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Impact of the 13-Valent Pneumococcal Conjugate Vaccine on Pneumococcal Carriage Among American Indians

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Abstract

Background—Community-wide impact of pneumococcal conjugate vaccines (PCV) is conferred by reductions in vaccine-type nasopharyngeal carriage. We evaluated the impact of PCV13 on carriage of PCV13-specific types (1, 3, 5, 6A, 7F, 19A) and 6C among American Indians.

Methods—A nasopharyngeal specimen was collected from community members of all ages between January 2010 and April 2012 (3 months before and 24 months after PCV13 introduction). Pneumococci were isolated by culture and serotyped using antisera. Monthly carriage prevalence and PCV13 coverage was calculated to identify the timing of vaccine impact relative to PCV13 introduction. Prevalence ratios (PR) were used to compare PCV13-specific carriage before and in Years 1 and 2 of PCV13 use. Coverage was calculated according to age and number of doses received.

Results—6645 participants (2859 <5 and 3786 ≥5 years) provided 6628 specimens. A decline in PCV13-specific and type 6C carriage among children <5 years of age was observed nine and 15 months after PCV13 introduction, respectively. Among under-immunized children, a decline in PCV13-specific carriage was observed 11 months after PCV13 introduction, when coverage in the community reached 58%. In Year 2 of PCV13 use, PCV13-specific and 6C carriage were reduced by 60% and 70%, respectively (PCV13-specific: PR=0.4, p<0.001; 6C: PR=0.3; p<0.001) among children <5 years of age. The reduction in PCV13-specific carriage among those 5–<8 years and 18+ years of age in Year 2 of PCV13 use was not statistically significant.

Conclusions—PCV13 reduced PCV13-specific and 6C carriage among children <5 years of age. Low pre-PCV13 carriage prevalence of PCV13-specific types limited confirming this reduction for adults.

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Keywords

pneumococcus; carriage; PCV13; American Indian; pneumococcal conjugate vaccine

Background

Routine use of 7-valent pneumococcal conjugate vaccine (PCV7), which began in October 2000 among American Indian (AI) communities in the United States (US), nearly eliminated PCV7-type invasive disease and nasopharyngeal (NP) carriage in the pediatric population age-eligible for vaccine use and among older age groups not targeted for vaccination [1, 2]. Although vaccine-type invasive pneumococcal disease (IPD) rates among AI children <5 years substantially decreased with PCV7 use, the residual all-serotype IPD rates remain disproportionately high compared to those of the general US population (49 versus 9 per 100,000 in 2012) [3,4].

A majority of AI children carry pneumococci in their nasopharynx and children are primarily responsible for introducing and transmitting pneumococci within households and communities [5-7]. Routine use of PCV in children provides indirect protection against vaccine-type carriage and disease among unimmunized individuals [8-10]. AI experienced partial replacement carriage with non-vaccine serotypes (NVTs) following PCV7 introduction, resulting in a net reduction in overall pneumococcal carriage [2], in contrast to other US populations that experienced complete PCV7 serotype carriage replacement [11, 12]. Among Navajo AI children in the PCV7-era, the rate of IPD increased for some NVT strains (i.e., 1, 3, 7F, 19A) and decreased for others (i.e., 5, 18B), but the overall NVT-IPD rate did not change [1]. In multiple US settings, serotypes 7F and 19A were leading causes of IPD and predominant colonizing serotypes in the PCV7-era [2, 13-15].

In March 2010, the United States Advisory Committee on Immunization Practices (ACIP) recommended replacing PCV7 with a 13-valent vaccine (PCV13) [16]. This switch provided the opportunity to evaluate the direct and indirect impact of PCV13 in Navajo and White Mountain Apache communities, a population at high-risk for carriage and disease, and to understand the pace of that impact relative to community vaccine coverage. In addition, the high prevalence of types 19A and 6C carriage among this population enabled an assessment of the impact of PCV13 on these added serotypes.

Methods

PCV13 Use

The study was conducted on the Navajo Nation and the White Mountain Apache reservation located in the southwestern United States. These communities are served by clinics and hospitals with staff from the US Indian Health Service (IHS), an agency of the US Department of Health and Human Services. The switchover from PCV7 to PCV13 occurred between March 25, 2010 and May 11, 2010; when PCV13 was received at a facility, unused PCV7 was returned to the manufacturer resulting in a rapid, complete switch to PCV13. PCV13 was used in three ways: to immunize the birth cohort; to complete the vaccine series

in infants who were partially immunized with PCV7; and as a one-dose catch-up for those <5 years of age who had already completed their 4-dose PCV7 schedule.

Nasopharyngeal carriage

A prospective, cross-sectional, household-based study of nasopharyngeal (NP) carriage was conducted over a 27-month period (January 2010 – March 2012) among AI families living on or around the Navajo and White Mountain Apache reservations. Children 7-<24 months of age (index participants) and their family members and close contacts were enrolled. Close contacts included relatives or friends residing in the same family compound or who lived elsewhere but had substantial contact with the index child. Index children were identified through preventive health care visits at IHS clinics and through IHS birth records. Household and individual characteristics, including the presence of respiratory symptoms, were documented at enrollment and participant's medical charts were reviewed for vaccination history and for antibiotic use occurring within 14 days before NP swab collection.

Laboratory analysis

NP specimens were collected in accordance with WHO recommendations [17] using Dacron swabs. Swabs were inoculated into 1 mL of skim milk, tryptone, glucose, glycerol (STGG) transport media. Specimens were snap-frozen in liquid nitrogen and stored at -70° C. STGG samples were transported to Case Western Reserve University, where they were cultured as follows: 200 µL of STGG was transferred to 5 mL of Todd Hewitt broth containing 0.5% yeast extract and 1 mL of rabbit serum and incubated for 6 hours at 37°C for broth enrichment. 10 µL of enriched broth was then plated onto gentamicin blood agar plates [18]. Pneumococci were identified using standard methods. A single colony of each morphology was selected, grown in pure culture and serotyped by the capsular swelling reaction using commercial antisera (Statens Serum Institut, Copenhagen, Denmark).

Vaccine coverage analysis

PCV13 coverage was estimated among study participants and for the full community of children <5 years of age using electronic health records for vaccination status, and the IHS User Population for the community denominator. Monthly coverage (coverage achieved on the last day in each month) was computed from March 2010 through March 2012 for children who were 7-<12 months, 12-<16 months and 16-<60 months of age at any time during the month. Fully-immunized children were defined as those age 7-<12 months with 3+ doses, age 12-<16 months with 3+ doses by <12 months or 1+ dose(s) if given after >12 months, age 16-<60 months with 1+ dose(s) if given after >12 months. Coverage was calculated by counting the number of PCV13 doses received by each child by the last day of each month divided by the total number of children in the age group at the end of that month. Age-standardized coverage estimates were calculated to reflect the age distribution of the community allowing coverage comparisons between study participants and children in the community <5 years of age.

Statistical analysis

Serotypes unique to PCV13 were defined as PCV13-specific types (i.e. 1, 3, 5, 6A, 7F and 19A). Differences in individual and household characteristics were compared by chi-square or t-tests. Monthly PCV13-specific type carriage prevalence and 95% confidence intervals (CI) were estimated to identify the timing of PCV13-specific type carriage reductions and the level of community coverage associated with the indirect effects of PCV13 use. Carriage prevalence for the pre-PCV13 period was estimated as the average carriage in January-March 2010. Carriage prevalence in the PCV13 period was estimated as the three-month rolling average (April 2010-March 2012) for study participants <5, 5-17 and 18+ years of age. The three-month rolling average was calculated using counts from the month of interest plus those from one month before and one month after divided by the total count of participants enrolled over that three-month period. The coverage level associated with a significant decline in PCV13-specific type carriage prevalence was defined as the community PCV13 coverage observed when the 95% confidence interval for the pre-PCV13 carriage prevalence no longer overlapped with the confidence intervals for the monthly carriage prevalence thereafter.

We calculated age-stratified prevalence ratios (PR) to compare the change in carriage prevalence before and after PCV13 introduction. The PR were calculated by comparing all-serotype and PCV13-specific type carriage from a previously published, PCV7-era 12-month observation period in this same population (September 2006-August 2007 [2]) to that of PCV13 Year 1 use (April 2010-March 2011) and PCV13 Year 2 use (April 2011-March 2012). This “before-after” annual prevalence comparison was used for analyses of serotype-specific changes and to account for seasonality, neither of which was possible using only the 3-month period immediately prior to the PCV13 switch. An insufficient number of children 8-17 years were enrolled in the PCV7-era study to allow comparison with the PCV13-era. We used carriage observed in the three-month period immediately preceding PCV13 introduction (January 2010-March 2010) to validate that carriage prevalence in the PCV7-era (2006-2007) had not substantially changed by 2010 (See Table, Supplemental Digital Content 1, for comparison of pre-PCV13 periods). Potential confounders were identified by testing the association between study population characteristics and study period by logistic regression; variables with a p-value of <0.1 were included in the model. Adjusted PR were estimated using log-binomial regression following a backward selection process using Akaike Information Criteria to select the best model. The adjusted prevalence ratios differed from the unadjusted values by less than 5%; therefore, the unadjusted ratios are presented. Correlations between household members were accounted for using a robust variance estimator. A Bonferroni correction was applied for multiple comparisons of individual serotypes and a p-value <0.003 was considered statistically significant. Analyses were completed in STATA (Version 12.0, StataCorp, College Station, Texas).

Ethical approvals

Approval was given by the Navajo and White Mountain Apache communities and from the Institutional Review Boards of Johns Hopkins Bloomberg School of Public Health, the IHS Phoenix Area and the Navajo Nation. Participating adults and parents of participating children provided written informed consent and children 7-17 years provided assent.

Results

Study population

From January 14, 2010 through April 2, 2012, 6682 participants from 1833 households were enrolled. Of these, 37 participants were excluded because individuals enrolled twice (we excluded the data from the second enrollment for an individual) or because they were the only participant enrolled in a household, leaving 6645 individuals from 1821 households in the analysis. Study participant characteristics are reported in Table 1; only the total household rooms and smoking prevalence varied by study period (Table 1).

A NP specimen was collected from 6628 (99.7%) of the 6645 participants; pneumococci were isolated from 2187 specimens (33%). Two serotypes were identified from 14 specimens (0.2%). Uptake of PCV13 was rapid; 80% of community children 7 months to <5 years of age were fully-immunized by the end of the study (March 2012; Figure 1). PCV13 coverage among study children and the overall community was similar (See Figure, Supplemental Digital Content 2, for a comparison of coverage among study children and the community).

Pneumococcal carriage

Children <5 years, but not those in older age groups, experienced a small but statistically significant decline in overall pneumococcal carriage from 56% during PCV7 use to 50% during Year 2 of PCV13 use (PR=0.88, $p=0.02$, Table 2). Fully-immunized children experienced the largest decrease in carriage from 56% to 48% (PR=0.85, $p=0.004$, Table 3). Associations between pneumococcal carriage and potential risk factors are presented in Table Supplemental Digital Content 3.

Among children <5 years, the carriage prevalence decreased in Year 2 of PCV13 use compared to the PCV7-era (2006-2007) for PCV13-specific types (PR=0.4, $p<0.0001$), type 19A (PR=0.4, $p=0.003$), type 3 (PR=0.1, $p=0.003$), and type 6C (PR=0.3, $p<0.001$) (Table 2). The decline in PCV13-specific type carriage became statistically significant nine months after PCV13 introduction (December 2010; Figure 1, upper panel) when PCV13 uptake among children <5 years in the community reached 50%. A significant reduction in carriage prevalence of serotypes 19A and 6C was observed 10 and 15 months after PCV13 introduction, respectively, when community PCV13 coverage was 55% and 69%, respectively (Figure 2). There were an insufficient number of type 3 colonization events to evaluate the specific month when reduced colonization was observed; only annual analyses were possible for type 3. No significant increase in the carriage prevalence of NVTs was observed (Year 2: PR=1.1, $p=0.5$).

The reduction in PCV13-specific type carriage was apparent in both fully-immunized and under-immunized children <5 years of age in Year 2 of PCV13 use (Table 3). Among under-immunized children, the decline in PCV13-specific type carriage prevalence became significant 11 months after PCV13-introduction when community coverage was 58% (Figure 1, lower panel). Among fully-immunized children <5 years ($n=1439$), PCV13-specific type carriage was rarely observed after PCV13 introduction ($n=44$; 3%; type 19A, $n=33$; type 3, $n=4$; type 7F, $n=2$); the same was true for under-immunized children in this

age group during Year 2 of PCV13 use (n=10; 3%; type 19A, n=8; type 3, n=1; type 7f, n=0) (Table 3).

Among older children and adults, a non-significant decline in the PCV13-specific type carriage prevalence was observed in Year 2 of PCV13 use (5-<8 years PR=0.7, p=0.3; 18+ years PR=0.4, p=0.1; Table 2). Children 5-<8 years experienced an increase in NVT carriage in Year 1 (PR=1.5, p=0.02), but this was not statistically significant in Year 2 (PR=1.3, p=0.08). No significant changes were observed in NVT carriage for adults (Year 1 PR=1.1, p=0.5; Year 2 PR=1.0, p=0.8).

Discussion

A reduction in PCV13-specific type carriage among under-immunized children (i.e., an indirect effect), was measurable by 11 months after PCV13-introduction when 58% of community children <5 years of age were fully-immunized. Two years following introduction of PCV13, there was a 60% reduction in PCV13-specific type carriage among all children <5 years of age, regardless of immunization status. Specifically, PCV13 use reduced carriage of types 3 and 19A and vaccine-associated 6C among children.

Our findings add to those from the other epidemiologic settings (Alaska, Massachusetts, South Africa and Kenya) where attempts have also been made to correlate PCV coverage with impact on carriage among vaccinated and unvaccinated individuals [19-23]. Although all studies noted reductions in VT carriage in unimmunized age groups within 6-24 months following introduction of an expanded valency PCV, when coverage ranged from 75%-100% with 1 PCV13 dose, only our study and the study in Massachusetts were designed to evaluate the earliest point and level of coverage when the indirect effects were first seen. In our study, indirect effects were observed when 50% of children in the community were fully immunized, whereas in the Massachusetts study, the indirect effects were first noted when 75% of children were “immune”, which was defined as children <12 months of age who received 2+ PCV13 doses or children 12+ months of age and received 3+ doses when <12 months or 1+ dose when 12+ months of age [20]. Differences in the definition of vaccine coverage and calculation of rolling averages limit direct comparison of these two studies; however, if we had applied the definition of coverage used in the Massachusetts study, we expect the calculated vaccine coverage values in the AI communities we studied would have been higher at the point when indirect effects were observed than the values using our method of calculation.

The decline in PCV13-specific type carriage outpaced the increase in NVT carriage, resulting in an 11% reduction in overall pneumococcal carriage among children <5 years of age following PCV13 use, but no change among older age groups. This reduction in overall carriage has also been noted in some African settings [22, 24, 25] while two other studies, both from the US, reported no change in overall carriage [20, 26]. All of these studies were observational, comparing carriage before and after PCV introduction, so attributing changes in overall pneumococcal carriage to PCV alone is not possible. The finding that pneumococcal carriage prevalence declined in some settings - generally those with high baseline carriage - but remained unchanged in other settings may relate to methods of

detection, reductions in acquisition of pneumococci, reductions in carriage density, or the characteristics of “replacement” strains circulating in these communities.

We observed a significant indirect effect of PCV13 among under-immunized children <5 years at the end of Year 1 of PCV13 use and similar sized effects in older children and adults during Year 2 when the proportion of children <5 years who were fully immunized increased to 80%; the low PCV13-specific type carriage prevalence in adults meant that the observed reduction did not reach statistical significance. We expect that continued high PCV coverage levels will likely lead to a further reduction in VT-colonization in older children and adults in the future. These indirect effects of PCV13 are aligned with observations following use of PCV7 in this and other indigenous populations [8, 27-29].

We were unable to compare PCV13-specific type prevalence among immunized and unimmunized children or adults living with and without an immunized child because of the rapid uptake of vaccine among children. A lower PCV13-specific type carriage prevalence in children <5 years was observed during the 12-month PCV7 period (2006-2007) compared to the three-month period immediately preceding PCV13 introduction. This may be attributable to seasonal variations in carriage or may reflect a true increase in PCV13-specific type carriage in the period immediately prior to PCV13 introduction compared to several years earlier. Using the PCV13-specific type carriage prevalence in 2006-2007 as the comparator period for assessing the impact of PCV13 introduction therefore likely yielded an under-estimate of the true reduction in PCV13-specific type carriage following the switch to PCV13 from PCV7.

There were several notable serotype-specific observations in this study, including what we believe to be the first documented impact of PCV13 on type 3 carriage among children. Additional years of monitoring will confirm whether this is likely to be a vaccine effect or secular trend. Other carriage studies have not reported an impact on type 3 carriage among children [30, 31] and there has been no consistent impact of PCV13 use on type 3 invasive pneumococcal disease [32-34]. As in previous studies [2] and other settings [19, 31], serotype 19A was a commonly carried serotype across all age groups in the PCV7 and early PCV13 periods. Even though type 19A carriage prevalence declined for children <5 years in the second year of PCV13 use, 19A continued to circulate; in Year 2 of PCV13 use, 2% of children <5 years of age were colonized with 19A, of whom 72% were fully immunized. Carriage of type 19A has been noted among children <5 years of age in other US settings who were either immunized or from a setting with high vaccine coverage [26, 31]. Immunogenicity data from a subset of study participants following the three-dose PCV13 primary series and booster dose demonstrated a strong immune response to the 19A conjugate [35]; all children achieved a 19A-specific IgG level ≥ 0.35 $\mu\text{g/mL}$, the non-inferiority threshold for comparison of PCV products, and 98% achieved levels ≥ 1.0 $\mu\text{g/mL}$, the 19A-specific threshold associated with protection from IPD [36, 37]. Furthermore, 79% achieved 19A IgG levels of ≥ 4.0 $\mu\text{g/mL}$, the putative threshold for colonization protection following PCV immunization [38]. Despite residual 19A carriage among vaccinated children <5 years of age, there were no cases of 19A IPD identified in this age group between 2011-2013 [4]. Less dense carriage of vaccine serotypes following PCV vaccination or carriage of a less invasive 19A clonal type might account for this observation.

Serotype 6C, first recognized in 2007 [40], was identified as the most common colonizing serotype in all ages from 2006-2008 among Navajo and White Mountain Apache populations [2]. Although the serotype 6C IPD rate increased after PCV7 introduction, it was not a leading cause of IPD [1]. Compared to the pre-PCV13 period, type 6C carriage prevalence decreased among children <5 years in Year 2 of PCV13 use supporting the idea that 6A antigen provides cross-protection against 6C [35, 40]. While a decline was noted in Year 2, type 6C prevalence returned to pre-introduction levels in the last quarter of the study. Additional time to monitor trends in type 6C carriage would be beneficial to more fully understand the vaccine's effect.

Despite evidence of a robust immune response and substantial reductions in 19F-IPD in these American Indian populations since the introduction of PCV, cases of type 19F IPD continue to occur [35]. There is no evidence from our study that use of PCV13 has had a further impact on 19F carriage than was seen with PCV7 in spite of our finding that PCV13 induced higher concentrations and functional activity against 19F than PCV7 induced in this same population [35]. Persistent 19F carriage and disease has been documented in other populations that have introduced a 19F-containing vaccine; reasons for this are not known but could relate to immunity waning more quickly for 19F than for other serotypes [41-44].

Although PCV use has reduced VT carriage prevalence, the overall prevalence of pneumococcal carriage (now predominantly NVT) remains high in these and other American Indian communities [45]. Large household size, households with two or more young children and households lacking piped water were the most important predictors of pneumococcal carriage. The link between these household characteristics and pneumococcal carriage likely reflects the relationship between lower socio-economic status and poorer health status.

The changes in PCV13-specific type carriage prevalence observed among children mirror the significant reductions in PCV13-specific type IPD detected by population-based active bacterial surveillance in this same population. The 60% reduction in PCV13-specific type carriage among children <5 years occurred concomitantly with an 89% decline in the PCV13-specific type IPD rate among community children in this age group during the study period (2010-2012) [4]. The rate of PCV13-specific type IPD also declined by 54% among adults 18-49 years following PCV13 introduction (2011-2013) compared to pre-PCV13 use (2007-2009), suggesting that the reductions in carriage we observed, while not statistically significant, were clinically meaningful [4]. The fact that PCV13-specific type IPD continues to occur among adults in this community suggests that carriage persists and that we may be underestimating true carriage prevalence by using culture-based methods and/or by using a nasopharyngeal swab alone in adults [29].

In conclusion, this post-introduction PCV13 assessment demonstrated a reduction in PCV13-specific type carriage among children in a population with high carriage prevalence and IPD rates. Indirect effects were observed when 58% of children less than 5 years of age in the community were fully immunized. The development of common definitions of vaccine coverage would facilitate evaluation of the level of coverage needed to achieve indirect effects across different settings. Reductions among children in overall

pneumococcal carriage prevalence were also observed in the two-year period after PCV13 introduction. Combining these carriage data with IPD data in this same community allows a more complete understanding of pneumococcal ecologic variation over time and offers opportunities to assess changes in propensity of strains to cause disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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All children <5 years of age^b

Under-immunized children <5 years of age

Figure 1.

Three-month moving average of PCV13-specific type carriage prevalence among Navajo and White Mountain Apache children <5 years of age and community PCV13 uptake and coverage^a

Figure 2.

Three-month moving average of serotype 19A (top) and 6C (bottom) carriage prevalence among Navajo and White Mountain Apache children <5 years of age across the study period

Characteristics of participating households and individuals during the three study periods: Pre-PCV13 and Years 1 and 2 of PCV13 use

Table 1

Household and Individual Characteristics	Pre-PCV13: September 2006-August 2007 (N=252 households, 865 individuals)	PCV13 Year 1: April 2010- March 2011 (N=794 households, 3016 individuals)	PCV13 Year 2: April 2011- March 2012 (N=897 households, 3146 individuals)	p-value ^a	p-value ^b
Total household members, mean (min, max)	5.8 (2, 20)	6.2 (2, 17)	5.7 (2, 18)	0.04	0.61
Children <6 years per household, mean (min, max)	2.1 (0, 7)	2.2 (1, 7)	1.9 (1, 8)	0.21	0.20
Total rooms, mean (min, max)	5.4 (1, 16)	5.9 (1, 16)	6.2 (1, 16)	0.002	<0.001
Sleeping rooms, mean (min, max)	2.6 (0, 6)	2.7 (1, 6)	2.7 (1, 6)	0.25	0.15
Running water, N (%)	212 (84)	666 (84)	779 (87)	0.95	0.22
Toilet facilities, N (%)	208 (83)	660 (83)	771 (86)	0.72	0.16
Smoker living in home, N (%)	63 (25)	145 (18)	116 (13)	0.02	<0.001
Wood, coal or pellet-burning stove, N (%)	151 (60)	522 (66)	534 (60)	0.06	0.91
Female gender, N (%)	533 (62)	1858 (62)	1948 (62)	0.99	0.87
Ever breastfed, <2 years, N (%)	138 (70)	576 (65)	729 (74)	0.26	0.19
Currently breastfeeding, <2 years, N (%)	53 (26)	204 (23)	214 (22)	0.43	0.30
Currently attends daycare ^c , <5 years, N (%)	8 (2)	34 (3)	48 (4)	0.44	0.13
Smoking tobacco use, 18 years, N (%)	31 (9)	140 (11)	91 (7)	0.27	0.37
Chewing tobacco use, 18 years, N (%)	12 (3)	62 (5)	48 (4)	0.27	0.68

^aComparison of Pre-PCV13 to PCV13 Year 1 as determined by chi-square or t-test.

^bComparison of Pre-PCV13 to PCV13 Year 2 as determined by chi-square or t-test.

^cDaycare attendance is defined as care received for 4 hours/week outside the home.

Table 2
Pneumococcal carriage prevalence among Navajo and White Mountain Apache children and adults before PCV13 and during Years 1 and 2 of PCV13 use

Serotype category ^a	Pre ^b N=402	Number colonized (%)				Pre N=107	5-8 years				Pre N=348	18+ years			
		Year 1 N=1260	Year 2 N=1365	PR (95% CI) ^c	Year 1 N=194		Year 2 N=282	PR (95% CI) ^c	Year 1 N=1277	Year 2 N=1222		PR (95% CI) ^c			
All types	226 (56)	676 (54)	680 (50) ^d	0.88 (0.80, 0.98)	45 (42)	97 (50)	131 (46)	1.10 (0.86, 1.43)	45 (13)	163 (13)	128 (10)	0.81 (0.59, 1.11)			
PCV7-type ^e															
6A	3 (1)	16 (1)	20 (1.5)	1.9 (0.6, 6.6)	3 (3)	1 (0.5)	4 (1)	0.5 (0.1, 2.2)	2 (0.5)	4 (0.3)	3 (0.3)	0.4 (0.1, 2.5)			
19F	0 (0)	3 (0.2)	5 (0.4)	--	1 (1)	1 (0.4)	2 (1)	0.7 (0.1, 8.3)	1 (0.3)	1 (0.1)	0 (0)	--			
	1 (0.3)	12 (1)	13 (1)	3.8 (0.5, 29.2)	2 (2)	0 (0)	2 (1)	0.4 (0.1, 2.6)	0 (0)	3 (0.2)	1 (0.1)	--			
PCV13-type															
3	31 (8)	101 (8)	54 (4) [*]	0.5 (0.3, 0.8)	11 (10)	10 (5)	18 (6)	0.6 (0.3, 1.3)	8 (2)	22 (2)	13 (1)	0.4 (0.2, 1.1)			
7F	28 (7)	88 (7)	39 (3) [*]	0.4 (0.3, 0.6)	9 (8)	10 (5)	16 (6)	0.7 (0.3, 1.5)	7 (2)	19 (1.5)	10 (1)	0.4 (0.1, 1.1)			
19A	7 (2)	15 (1)	3 (0.2) [*]	0.1 (0.03, 0.5)	2 (2)	4 (2)	6 (2)	1.1 (0.2, 5.5)	1 (0.3)	6 (0.5)	5 (0.5)	1.4 (0.2, 12.1)			
	1 (0.3)	2 (0.2)	2 (0.2)	0.6 (0.1, 6.5)	2 (2)	1 (0.4)	2 (1)	0.4 (0.1, 2.6)	1 (0.3)	1 (0.1)	1 (0.1)	0.3 (0.01, 4.5)			
	20 (5)	67 (5)	29 (2) [*]	0.4 (0.2, 0.7)	4 (4)	4 (2)	7 (2)	0.6 (0.2, 2.2)	4 (1)	9 (0.7)	4 (0.3)	0.3 (0.1, 1.1)			
6C	23 (6)	59 (5)	27 (2) [*]	0.3 (0.2, 0.6)	5 (5)	4 (2)	9 (3)	0.7 (0.2, 2.0)	5 (1)	7 (0.6)	4 (0.3)	0.2 (0.1, 0.8)			
NVT	172 (43)	575 (46)	626 (46)	1.1 (0.9, 1.2)	32 (30)	87 (45) [*]	113 (40)	1.4 (1.0, 1.9)	32 (9)	141 (11)	115 (9)	1.0 (0.7, 1.4)			
35B ^g	8 (2)	47 (4)	61 (5)	2.2 (1.1, 4.6)	2 (2)	7 (3)	11 (3)	2.1 (0.5, 9.3)	4 (1)	10 (1)	16 (1)	1.1 (0.4, 3.4)			
23B	16 (4)	53 (4)	51 (4)	0.9 (0.5, 1.6)	2 (2)	9 (4)	8 (2)	1.5 (0.3, 7.0)	1 (0.3)	5 (0.4)	7 (0.6)	2.0 (0.2, 16.1)			
16F	8 (2)	25 (2)	19 (1)	0.7 (0.3, 1.6)	0 (0)	5 (2)	7 (2)	--	0 (0)	5 (0.4)	3 (0.3)	--			
15B	11 (3)	34 (3)	45 (3)	1.2 (0.6, 2.3)	4 (4)	6 (3)	6 (2)	0.5 (0.1, 1.7)	1 (0.3)	5 (0.4)	5 (0.4)	1.4 (0.2, 12.1)			
22F	12 (3)	28 (2)	48 (4)	1.2 (0.6, 2.2)	3 (3)	7 (3)	9 (3)	1.1 (0.3, 4.1)	3 (1)	5 (0.4)	10 (1)	1.4 (0.3, 6.5)			
23A	17 (4)	40 (3)	33 (2)	0.6 (0.3, 1.0)	0 (0)	6 (3)	6 (2)	--	3 (1)	7 (1)	4 (0.3)	0.3 (0.1, 1.7)			
11A	12 (3)	32 (3)	30 (2)	0.7 (0.4, 1.4)	1 (1)	5 (2)	7 (2)	2.6 (0.3, 21.3)	0 (0)	10 (1)	8 (1)	--			
7C	0 (0)	25 (2)	37 (3)	--	0 (0)	7 (3)	2 (0.6)	--	1 (0.3)	14 (1)	9 (1)	2.6 (0.3, 20.2)			
15C	5 (1)	29 (2)	28 (3)	2.2 (0.9, 5.6)	1 (1)	3 (1)	7 (2)	2.6 (0.3, 21.3)	1 (0.3)	5 (0.4)	3 (0.3)	0.9 (0.1, 8.2)			
31	0 (0)	12 (1)	27 (2)	--	1 (1)	4 (2)	7 (2)	1.9 (0.2, 16.1)	3 (1)	4 (0.3)	4 (0.3)	0.4 (0.1, 1.7)			
NT	11 (3)	39 (3)	31 (2)	0.8 (0.4, 1.6)	1 (1)	9 (4)	9 (3)	3.4 (0.4, 26.6)	2 (1)	26 (2)	9 (1)	1.0 (0.7, 1.4)			

^aNo isolates were recovered for PCV13 serotypes 4, 5, 14 and 18C. Only three serotype 1 isolates were recovered after PCV13 introduction (in Year 1). One isolate was from a child <5 years and two isolates were from adults 18-49 years.

^bTime periods: Pre-PCV13 introduction = September 2006-August 2007, Year 1 of PCV13 use = April 2010-March 2011 and Year 2 of PCV13 use April 2011-March 2012.

^cPR = prevalence ratio comparing prevalence in Year 2 of PCV13 introduction vs. prevalence Pre-PCV13 introduction as calculated by log-binomial regression; CI = confidence interval.

^dA statistically significant decrease in prevalence compared to Pre-PCV13 introduction prevalence is identified by an asterisk (*). There were no significant increases in prevalence for any individual serotypes in Years 1 or 2 of PCV13 use. A Bonferroni correction was applied to account for multiple comparisons of individual serotypes resulting in a corrected p-value of 0.003, otherwise p<0.05 was used. The p-value was reported in the text for some prevalence comparisons to aid in understanding the level of statistical significance.

^ePCV7-type = serotypes 4, 6B, 9V, 14, 18C, 19F, 23F.

^fPCV13-specific type = serotypes 1, 3, 5, 6A, 7F, 19A.

^g10 most common non-vaccine serotypes for the study population are presented.

Table 3

Pneumococcal carriage prevalence among Navajo and White Mountain Apache fully-immunized and under-immunized children <5 years of age before PCV13 and during Years 1 and 2 of PCV13 use

Serotype category	Pre-PCV13 N=402	Number colonized (%)					
		Fully-immunized ^a			Under-immunized ^a		
		Year 1 N=396	Year 2 N=1043	PR (95% CI) ^b	Year 1 N=864	Year 2 N=322	PR (95% CI) ^b
All types	226 (56)	202 (51)	502 (48)*	0.85 (0.77, 0.95)	474 (55)	178 (55)	0.98 (0.86, 1.12)
PCV13-specific type ^c	28 (7)	15 (4)	29 (3)* ^d	0.4 (0.2, 0.6)	73 (8.5)	10 (3)*	0.4 (0.2, 0.9)
3	7 (2)	2 (0.5)	2 (0.2)*	0.1 (0.02, 0.5)	13 (1.5)	1 (0.3)	0.2 (0.02, 1.4)
19A	20 (5)	12 (3)	21 (2)*	0.4 (0.2, 0.7)	55 (6)	8 (2)	0.5 (0.2, 1.1)
6C	23 (6)	23 (4)	17 (2)*	0.3 (0.1, 0.5)	39 (4.5)	10 (3)	0.5 (0.3, 1.1)

^aFully-immunized = Children who met the following PCV13 dose criteria: age 7-<12 months: 3+ PCV13 doses, age 12-<16 months: 3+ doses <12 months or 1+ dose(s) >12 months, age 16-<60 months: 1+ dose(s) when received aged 12 months. Under-immunized = Children who did not meet the definition of fully-immunized.

^bPR = prevalence ratio comparing prevalence in Year 2 of PCV13 introduction vs. prevalence pre-PCV13 introduction as calculated by log-binomial regression; CI = confidence interval.

^cPCV13-specific type = serotypes 1, 3, 5, 6A, 7F, 19A.

^dA statistically significant change in prevalence compared to pre-PCV13 prevalence is identified by an asterisk (*).