

Nasopharyngeal versus Oropharyngeal Sampling for Detection of Pneumococcal Carriage in Adults

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Several studies have shown that nasopharyngeal sampling is more sensitive than oropharyngeal sampling for the detection of pneumococcal carriage in children. The data for adults are limited and conflicting. This study was part of a larger study of pneumococcal carriage on the Navajo and White Mountain Apache Reservation following a clinical trial of a seven-valent pneumococcal conjugate vaccine. Persons aged 18 years and older living in households with children enrolled in the vaccine trial were eligible. We collected both nasopharyngeal and oropharyngeal specimens by passing a flexible calcium alginate wire swab either nasally to the posterior nasopharynx or orally to the posterior oropharynx. Swabs were placed in skim milk-tryptone-glucose-glycerin medium and frozen at -70°C . Pneumococcal isolation was performed by standard techniques. Analyses were based on specimens collected from 1,994 adults living in 1,054 households. Nasopharyngeal specimens (11.1%; 95% confidence interval [CI], 9.8 and 12.6%) were significantly more likely to grow pneumococci than were oropharyngeal specimens (5.8%; 95% CI, 4.8 to 6.9%) ($P < 0.0001$). Few persons had pneumococcal growth from both specimens (1.7%). Therefore, both tests together were more likely to identify pneumococcal carriage (15.2%; 95% CI, 13.7 to 16.9%) than either test alone. Although we found that nasopharyngeal sampling was more sensitive than oropharyngeal sampling, nasopharyngeal sampling alone would have underestimated the prevalence of pneumococcal carriage in this adult population. Sampling both sites may give more accurate results than sampling either site alone in studies of pneumococcal carriage in adults.

Infection with *Streptococcus pneumoniae* (pneumococcus) can result in a range of illnesses, including bacteremia, meningitis, pneumonia, otitis media, and sinusitis, which together cause substantial morbidity and mortality worldwide. The ascertainment of pneumococcal colonization is important for many types of studies. Several investigators have demonstrated that nasopharyngeal (NP) sampling is more sensitive than oropharyngeal (OP) sampling for detecting pneumococcal carriage in children (4, 8, 10, 14), although contrary results exist (3). The optimal sampling method for identifying pneumococcal colonization in adults is not clear for several reasons. First, few studies have compared the yields from different sampling sites. Second, in some studies the sampling methods are not clearly specified. Third, the optimal sampling method may vary depending on the type of medium and the microbiologic methods used. Four studies that have compared NP and OP sampling in adults have come to different conclusions (2, 6, 9, 10). To our knowledge, no studies have been published that compared sampling sites when skim milk-tryptone-glucose-glycerin (STGG) medium was used to collect and store specimens. The inoculation of STGG medium is at least as sensitive as direct plating (12) and is a commonly used methodology that can be

used under a wide variety of conditions. The objective of this study was to evaluate the sensitivity of NP sampling versus OP sampling with inoculation into STGG medium for the detection of pneumococcal carriage in adults.

MATERIALS AND METHODS

Study site. This study was performed in the context of a group-randomized clinical trial of a pediatric seven-valent pneumococcal conjugate vaccine on the Navajo and White Mountain Apache Reservation in the southwestern United States. Descriptions of the vaccine trial have been published elsewhere (11, 13). During the vaccine trial, a study was done to evaluate the impact of conjugate vaccine use on pneumococcal carriage in children (K. L. O'Brien, M. A. Bronsdon, G. M. Carlone, R. R. Facklam, B. Schwartz, R. Reid, and M. Santosham, Abstr. Soc. Ped. Res., abstr. 1463, 2001). A study of adult carriage was performed between the conclusion of the vaccine trial and the initiation of widespread use of the pneumococcal conjugate vaccine for children. The present study was nested within the adult carriage study.

Enrollment. First, the 513 households with children enrolled in the childhood carriage study were approached. To achieve the target sample size, we selected additional households with children who participated in the conjugate vaccine trial. Because the Navajo and White Mountain Apache Reservation includes large, sparsely populated areas, the selection of additional households was based on their proximity to one of five field offices. Ordered lists of communities by distance were prepared, and communities were visited in order until the necessary sample size for the adult carriage study was reached. Within each selected community, all households with a child enrolled in the vaccine trial were visited at least twice. If an adult family member consented, the household was enrolled. Households were excluded if the vaccine trial participant had moved.

Specimen collection. Specimens were collected by trained community health workers who were periodically observed in the field by study nurses and physicians. An NP specimen and an OP specimen were collected from all consenting

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persons aged 18 years and over in enrolled households. For NP specimen collection, a flexible, aluminum-shaft calcium alginate swab was gently passed through the nostril until resistance was encountered. If the insertion depth was approximately the distance from the tip of the nose to the ear, the swab was assumed to have reached the posterior nasopharynx. If the swab could not be passed to this depth it was withdrawn and passed through the other nostril. For OP specimen collection, similar swabs were passed through the mouth and rubbed over the tonsils and posterior oropharynx. Swabs were immediately inoculated into STGG medium (12) produced by the Centers for Disease Control and Prevention and then placed in an ice pack. The same day, the specimens were taken to a field office, vortexed thoroughly, and frozen at -70°C .

Pneumococcal isolation. Specimens were transported on dry ice to the Centers for Disease Control and Prevention. Specimens were then thawed and streaked onto gentamicin-tryptic soy agar-5% sheep blood agar plates (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 pneumococcal colonies. Pneumococcal identification was confirmed by optochin susceptibility and bile solubility assays. When pneumococci were identified, a single colony was selected from each plate and the serotype was determined by the Quellung reaction. If pneumococcal colonies of multiple morphologies were present, each morphological type was serotyped.

Data analysis. Analyses were performed by use of the SAS software package (version 8.0; SAS, Cary, N.C.). Confidence intervals around proportions were calculated by the exact binomial test. McNemar's test was used to compare correlated proportions.

Ethical considerations. This 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. Approval was also given by the leadership of the White Mountain Apache Tribe and community health boards in the study areas. Written informed consent was obtained from all participants after the study was explained to them in English or in their native language.

RESULTS

Participation. A total of 2,690 households were approached for this study. Of these, 878 were excluded. For 516 households, no one was home on at least two visits. Contact was made with 1,296 households, 1,119 of which (86%) enrolled in the study. The mean number of adults living within enrolled households was 2.35 (range, 1 to 10). On average, 1.85 (range, 0 to 10) adults per household consented to participate, 0.37 (range, 0 to 6) adults per household refused, and 0.13 (range, 0 to 4) adults per household could not be located. Both NP and OP swabs were obtained from 1,994 adults living in 1,054 households. For the remaining 65 households, we were unable to collect both specimens from an adult household member. The median age of participants was 31.5 years, and 69.6% of the participants were female.

Carriage rates. Pneumococci were recovered from 304 (15.2%) participants. Pneumococcal carriage rates varied by age from 17.7% (18 to 29 years) to 10.3% (40 to 49 years) but did not follow a clear pattern of increase or decrease with age. Pneumococci were isolated more frequently from NP specimens than from OP specimens, and the overall carriage rates were higher when both specimens were considered (Table 1). For 33 persons for whom there was isolation of pneumococci from both NP and OP specimens, the serotypes of the isolates obtained from the two sampling sites were the same in 23 (69.7%) cases. The hierarchy of isolation (both sites > NP > OP) did not differ by age, gender, whether the subject smoked, whether the subject lived with a smoker, whether the subject was the parent of a child in the household, or whether the household used a wood or coal stove for heating (a proxy for indoor air pollution). There was no difference in the hierarchy

TABLE 1. Pneumococcal growth by sampling site ($N = 1,994$)

Site	No. (%) of samples with pneumococcal growth	95% Confidence interval (lower-upper [%])
NP	222 (11.1)	9.8–12.6
OP	115 (5.8)	4.8–6.9
NP or OP	304 (15.2)	13.7–16.9
NP and OP	33 (1.7)	1.1–2.3

of isolation by month of data collection, whether the data were collected before or after a mid-study review of sampling technique, or among the five study sites.

A total of 40 pneumococcal serotypes were identified in addition to the nontypeable specimens. An analysis of the sites of isolation was done for the four serotypes with at least 20 isolates (Table 2). The rates of isolation were only significantly different for one serotype, type 35B, which was more frequently isolated from NP specimens. As a group, the seven serotypes contained in the pneumococcal conjugate vaccine (i.e., 4, 6B, 9V, 14, 18C, 19F, and 23F) followed the same hierarchy of isolation as all other pneumococci. The isolation of vaccine-type organisms was significantly more common when both NP and OP specimens were considered (3.4%) than when the NP specimen alone was considered (3.4% versus 2.3% [$P < 0.001$]), and isolation from the NP specimen was significantly more common than isolation from the OP specimen (2.3% versus 1.4% [$P = 0.02$]).

DISCUSSION

We found that NP sampling was more sensitive than OP sampling for detecting pneumococcal carriage in adults. Carriage at both sites was uncommon, and an analysis of both specimens was significantly more likely to identify pneumococcal carriage than an analysis of either specimen alone. There are at least three possible explanations for our results: there may have been differential colonization in the patients, the test may have had some insensitivity, or there may have been some inconsistent sampling technique in the study.

Differential colonization. Environmental, host, or microbial factors could result in differential NP or OP colonization. We did not find any differences based on the environmental or host factors evaluated in this study. The only microbial factor that we evaluated was serotype. Most serotypes occurred rarely, but for the four serotypes with at least 20 isolates, one serotype was isolated significantly more frequently from one site than from the other. Of the subjects with pneumococcal isolation from both sites, two-thirds had the same serotype isolated from both

TABLE 2. Numbers of pneumococcal isolates for serotypes with at least 20 isolates by site of isolation ($N = 1,994$)

Serotype	No. (%) of isolates	
	NP (%)	OP (%)
3	16 (0.8)	16 (0.8)
19F	12 (0.6)	11 (0.6)
35B ^a	20 (1.0)	6 (0.3)
NT	8 (0.4)	17 (0.9)

^a $P < 0.01$ (McNemar's test).

sites, suggesting that most strains were able to colonize either site. Microbial factors that are unrelated to serotype could play a role in determining the location of colonization. Many microbial characteristics have been associated with pneumococcal adhesion to respiratory epithelial cells, including interactions with other microorganisms in the pharynx (7). Also, different pneumococcal strains of the same serotype have been shown to adhere differently to epithelial cells in vitro (1, 5).

Test insensitivity. Another possible explanation for our findings is that study subjects carried pneumococci in both sites but that the sampling method had a low sensitivity. This explanation would account for our observation that the isolation of pneumococci from both NP and OP specimens was uncommon. Other studies have found that methods to increase sensitivity were associated with an increased recovery of pneumococci from adults. Masters et al. found that broth enrichment was particularly useful for the isolation of pneumococci from adult respiratory specimens and that the presence of nasal discharge, which may be associated with a higher colonization density, increased the yield of pneumococci from adult NP specimens (10). Two studies found that the use of both mouse inoculation and direct plating on blood agar plates resulted in a higher rate of pneumococcal isolation than either test alone, suggesting that test sensitivity was an important factor in their results (6, 9). One approach to evaluate the sensitivity of this sampling method would be to compare the results for two NP samples with that for a single NP sample. If NP sampling has a low sensitivity, then more carriers would be identified when two samples are taken. Broth enrichment could also be evaluated as a technique to increase pneumococcal isolation from NP and OP samples.

Inconsistent sampling technique. We do not believe that our results can be explained by inconsistent sampling technique. If any of the community health workers had an inconsistent technique, we would have expected to see different results at different study sites and as the study progressed. However, the hierarchy of isolation by anatomic site was similar across all five of our study sites and over time.

Our finding that collecting both NP and OP swabs resulted in a higher rate of observed carriage in adults is consistent with that of Masters et al., who concluded that "both swabs should be taken for the detection of pneumococci" (10). Our results differ from those of three other studies (2, 6, 9). First, Hendley et al. concluded that OP sampling was superior to NP sampling. This study used "wooden swabs passed along the floor of the nose to the nasopharynx" (9). It is possible that the use of rigid swabs limited the depth of sampling. The importance of sampling deep in the nasal cavity is supported by another study that compared pneumococcal isolation rates from NP specimens collected orally by an "ENT specialist" with nasal specimens collected by anterior rhinoscopy. The number of specimens in this study was small, but nasopharyngeal sampling yielded more pneumococcal isolates than nasal sampling (15). Second, Boersma et al. found that NP cultures had a lower yield of pneumococci than OP cultures and added little to the results for OP cultures alone (2). Conversely, Foy et al. found that "nasal" specimens were superior to throat swabs and that throat swabs identified few additional carriers (6).

In summary, we found that NP sampling detected more pneumococcal carriers than OP sampling in Navajo and White

Mountain Apache adults. Furthermore, sampling both the NP and OP sites significantly increased the yield over sampling either site alone. We suggest collecting both NP and OP swabs for more accurate detection of *S. pneumoniae* colonization rates in adults. When it is not feasible to collect both NP and OP swabs, then NP swabs alone should be used. Our study suggests that studies that use a single sample to identify carriage may underestimate pneumococcal carriage rates in adults and that carriage rates in adults may be higher than previously thought.

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REFERENCES

- Andersson, B., B. Eriksson, E. Falsen, A. Fogh, L. A. Hanson, O. Nylan, H. Peterson, and E. C. Svanborg. 1981. Adhesion of *Streptococcus pneumoniae* to human pharyngeal epithelial cells in vitro: differences in adhesive capacity among strains isolated from subjects with otitis media, septicemia, or meningitis or from healthy carriers. *Infect. Immun.* **32**:311–317.
- Boersma, W. G., A. Lowenberg, Y. Holloway, H. Kuttscrutter, J. A. Snijder, and H. Koeter. 1993. The role of antigen detection in pneumococcal carriers: a comparison between cultures and capsular antigen detection in upper respiratory tract secretions. *Scand. J. Infect. Dis.* **25**:51–56.
- Box, Q. T., R. T. Cleveland, and C. Y. Willard. 1961. Bacterial flora of the upper respiratory tract. *Am. J. Dis. Child* **102**:293–301.
- Capeding, M. R., H. Nohynek, L. T. Sombrero, L. G. Pascual, E. S. Sunico, G. A. Esparar, E. Esko, M. Leinonen, and P. Ruutu. 1995. Evaluation of sampling sites for detection of upper respiratory tract carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae* among healthy Filipino infants. *J. Clin. Microbiol.* **33**:3077–3079.
- Cundell, D., J. N. Weiser, J. Shen, A. Young, and E. I. Toumanen. 1995. Relationship between colonial morphology and adherence of *Streptococcus pneumoniae*. *Infect. Immun.* **63**:757–761.
- Foy, H. M., B. Wentworth, G. E. Kenny, J. M. Kloeck, and J. T. Grayston. 1975. Pneumococcal isolation from patients with pneumonia and control subjects in a prepaid medical care group. *Am. Rev. Respir. Dis.* **111**:595–603.
- Garcia-Rodriguez, J. A., and M. J. F. Martinez. 2002. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J. Antimicrob. Chemother.* **50**:59–73.
- Gray, B. M., G. M. Converse, and H. C. Dillon. 1980. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. *J. Infect. Dis.* **142**:923–933.
- Hendley, J. O., M. A. Sande, P. M. Stewart, and J. M. Gwaltney, Jr. 1975. Spread of *Streptococcus pneumoniae* in families. I. Carriage rates and distribution of types. *J. Infect. Dis.* **132**:55–61.
- Masters, P. L., W. Brumfitt, and R. L. Mendez. 1958. Bacterial flora of the upper respiratory tract in Paddington families, 1952–1954. *Br. Med. J.* **1**:1200–1205.
- Moulton, L. H., K. L. O'Brien, R. Kohberger, I. Chang, R. Reid, R. Weatherholtz, J. G. Hackell, G. R. Siber, and M. Santosham. 2001. Design of a group-randomized *Streptococcus pneumoniae* vaccine trial. *Control Clin. Trials* **22**:438–452.
- O'Brien, K. L., M. A. Bronsdon, R. Dagan, P. Yagupsky, J. Janco, J. Elliott, C. G. Whitney, Y. H. Yang, L. G. Robinson, B. Schwartz, and G. M. Carlone. 2001. Evaluation of a medium (STGG) for transport and optimal recovery of *Streptococcus pneumoniae* from nasopharyngeal secretions collected during field studies. *J. Clin. Microbiol.* **39**:1021–1024.
- O'Brien, K. L., L. H. Moulton, R. Reid, R. Weatherholtz, J. Oski, L. Brown, G. Kumar, A. Parkinson, D. Hu, J. Hackell, I. Chang, R. Kohberger, G. Siber, and M. Santosham. 2003. Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. *Lancet* **362**:355–361.
- Rapola, S., E. Salo, P. Kiiski, M. Leinonen, and A. K. Takala. 1997. Comparison of four different sampling methods for detecting pharyngeal carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae* in children. *J. Clin. Microbiol.* **35**:1077–1079.
- Ylikoski, J., S. Savolainen, and H. Jousimies-Somer. 1989. Bacterial flora in the nasopharynx and nasal cavity of healthy young men. *ORL J. Otorhinolaryngol. Relat. Spec.* **51**:50–55.