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Invasive disease potential of *Streptococcus pneumoniae* serotypes before and after 10-valent pneumococcal conjugate vaccine introduction in a rural area, southern Mozambique

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ABSTRACT

Background: Invasive pneumococcal disease (IPD) is a significant cause of morbidity and mortality among children worldwide. In April 2013, Mozambique introduced 10-valent PCV (PCV10) into the National Expanded Program on immunization using a three-dose schedule at 2, 3, and 4 months of age. We aimed to evaluate the invasive disease potential of pneumococcal serotypes among children in our region before and after PCV10 introduction.

Methods: We used data from ongoing population-based surveillance for IPD and cross-sectional pneumococcal carriage surveys among children aged <5 years in Manhiça, Mozambique. To determine the invasive disease potential for each serotype pre- and post-PCV10 introduction, odds ratios (OR) and 95% confidence intervals (95% CI) were calculated comparing serotype-specific prevalence in IPD and in carriage. For each serotype, OR and 95% CI > 1 indicated high invasive disease potential and OR and 95% CI < 1 indicated low invasive disease potential.

Results: In the pre-PCV10 period, 524 pneumococcal isolates were obtained from 411 colonized children and IPD cases were detected in 40 children. In the post-PCV10 period, 540 pneumococcal isolates were obtained from 507 colonized children and IPD cases were detected in 30 children. The most prevalent serotypes causing IPD pre-PCV10 were 6A (17.5%), 6B (15.0%), 14 (12.5%), 23F (10.0%) and 19F (7.5%), and post-PCV10 were 6A (36.7%), 13 (10%), 1 (10.0%), 6B (6.7%) and 19A (6.7%). Serotypes associated with high invasive disease potential pre-PCV10 included 1 (OR:22.3 [95% CI 2.0; 251.2]), 6B (OR:3.1 [95% CI 1.2; 8.1]), 14 (OR: 3.4 [95% CI 1.2; 9.8]) and post-PCV10 included serotype 6A (OR:6.1[95% CI 2.7; 13.5]).

Conclusion: The number of serotypes with high invasive disease potential decreased after PCV10 introduction. Serotype 6A, which is not included in PCV10, was the most common cause of IPD throughout the study and showed a high invasive potential in the post-PCV10 period.

1. Introduction

Streptococcus pneumoniae (pneumococcus) is a major cause of disease in children, and is associated with high morbidity and mortality worldwide [1]. In 2015, invasive pneumococcal disease (IPD) was responsible for an estimated 318,000 deaths in children under 5 years of age, with most of these deaths occurring in Africa and Southeast Asia [2]. Pneumococcus causes a wide spectrum of disease, ranging from non-invasive disease such as sinusitis, acute

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otitis media, and conjunctivitis to more severe disease such as pneumonia, bacteremia and meningitis [3]. Data from rural Mozambique prior to the introduction of 10-valent pneumococcal conjugate vaccine (PCV10) show an invasive pneumococcal disease (IPD) incidence of 245 cases per 100,000 child-years and a case fatality proportion of approximately 14% in children aged <5 years [4].

Pneumococcal carriage precedes disease and plays an important role in transmission [5]. In rural Mozambique, the prevalence of pneumococcal carriage was 80.5% in children <5 years of age before PCV10 introduction [6]. The polysaccharide capsule of the pneumococcus, which is the most important virulence factor and the target of pneumococcal vaccines, is highly heterogeneous, and based on its antigenic composition, more than 90 pneumococcal serotypes have been described to date [7]. Pneumococcal serotypes differ in their prevalence and invasive disease potential (capacity to cause invasive disease) [8,9]. Several studies from developed countries have calculated case to carrier ratio as a surrogate of invasive disease potential [10–12]. However, the study of invasive disease potential of pneumococcal serotype is highly complex, and robust surveillance of IPD and carriage is required for better understanding of the epidemiology of pneumococcal serotypes before and after PCV introduction [13].

Declines in both IPD and vaccine-type pneumococcal colonization have been observed after PCV introduction [14,15]. Despite these declines, pneumococcal colonization has remained stable after PCV introduction with a change in serotype distribution [16]. Data from the United Kingdom and Finland prior to PCV introduction have demonstrated that vaccine-types 1, 5, 6B, 7F, 14, 18C and 19A had higher invasive potential than non-vaccine types [10,17]. However, with the widespread use of high valent PCVs such as PCV10 and PCV13, it is possible that other non-vaccine serotypes will emerge with a high degree of invasive disease potential. Therefore, understanding the distribution of serotypes causing colonization and disease pre- and post-PCV introduction is important for evaluating disease potential of emerging serotypes. This information can be helpful for development of future vaccines.

Mozambique introduced PCV10 into the national routine infant immunization schedule in April 2013 using a 3-dose primary series at ages 2, 3 and 4 months with no booster or catch-up campaign. Coverage of three doses based on WHO-UNICEF estimates for 2014 and 2015 were 73% and 80%, respectively [18]. Using data from our ongoing population-based surveillance for IPD and cross-sectional pneumococcal carriage surveys in Manhiça, we evaluated the invasive disease potential of pneumococcal serotypes and changes in serotype distribution pre- and post-PCV10 introduction.

2. Methods

2.1. Study population and design

We used data from ongoing pediatric population-based surveillance for IPD among children aged <5 years in Manhiça District, a rural area in southern Mozambique with a high Human Immunodeficiency Virus (HIV) and malaria prevalence [19,20], to calculate the incidence rate by dividing the number of IPD cases caused by each serotype by the number of children aged <5 years in the demographic surveillance area. We used IPD data from April 2012 through March 2013 (pre-PCV10) and from April 2014 through March 2015 (post-PCV10), and two cross-sectional carriage surveys (October 2012–March 2013 and October 2014–March 2015) to calculate the invasive disease potential of individual serotypes. For the cross-sectional surveys, we randomly recruited HIV-uninfected children from the community using the Manhiça district Demographic Surveillance System (DSS) [21], while HIV-infected children were recruited sequentially from outpatient clinics at the Manhiça District Hospital until target enrollment number was met. The methods of the pediatric population-based surveillance for IPD and cross-sectional surveys have been previously described [4,6,22]. Data on PCV10 receipt were obtained from vaccination cards for children with IPD and those enrolled in the pneumococcal carriage survey post-PCV10 introduction.

2.2. Laboratory procedures

For the pneumococcal carriage surveys, a nasopharyngeal specimen (NP) using sterile calcium alginate swabs (Fisherbrand, catalog number 14-959-77 and 14959-78) was obtained from all eligible children whose parent or guardian provided written informed consent. Children were not eligible to be enrolled if they had signs of severe acute respiratory illness at the time of enrollment; defined by increased respiratory rate and chest wall indrawing [23]. After collection, NP swabs were immediately placed in 1.0 ml skim milk-tryptone-glucose-glycerol (STGG) medium and kept on ice packs in a cooler box for up to 4 h until storage at −70 °C for subsequent processing. The NP specimens inoculated in STGG were processed using methods previously described [23].

Blood samples obtained from all children aged <2 years requiring hospitalization and from children aged 2-<15 years with axillary temperature ≥39 °C or any signs of severity except trauma were sent to Centro de Investigação em saúde de Manhica (CISM) laboratory for culture [24]. Cerebral spinal fluid (CSF) was collected via lumbar puncture for all children with suspected meningitis and admitted infants aged <29 days. CSF specimens were processed at CISM laboratory for culture and at the US Centers for Disease Control and Prevention (CDC) *Streptococcus* laboratory by quantitative PCR (qPCR) for the detection of pneumococcal *lytA* gene [25].

Suspected pneumococcal colonies from both IPD surveillance and carriage surveys were tested by optochin susceptibility (BBL Taxo; Becton Dickinson) and bile solubility 2% (Sodium deoxycholate, SIGMA-ALDRICH, Steinheim, Germany). Pneumococcal isolates were serotyped by Quellung reaction at CDC's *Streptococcus* Laboratory. Nontypeable pneumococcal isolates from carriage surveys underwent qPCR for pneumococcal *lytA* gene detection followed by conventional multiplex PCR for pneumococcal serotyping [23,25].

2.3. Definitions

An IPD case was defined as isolation of pneumococcus from a normally sterile site (blood or CSF) in a child aged <5 years admitted to Manhiça District Hospital who was a resident of the surveillance catchment area. IPD cases with pneumococcus detected in a CSF specimen (CSF only or in both CSF and blood) were classified as pneumococcal meningitis; all other cases with pneumococcus isolated from blood were classified as bacteremia. Among IPD cases classified as bacteremia, those in children with cough and/or breathing difficulties plus tachypnea were further determined to be bacteremic pneumonia. Isolates of serotype 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F were defined as PCV10-serotypes; isolates of serotype 3, 6A and 19A were defined as PCV13 unique serotypes, and all other isolates were defined as non-vaccine serotypes. Anthropometric measurements were taken for all children. Weight for age Z-score (waz) was calculated using WHO Anthro Software and Macro [26]. Malnutrition status was defined based WHO child growth chart standards [27].

2.4. Data analysis

We used STATA v.13 software program (SataCorp, College Station, TX, USA) and MS Excel 2016 (Microsoft Corp. Seattle, WA) to compare characteristics of children with IPD and pneumococcal carriage in the pre- and post-PCV10 period. Proportions were compared using chi-square or Fisher exact tests, when appropriate. Incidence of IPD by serotype for each PCV10 period was calculated using child-years as the denominator and 95% confidence intervals (95%CI) were obtained using Poisson distribution. The invasive disease potential of serotypes was estimated using odds ratios (ORs) and 95% CI. ORs and 95%CIs were calculated for each individual serotype using Open Source Epidemiologic Statistics; individual serotypes were treated as a binary outcome. The formula for the OR calculation was *ad/bc* where *a* = number of serotype-specific invasive isolates, *b* = number of serotype-specific carriage isolates, *c* = other invasive isolates, and *d* = other carriage isolates. For each serotype, OR > 1 indicated high probability to cause invasive disease and OR < 1 indicated low probability to cause invasive disease. An OR was considered statistically significant when CIs limits did not include 1 [17,28].

2.5. Ethical considerations

The Institutional Review Boards (IRB) of the Mozambican bioethics committee and the US Centers for Disease control and Prevention reviewed and approved the protocols for the pneumococcal carriage surveys. The IPD surveillance was reviewed in accordance with CDC human research protection procedures and was determined to be non-research, public health surveillance, while IRB approval for IPD surveillance was obtained from the Mozambique bioethics committee. Written informed consent was required for all carriage survey participants.

3. Results

3.1. Patient characteristics

A total of 1098 children were enrolled in the pneumococcal carriage surveys, including 506 during the pre- and 592 during the post-PCV period. The prevalence of pneumococcal carriage was 81.2% (411/506) and 85.6% (507/592) in the pre- and post-PCV periods, respectively; for a total of 424 and 540 pneumococcal isolates. Some colonized children had more than one pneumococcal isolate. A total of 70 pneumococcal isolates were obtained from children with IPD. Of those, 40 (57.1%) were obtained in the pre-PCV period and 30 (42.9%) in the post-PCV period. Bacteremic pneumonia represented 19 (47.5%) and 14 (46.7%) of the IPD cases pre- and post-PCV, respectively. Among 27 cases of IPD in children for whom HIV status was known, 38.5% (5/13) and 35.7% (5/14) were in HIV-infected children in the pre-PCV and post-PCV periods, respectively. Among children with pneumococcal colonization detected in the carriage surveys, 165 (40.2%) and 336 (66.3%) were HIV-infected in the pre- and post-PCV periods, respectively. The case fatality ratio among children with IPD was 15.0% (6/40) in the pre- and 3.0% (1/30) in the post-PCV period (Table 1).

3.2. Incidence and serotype distribution

Changes in IPD incidence caused by PCV10 serotypes were observed from the pre- to post-PCV period. After PCV10 introduction, no IPD cases caused by serotypes 4, 9V, 14 and 18C, which are included in PCV10, were observed. Incidence of IPD caused by serotype 1 increased. However, this increase was not statistically significant. Incidence of IPD caused by serotypes 6A and 19A, which are not included in PCV10, increased in the post-PCV period (Fig. 1).

Table 1
Demographic characteristics of children with invasive pneumococcal disease (IPD) and pneumococcal colonization pre- and post-PCV10 introduction in Mozambique.

Characteristics	Pre-PCV10			Post-PCV10		
	IPD N = 40 n (%)	Pneumococcal colonization N = 411 n (%)	*P value*	IPD N = 30 n (%)	Pneumococcal colonization N = 507 n (%)	*P value*
Sex, male[1]	25 (62.5)	211 (51.3)	0.177	18 (62.1)	247 (48.7)	0.230
Age (months)						
Median age (interquartile range)	12 (7–27.5)	25.1 (13.1–42.2)	<0.001	19.5 (13–30)	24.5 (13–41.2)	0.245
Age group[2]			0.001			0.275
0–11	20 (50.0)	93 (23.1)		7 (20.7)	110 (22.0)	
12–23	8 (20.0)	103 (25.6)		12 (41.4)	139 (27.7)	
24–59	12 (30.0)	207 (51.4)		11 (37.9)	252 (50.3)	
Malnutrition[3]			<0.001			<0.001
None	23 (59.0)	343 (85.0)		15 (48.4)	453 (89.9)	
Moderate	6 (15.4)	44 (10.9)		8 (22.6)	42 (8.3)	
Severe	10 (25.6)	18 (4.4)		7 (29.0)	9 (3.0)	
HIV status[4]			0.903			0.018
Positive	5 (38.5)	165 (40.2)		5 (35.7)	336 (66.3)	
Negative	8 (61.5)	246 (59.9)		9 (64.3)	171– (33.7)	
PCV10 Doses[5]						0.354
0 Doses	NA	NA		2 (14.3)	19 (8.2)	
1–2 Doses	NA	NA		3 (21.4)	32 (13.7)	
3 Doses	NA	NA		9 (64.3)	182 (78.1)	
Clinical manifestation						NA
Bacteremic pneumonia	19 (47.5)	NA		14 (46.7)	NA	
Bacteremia without pneumonia	18 (45.0)	NA		15 (50)	NA	
Meningitis	3 (7.5)	NA		1 (3.3)	NA	
Mortality	6 (15.0)	NA		1 (3.3)	NA	

NA: Not applicable
[1] Post-PCV10: One child with IPD missing sex information.
[2] Pre-PCV10: Eight children with pneumococcal colonization missing age/Post-PCV10: Six children with pneumococcal colonization missing age.
[3] Pre-PCV10: One children with IPD and 6 with pneumococcal colonization missing weight for age Z-score (waz) calculation /Post-PCV: Three children with pneumococcal colonization missing weight for age Z-score (waz) calculation.
[4] Pre-PCV10: Twenty-seven children with IPD missing HIV status/PostPCV10: Sixteen children with IPD missing HIV status.
[5] Post-PCV10: Sixteen children with IPD and 274 children with pneumococcal colonization not age eligible for PCV10.

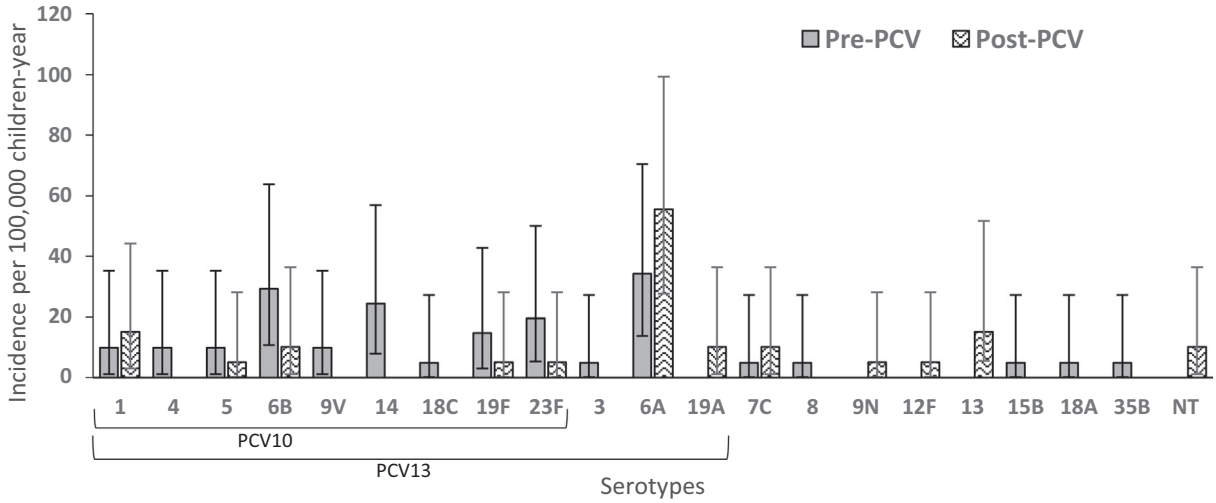

Fig. 1. Incidence of invasive pneumococcal disease by serotype pre-PCV10 (2012–2013) and post-PCV10 (2014–2015) introduction in Mozambique.

Of the 424 pneumococcal carriage isolates from the pre-PCV period, 42 different serotypes were obtained. The most frequent serotypes were 19F (13.9%), 23F (12.3%), 6A (10.14%), 13 (5.9%) and 6B (5.4%). Of the 540 pneumococcal carriage isolates obtained during the post-PCV period, 41 different serotypes were identified. The most frequent serotypes identified post-PCV were 6A (8.7%), 23F (8.0%), non-typeable (NT) (8.0%), 19A (7.0%) and 19F (6.9%). Carriage prevalence of serotypes 19F ($p = 0.001$) and serotype 23F ($p = 0.03$) declined significantly from the pre- to post-PCV period. Carriage prevalence of 19A significantly increased from 4.0% in the pre- to 7.0% in the post-PCV period ($p = 0.04$). Other serotypes with significant increases in prevalence post-PCV10 were 7C (5.4% versus 1.9%; $p = 0.005$), 10A (3.3% versus 0.7%; $p = 0.01$) and 11A (3.5% versus 1.2%; $p = 0.02$).

Among IPD cases, 16 different serotypes were identified in the pre-PCV period and 12 in the post-PCV period. In the pre-PCV period, the most frequent serotypes were 6A (17.5%), 6B (15.0%), 14 (12.5%), 23F (10.0%) and 19F (7.5%), while in the post-PCV period, serotypes 6A (36.7%), 13 (10.0%), 1 (10.0%), 6B (6.7%) and 19A (6.7%) were the most prevalent (Fig. 2).

3.3. Invasive disease potential

Among the serotypes identified in the pre-PCV period, high invasive potential was found for serotype 1 (OR: 22.3 [95% CI 2.0; 251.2]), 6B (OR: 3.1 [95% CI 1.2; 8.1]), and 14 (OR: 3.4 [95% CI 1.2; 9.8]). Among the serotypes identified in the post-PCV period, high invasive potential was found only for serotype 6A (OR: 6.1[95% CI 2.7; 13.5]) (Table 2).

4. Discussion

Two years after PCV10 introduction in Mozambique, the number of serotypes with high invasive disease potential decreased from three to one. All three serotypes with high invasive disease potential pre-PCV (1, 6B, 14) are included in the PCV10 formulation. The introduction of the vaccine was associated with a decline in these serotypes, and in the post-PCV10 period, serotype 6A (included in PCV13 but not PCV10) became the sole serotype with high invasive disease potential. Although increases in carriage and IPD caused by serotype 6A were observed after PCV10 introduction, these increases were not statistically significant. Immunogenicity studies have shown that PCV10 induces functional antibody against serotype 6A following the primary series, however; IgG geometric mean concentrations (GMCs) to serotype 6A was lower among children who received PCV10 compared to those who received PCV13 pre- and post-PCV booster dose [29]. In a recent study in Austria, no declines in IPD caused by serotype 6A were observed among children using a PCV10 schedule of two primary doses with a booster dose at 12 months of age [29,30]. Serotype 19A, which is also not included in PCV10, increased significantly in both carriage and IPD post-PCV10 introduction. An increase in 19A pneumococcal colonization in Mozambique after PCV10 introduction has been previously reported among children who received three primary doses of PCV10 [31]. No 19A IPD was observed in the pre-PCV10 period, however, from 2014 to 2015, the incidence of 19A IPD increased from zero to 10.1 per 100,000 child-years at risk. Despite these increases, serotype 19A was not associated with high invasive disease potential in the post-PCV10 period. Prior studies have reported 19A as a highly invasive strain and linked to antimicrobial resistance, which is a global patient safety concern [32,33].

The reduction in the number of serotypes associated with high invasive disease potential is concordant with findings from studies conducted in France and in the United States, which described a similar decrease in the number of serotypes with high invasiveness post-PCV introduction [13,34]. Several studies conducted in high- and low-resource countries showed that PCV introduction results in a sustained reduction of vaccine-type colonization and disease [15,35–37]. However, increases in non-vaccine type IPD after PCV introduction have been reported in Europe and have raised concerns [38-40]. Although we observed an increase in incidence of non-vaccine serotypes causing IPD post-PCV, especially serotypes 13, 12F and 9N, these serotypes were not associated with high invasive disease potential. Of these serotypes, 12F is of particular concern because other studies have shown that this serotype is highly invasive and is usually associated with invasive disease and meningitis outbreaks [32,41].

The serotypes with high invasive disease potential found in our study pre-PCV10 are similar to those reported by previous pre-PCV studies of *Streptococcus pneumoniae* conducted in Finland from 1995 to 1999 and in Portugal from 2001 to 2003, which also concluded that serotype 1, 6B and 14 were associated with high invasive disease potential [11,17]. We found serotype 6A to be the only serotype associated with high invasive disease potential in the post-PCV period. In contrast, studies from high-resource settings pre-PCV have shown that 6A has low invasive disease potential and is highly associated with carriage [17,42,43]. In a recent

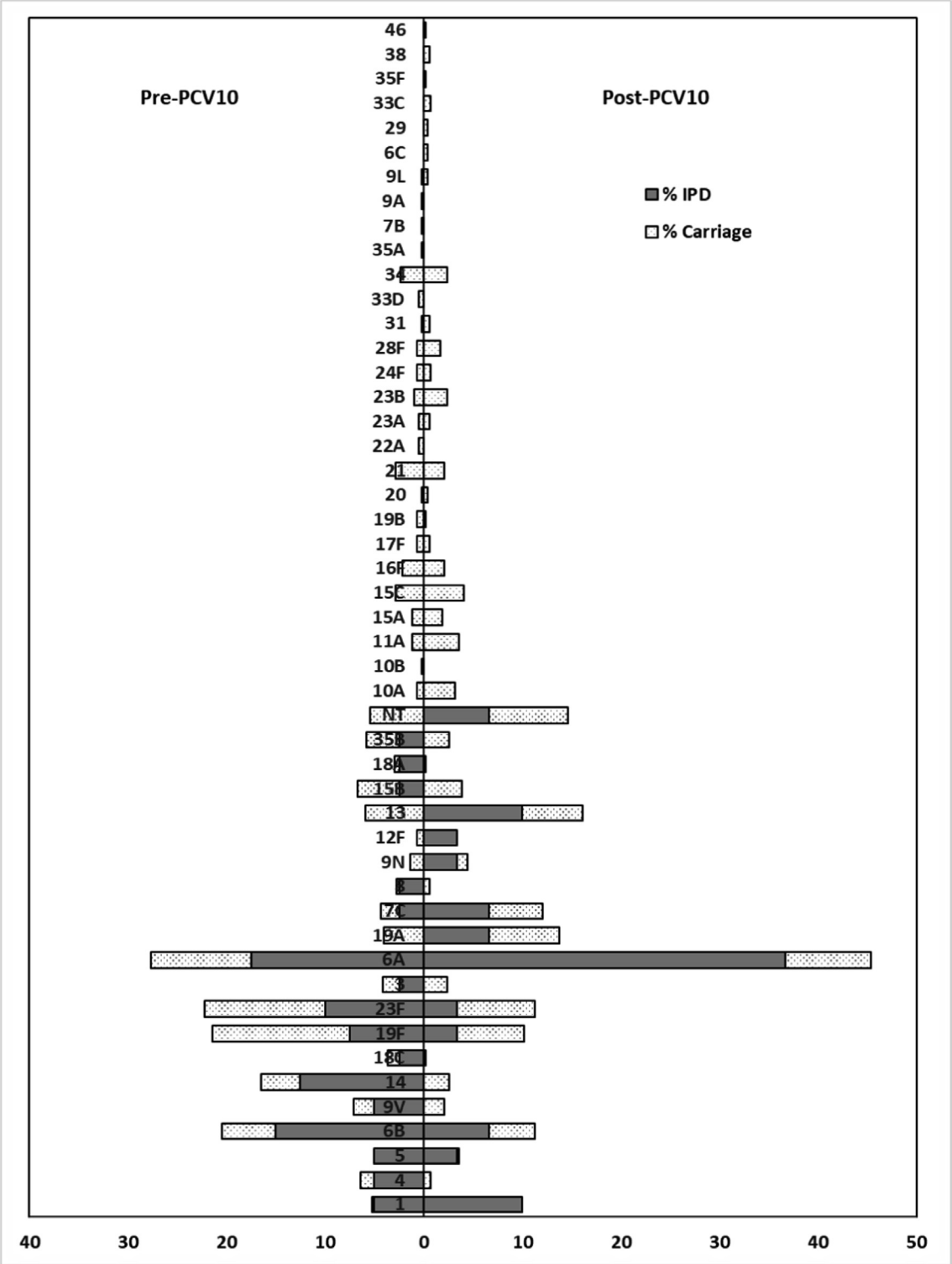

Fig. 2. Serotype distribution of nasopharyngeal pneumococcal colonization and invasive pneumococcal disease isolates pre-PCV10 (2012–2013 and post-PCV10 (2014–2015) introduction in Mozambique. (PCV10 serotypes: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F/PCV13 serotypes: 3, 6A, 19A, and PCV10 serotypes).

meta-analysis using studies conducted after PCV introduction, 6A was also found to have a low invasive disease potential [44]. However, this meta-analysis included countries using other PCV formulations and different vaccine schedules. In our study, serotype 6A was the predominant serotype causing IPD post-PCV10 period, which likely explain the association with high invasive disease potential we observed for this serotype. In 2017, Mozambique Ministry of Health recommended a switch from PCV10 to PCV13 for routine infant vaccination, which will provide coverage for both serotypes 6A and 19A [45]. It will be important to monitor the changes in serotype-specific disease incidence after PCV13 is nationally implemented into the infant immunization program.

We observed that several serotypes, especially non-vaccine types, were not associated with invasive disease. Several studies

Table 2
Invasive disease potential of pneumococcal serotypes among children aged <5 years pre- and post-PCV10 introduction in Mozambique.

Serotype	Pre-PCV10					Post-PCV10				
	Invasive	Carriage	OR	95% CI	*P value*	Invasive	Carriage	OR	95% CI	*P value*
1	2	1	**22.3**	**2.0–251.2**	**0.041**	3	0	NA	NA	NA
4	2	6	3.7	0.7–18.8	0.291	0	4	NA	NA	NA
5	2	0	NA	NA	NA	1	1	18.6	1.1–304.6	0.205
6B	6	23	**3.1**	**1.2–8.1**	**0.017**	2	25	1.5	0.3–6.5	0.845
9V	2	9	2.4	0.5–11.6	0.487	0	11	NA	NA	NA
14	5	17	**3.4**	**1.2–9.8**	**0.016**	0	14	NA	NA	NA
18C	1	5	2.1	0.2–18.9	0.839	0	1	NA	NA	NA
19F	3	59	0.5	0.1–1.7	0.375	1	37	0.5	0.1–3.5	0.779
23F	4	52	0.8	0.3–2.3	0.911	1	43	0.4	0.1–3.0	0.615
3	1	7	1.5	0.2–12.7	>0.910	0	13	NA	NA	NA
6A	7	43	1.9	0.8–4.5	0.152	**11**	**47**	**6.1**	**2.7–13.5**	**0.001**
19A	0	17	NA	NA	NA	2	38	0.9	0.2–4.1	>0.910
7C	1	8	1.3	0.2–10.9	>0.910	2	29	1.3	0.3–5.5	0.992
8	1	1	10.9	0.7–176.8	0.330	0	3	NA	NA	NA
9N	0	6	NA	NA	NA	1	6	3.1	0.4–26.3	0.633
12F	0	3	NA	NA	NA	1	0	NA	NA	NA
13	0	25	NA	NA	NA	3	33	1.7	0.5–5.9	0.585
15B	1	18	0.6	0.1–4.4	0.910	0	21	NA	NA	NA
18A	1	2	5.4	0.5–61	0.475	0	1	NA	NA	NA
35B	1	14	0.8	0.1–5.9	>0.910	0	14	NA	NA	NA
NT	0	23	NA	NA	NA	2	43	0.8	0.2–3.6	>0.910
Other[1]	0	85	NA	NA	NA	0	156	NA	NA	NA
Total	40	424				30	540			

95% CI: 95% confidence interval.
OR: Odds ratio.
NA: Not applicable.
[1] Pre-PCV10 Include serotypes: 7B, 9A, 9L, 10A, 10B, 11A, 15A, 15C, 16F, 17F, 19B, 20, 21, 22A, 23A, 23B, 24F, 28F, 31, 33D, 34, and 35A. Post-PCV10 include serotypes: 6C, 9L, 10A, 11A, 15A, 15C, 16F, 17F, 19B, 20, 21, 23A, 23B, 24F, 28F, 29, 31, 33C, 34, 35F, 38, and 46.

have shown that non-vaccine serotypes 11A, 15A, 15B/C, 16F, 23A, 23B, 34, 35F, 35B and 37 have a low invasive disease potential [11–13,34]. However, the emergence of non-vaccine serotypes with high invasive disease potential is possible, as was demonstrated in a recent Swedish study where serotype 8 and 22F were found to have high invasive disease potential 8 years after PCV introduction in the country [16].

Changes in the incidence of PCV10 IPD were observed, with no cases of IPD caused by serotypes 4, 9V, 14 and 18C in the post-PCV introduction and increases in incidence of IPD caused by serotypes 6A and 19A, which are not included in the PCV10. Another notable observation in our study was the significant reduction in the carriage prevalence of vaccine-types 19F and 23F in the post-PCV period. These findings are in concordance with previous studies conducted in England, which found a significant reduction of carriage of serotypes 19F and 23F after PCV7 implementation [38]. The reduction in carriage of vaccine-types likely contributed to the reduction in IPD incidence caused by these serotypes.

Our study has some limitations. First, we compared a relatively short period pre- and post-PCV10 introduction to align with our cross-sectional carriage surveys, which led to a small number of IPD isolates being included in the analysis. Second, we only have data on IPD from a rural site in Mozambique, and, therefore, our findings may not be generalizable to the entire country. Third, the use of OR only allows for comparison within the population and it is not an absolute value that allows comparison between population. Therefore, the high OR for 6A found in the post-PCV10 period may be related to the increase in prevalence of non-vaccine types with lower invasive disease potential. Finally, we did not assess invasive disease potential among HIV-infected compared with HIV-uninfected children because we did not have enough power. However, there was no difference in HIV status among those with IPD and pneumococcal colonization.

In conclusion, we have shown that after PCV10 introduction the number of serotypes associated with high invasive disease potential declined. Serotype 6A, which is not included in PCV10, became the main cause of pneumococcal colonization and disease post-PCV10 and had high invasive disease potential. Surveillance of both carriage and IPD can provide good insight into the invasive disease potential of pneumococcal serotypes pre- and post-PCV introduction, and can help inform future formulations of pneumococcal vaccines.

Disclaimer

The findings and conclusions of this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). The authors have no conflicts of interest to disclose.

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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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References

[1] O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. Lancet 2009;374:893–902. https://doi.org/10.1016/S0140-6736(09)61204-6.

[2] Wahl B, O'Brien KL, Greenbaum A, Majumder A, Liu L, Chu Y, et al. Burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000–15. Lancet Glob Heal 2018;6:e744–57. https://doi.org/10.1016/S2214-109X(18)30247-X.

[3] Simell B, Auranen K, Käyhty H, Goldblatt D, Dagan R, O'Brien KL. The fundamental link between pneumococcal carriage and disease. Exp Rev Vacc 2012;11:841–55. https://doi.org/10.1586/erv.12.53.

[4] Sigauque B, Verani J, Massora S, Vubil D, Acacio S, Nhampossa T, et al. Burden of invasive pneumococcal disease among children in rural Mozambique: 2001–2012. PLoS ONE 2018;13:1–13.

[5] Bogaert D, De Groot R, Hermans PWM. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. Lancet Infect Dis 2004;4:144–54. https://doi.org/10.1016/S1473-3099(04)00938-7.

[6] Verani JR, Massora S, Aca S, Santos RT, Vubil D, Pimenta F, et al. Nasopharyngeal carriage of Streptococcus pneumoniae among HIV-infected and – uninfected children <5 years of age before introduction of pneumococcal conjugate vaccine in Mozambique. PLoS ONE 2018;13:1–12. https://doi.org/10.1371/journal.pone.0191113.

[7] Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, et al. Pneumococcal capsules and their types: past, present, and future. Clin Microbiol Rev 2015;28:871–99. https://doi.org/10.1128/CMR.00024-15.

[8] Brueggemann AB, Griffiths DT, Meats E, Peto T, Crook DW, Spratt BG. Clonal relationships between invasive and carriage *Streptococcus pneumoniae* and serotype- and clone-specific differences in invasive disease potential. J Infect Dis 2003;187:1424–32. https://doi.org/10.1086/374624.

[9] del Amo E, Selva L, de Sevilla MF, Ciruela P, Brotons P, Triviño M, et al. Estimation of the invasive disease potential of Streptococcus pneumoniae in children by the use of direct capsular typing in clinical specimens. Eur J Clin Microbiol Infect Dis 2015;34:705–11. https://doi.org/10.1007/s10096-014-2280-y.

[10] Brueggemann AB, Peto TEA, Crook DW, Butler JC, Kristinsson KG, Spratt BG. Temporal and geographic stability of the serogroup- specific invasive disease potential of. J Infect Dis 2004;190:1203–11.

[11] Sá-Leao R, Pinto F, Aguiar S, Nunes S, Carriço Ja, Frazao N, et al. Analysis of invasiveness of pneumococcal serotypes and clones circulating in portugal before widespread use of conjugate vaccines reveals heterogeneous behavior of clones expressing the same serotype. J Clin Microbiol 2011;49:1369–75. doi:10.1128/JCM.01763-10.

[12] del Amo E, Brotons P, Monsonis M, Triviño M, Iñigo M, Selva L, et al. High invasiveness of pneumococcal serotypes included in the new generation of conjugate vaccines. Clin Microbiol Infect 2014;20:684–9. https://doi.org/10.1111/1469-0691.12422.

[13] Yildirim I, Little BA, Finkelstein J, Lee G, Hanage WP, Shea K, et al. Surveillance of pneumococcal colonization and invasive pneumococcal disease reveals shift in prevalent carriage serotypes in Massachusetts' children to relatively low invasiveness. Vaccine 2017;35:4002–9. https://doi.org/10.1016/j.vaccine.2017.05.077.

[14] von Gottberg A, de Gouveia L, Tempia S, Quan V, Meiring S, von Mollendorf C, et al. Effects of vaccination on invasive pneumococcal disease in South Africa. N Engl J Med 2014;371:1889–99. https://doi.org/10.1056/NEJMoa1401914.

[15] Desai AP, Sharma D, Crispell EK, Baughman W, Thomas S, Tunali A, et al. Decline in Pneumococcal Nasopharyngeal carriage of vaccine serotypes after the introduction of the 13-valent pneumococcal conjugate vaccine in children in Atlanta, Georgia. Pediatr Infect Dis J 2015;34:1168–74. https://doi.org/10.1097/INF.0000000000000849.

[16] Lindstrand A, Galanis I, Darenberg J, Morfeldt E, Naucler P, Blennow M, et al. Unaltered pneumococcal carriage prevalence due to expansion of non-vaccine types of low invasive potential 8 years after vaccine introduction in Stockholm, Sweden. Vaccine 2016;34:4565–71. https://doi.org/10.1016/j.vaccine.2016.07.031.

[17] Hanage WP, Kaijalainen TH, Syrjänen RK, Auranen K, Leinonen M, Mäkelä PH, et al. Invasiveness of serotypes and clones of Streptococcus pneumoniae among children in Finland. Infect Immun 2005;73:431–5. https://doi.org/10.1128/IAI.73.1.431-435.2005.

[18] WHO. Summary, WHO vaccine-preventable diseases: monitoring system. 2018 global summary; 2018.

[19] González R, Munguambe K, Aponte JJ, Bavo C, Nhalungo D, Macete E, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. HIV Med 2012;13:581–8. https://doi.org/10.1111/j.1468-1293.2012.01018.x.

[20] Saúte F, Aponte J, Almeda J, Ascaso C, Abellana R, Vaz N, et al. Malaria in southern Mozambique: definition in Manhica district malariometric indicators and malaria case. Trans R Soc Trop Med Hyg 2003:661–6.

[21] Sacoor C, Nhacolo A, Nhalungo D, Aponte JJ, Bassat Q, Augusto O, et al. Profile: Manhiça health research centre (Manhiça HDSS). Int J Epidemiol 2013;42:1309–18. https://doi.org/10.1093/ije/dyt148.

[22] Roca A, Sigaúque B, Quintó L, Mandomando I, Vallès X, Espasa M, et al. Invasive pneumococcal disease in children<5 years of age in rural Mozambique. Trop Med Int Health 2006;11:1422–31. https://doi.org/10.1111/j.1365-3156.2006.01697.x.

[23] Carvalho MDG, Pimenta FC, Jackson D, Roundtree A, Ahmad Y, Millar EV, et al. Revisiting pneumococcal carriage by use of broth enrichment and PCR techniques for enhanced detection of carriage and serotypes. J Clin Microbiol 2010;48:1611–8. https://doi.org/10.1128/JCM.02243-09.

[24] Sigaúque B, Roca A, Mandomando I, Morais L, Quintó L, Sacarlal J, et al. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. Pediatr Infect Dis J 2009;28:108–13. https://doi.org/10.1097/INF.0b013e318187a87d.

[25] Carvalho GS, Tondella ML, Mccaustland K, Weidlich L, Mcgee L, Mayer LW, et al. Evaluation and improvement of real-time PCR assays targeting lytA, ply, and psaA Genes for Detection of Pneumococcal DNA. J Clin Microbiol 2007;45:2460–6. https://doi.org/10.1128/JCM.02498-06.

[26] World Health Organization. Child growth standards. WHO Anthro and macros 2011:Available from: <http://www.who.int/nutgrowthdb/software/en/> [accessed March 10, 2019].

[27] WHO. WHO child growth standards and the identification of severe acute malnutrition in infants and children; 2009. p. 1–12.

[28] Shouval DS, Greenberg D, Givon-Lavi N, Porat N, Dagan R. Site-specific disease potential of individual Streptococcus pneumoniae serotypes in pediatric invasive disease, acute otitis media and acute conjunctivitis. Pediatr Infect Dis J 2006;25:602–7. https://doi.org/10.1097/01.inf.0000220231.79968.f6.

[29] Pomat WS, van den Biggelaar AHJ, Wana S, Francis JP, Solomon V, Greenhill AR, et al. Safety and immunogenicity of pneumococcal conjugate vaccines in a high-risk population: a randomized controlled trial of 10-valent and 13-valent pneumococcal conjugate vaccine in Papua New Guinean Infants. Clin Infect Dis 2018:1–10. https://doi.org/10.1093/cid/ciy743.

[30] Richter IdL, Schmid D, Kanitz EE, Zwazl I, Pö Llabauer E, Jasinska J, et al. Invasive pneumococcal diseases in children and adults before and after introduction of the 10-valent pneumococcal conjugate vaccine into the Austrian national immunization program. PLoS ONE 2019;14:1–21. https://doi.org/10.1371/journal.pone.0210081.

[31] Sigaúque B, Moiane B, Massora S, Pimenta F, Verani JR, Mucavele H, et al. Declines in vaccine type pneumococcal carriage in children less than 5 years old after introduction of 10-valent. Pediatr Infect Dis J 2018;37:1054–60. https://doi.org/10.1097/INF.0000000000002134.

[32] Song JY, Nahm MH, Moseley MA. Clinical implications of pneumococcal serotypes: invasive disease potential, clinical presentations, and antibiotic resistance. J Korean Med Sci 2013;28:4–15. https://doi.org/10.3346/jkms.2013.28.1.4.

[33] Isturiz R, Sings HL, Hilton B, Arguedas A, Reinert R-R, Jodar L. Streptococcus pneumoniae serotype 19A: worldwide epidemiology. Expert Rev Vaccines 2017;16:1007–27. https://doi.org/10.1080/14760584.2017.1362339.

[34] Varon E, Cohen R, Béchet S, Doit C, Levy C. Invasive disease potential of pneumococci before and after the 13-valent pneumococcal conjugate vaccine implementation in children. Vaccine 2015;33:6178–85. https://doi.org/10.1016/j.vaccine.2015.10.015.

[35] Navne JE, Koch A, Slotved HC, Andersson M, Melbye M, Ladefoged K, et al. Effect of the 13-valent pneumococcal conjugate vaccine on nasopharyngeal carriage by respiratory pathogens among greenlandic children. Int J Circumpolar Health 2017;76:1–8. https://doi.org/10.1080/22423982.2017.1309504.

[36] Hammitt LL, Akech DO, Morpeth SC, Karani A, Kihuha N, Nyongesa S, et al. Population effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of Streptococcus pneumoniae and non-typeable Haemophilus influenzae in Kilifi, Kenya: findings from cross-sectional carriage studies. Lancet Glob Heal 2014;2:e397–405. https://doi.org/10.1016/S2214-109X(14)70224-4.

[37] Mackenzie GA, Hill PC, Jeffries DJ, Hossain I, Uchendu U, Ameh D, et al. Effect of the introduction of pneumococcal conjugate vaccination on invasive pneumococcal disease in The Gambia: a population-based surveillance study. Lancet Infect Dis 2016;16:703–11. https://doi.org/10.1016/S1473-3099(16)00054-2.

[38] Flasche S, van Hoek AJ, Sheasby E, Waight P, Andrews N, Sheppard C, et al. Effect of pneumococcal conjugate vaccination on serotype-specific carriage and invasive disease in England: a cross-sectional study. PLoS Med 2011;8:1–9. https://doi.org/10.1371/journal.pmed.1001017.

[39] Miller E, Andrews NJ, Waight P a., Slack MPE, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate

vaccination in England and Wales: an observational cohort study. Lancet Infect Dis 2011;11:760–8. doi:10.1016/S1473-3099(11)70090-1.

[40] Ladhani SN, Collins S, Djennad A, Sheppard CL, Borrow R, Fry NK, et al. Rapid increase in non-vaccine serotypes causing invasive pneumococcal disease in England and Wales, 2000–17: a prospective national observational cohort study. Lancet Infect Dis 2018;18:441–51. https://doi.org/10.1016/S1473-3099(18)30052-5.

[41] Rakov AV, Ubukata K, Robinson DA. Population structure of hyperinvasive serotype 12F, clonal complex 218 Streptococcus pneumoniae revealed by multilocus boxB sequence typing. Infect Genet Evol 2011;11:1929–39. https://doi.org/10.1371/journal.pone.0150442.

[42] Zemlickova H, Jakubu V, Urbaskova P, Motlova J, Musilek M, Adamkova V. Serotype-specific invasive disease potential of Streptococcus pneumoniae in Czech children. J Med Microbiol 2010;59:1079–83. https://doi.org/10.1099/jmm.0.018390-0.

[43] Kronenberg A, Zucs P, Droz S, Mühlemann K. Distribution and invasiveness of Streptococcus pneumoniae serotypes in Switzerland, a country with low antibiotic selection pressure, from 2001 to 2004. J Clin Microbiol 2006;44:2032–8. https://doi.org/10.1128/JCM.00275-06.

[44] Balsells E, Dagan R, Yildirim I, Gounder PP, Steens A, Muñoz-Almagro C, et al. The relative invasive disease potential of Streptococcus pneumoniae among children after PCV introduction: a systematic review and meta-analysis. J Infect 2018;77:368–78. https://doi.org/10.1016/j.jinf.2018.06.004.

[45] Ministério da Saúde. Comité de Peritos de imunização (CoPI) Décima Primeira Reunião. Recomendacao Sobre a mudanca da vacina PCV10 para PCV13. Recomendacao R01/11/2016; 2016. p. 0–2.