is still a concern. Replacing PCV10 with other pneumococcal conjugate vaccines, particularly PCV13, which is already available in Brazil, should reduce the proportion of ERY-R isolates and PNSP by at least 14% and 18%, respectively. We also developed a statistic model that is able to predict, with a good level of accuracy, risk factors for pneumococcal carriage in our region.

CRediT authorship contribution statement

Letícia B.D.P. Fortuna: Formal analysis, Investigation, Methodology, and Writing – orginal draft. **Filipe M. Miranda:** Data curation, Formal analysis, Investigation, Methodology, and Writing – review & editing. **Isa M.F. Antunes:** Investigation, Methodology, and Writing – review & editing. **Amanda B. Silva:** Investigation, Methodology, and Writing – review & editing. **Amanda S. Cabral:** Investigation, Methodology, and Writing – review & editing. **Ítalo M. Dolores:** Data curation, Formal analysis, and Writing – review & editing. **Nayara T. Cardoso-Marques:** Formal analysis, Investigation, Methodology, and Writing – review & editing. **Lúcia M. Teixeira:** Conceptualization, Funding acquisition, Resources, and Writing – review & editing. **Felipe P.G. Neves:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, and Writing – orginal draft.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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