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Direct effect of the 13-valent pneumococcal conjugate vaccine use on pneumococcal colonization among children in Brazil

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ABSTRACT

Background: The 13-valent pneumococcal conjugate vaccine (PCV13) has been commercially available in Brazil since 2010. We investigated the carriage prevalence, capsular types, and antimicrobial resistance among pneumococci isolated from children immunized with PCV13 in Brazil.
Methods: We analyzed 500 children < 6 years old attending public (n = 270) and private (n = 230) clinics in Niterói/RJ, Brazil, in 2014. We determined the antimicrobial susceptibility and capsular types for all isolates.
Results: Thirty-eight (7.6%) of 500 children had received at least one PCV13 dose. Since only two (0.7%) of 270 children at the public clinic were vaccinated with PCV13, major analyses focused on 36 (15.7%) of 230 children attending private clinics. Nine (25%) of 36 children were pneumococcal carriers. Characteristics associated with carriage were age ≥ 2 years, cough/expectoration, and childcare center attendance ($p \leq 0.01$). The capsular types found were 15B/C (n = 2), 6C, 11A/D, 16F, 23A, and 23F. Two isolates were non-typeable (NT). Three (33.3%) isolates were multidrug resistant. We found four (44.4%) penicillin non-susceptible pneumococci, with penicillin and ceftriaxone MICs ranging from 0.12 to 4.0 μg/ml and 0.023–0.5 μg/ml, respectively. We also detected two (22.2%) erythromycin-resistant isolates (MICs of 3.0 and 256 μg/ml).
Conclusions: Colonization with PCV13 serotype was rare among the vaccinated children. Increasing PCV13 coverage might help reduce the frequency of major serotypes currently associated with invasive pneumococcal diseases in Brazil, such as 3 and 19A. The isolation of multidrug-resistant serotype 6C and NT isolates in carriage, however, requires close monitoring.

1. Introduction

Streptococcus pneumoniae is the most common bacterial agent of community-acquired pneumonia. It also causes other serious diseases, including bacteremia and meningitis. Pneumococcal diseases are a major cause of deaths worldwide, especially among children < 5 years old, despite public health efforts directed at expansion of vaccination programs [1].

In Brazil, the 10-valent pneumococcal conjugate vaccine (PCV10) was introduced free of charge via the National Immunization Program (NIP) in 2010. Despite significant decrease in pneumonia hospitalization estimates in almost all age groups following PCV10 implementation, the average annual rate of hospitalizations due to pneumonia remained high (around 700,000) between 2005 and 2015. Such hospitalizations have been affecting mostly young children, with an average annual incidence of 7900 cases in children aged<5 years per 100,000 population [2]. Additionally, the average annual rates of pneumococcal meningitis in

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; $cMLS_B$, constitutive macrolide lincosamide and streptogramin B resistance phenotype; ERY-R, erythromycin-resistant; IPD, invasive pneumococcal disease; IQR, interquartile range; M, macrolide resistance phenotype; MDR, multidrug resistant; MIC, minimum inhibitory concentration; NT, non-typeable; PCV, pneumococcal conjugate vaccines; PCR, polymerase chain reaction; PNSP, penicillin non-susceptible pneumococci; ST, sequence type.

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the pre-PCV10 period (2007–2009; 1080 cases) and in the PCV10 period (2011–2015; 1060 cases) are very similar [3].

One of the reasons that may help explain the persistently high incidence of pneumococcal diseases in Brazil even after PCV10 introduction is the serotype replacement phenomenon [4]. Prevalent serotypes associated with invasive pneumococcal diseases (IPD) among children < 5 years old in 2010 [5] were 14 (29.8%), 6B (15.3%), 6A, 23F (7.9% each), and 19A (6%). In 2014 [6], other serotypes became prevalent: 19A (28.2%), 3 (7.6%), 5, 12F (6.1% each), and 6C (4.6%). Three (3, 5, and 19A) of the five major serotypes that emerged in IPD in Brazil after PCV10 routine vaccination are found in the 13-valent PCV (PCV13), although serotype 5 is also present in PCV10 formulation. Additionally, PCV13 includes serotype 6A, which is believed to cross-protect against serotype 6C [7].

PCV13 has been available in Brazil since 2010 in private immunization clinics, but only by out-of-pocket payment. Due to its high cost, PCV13 coverage is low among Brazilian children, contrasting with PCV10, which reached high (>85%) coverage in the age-eligible population in Brazil by 2013 [8,9]. Here, we report the direct effect of PCV13 immunization on pneumococcal carriage, capsular type distribution and antimicrobial resistance of pneumococcal isolates recovered from children living in a large metropolitan area in southeastern Brazil.

2. Material and methods

2.1. Population and study design

Between September 29 and December 5, 2014, we interviewed legal guardians of 500 children age-eligible for pneumococcal conjugate vaccination (aged between ≥2 months and <6 years) who attended two private (n = 230) and one public (n = 270) pediatric clinics for routine check-up or sick visits in Niterói city, Rio de Janeiro, Brazil. The city has an estimated population of approximately 32,000 children under 6 years of age [10]. Children who had received at least one dose of the PCV13 were enrolled in the study. We then collected a single nasopharyngeal swab from each child. Their legal guardians responded to a questionnaire, which included pneumococcal vaccination history, demographic and clinical characteristics, using the mobile data collection application Magpi (Magpi, Washington, DC).

2.2. Pneumococcal isolates

Collection and transport of nasopharyngeal specimens, as well as isolation and identification of the pneumococcal isolates were performed as previously described [9].

2.3. Determination of capsular types

We deduced the capsular types of the pneumococcal isolates by sequential multiplex PCR [11]. Serotypes within serogroup 6 isolates were identified by Quellung reaction with antisera kindly provided by the Centers for Disease Control and Prevention.

2.4. Antimicrobial susceptibility testing

We used the disk-diffusion method to determine the antimicrobial susceptibility profile of the pneumococcal isolates [12]. Nine antimicrobial agents were tested: chloramphenicol (30 μg), clindamycin (2 μg), erythromycin (15 μg), levofloxacin (5 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). Minimum inhibitory concentrations (MICs) were determined by E-test® (BioMérieux, Marcy l'Etoile, France). We determined penicillin and ceftriaxone MICs among penicillin non-susceptible pneumococci (PNSP), as well as erythromycin MICs among erythromycin-resistant (ERY-R) isolates. Macrolide resistance phenotypes were investigated by the double-disk test [12].

2.5. Detection of erythromycin resistance genes

We investigated the presence of the *erm*(A), *erm*(B) and *mef*(A/E) genes by PCR among ERY-R isolates [13].

2.6. Statistical analyses

We evaluated the association of demographic and clinical characteristics with pneumococcal carriage with the Fisher's exact test using Epi Info™ version 7.2.2.6. The association was considered statistically significant when *p* value was <0.05.

2.7. Ethical considerations

This study was approved by the Ethics Committee of the *Universidade Federal Fluminense* (CAAE 26823614.2.0000.5243). Legal guardians of all participants provided a written informed consent.

3. Results

3.1. Study population

Thirty-eight (7.6%) of 500 children had received at least one dose of the PCV13. The majority (n = 36; 94.7%) attended private clinics. Twenty (52.6%) children were male and the overall median age was 1.9 years (IQR: 0.6 and 2.9 years old). The two children at the public clinic were male and aged 3.9 years and 5.7 years.

Number of doses and/or vaccination schedules varied among the participants. One child at the public clinic received three primary doses (3p) with PCV10 plus one booster dose (+1) with PCV13 and the other one got the full schedule (3p+1) with PCV13. Among the 36 children at the private clinics, 15 (41.7%) received the full schedule with PCV13, 12 (33.3%) received 3p with PCV13, three (8.3%) received 3p with PCV10+1 with PCV13, four (11.1%) received 2p with PCV13, one (2.8%) received 1p with PCV13 followed by two additional primary doses with PCV10, and one (2.8%) received 1p with PCV13. The 23 (63.9%) children who had not received the full vaccination schedule (3p+1) were not old enough for additional doses. All but one child got their respective last vaccine dose two weeks or earlier before the specimen collection; the only exception was a 6-month-old child who had received the third PCV13 primary dose within a week before the collection and was not a pneumococcal carrier.

3.2. Pneumococcal carriage

Since only two (0.7%) of the 270 children attending the public clinic received PCV13 doses, the main analyses focused on children who attended the private clinics, where 36 (15.7%) of 230 children were immunized with at least one dose of the PCV13.

Nine (25%) of the 36 children were colonized with *S. pneumoniae*. Additionally, all pneumococcal carriers had received the full vaccination schedule (3p+1); seven received all four doses with PCV13 and two received PCV13 only for the booster dose.

Characteristics independently associated with pneumococcal carriage were age ≥ 2 years (p = 0.01), presence of cough/expectoration (p = 0.01), and childcare center attendance (p < 0.01). Table 1 shows the demographic and clinical characteristics of the 36 children included in the analysis.

Table 1
Demographic and clinical characteristics of 36 children who had received at least one dose of the 13-valent pneumococcal conjugate vaccine out of 230 children attending private pediatric clinics in Niterói, RJ, Brazil, 2014.

Characteristic (n)	Pneumococcal carriage		*p*-value
	Yes (n = 9)	No (n = 27)	
Sex			0.12
Male (18)	2	16	
Female (18)	7	11	
Age			0.01
0–<2 years (22)	2	20	
2–<6 years (14)	7	7	
Self-reported ethnicity			1
White (33)	8	25	
Parda[a] (3)	1	2	
Symptomatology at time of interview[b]			0.44
Yes (18)	6	12	
No (18)	3	15	
Coryza/sneezing			0.27
Yes (14)	5	9	
No (22)	4	18	
Cough/expectoration			0.01
Yes (11)	6	5	
No (25)	3	22	
Asthma/bronchitis			1
Yes (2)	0	2	
No (34)	9	25	
Rhinitis			1
Yes (8)	2	6	
No (28)	7	21	
Antibiotic use in previous 2 weeks			1
Yes (4)	1	3	
No (32)	8	24	
Childcare center attendance			< 0.01
Yes (16)	8	8	
No (20)	1	19	
Has at least one sibling < 6 years old			1
Yes (4)	1	3	
No (32)	8	24	

[a] The closest translation for *parda* is 'mixed' in English.
[b] Fever, coryza/sneezing, cough/expectoration, fatigue/breathlessness, hypoactivity, vomit, and/or diarrhea.

3.3. Capsular types and antimicrobial susceptibility

Seven (77.8%) of the nine pneumococcal isolates were typed. They belonged to six different capsular types: 15B/C (two isolates), 6C, 11A/D, 16F, 23A, and 23F. Two isolates were non-typeable (NT).

All nine isolates were susceptible to chloramphenicol, levofloxacin, rifampicin, and vancomycin. Three (33.3%) isolates, belonging to serotypes 15B/C, 23A or NT, were susceptible to all antimicrobial agents tested. Three (33.3%) isolates were multidrug resistant (MDR) and they were resistant to three or four classes of antimicrobial agents. Two isolates (serotypes 11A/D and 15B/C) were resistant only to sulfamethoxazole/trimethoprim. Capsular types and resistance profiles of the four isolates that were resistant to at least two antimicrobial agents are shown in Table 2.

The four (44.4%) PNSP had penicillin and ceftriaxone MICs ranging from 0.12 to 4.0 μg/ml and 0.023–0.5 μg/ml, respectively. Following CLSI criteria, all isolates would be resistant to parenteral penicillin (meningitis breakpoint), one isolate would be intermediate to parenteral penicillin (nonmeningitis breakpoint), and two isolates would be intermediate and two would be resistant to oral penicillin V. On the other hand, all four isolates would be susceptible for both ceftriaxone meningitis and nonmeningitis breakpoints.

We found two (22.2%) ERY-R isolates. One displayed the $cMLS_B$ phenotype, with MIC of 256 μg/ml and the *erm*(B) gene. The other isolate had the M phenotype, MIC of 3 μg/ml and the *mef*(A/E) gene.

4. Discussion

We evaluated the direct effect of PCV13 vaccination on pneumococcal colonization among children attending one public and two private pediatric clinics in a large metropolitan area of Rio de Janeiro, Brazil. PCV13 coverage (around 8%) was low and the vaccine was almost exclusively used by children who attended the private clinics. Although immunization with PCV10 is free of charge via Brazilian NIP, pediatricians may recommend PCV13 vaccination if the parents can afford it, due to its higher serotype coverage. Despite the low number of children immunized with PCV13, the prevalence of colonization among children attending the private clinics analyzed here (9/36; 25%) was very similar to that observed among children vaccinated with PCV10 (115/422; 27%) from the same period and geographical region [9]. Frequencies of non-susceptibility to penicillin and erythromycin were also comparable.

Carriage was more likely among children ≥ 2 years old, who attended childcare centers and who presented with cough/expectoration. These factors have already been associated with pneumococcal colonization among Brazilian children after PCV10 introduction in Brazil [9,14]. Colonization was also much more common among female children (39% vs. 11%), but it was strongly related to a higher frequency of cough/expectoration among female (7/18; 38.9%) compared to male (4/18; 22%) children.

Only one child carried a PCV13 serotype (23F) and the isolate was non-susceptible to penicillin. In addition, a MDR serotype 6C, which is antigenically related to the PCV13 serotype 6A, was detected in another child. Of note, both children had received the full schedule (3p+1) of the PCV13.

Serotype 6C has emerged worldwide following PCV7 or PCV10 introduction [15,16], including in Brazil, associated with colonization among children [9,14] as well as IPD in both children and adults [17]. The emergence of serotype 6C associated with carriage in our geographical area was mainly due to the clonal expansion of the ST386 [18], which had exactly the same MDR profile observed in the isolate found here.

Other serotypes observed among the children investigated, such as 15B/C, 11A/D, and 23A have been commonly found in carriage and noninvasive pneumococcal diseases before and after PCV introduction worldwide [19–21], since they are not present in

Table 2
Characteristics of four pneumococcal isolates resistant to two or more antimicrobial agents recovered from children who received at least one dose of the 13-valent pneumococcal conjugate vaccine.

Isolate	Capsular type	Resistance (R+I) profile	MIC (μg/ml)			ERY Resistance	
			PEN	CRO	ERY	Phenotype	Genotype
027	23F	PEN+SXT	0.12	0.064	–	–	–
056	NT	ERY+PEN+SXT	4.0	0.5	3.0	M	*mef*(A/E)
094	16F	PEN+SXT+TET	2.0	0.047	–	–	–
146	6C	CLI+ERY+PEN+TET	0.5	0.023	>256	$cMLS_B$	*erm*(B)

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.

any PCV formulation available at the moment. However, their association with IPD is rare [6].

The PCV13 serotypes 3, 6A, and 19A, which are not included in the PCV10, were not detected in the population analyzed. Serotype 6A IPD cases have been decreasing in prevalence, even in countries using PCV10 [16,17]. However, it was the third most common (8.7%) serotype found among 115 children colonized with *S. pneumoniae* that had been immunized with PCV10 [9] in the same city and period investigated here; the prevalent serotypes in that study were 6C (15.7%) and 15B/C (11.3%).

In contrast, IPD caused by serotypes 3 and 19A increased in countries that implemented PCV7 or PCV10 [16,22–24], including Brazil, where they represent major causes of IPD, along with serotype 6C [17]. In addition, MDR serotype 19A isolates have been found colonizing children immunized with PCV10 in the study area [9]. Countries that replaced PCV7 by PCV13 reported a significant decrease in IPD caused by 19A and 6C. No effect, however, has been observed against serotype 3 [16,24,25].

Additionally, the detection of two (22.2%) NT isolates highlights the ability of pneumococci to evade all vaccine formulations currently available against this microorganism, since all of them are based on the polysaccharide capsule. This scenario gets even worst with one of the MDR NT isolates with the highest MICs for penicillin (4.0 μg/ml) and ceftriaxone (0.5 μg/ml) observed among the isolates.

The major limitation of the present study is the low frequency of children immunized with PCV13. However, it is a condition already expected among Brazilian populations due to the high costs of PCV13 doses. In addition, to the best of our knowledge, this is the first study to demonstrate the direct effect of the PCV13 on pneumococcal colonization among children in Brazil. Also, the children received different combination of vaccines and dosages and this may have impacted the colonization frequency.

5. Conclusions

Colonization with PCV13 serotype was rare. Despite similar direct impact of PCV10 and PCV13 on carriage prevalence and antimicrobial resistance frequencies, serotypes 6A and 19A were only found in children vaccinated with PCV10 in the study geographical region [9]. In addition, serotype 3, one of the most common IPD causes in Brazil at present along with serotype 19A, was not found. Increasing PCV13 coverage might help eliminate or reduce the frequency of major serotypes associated with IPD in Brazil. Carriage with MDR serotype 6C and NT isolates among PCV13-vaccinated children, however, requires close monitoring.

Authors' contributions

FPGN, LMT, and LWR contributed to the overall design of the study. CAAC, FPGN, and NTC participated in field and clinical aspects of the study. FPGN and NTC performed major experimental analyses. FPGN drafted the manuscript. CAAC, LMT, and LWR helped draft the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

None.

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