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Evaluation of Sampling Sites for Detection of Upper Respiratory Tract Carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae* among Healthy Filipino Infants

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Two sampling techniques, nasal swabbing and oropharyngeal swabbing, for detection of the upper respiratory tract carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae* were studied prospectively with 296 healthy Filipino infants at various ages: 6 to 8, 10 to 12, 14 to 17, 18 to 22, 32 to 39, and 46 to 65 weeks. In all age groups *S. pneumoniae* was isolated significantly more often ($P < 0.0001$) from the nasal site than from the oropharyngeal site. *H. influenzae* was found equally often at both sites.

Streptococcus pneumoniae and *Haemophilus influenzae* are the most common lower respiratory tract pathogens in children in developing countries (13, 15). Isolation and serotyping of these bacteria from the upper respiratory tract (URT) are of little diagnostic value per se. However, these procedures may give information on the type-specific etiology of pneumonia when combined with acute-phase serum or urinary antigen findings and significant increases in the titers of antibodies to species-specific antigens of *S. pneumoniae* or *H. influenzae*. URT carriage studies are also important in monitoring the resistance patterns of antimicrobial agents (10) and measuring the efficacy of the *H. influenzae* type b and, possibly, the new pneumococcal conjugate vaccines (11, 14).

The results of previous studies on URT carriage evaluating isolation techniques and sampling sites have varied (4–9). Only one study was done in a developing country (12). Few studies have looked at nasal (NS) and oropharyngeal (OP) sites simultaneously in the same subjects. The objective of this study was to find out which of the sampling techniques, NS swabbing or OP swabbing, when performed simultaneously, would yield the highest rate of isolation of *S. pneumoniae* and *H. influenzae.*

Infants residing in two selected periurban villages were enrolled in the study between April 1992 and October 1993 in connection with an immunogenicity study on *H. influenzae* type b conjugate vaccines when they were brought to the local health station for their routine vaccinations at the age of 6 to 8 weeks. Each parent or guardian gave their informed consent. This study was approved by the Ethical Review Board of the Research Institute for Tropical Medicine in Manila, Philippines.

The vaccination schedule was based on the Expanded Programme on Immunization of the Department of Health. The participants were monitored until 1 year of age. The parents were asked about the history of antibiotic use during the 2 weeks prior to each visit. At each visit, NS and OP swabs were taken simultaneously prior to each immunization at 6 to 8, 10 to 12, 14 to 17, 32 to 39, and 46 to 65 weeks of age. In addition, NS and OP swabs were taken 1 month after the primary immunization series at 18 to 22 weeks of age and 1 month after the booster vaccination at 10 months of age. All visits due to illness (5%) were excluded from this analysis.

The NS swab was taken by a medical technologist with a cotton-tipped wooden applicator from the nostrils, and the OP swab was taken from the posterior wall and tonsillar areas of the oropharynx. Each applicator was then placed into the Amies transport medium for transport to the bacteriology laboratory and inoculated on agar plates, with a transit time of 6 to 7 h. This routine was done for 8 months, after which inoculation on culture plates was done on site.

Swabs were cultured on Trypticase soy agar plates containing 5% sheep blood and 5 μg of gentamicin sulfate per ml to isolate *S. pneumoniae* and on chocolate agar plates containing 300 μg of bacitracin per ml for isolation of *H. influenzae*. Plates were incubated at 35 to 37°C in a 5% CO_2 incubator for 18 to 24 h. *S. pneumoniae* organisms were identified by their alpha-hemolytic colony morphology, Gram stain, and susceptibility to the optochin disc; *H. influenzae* organisms were identified by X and V factor requirements and the porphyrin test.

Carriage rate was defined as the percentage of swabs, i.e., NS alone, OP alone, or both, which yielded *S. pneumoniae* or *H. influenzae* per child.

The data were entered by using the epidemiologic software Epiinfo, version 5. Frequency distributions and cross-tabulations were generated by using SAS statistical software. For the stratified analysis, the differences in the isolation rates of *S. pneumoniae* and *H. influenzae* at different sampling sites, with control for the effect of the confounders, were tested for statistical significance by using McNemar's paired chi-square test of the EPISTAT software. The level of significance was set at 0.05.

A total of 296 children were enrolled in the study; 176 (59%) were males and 120 (41%) were females. These children visited the health center regularly until 1 year of age.

There were 1,567 NS and OP swabs collected. The URT carriage rates of *S. pneumoniae* and *H. influenzae* at NS and OP sites for various age groups are shown in Tables 1 and 2. *S. pneumoniae* was isolated more frequently from the NS site than from the OP site, and the difference was statistically significant in all age groups. On the other hand, *H. influenzae*

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TABLE 1. URT carriage rates of *S. pneumoniae* in infants by age and anatomical site

Age (wk) [*n*]	No. positive[a] (%)		
	Both sites	OP[b]	NS[b]
6–8 [295]	30	34 (11.5)	82 (27.8)
10–12 [270]	41	48 (17.8)	108 (40.0)
14–17 [240]	36	44 (18.3)	113 (47.1)
18–22 [229]	34	44 (19.2)	124 (54.1)
32–39 [158]	26	29 (18.4)	94 (59.5)
46–65 [158]	23	23 (14.5)	86 (54.4)

[a] All NS values are significantly different from the corresponding OP values.
[b] The data include organisms isolated at both sites simultaneously.

was detected somewhat more often at the OP site than at the NS site, but the difference was not statistically significant.

After transport from the study site, 479 swabs were inoculated at the laboratory. Plating at the study site was done for 1,088 swabs. For the purpose of comparing the rates of isolation from the two inoculation sites, stratification of data was done. Nine hundred eighty-three swabs, 564 swabs inoculated at the laboratory and 419 swabs from the study site, were analyzed. When inoculation was done in the laboratory, the NS swab yielded *S. pneumoniae* significantly more often than did the OP swab (201 [35.6%] versus 13 [2.3%]; $P < 0.0001$). When plating was done on site, NS and OP swabs were positive in 84 (20%) and 12 (2.8%) cases, respectively ($P < 0.0001$). Isolation of *H. influenzae* in the NS and OP areas when inoculation was done in the laboratory was not significant (81 [14.3%] and 66 [11%], respectively). When plating was done on site, there were more isolates from the OP site (64 [15.2%]) than from the NS site (45 [10.7%]); however, the overall difference was not statistically significant.

The effects of feeding pattern (breast-feeding, formula, or both), number of siblings, season, and prior antibiotic usage were analyzed as potential confounders of the difference in bacterial yield between anatomical sites. When stratified analysis was performed, the difference in carriage rates of *S. pneumoniae* and *H. influenzae* between OP and NS swabs remained unaffected.

In this longitudinal prospective study of healthy Filipino infants, we were able to show a significantly higher carriage rate for *S. pneumoniae* with NS swabs than with OP swabs. To our knowledge, this is the first time that the two anatomical sites have been swabbed concurrently to monitor the carriage of these pathogens in a large number of healthy children.

A number of studies have suggested that *S. pneumoniae* may be found in NS or OP aspirates more frequently than by throat or OP swabbing (5, 6, 9), but there are contrary results suggesting a higher rate of recovery by the latter method for *S. pneumoniae* (4, 7). The earlier studies were conducted with children of a wider age range (0 to 5 years), the children were monitored not longitudinally but cross-sectionally in various age groups, and the numbers of children were smaller. The differences in study populations, exact sampling sites, and culture methods may explain the variable results.

TABLE 2. URT carriage rates of *H. influenzae* among infants by anatomical site and age

Age (wk) [*n*]	No. positive (%)			*P*[b]
	Both sites	OP[a]	NS[a]	
6–8 [295]	44	73 (24.7)	61 (20.7)	0.1048
10–12 [270]	55	90 (33.3)	81 (30.0)	0.3057
14–17 [240]	69	104 (43.3)	93 (38.8)	0.1930
18–22 [229]	50	87 (38.0)	77 (33.6)	0.2606
32–39 [158]	43	75 (47.5)	79 (50.0)	0.6885
46–65 [158]	34	66 (41.8)	64 (40.5)	1.0000

[a] The data include organisms isolated at both sites simultaneously.
[b] OP versus NS.

The significant difference between the NS carriage and OP carriage of *S. pneumoniae* indicates the paramount importance of the choice of the swabbing site. Using the NS swab alone would have missed only 5% of all the *S. pneumoniae* findings, whereas using the OP swab alone would have missed approximately 66%. The significant difference between the rates of *S. pneumoniae* URT carriage at the two sites was seen across all age groups. The recovery rates of *S. pneumoniae* and *H. influenzae*, which are both fastidious and fragile bacteria, obtained when the inoculation was done in the laboratory did not differ significantly from the rates obtained when the inoculation was done at the study site. The effects of a number of other confounding factors which might influence the intensity or site of carriage were analyzed, but none of them proved significant.

Both *S. pneumoniae* and *H. influenzae* attach to buccal and OP epithelial cells (2, 3), evidently through the interaction of bacterial surface adhesins with a glycoconjugate receptor. *S. pneumoniae* binds to the GlcNAcB1-3Gal moiety of the lacto and neolacto series of glycolipids (1). It is possible that the density of receptor molecules for *S. pneumoniae* in the nasal cavity is higher than in the oropharynx, leading to higher culture yields for NS swabs.

The recovery rates of *H. influenzae* by NS swabbing and OP swabbing did not differ significantly. Using the OP or NS swab alone, we would have missed approximately a quarter of all the *H. influenzae* isolates.

In summary, *S. pneumoniae* is detected significantly more frequently by NS swabs than by OP swabs in healthy children less than 1 year of age, whereas the carriage rates of *H. influenzae* at NS and OP sites are similar. Therefore, in studying URT carriage of *S. pneumoniae* with a single swab, NS swabbing should be preferred.

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