JOURNAL OF CLINICAL MICROBIOLOGY, May 1997, p. 1077–1079 Vol. 35, No. 5
0095-1137/97/$04.00+0


# Comparison of Four Different Sampling Methods for Detecting Pharyngeal Carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae* in Children

S. RAPOLA,[1]* E. SALO,[2] P. KIISKI,[1] M. LEINONEN,[3] AND A. K. TAKALA[1]

*Department of Vaccines[1] and Department of Oulu,[3] National Public Health Institute, and Aurora Hospital,[2] Helsinki, Finland*

Received 4 October 1996/Returned for modification 9 December 1996/Accepted 31 January 1997

**Samples from 96 children with acute respiratory infection were obtained simultaneously with nasal, nasopharyngeal, and oropharyngeal swabs and by nasopharyngeal aspiration and were cultured on chocolate and blood agar plates. The rates of isolation of *Streptococcus pneumoniae* and *Haemophilus influenzae* detected by the four sampling methods were compared. Nasopharyngeal aspirates were optimal for the detection of both *S. pneumoniae* (isolation rate, 33%) and *H. influenzae* (isolation rate, 31%). When a nasopharyngeal aspirate is not available, such as for healthy children or children with no obtainable secretions, the nasopharyngeal swab seems optimal for the detection of both *S. pneumoniae* and *H. influenzae* among children younger than 13 months of age. Among older children, similarly, the nasopharyngeal swab seems optimal for the detection of *S. pneumoniae*; however, for *H. influenzae*, the oropharyngeal swab seems optimal.**

*Streptococcus pneumoniae* and *Haemophilus influenzae* are commonly present on the mucosal membrane of the nasopharynges and throats of healthy children (14, 16). They are also the most important bacterial pathogens causing mucosal infections such as otitis media, maxillary sinusitis, and bronchitis. Colonization of the nasopharynges, the role of mucosal carriage of *S. pneumoniae* and *H. influenzae*, and the role of cofactors (e.g., concurrent viral infections) are central issues in research on the pathogenesis of clinical infections caused by these organisms (7, 10, 16, 17).

It is also important to evaluate mucosal carriage of *S. pneumoniae* and *H. influenzae* in vaccination trials. *H. influenzae* type b conjugate vaccines have been shown not only to protect individuals from invasive *H. influenzae* type b infections but also to reduce carriage of *H. influenzae* type b (15). Preliminary data with pneumococcal conjugate vaccines have shown a significant reduction of nasopharyngeal carriage of vaccine-type *S. pneumoniae* (6); the reduced mucosal carriage is anticipated to decrease the incidence of mucosal infections.

The observed rate of asymptomatic carriage of *S. pneumoniae* and *H. influenzae* is highest in children of preschool age and tends to decrease with increasing age (7, 12). The rates of carriage of *S. pneumoniae* and *H. influenzae* have been reported to vary from 7 to 99% (3, 8) and 5 to 87% (2, 3), respectively, depending on the age, health, and socioeconomic status of the child and the study population. Variations in sampling methods, delays prior to culturing and differences in culture techniques may affect the detected isolation rates. Microbiologic methods for culturing these bacteria are quite well documented, but data concerning the influence of the sampling methods are sparse and controversial. Nasopharyngeal sampling has been considered better than oropharyngeal sampling for detecting *S. pneumoniae* in most studies (4, 7, 12), but there are also contradictory results (3). In detecting *H. influenzae*, oropharyngeal sampling has been reported to be superior to nasopharyngeal sampling (3, 12). Only a few carriage studies, however, have directly compared different sampling methods for the detection of *S. pneumoniae* and/or *H. influenzae* (3, 4, 12).

In this study samples were obtained simultaneously from 96 children by four different methods in order to optimize the detection of *S. pneumoniae* and *H. influenzae* carried on the mucosa of the upper respiratory tract.

## MATERIALS AND METHODS

The samples were collected from June 1993 through January 1994 at the Aurora Hospital of the Helsinki Health Center, a primary care and referral center for children in the Helsinki, Finland, area. We offered participation in the study to children with symptoms of acute respiratory infection, defined as an illness having a sudden onset with rhinorrhea, pharyngitis, or cough, indicating mucosal involvement of the nose, throat, or bronchus, anticipating that all four samples could easily be obtained with minor inconvenience to the child. Children who were receiving antimicrobial treatment were excluded from the study. Altogether, 101 children younger than 7 years of age were enrolled in the study.

The study design was approved by the Ethics Committee of the Helsinki Health Center and the Ethical Issues Committee of the National Public Health Institute. Informed consent was obtained from the parents.

Four different samples were obtained from the upper respiratory tract of each child in the following order (the nasal samples were all obtained from the same nostril). (i) A nasal swab (N) specimen was obtained from a depth of 1 cm in the nostril with a cotton-tipped wooden swab (diameter, 2 mm). (ii) To obtain a nasopharyngeal aspirate (NPA), a catheter (diameter, 3.3 mm) of the Pediatric Mucus Extractor (Orion Diagnostica, Espoo, Finland) was guided through the nostril to a distance equal to that from the child's nose to the outer ear to ensure that the tip was in the nasopharynx, and the catheter was pulled back by applying gentle suction with an electric suction device. (iii) To obtain a nasopharyngeal swab (NP) specimen, a calcium alginate-tipped aluminum wire swab (Calgiswab1; Orion Diagnostica) was inserted in the nasopharynx through the nostril as described above. Once in the nasopharynx, the swab was left in place for 5 s and was then removed gently. (iv) An oropharyngeal swab (OP) specimen was obtained from the posterior wall of the oropharynx with a cotton-tipped wooden swab.

All specimens were placed in Stuart transport medium (Transpocult; Orion Diagnostica), stored overnight at 4°C, and inoculated the next morning in the laboratory of the National Public Health Institute.

To isolate *H. influenzae* the swabs were cultured on chocolate agar plates, with oleandomycin (50 μg) disks (Biodisk, Solna, Sweden) placed on the first and second streaked quarters of the plate to inhibit the growth of normal flora (9). For detection of *S. pneumoniae* the swabs were cultured on chocolate agar plates and on blood agar plates containing 5 μg of gentamicin per ml (5). The plates were incubated for 24 to 48 h at 35°C in an atmosphere containing 5% $CO_2$. Colonies appearing around the oleandomycin disks were considered probable *H.*

* Corresponding author. Mailing address: Department of Vaccines, National Public Health Institute, Mannerheimintie 166, 00300 Helsinki, Finland. Phone: 358-9-4744680. Fax: 358-9-4744675. E-mail: Satu.Rapola@ktl.fi.

TABLE 1. Comparison of the four sampling methods for the detection of *S. pneumoniae* and *H. influenzae*

| Sample | No. (%) of sample infected with *S. pneumoniae* | *P* value for *S. pneumoniae*[a] | No. (%) of samples infected with *H. influenzae* | *P* value for *H. influenzae*[a] |
|---|---|---|---|---|
| N | 31 (32) | 0.0009 | 15 (16) | 0.005 |
| NP | 29 (30) | 0.0067 | 18 (19) | 0.039 |
| NPA | 32 (33) | 0.0005 | 30 (31) | 0.491 |
| OP | 19 (20) | Reference | 27 (28) | Reference |

[a] The *P* values refer to differences in comparisons of N specimens, NP specimens, or NPAs with OP specimens. Calculations were done by using the test for paired proportions (McNemar's test).

*influenzae* isolates, and they were further analyzed for their X (hemin) and V (NAD) factor requirements. Production of β-lactamase was tested by the cloverleaf method (11). *S. pneumoniae* isolates were identified by their α-hemolytic colony morphology and susceptibility to an optochin disc (Biodisc), and they were serotyped by counterimmunoelectrophoresis with antisera from the State Serum Institute, Copenhagen, Denmark. The latex agglutination technique was used to identify serotypes 7 and 14 (15). The penicillin resistance of the isolated *S. pneumoniae* organisms was determined by the agar dilution method (13).

**Statistical methods.** The $\chi^2$ test with two-by-two tables was used when age groups were compared. Paired comparisons of the sampling methods were done by the test for paired proportions (McNemar's test). Kappa statistics were used to evaluate the level of agreement for pairs of sampling methods.

## RESULTS

All four types of samples (N, NP, and OP specimens and NPAs) were obtained from 96 children. Thirty-four percent of the children were girls, and 66% were boys. The median age was 3.9 months (range, 0.9 to 73.9 months), and 72% of the children were less than 13 months of age.

*S. pneumoniae* was found in at least one of the four samples from 32 (33%) children and *H. influenzae* was found in at least one of the four samples from 39 (41%) children. Both organisms were found in 13 (14%) of the children; 38 (40%) children carried neither organism. Of the children with positive *S. pneumoniae* culture results, 41% were also positive for *H. influenzae. S. pneumoniae* was cultured somewhat less frequently (33%) from children positive for *H. influenzae.*

The isolation rates differed according to the sampling site. The highest rate of isolation of *S. pneumoniae* (33%) was found with the NPAs. With the N specimens the isolation rate was 32%, and with the NP specimens the isolation rate was 30% (the differences were not significant). The lowest isolation rate was with the OP specimens (20%), which differed significantly from rates with the other samples (Table 1). Penicillin-resistant *S. pneumoniae* (MIC, ≥0.06 μg/ml) was found in the samples from one child.

A single *S. pneumoniae* serotype or serogroup was detected among 27 of the 32 carriers of *S. pneumoniae*, while two pneumococcal strains were cultured simultaneously from the samples from five children. Overall, the most frequent serotypes or serogroups were 19, 6, 23, and 14, representing 72% of the strains.

*H. influenzae* was also most often detected in NPAs (31%). For N specimens (16%) and NP specimens (19%), isolation rates were significantly lower than those for either NPAs or the OP specimens (28%) (Table 1). *H. influenzae* was found in only one of the four samples from 15 children: in eight OP specimens, in six NPAs, and in one N specimen. The *H. influenzae* organisms isolated from three children produced β-lactamase.

The highest rates of isolation of both *S. pneumoniae* and *H. influenzae* were for NPAs. For the detection of *S. pneumoniae*, the agreement between the rates for NPAs and NP specimens, the most often used method for the detection of *S. pneumoniae*, was excellent (kappa statistic, 0.93). For the detection of *H. influenzae*, the agreement between the rates for NPAs and OP specimens, the most often used method for the detection of *H. influenzae*, was good (kappa statistic, 0.53).

In order to determine whether there was a correlation between the age of the child and the site positive for bacteria, we looked at the rates of isolation of *S. pneumoniae* and *H. influenzae* for children less than 13 months of age. For children in this age group, the highest rates of isolation of *S. pneumoniae* (29%) and *H. influenzae* (28%) were with the NPAs. The overall rate of isolation of *S. pneumoniae* was 63%. The rates of isolation of *S. pneumoniae* were significantly higher with nasal (N and NP) specimens than with OP specimens (28% with N specimens and 26% with NP specimens compared to 14% with OP specimens; $P = 0.0027$ and $P = 0.0047$, respectively). *H. influenzae* was detected in 67% of the children, with no significant differences between the rates of isolation with nasal specimens and OP specimens (14% with N specimens and 17% with NP specimens compared to 25% with OP specimens; $P = 0.052$ and $P = 0.197$, respectively).

## DISCUSSION

Our results indicate that NPAs are optimal for the detection of both *S. pneumoniae* and *H. influenzae*. However, for children with no secretions obtainable by nasopharyngeal aspiration or N specimens, the NP specimen seems optimal for the detection of *S. pneumoniae*, whereas the OP specimen seems optimal for the detection of *H. influenzae.*

The results of our study indicate that *S. pneumoniae* is more often found in cultures of nasal (N and NP) specimens or NPAs and that *H. influenzae* is more often found in cultures of OP specimens, a result similar to those found in previous studies (3, 7). The difference in the preferred sites may reflect the ability of these bacteria to adhere to different mucosal cell types, or it may be connected with receptor density and the receptivity of the mucosal cells in the nasopharyngeal area for attaching *S. pneumoniae* and *H. influenzae* (1, 4).

Capeding et al. (4) evaluated sampling sites for the detection of upper respiratory tract carriage of *S. pneumoniae* and *H. influenzae* among younger healthy study subjects, Filipino infants aged less than 65 weeks. They reported significantly higher rates of isolation of *S. pneumoniae* from the nasal site than from the oropharyngeal site, but *H. influenzae* was found equally often at both sites. In our study, the results were similar for children in the same age group (children less than 13 months of age), i.e., a significantly higher rate of isolation of *S. pneumoniae* with nasal sampling than with OP sampling and no statistically significant difference in the detection of *H. influenzae* between nasal specimens and OP specimens. These parallel findings indicate that the rate of isolation of these bacteria is not affected by the acute respiratory infection of the child but rather by the method of sampling.

Sixteen percent of the children from whom *S. pneumoniae* was isolated were found to carry two serotypes at the same time. Serotypes 19, 6, 23, and 14 comprised 72% of all sero-

types detected. The strains of these serotypes associated with mucosal infections have been shown to have higher adhesive capacities than the strains causing invasive diseases (1).

In this study group, i.e., infants and children with respiratory infection and secretions, the NPAs were superior for the simultaneous detection of *S. pneumoniae* and *H. influenzae*. The agreement of the results obtained with NPAs with those obtained with NP specimens for the detection of *S. pneumoniae* was excellent, and the agreement of the results obtained with NPAs with those obtained with OP specimens for the detection of *H. influenzae* was good.

When NPAs are not available, such as for healthy children or children with no obtainable secretions, use of the NP specimen seems optimal for the detection of both *S. pneumoniae* and *H. influenzae* among children less than 13 months of age. Among older children, use of the NP specimen seems optimal for the detection of *S. pneumoniae* but is inadequate for the detection of *H. influenzae*. For the detection of *H. influenzae* among older children, use of the OP specimen seems optimal.

## ACKNOWLEDGMENTS

We thank the families of the study children and the personnel at Aurora Children's Hospital. We also thank Alice Kyyriäinen for excellent technical work. Juhani Eskola and P. Helena Mäkelä are acknowledged for critical reading of the manuscript, and Janne Rapola is acknowledged for supportive criticism and assistance in the management of the data.

## REFERENCES

1. **Andersson, B., B. Eriksson, E. Falsen, A. Fogh, L. Å. Hanson, O. Nylen, H. Peterson, and C. Svanborg Eden.** 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.
2. **Aniansson, G., B. Alm, B. Andersson, P. Larsson, O. Nylen, H. Peterson, P. Rigner, M. Svanborg, and C. Svanborg.** 1992. Nasopharyngeal colonization during the first year of life. J. Infect. Dis. **165**(Suppl. 1)**:**S38–S42.
3. **Box, Q. T., R. T. Cleveland, and C. Y. Willard.** 1961. Bacterial flora of the upper respiratory tract. Am. J. Dis. Child. **102:**39–47.
4. **Capeding, M. R. Z., 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.
5. **Converse, G. M., III, and H. C. Dillon, Jr.** 1977. Epidemiological studies of *Streptococcus pneumoniae* in infants: methods of isolating pneumococci. J. Clin. Microbiol. **5:**293–296.
6. **Dagan, R., R. Melamed, M. Muallem, L. Piglansky, D. Greenberg, O. Abramson, P. M. Mendelman, N. Bohidar, and P. Yagupsky.** 1996. Reduction of nasopharyngeal carriage of pneumococci during the second year of life by a heptavalent conjugate pneumococcal vaccine. J. Infect. Dis. **174:** 1271–1278.
7. **Gray, B. M., G. M. Converse III, 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.
8. **Hable, K. A., J. A. Washington II, and E. C. Herrmann, Jr.** 1971. Bacterial and viral throat flora. Comparison of findings in children with acute upper respiratory tract disease and in healthy controls during winter. Clin. Pediatr. **10:**199–203.
9. **Hallmann, L., and F. Burghardt.** 1974. *Haemophilus influenzae*, p. 151–154. *In* L. Hallmann and F. Burghardt (ed.), Clinical microbiology. Georg Thieme Verlag, Stuttgart, Germany. (In German.)
10. **Henderson, F. W., A. M. Collies, M. A. Sanyal, J. M. Watkins, D. L. Fairclough, W. A. Clyde, Jr., and F. W. Denny.** 1982. A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. N. Engl. J. Med. **306:**1377–1383.
11. **Kjellander, J., and K. E. Myrbäck.** 1964. A simple test for penicillinase-production. Acta Pathol. Microbiol. Scand. **61:**494.
12. **Masters, P. L., W. Brumfitt, R. L. Mendez, and M. Likar.** 1958. Bacterial flora of the upper respiratory tract in Paddington families, 1952–4. Br. Med. J. **1:**1200–1205.
13. **Nissinen, A., P. Grönroos, P. Huovinen, E. Herva, M.-L. Katila, T. Klaukka, S. Kontiainen, O. Liimatainen, S. Oinonen, and P. H. Mäkelä.** 1995. Development of beta-lactamase-mediated resistance to penicillin in middle-ear isolates of *Moraxella catarrhalis* in Finnish children, 1978–1993. Clin. Infect. Dis. **21:**1193–1196.
14. **Riley, I. D., and R. M. Douglas.** 1981. An epidemiologic approach to pneumococcal disease. Rev. Infect. Dis. **3:**233–245.
15. **Takala, A. K., J. Eskola, M. Leinonen, H. Käyhty, A. Nissinen, E. Pekkanen, and P. H. Mäkelä.** 1991. Reduction of oropharyngeal carriage of *Haemophilus influenzae* type B (Hib) in children immunized with an Hib conjugate vaccine. J. Infect. Dis. **164:**982–986.
16. **Teele, D. W.** 1992. Pneumococcal infections, p. 1223–1228. *In* R. D. Feigin and J. D. Cherry (ed.), Textbook of pediatric infectious diseases, 3rd ed. The W. B. Saunders Company, Philadelphia, Pa.
17. **Watson, D. A., D. M. Musher, and J. Verhoef.** 1995. Pneumococcal virulence factors and host immune responses to them. Eur. J. Clin. Microbiol. Infect. Dis. **14:**479–490.