Nasopharyngeal Carriage of *Streptococcus pneumoniae* in Navajo and White Mountain Apache Children Before the Introduction of Pneumococcal Conjugate Vaccine

Eugene V. Millar, PhD, Katherine L. O'Brien, MD, MPH,* Elizabeth R. Zell, MS,† Melinda A. Bronsdon, MS, RM(AAM),* Raymond Reid, MD, MPH,* and Mathuram Santosham, MD, MPH**

Background: Infants and children are frequently colonized with pneumococcus. Recent nasopharyngeal acquisition of pneumococcus is thought to precede disease episodes. The increased risk of pneumococcal disease among Navajo and White Mountain Apache populations has been documented. Little is known about the dynamics of pneumococcal carriage in these populations.

Methods: A group randomized, controlled trial of 7-valent conjugate pneumococcal vaccine (PnCRM7, Wyeth) was conducted on the Navajo and Apache reservations. A nasopharyngeal (NP) carriage study was nested in the trial to evaluate the impact of PnCRM7 on carriage. Children <6 years of age had NP swabs collected at enrollment and at 6 and 12 months following enrollment. We analyzed carriage data from children in control vaccine randomized communities to describe the epidemiology of pneumococcal carriage.

Results: Of the 410 participants enrolled, 92% were colonized with pneumococcus at least once during the course of the study. Sixty-three percent of NP specimens were positive for pneumococcus. The most common serotypes were 6A, 6B, nontypable, 23F, 14, 19F, 19A, and 9V. Thirty-eight percent of isolates were vaccine serotypes. Age <2 years, male sex, daycare attendance, and having a sibling colonized with pneumococcus were associated with an increased risk of carriage.

Conclusions: The high carriage prevalence among Navajo and Apache children reflects an intense exposure to pneumococcus. The lack of modifiable risk factors for carriage highlights the importance of preventive strategies for disease control.

Key Words: pneumococcus, *Streptococcus pneumoniae*, nasopharyngeal carriage, American Indian

(*Pediatr Infect Dis J* 2009;28: 711–716)

Streptococcus pneumoniae (pneumococcus) is a leading cause of invasive and noninvasive disease worldwide, and infants and young children are at particularly high risk. Nasopharyngeal (NP) carriage plays an important role in the epidemiology of pneumococcus, as recent acquisition of a new serotype is thought to precede episodes of pneumococcal disease.[1]

Carriage prevalence is highest among infants and young children and generally decreases with age.[2] In the general US population and other industrialized countries, the mean age of acquisition of pneumococcus is approximately 6 months,[1,3] whereas children in developing countries are colonized as early as 2 to 3 months of age.[4,5] A number of factors have been associated with increased prevalence of carriage, including younger age, crowding, day-care attendance, and exposure to cigarette smoke.[4,6,7]

Previous studies have described the epidemiology of pneumococcal carriage among children in the general US population.[1,8,9] However, no studies have been conducted among Navajo and White Mountain Apache (WMA) populations in the southwest United States. In the preconjugate pneumococcal vaccine era, these populations experienced high rates of invasive pneumococcal disease.[10,11] A group-randomized efficacy trial of a 7-valent conjugate pneumococcal vaccine (PnCRM7) was conducted on the Navajo and WMA reservations from May 1997 to October 2000.[12] An investigational group C meningococcal conjugate vaccine (MnCC) was used as the control vaccine. A carriage study was nested within the trial to evaluate the impact of PnCRM7 on pneumococcal carriage among vaccinees and their siblings.[13] To describe the natural history and risk factors for pneumococcal carriage among Navajo and WMA children in the absence of PnCRM7, we analyzed carriage data from children living in MnCC-randomized communities.

MATERIALS AND METHODS

A group-randomized efficacy trial of a 7-valent conjugate pneumococcal vaccine (PnCRM7; Wyeth Vaccines; serotypes: 4, 6B, 9V, 14, 18C, 19F, and 23F) was conducted on the Navajo and WMA reservations from April 1997 to October 2000.[12] The study area and population as well as the randomization procedures for the trial have been described previously.[14] Briefly, randomization units on the Navajo and Apache reservations were defined by geography and population size, so as to minimize the social interactions of adults and children in a given randomization unit with those of others in a different randomization unit during the course of the trial.

Trial participants received either PnCRM7 vaccine or *Neisseria meningitidis* group C protein conjugate vaccine (MnCC, Wyeth Vaccines) as a control vaccine. MnCC contains group C oligosaccharides coupled to the CRM_{197} protein, a nontoxic variant of diphtheria toxin. Each dose of study vaccine also contained 0.5 mg of aluminum phosphate as an adjuvant.

All children less than 2 years of age were eligible to participate in the trial. Primary efficacy subjects were defined as those who were enrolled between 6 weeks and 7 months of age, received 3 doses of vaccine 2 months apart (a minimum of 4 weeks apart) and a booster dose at 12 to 15 months of age (at least 2 months after the third dose). A total of 5792 children were enrolled in the primary efficacy group.

A subset of infants from the primary efficacy group and their household siblings <6 years of age were concurrently en-

Accepted for publication February 10, 2009.
From the *Center for American Indian Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; and †Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA.
Address for Correspondence: Eugene V. Millar, PhD, 621 N Washington Street, Baltimore, MD 21205. E-mail: emillar@jhsph.edu.
Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

ISSN: 0891-3668/09/2808-0711
DOI: 10.1097/INF.0b013e3181a06303

rolled in a carriage study to evaluate the impact of PnCRM7 on pneumococcal carriage among vaccinees and their household contacts.[13] Children with congenital anomalies of the nasopharynx were excluded from the carriage study. NP swabs were collected from vaccinees during 3 visits, at approximately 7, 12 to 15, and 18 to 24 months of age. In addition, household siblings ≤6 years of age who consented to participate were also swabbed during these visits. If any child in a given household tested positive for pneumococcus at 1 of the 3 regularly scheduled study visits, follow-up visits were made at 1- and 3-months after the scheduled visit to assess duration of carriage.

Trained nurses and field workers collected NP specimens. A small, flexible calcium alginate swab (Fisher Scientific) was inserted into the posterior nasopharynx and rotated 180 degrees. The swabs were inoculated into STGG transport media,[15] frozen at—70°C and transported on dry ice to the Centers for Disease Control and Prevention (CDC; Atlanta, Georgia) for isolation and serotyping. A 10-μL aliquot of each specimen was streaked onto a gentamicin-TSA 5% sheep blood agar plate (Becton Dickinson, Cockeysville, MD) and incubated overnight at 37°C in 5% CO_2. Phenotypic characteristics (morphology and α-hemolysis) were used for the presumptive identification of pneumococcus. Pneumococci were confirmed by optochin susceptibility and bile solubility assays.

Pneumococci were serotyped by a novel immunoblot method, described previously.[16] This method was developed to increase the sensitivity for detecting multiple pneumococcal serotypes in nasopharyngeal specimens. For specimens with <25 colonies, 4 colonies were picked from the plate and serotyped by Quellung reaction.

Information on demographics and risk factors for carriage were obtained at each study visit. Daycare attendance was defined as any place where the child spent at least 3 days per week for 4 hours per day and where 5 or more children were also present.

The duration of a given carriage episode was calculated as the number of days between the first and last consecutive specimen that was positive for a given serotype. For episodes in which the last specimen was negative for a given serotype, the duration of carriage was calculated as the midpoint between the last positive specimen and the next specimen that was negative.

To describe the epidemiology of carriage in the absence of conjugate pneumococcal vaccine, we restricted our analysis to children living in MnCC-randomized communities during the course of the trial. Analysis was carried out with the SAS software package (SAS version 9.0; Cary, NC). Odds ratios (OR) and 95% confidence intervals were calculated to identify potential risk factors for carriage. Risk factors having $P \leq 0.10$ in bivariable tests of association were selected for inclusion in the final model. We controlled for the within-subject correlation between multiple study visits by using generalized estimating equations (GEE),[17] constructed in the PROC GENMOD procedure. Results were considered statistically significant if the 2-tailed P value was less than 0.05. Serotype-specific estimates of the duration of carriage were calculated within each round.

The study was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health, the Centers for Disease Control and Prevention, the Navajo Nation, the Phoenix Area Indian Health Service, and the National Indian Health Service. Tribal approval was given by the Navajo Nation and the WMA tribe. Parents or guardians of study children signed a written informed consent document after reading the document or having the consent document read and explained to them in English or in their native language.

RESULTS

A total of 566 vaccinees (294 PnCRM7, 272 MnCC) and 286 siblings (144 PnCRM7, 142 MnCC) from 511 households (264 PnCRM7, 247 MnCC) were enrolled in the NP carriage study. A median of 2 children (range, 1–4 children) from each household was enrolled. Three children residing in MnCC-randomized communities each received 3 doses of PnCRM7 in error. The carriage results from these 3 households (3 vaccinees and 1 sibling) were excluded from the final analysis, leaving 410 participants (269 MnCC vaccinees and 141 siblings of MnCC vaccinees) in the current analysis.

The baseline characteristics of the 410 carriage study participants are presented in Table, Supplemental Digital Content, http://links.lww.com/A1438. Three-hundred thirty (80.5%) were Navajo. The median age of vaccinees and siblings at the first visit was 8.2 months (range, 6–20 months) and 44.9 months (range, 1–81 months), respectively. In the month before the first NP visit, 52 (12.6%) children had attended daycare, 80 (19.4%) had an episode of otitis media, and 84 (20.4%) had received antibiotics. Thirty-one (7.5%) children were receiving antibiotics at the time of the first NP swab. Antibiotic use, whether at the first visit or in the month prior, was associated with reported episodes of acute otitis media in the earlier month (data not shown). Amoxicillin and trimethoprim-sulfamethoxazole were the most frequently reported antibiotics. Including the vaccinees, a median of 2 children <6 years of age (range, 1–5 children) lived in the household. Sixty-seven (16.3%) children lived in a household with a cigarette smoker and 235 (57%) lived in a household that had a wood/coal-burning stove.

The average length of follow-up for the 410 participants was 12.1 months (range, 1 day-19.1 months). Twelve (2.9%) children were withdrawn from the study before completing all study visits; 5 moved away from the study area, 4 were withdrawn at the parents' request, and 3 reached the maximum age for eligibility before the conclusion of the study.

A total of 1068 NP specimens were collected; 677 (63.4%) were positive for pneumococcus. Pneumococcus was isolated at least once from 381 (92.9%) of 410 enrolled children. The prevalence of overall pneumococcal carriage was >50% in all age groups but was highest among children of 2 years of age (75%) (Fig. 1). The frequency of carriage did not vary by season (data not shown).

A total of 61 serotypes from 32 different serogroups were isolated. The carriage prevalence of vaccine serotypes (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) as well as the 10 most common nonvaccine serotypes are shown in Table 1. Thirty-nine percent of all pneumococcal isolates were vaccine serotypes. Each child carried an average of 2 (range, 0–6) pneumococcal serotypes. The distribution of the number of serotypes carried per child was as follows: 0 (8.4%), 1 to 2 (47.2%), 3 to 4 (36.2%), and 5 to 6 (7.2%). Simultaneous carriage with more than one serotype was detected in 65 (9.6%) specimens; 63 (9.3%) contained 2 serotypes, and 2 (0.3%) contained 3 serotypes.

At the first visit, 269 (65.3%) of 410 children carried pneumococcus. The carriage prevalence was not significantly different between Navajo and Apache children (63.6% vs. 65.8%, respectively; $P = 0.71$). Children who were receiving antibiotics at the first visit, or who had received antibiotics or had an episode of otitis media in the previous month were less likely to carry pneumococcus ($P = 0.01$). Among positive specimens, the proportion of vaccine-type isolates was higher among children who had received antibiotics in the last month (44.1% vs. 35.9%; $P = 0.05$), but not among those currently receiving antibiotics (32.8% vs. 36.7%; $P = 0.52$).

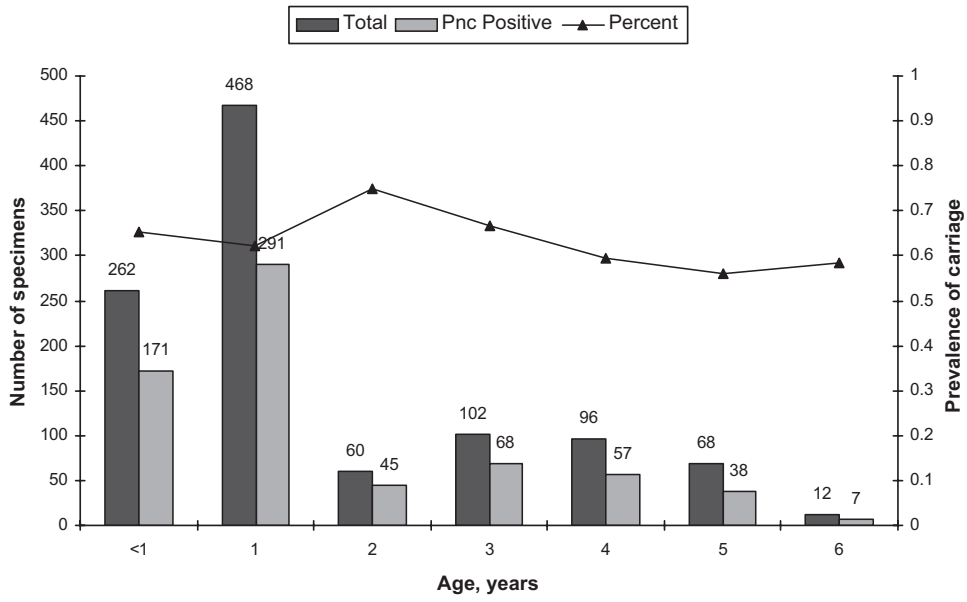

FIGURE 1. Age-specific prevalence of pneumococcal carriage among American Indian study children.

TABLE 1. Serotype Distribution of Pneumococcal Carriage Isolates From American Indian Children; Only the 10 Most Prevalent Nonvaccine Serotypes are Shown

Serotype	Number (%)
6A	104 (15.4)
*6B**	*68 (10)*
NT	62 (9.2)
23F	*59 (8.7)*
14	*47 (6.9)*
19F	*40 (5.9)*
19A	32 (4.7)
9V	*30 (4.4)*
22F	24 (3.5)
11A	23 (3.4)
35B	20 (2.9)
35F	18 (2.7)
31	15 (2.2)
15A	15 (2.2)
4	*15 (2.2)*
15A	14 (2.1)
18C	*8 (1.2)*

*Serotypes contained in the 7-valent pneumococcal conjugate vaccine (PnCRM7) are in italics.

NT indicates nontypable.

The frequency of overall carriage was higher among children who lived in a household with a smoker, but this did not reach statistical significance (73.1% vs. 62.3%; $P = 0.09$). There was no association between pneumococcal carriage and the presence of a wood- or coal-burning stove in the household.

Age-stratified, serotype-specific estimates of the duration of carriage are presented in Table 2. Type 6A had the longest carriage episodes, with median duration ranging from 35 to 63 days. Among the vaccine serotypes, carriage episodes were longest for serotypes 23F, 6B, and 14.

We constructed a multivariable model to evaluate potential risk factors for carriage, using GEE to control for within-subject correlations. In the model, age <2 years (odds ratio [OR]: 2.22, 95% CI: 1.42–3.49), male sex (OR: 1.52, 95% CI: 1.05–2.21), daycare attendance in the previous month (OR: 1.94, 95% CI: 1.16–3.24), and having a sibling colonized with pneumococcus (OR: 4.31, 95% CI: 2.79–6.66) were associated with an increased risk of carriage (Table 3). Children who were currently receiving antibiotics (OR: 0.51, 95% CI: 0.27–0.94) or had received antibiotics in the previous month (OR: 0.53, 95% CI: 0.22–1.27) were less likely to be colonized with pneumococcus. The presence of a cigarette smoker and the presence of a wood/coal-burning stove in the household were not significantly associated with pneumococcal carriage in the multivariable model.

DISCUSSION

The increased risk of invasive pneumococcal disease among Navajo and WMA children in the preconjugate vaccine era has been documented previously.[10,11] However, before this study, the dynamics of pneumococcal carriage in these high-risk populations has never been evaluated.

The prevalence of pneumococcal carriage (>60%) among Navajo and Apache children is 2- to 3-fold higher than that of similarly aged US children[8,9] and comparable to that of children living in developing countries.[4,18] Nearly all children (92%) carried pneumococcus at least once and >60% acquired 2 or more serotypes during the study period. In a previous carriage study among Navajo and Apache infants, ~50% were colonized by 2 months of age.[13] Taken together, these carriage data indicate an early and intense exposure of Navajo and WMA children to pneumococcus, which may explain in part the high rates of pneumococcal disease observed in these populations before the availability of conjugate pneumococcal vaccine.

We examined a number of potential risk factors for pneumococcal carriage in this population. In a multivariable model, age <2 years, day care attendance, and having a household sibling who carried pneumococcus were associated with an increased risk of carriage. These findings are consistent with a number of studies[2,9,18–20] and lend further evidence for the importance of young children in the transmission of pneumococcus in the household and in the community. Because they are not modifiable risk factors, they highlight the importance of conjugate pneumococcal vaccine

TABLE 2. Serotype-Specific Duration of Pneumococcal Carriage Episodes by Age Group

		Round 1			Round 2			Round 3		
Serotype	Age Group, Yr	N	Median	Range	N	Median	Range	N	Median	Range
6A	<2	19	35	13.5–81	32	58	13–11.50	26	56.8	14.5–112
	≤2	15	41	10.5–83	16	38.8	9–107	16	63	16.5–96
23F	<2	22	28	11.5–102	16	49.3	10.5–86	17	28	12–106
	≤2	7	40.5	12–102	8	55.3	12–67.5	7	46	10.5–78
6B	<2	20	31.5	14–92	17	21.5	10.5–163	16	27.8	14.5–122.5
	≤2	4	37	17.5–85	6	31.5	16–84	12	31.8	12.5–92
19F	<2	17	28	13.5–77	11	20.5	10.5–116	8	19.3	14–69
	≤2	4	18.8	7–27	2	31	10.5–51.5	2	43	32.5–53.5
14	<2	13	32	16–77	11	58.5	13.5–92	6	40	18.5–135.5
	≤2	8	31.5	10.5–104.5	6	29.8	18–85	9	31	13–116.5
NT	<2	14	29.3	11.5–71	12	45.5	17.5–75	17	41.5	12.5–169
	≤2	7	35	15–85	9	20	11.5–91	9	23	13–72.5
31	<2	9	24.5	16.5–67	2	63.3	50–76.5	2	38.5	12.5–64.5
	≤2	5	40	7–52	2	17	12–22	—	—	—
9V	<2	5	20	17–22.5	7	15.5	13.5–66	10	28.5	13–78
	≤2	4	26.5	16.5–35	4	17.3	13–39	8	33.5	10.5–84.5
10A	<2	8	23.5	11–80.5	5	50.5	27.5–84	1	13	—
	≤2	3	70.5	18–84	3	27.5	21–101	—	—	—
19A	<2	7	25	13.5–87	10	30	12–110	8	32.5	16.5–83
	≤2	5	23	19–45	8	35.3	17.5–87	3	62	13–63

NP swabs were collected from vaccinees during 3 visits, at approximately 7 (Round 1), 12 to 15 (Round 2), and 18 to 24 (Round 3) months of age. In addition, household siblings ≤6 year of age who consented to participate were also swabbed during these visits.

TABLE 3. Multivariable Analysis of Risk Factors for Pneumococcal Carriage Among American Indian Children

Risk Factor	Odds Ratio (95% CI)
<2 yr of age	2.22 (1.42–3.49)
Male	1.52 (1.05–2.21)
Ever breast fed	0.82 (0.57–1.18)
Current antibiotic use	0.51 (0.27–0.94)
Antibiotic use in last mo	0.53 (0.22–1.27)
Attended day care in last mo	1.94 (1.16–3.24)
Smoker lives in household	1.14 (0.74–1.77)
Wood/coal burning stove in household	1.31 (0.95–1.83)
Sibling colonized with pneumococcus	4.31 (2.79–6.66)

CI indicates confidence interval.

as a strategy for prevention of carriage and disease due to vaccine serotypes.

Boys were at slightly higher risk for pneumococcal carriage in our study. To our knowledge, only 1 other study has reported an increased risk of pneumococcal carriage among boys as compared with girls.[21] Data from Navajo as well as from the Active Bacterial Core Surveillance Program of the Centers for Disease Control and Prevention have shown higher rates of invasive pneumococcal disease among boys as compared with girls <5 years of age (R. Weatherholtz, manuscript in preparation and Cynthia Whitney, personal communication).

Children who had received antibiotics at the time of nasopharyngeal swab or in the month prior were less likely to carry pneumococcus.[22,23] Amoxicillin was the most commonly reported antibiotic in this study. In other studies, antibiotic use has been cited as a risk factor for carriage of drug-resistant pneumococci.[24,25] Although we did not assess antibiotic susceptibility among these isolates, there were a higher proportion of vaccine-type pneumococci among colonized children who had received antibiotics in the last month (44.1% vs. 35.9%; $P = 0.05$). The majority of drug-resistant pneumococcal isolates in the United States belong to serotypes included in PnCRM7 (eg, serotypes 6B, 9V, 14, 19F, and 23F).[26]

We did not observe an increased risk of carriage among children in households with a wood- or coal-burning stove or in households where a cigarette smoker also resided. Household exposure to cigarette smoke has been associated with increased risk of pneumococcal carriage in children.[4] Previous studies among Navajo children have reported an increased risk of acute lower respiratory illness among children living in houses that cooked with wood versus those that cooked with gas or electricity alone.[27] Additional information, such as the frequency and duration of these exposures to indoor air pollution, is needed to assess the impact of these environmental factors on the risk of pneumococcal carriage.

No seasonality of carriage was apparent in our study. Among Papua New Guinean children, another population in whom high rates of pneumococcal carriage and disease have been documented, carriage patterns do not follow a seasonal trend.[28] By contrast, among populations with a lower prevalence of overall pneumococcal carriage, increased rates of carriage have been observed during the winter months.[1,29] The reasons for this seasonality are poorly understood, but may be attributed to an increase in viral respiratory infections, crowding, or other environmental factors.[30,31]

The most frequently carried pneumococci were serotypes 6A, 6B, nontypable, 23F, 14, 19F, 19A, and 9V, accounting for ~65% of all isolates. Thirty-eight percent of pneumococcal isolates were serotypes included in PnCRM7 and an additional 23% belonged to vaccine-associated serotypes (ie, 6A, 19A, 23B, etc.). Among European children, who have lower overall rates of carriage (~25%–30%), prevalence of PnCRM7 serotype carriage ranged from 52% to 63% prior to the availability of conjugate pneumococcal vaccines.[3,19,32] Serotype differences between Navajo and US children have been shown among invasive pneumococcal isolates; in the preconjugate vaccine era, ~50% of all invasive pneumococcal disease among Navajo children <2 years of age were vaccine-type pneumococci, whereas in the general US population, this proportion was >85%.[11,33]

Conjugate pneumococcal vaccines have been shown to protect against nasopharyngeal acquisition of VT pneumococci and, in some cases, to simultaneously increase carriage of nonva-

ccine type pneumococci (ie, serotype replacement carriage).[13,34,35] Since the introduction of conjugate pneumococcal vaccines in the United States, increases in rates of nonvaccine type invasive disease have been reported among the general US population and among an Alaska Native population.[36–38] Furthermore, a number of studies have reported significant increases in the prevalence of certain serotypes, particularly serotype 19A, among invasive disease isolates.[36,39] We continue to monitor active, laboratory-based surveillance data from the Navajo and Apache reservations for trends in invasive pneumococcal disease in the conjugate pneumococcal vaccine era.

There are some limitations to this study. Because of the timing of specimen collection, it is possible that carriage episodes were missed. As a result, the reported prevalence may be underestimated. Because it is difficult to distinguish between persistent carriage and serial acquisitions for any given serotype, the duration of carriage estimates may be overestimated. We do not have longitudinal data on carriage in the first 6 months of age, when the first exposures to pneumococcus likely occurred. We did not collect detailed information on the frequency and duration of certain environmental exposures (ie, cigarette smoke, wood-burning stoves) that may have impacted carriage status. Finally, for any given serotype, further evaluation of the relationship between carriage and likelihood of invasive disease ("invasiveness") is warranted.

The present study documents a high prevalence of pneumococcal carriage among Navajo and WMA children. The intense exposure of these Native American children to pneumococcus may explain the higher rates of invasive pneumococcal disease observed in these communities when compared with the general US population. The high carriage prevalence, combined with a broad diversity of carriage serotypes, provides a dynamic reservoir for pneumococcal infection in these high-risk populations. Finally, continued population-based laboratory surveillance is essential to monitor trends in vaccine-type and nonvaccine-type pneumococcal disease in the current PnCRM7 era.

ACKNOWLEDGMENTS

The authors would like to thank the Navajo and Apache children and parents for their participation in this study, and the fieldworkers, nurses, and staff of the Center for American Indian Health of the Johns Hopkins University for their work on this study.

REFERENCES

1. Gray BM, Converse GM, Dillon HC. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. *J Infect Dis*. 1980;142:923–933.
2. Hendley JO, Sande MA, Stewart PM, et al. Spread of *Streptococcus pneumoniae* in families. I. Carriage rates and distribution of types. *J Infect Dis*. 1975;132:55–61.
3. Syrjanen RK, Kilpi TM, Kaijalainen TH, et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Finnish children younger than 2 years old. *J Infect Dis*. 2001;184:451–459.
4. Coles C, Kanungo R, Rahmathullah L, et al. Pneumococcal nasopharyngeal colonization in young South Indian Infants. *Pediatr Infect Dis J*. 2001;20:289–295.
5. Gratten M, Gratten H, Poil A, et al. Colonisation of influenzae and *Streptococcus pneumoniae* in the upper respiratory tract of neonates in Papua New Guinea: primary acquisition, duration of carriage, and relationship to carriage in mothers. *Biol Neonate*. 1986;50:114–120.
6. Henderson F, Gilligan P, Wait K, et al. Nasopharyngeal carriage of antibiotic-resistant pneumococci by children in group day care. *J Infect Dis*. 1988;157:256–263.
7. Sung RY, Ling JM, Fung SM, et al. Carriage of *Haemophilus influenzae* and *Streptococcus pneumoniae* in healthy Chinese and Vietnamese children in Hong Kong. *Acta Paediatr*. 1995;84:1262–1267.
8. Faden H, Duffy L, Wasielewski R, et al. Relationship between nasopharyngeal colonization and the development of otitis media in children. Tonawanda/Williamsville Pediatrics. *J Infect Dis*. 1997;175:1440–1445.
9. Samore MH, Magill MK, Alder SC, et al. High rates of multiple antibiotic resistance in *Streptococcus pneumoniae* from healthy children living in isolated rural communities: association with cephalosporin use and intrafamilial transmission. *Pediatrics*. 2001;108:856–865.
10. Cortese MM, Wolff M, Almeido-Hill J, et al. High incidence rates of invasive pneumococcal disease in the White Mountain Apache population. *Arch Intern Med*. 1992;152:2277–2282.
11. O'Brien KL, Shaw J, Weatherholtz R, et al. Epidemiology of invasive *Streptococcus pneumoniae* among Navajo children in the era before use of conjugate pneumococcal vaccines, 1989–1996. *Am J Epidemiol*. 2004;160:270–278.
12. O'Brien KL, Moulton LH, Reid R, et al. Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. *Lancet*. 2003;362:355–361.
13. O'Brien KL, Millar EV, Zell ER, et al. Effect of pneumococcal conjugate vaccine on nasopharyngeal colonization among immunized and unimmunized children in a community-randomized trial. *J Infect Dis*. 2007;196:1211–1220.
14. Moulton LH, O'Brien KL, Kohberger R, et al. Design of a group-randomized *Streptococcus pneumoniae* vaccine trial. *Control Clin Trials*. 2001;22:438–452.
15. O'Brien KL, Bronsdon MA, Dagan R, et al. Evaluation of a medium (STGG) for transport and optimal recovery of *Streptococcus pneumoniae* from nasopharyngeal secretions collected during field studies. *J Clin Microbiol*. 2001;39:1021–1024.
16. Bronsdon MA, O'Brien KL, Whitney CJ, et al. Detection of multiple serotypes of *Streptococcus pneumoniae* in nasopharyngeal secretions using an immunoblot method. *J Clin Micro*. 2004;42:1596–1600.
17. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*. 1986;42:121–130.
18. Lloyd-Evans N, O'Dempsey TJ, Baldeh I, et al. Nasopharyngeal carriage of pneumococci in Gambian children and in their families. *Pediatr Infect Dis J*. 1996;15:866–871.
19. Dagan R, Melamed R, Muallem M, et al. Nasopharyngeal colonization in southern Israel with antibiotic-resistant pneumococci during the first 2 years of life: relation to serotypes likely to be included in pneumococcal conjugate vaccines. *J Infect Dis*. 1996;174:1352–1355.
20. Leino T, Auranen K, Jokinen J, et al. Pneumococcal carriage in children during their first two years: important role of family exposure. *Pediatr Infect Dis J*. 2001;20:1022–1027.
21. Dowling JN, Sheehe PR, Feldman HA. Pharyngeal pneumococcal acquisitions in "normal" families: a longitudinal study. *J Infect Dis*. 1971;124:9–17.
22. Cohen R, Bingen E, Varon E, et al. Change in nasopharyngeal carriage of *Streptococcus pneumoniae* resulting from antibiotic therapy for acute otitis media in children. *Pediatr Infect Dis J*. 1997;16:555–560.
23. Ghaffar F, Muniz LS, Katz K, et al. Effects of large dosages of amoxicillin/clavulanate or azithromycin on nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae*, nonpneumococcal alpha-hemolytic streptococci, and *Staphylococcus aureus* in children with acute otitis media. *Clin Infect Dis*. 2002;34:1301–1309.
24. Abdel-Haq N, Abuhammour W, Asmar B, et al. Nasopharyngeal colonization with *Streptococcus pneumoniae* in children receiving trimethoprim-sulfamethoxazole prophylaxis. *Pediatr Infect Dis J*. 1999;18:647–648.
25. Leach AJ, Shelby-James TM, Mayo M, et al. A prospective study of the impact of community-based azithromycin treatment of trachoma on carriage and resistance of *Streptococcus pneumoniae*. *Clin Infect Dis*. 1997;24:356–362.
26. Whitney CG, Farley MM, Hadler J, et al. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. *N Engl J Med*. 2000;343:1917–1924.
27. Robin LF, Lees PS, Winget M, et al. Wood-burning stoves and lower respiratory illnesses in Navajo children. *Pediatr Infect Dis J*. 1996;15:859–865.
28. Montgomery JM, Lehmann D, Smith T, et al. Bacterial colonization of the upper respiratory tract and its association with acute lower respiratory tract infections in Highland children of Papua New Guinea. *Rev Infect Dis*. 1990;12:S1006–S1016.
29. Jebaraj R, Cheran T, Raghupathy P, et al. Nasopharyngeal colonization of infants in southern India with Streptococcus pneumoniae. *Epidemiol Infect*. 1999;123:383–388.
30. Dowell SF, Whitney CG, Wright C, et al. Seasonal patterns of invasive pneumococcal disease. *Emerg Infect Dis*. 2003;9:573–579.

31. Ghaffar F, Friedland IR, McCracken GH Jr. Dynamics of nasopharyngeal colonization by Streptococcus pneumoniae. *Pediatr Infect Dis J*. 1999;18:638–646.
32. Marchisio P, Esposito S, Schito GC, et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy children: implications for the use of heptavalent pneumococcal conjugate vaccine. *Emerg Infect Dis*. 2002;8:479–484.
33. Centers for Disease Control and Prevention. Preventing Pneumococcal Disease Among Infants and Young Children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep.* 2000;49(RR-9):1–35.
34. Dagan R, Givon-Lavi N, Zamir O, et al. Reduction of nasopharyngeal carriage of *Streptococcus pneumoniae* after administration of a 9-valent pneumococcal conjugate vaccine to toddlers attending day care centers. *J Infect Dis*. 2002;185:927–936.
35. Mbelle N, Huebner RE, Wasas AD, et al. Immunogenicity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. *J Infect Dis*. 1999;180:1171–1176.
36. Hicks LA, Harrison LH, Flannery B, et al. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998–2004. *J Infect Dis*. 2007;196:1346–1354.
37. Messina AF, Katz-Gaynor K, Barton T, et al. Impact of the pneumococcal conjugate vaccine on serotype distribution and antimicrobial resistance of invasive *Streptococcus pneumoniae* isolates in Dallas, TX, children from 1999 through 2005. *Pediatr Infect Dis J*. 2007;26:461–467.
38. Singleton RJ, Hennessy TW, Bulkow LR, et al. Invasive pneumococcal disease caused by nonvaccine serotypes among Alaska native children with high levels of 7-valent pneumococcal conjugate vaccine coverage. *JAMA*. 2007;297:1784–1792.
39. Pelton SI, Huot H, Finkelstein JA, et al. Emergence of 19A as virulent and multidrug resistant Pneumococcus in Massachusetts following universal immunization of infants with pneumococcal conjugate vaccine. *Pediatr Infect Dis J*. 2007;26:468–472.