Influence of Sampling Technique on Detection of Potential Pathogens in the Nasopharynx

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Objectives: To determine the optimal approach for nasopharyngeal culture and to establish which approach children tolerate best.

Design: Cross-sectional study.

Setting: A pediatric otolaryngology department of a Dutch tertiary care hospital.

Patients: A cohort of 42 children with chronic suppurative otitis media.

Intervention: Paired nasopharyngeal samples were collected transorally and transnasally and cultured for potential aerobic pathogens.

Main Outcome Measures: The isolation rate of both samples and the amount of discomfort measured by the visual analog scale.

Results: Forty-six (87%) of 53 samples obtained transnasally were culture positive vs 40 (75%) of 53 samples obtained transorally (P = .20). Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Staphylococcus aureus were found more frequently with the transnasal than with the transoral approach: 34% vs 13% (P = .003), 62% vs 51% (P = .20), 30% vs 19% (P = .15), and 21% vs 11% (P = .18), respectively. Mean (SD) visual analog scale scores were 5.3 (1.0) and 3.4 (1.7) (P < .001) for the transnasal and transoral approaches, respectively.

Conclusions: Although the transoral approach is better tolerated in children, the isolation rate of the transnasal approach is higher, especially for S pneumoniae. The transnasal sampling technique should therefore be the preferred approach for detection of potential pathogens in the nasopharynx in children.


The nasopharynx is the assembly point of clearance of secretions from the nasal cavity, paranasal sinuses, and middle ear. Both in children and adults the nasopharynx is colonized by a variety of microorganisms, including commensals and potential pathogens such as Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis. Detection of potential pathogens in the nasopharynx is important in understanding the etiology of middle ear disease, the evaluation of antibiotic resistance before and after treatment, and the development of new vaccines. Reported carriage varies strongly across studies. Differences in study populations, sampling and culture techniques, and the frequency of specimen collection may explain this variation. Although variables in genetic background, socioeconomic conditions, and culture techniques have been studied extensively, little is known about the influence of different nasopharyngeal sampling techniques on the culture results, especially in children.

The nasopharynx can be approached through the nose and the mouth. In most studies, samples are obtained transnasally because this technique is presumed to be the best way to obtain a representative sample of the nasopharyngeal flora. However, transnasal sampling is unpleasant, and the transoral approach may be preferred, especially when samples have to be obtained repeatedly from children. So far, to our knowledge, comparisons of these 2 approaches in children have not been published. The purposes of this study are (1) to compare the isolation rate of paired nasopharyngeal samples obtained transorally and transnasally in children and (2) to establish which approach is tolerated best.
key agar plates for the isolation of potential aerobic pathogens. Swabs were transported to the microbiology laboratory and plated within 18 hours of sampling onto sheep blood (5%), Stuart transport medium at room temperature. Swabs were immediately stored in Stuart transport medium at room temperature.

The nasopharyngeal swabs were immediately stored in Stuart transport medium at room temperature. Swabs were transported to the microbiology laboratory and plated within 18 hours of sampling onto sheep blood (5%), Haemophilus, and MacConkey agar plates for the isolation of potential aerobic pathogens. The culture plates were incubated aerobically at 37°C (MacConkey agar) and less than 5% carbon dioxide (blood and Haemophilus agars). They were examined at 24 and 48 hours. Colonies suspected to be Staphylococcus, Streptococcus, Candida albicans, and other aerobic gram-negative bacteria were identified by previously described methods.

### METHODS

**POPULATION CHARACTERISTICS**

From October 11, 2004, until February 21, 2005, paired nasopharyngeal samples were collected from children visiting the Department of Pediatric Otolaryngology of the University Medical Center Utrecht. All children had chronic suppurative otitis media and participated in a trial on the effectiveness of prolonged treatment with a combination product of sulfamethoxazole-trimethoprim. Inclusion criteria for this study were age between 1 and 12 years and otorrhea for more than 3 months. Exclusion criteria were cholesteatoma, known immune deficiency other than for IgA or IgG, Down syndrome, craniofacial anomalies, cystic fibrosis, immotile cilia syndrome, allergy to sulfamethoxazole-trimethoprim, or continuous use of antibiotics for more than 6 weeks in the past 6 months.

In total, 53 paired nasopharyngeal samples were collected from 42 children at baseline or at one of the follow-up visits. The mean age of the children was 5.5 years (range, 1.3-11.7 years); 20 were boys, and 22 were girls.

Forty-six (87%) of the samples obtained transnasally were culture positive vs 40 (75%) of the samples obtained transorally. Since the results were similar, the results of the general known analyses are presented.

**COLLECTION OF SPECIMENS**

Both at baseline and during follow-up visits, study physicians obtained nasopharyngeal samples transnasally and transorally, using flexible, sterile, rayon-tipped swabs (Medical Wire & Equipment Co, Corsham, England). For the transnasal technique, the nasal cavity was inspected using a nasal speculum. A culture swab was inserted under the inferior turbinate along the floor of the nose until the nasopharynx was reached. When resistance was felt, the swab was rotated and subsequently removed. For the transoral technique, the throat was inspected under direct light and depression of the tongue. A culture swab was inserted in the oropharynx and, using the flexibility of the wire, curved upward behind the soft palate, where it was swept over the posterior wall of the nasopharynx. Contact of the swab with the pharyngeal tonsils and the mucosa of the oral cavity were avoided.

**MICROBIOLOGICAL INVESTIGATION**

The nasopharyngeal swabs were immediately stored in Stuart transport medium at room temperature. Swabs were transported to the microbiology laboratory and plated within 18 hours of sampling onto sheep blood (5%), Haemophilus, and MacConkey agar plates for the isolation of potential aerobic pathogens. The culture plates were incubated aerobically at 37°C (MacConkey agar) and less than 5% carbon dioxide (blood and Haemophilus agars). They were examined at 24 and 48 hours. Colonies suspected to be Staphylococcus, Streptococcus, Candida albicans, and other aerobic gram-negative bacteria were identified by previously described methods.

**STATISTICAL ANALYSIS**

The total percentage of culture-positive samples obtained transnasally was compared with the percentage obtained transorally. The isolation rate of each microorganism for both samples was compared. Statistical significance was tested by the McNemar test. Means with standard deviations and median scores were calculated for the VAS scores of both nasopharyngeal approaches, and statistical significance was tested by the Wilcoxon signed rank test. We also performed repeated-measures analyses because some children had samples obtained more than once. Since the results were similar, the results of the generally known analyses are presented.

In total, 53 paired nasopharyngeal samples were collected from 42 children at baseline or at one of the follow-up visits. The mean age of the children was 5.5 years (range, 1.3-11.7 years); 20 were boys, and 22 were girls.

Forty-six (87%) of the samples obtained transnasally were culture positive vs 40 (75%) of the samples obtained transorally ($P=.20$; Figure). Seven different species groups were detected by both approaches. Multiple microorganisms were isolated in 26 (49%) of the samples.

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**RESULTS**

<table>
<thead>
<tr>
<th>Species</th>
<th>Transoral</th>
<th>Transnasal</th>
<th>Both Transoral and Transnasal</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>33</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>7</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em></td>
<td>18</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>16</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Pseudomonas species</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><em>Haemophilus species</em></td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No Organisms Cultured</td>
<td>6</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Multiple Organisms</td>
<td>14</td>
<td>12</td>
<td>26</td>
</tr>
</tbody>
</table>

Figure: Number of positive nasopharyngeal cultures obtained by the transnasal and transoral approach and the combination of these approaches per species (N=53).

For children older than 4 years, the 6-smiles visual analog scale (VAS) was used to establish which approach was tolerated best. Children were asked to point out which smile best described the feeling associated with sampling the nasopharynx through the nose and through the mouth. The first smile indicated no pain, whereas the sixth smile indicated worst possible pain. Finally, the children were asked which technique they felt was most unpleasant.
obtained transnasally and in 14 (26%) obtained transorally ($P=0.004$).

*S. pneumoniae* was isolated significantly more frequently with the transnasal approach than with the transoral approach (34% vs 13%; $P=0.003$; Figure). In 6 (11%) of the samples, both samples were positive for *S. pneumoniae*. For *H. influenzae*, *M. catarrhalis*, and *S. aureus*, both samples were positive in 22 (42%), 7 (13%), and 4 (8%) of the samples, respectively. These potential pathogens were also cultured more frequently with the transnasal approach than with the transoral approach: 62% vs 51% ($P=0.20$), 30% vs 19% ($P=0.15$), and 21% vs 11% ($P=0.18$), respectively.

For detection of *S. pneumoniae*, the transnasal approach alone would have missed 1 of 19 positive *S. pneumoniae* culture results, and the transoral approach alone would have missed 12. For *H. influenzae*, *M. catarrhalis*, and *S. aureus*, the transnasal and transoral approaches would have missed 5 vs 11 of 38, 3 vs 9 of 19, and 2 vs 7 of 13 positive culture results, respectively. The other potential pathogens were isolated in less than 6% of the nasopharyngeal samples, and isolation rates were similar for the transnasal and transoral approaches.

Nineteen of the 22 children older than 4 years were evaluated with the VAS. Mean (SD) VAS scores were 5.3 (1.0) (median, 6.0; range, 3–6) and 3.4 (1.7) (median, 4.0; range, 1–6) for the transnasal and transoral approaches, respectively ($P<0.001$). Sixteen children indicated that the transnasal approach was more unpleasant than the transoral approach, 2 children felt that both approaches were equally unpleasant, and only 1 child believed the transoral approach to be the most unpleasant.

### COMMENT

In children, sampling the nasopharynx by a transnasal approach gave a higher detection rate of potential pathogens than by a transoral approach, especially for *S. pneumoniae*. The transoral technique, however, was better tolerated.

Chi et al. performed a similar study in 99 healthy adults. In contrast to our findings, they found that the transoral technique gave the best detection of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*. This discrepancy may be explained by the fact that nasopharyngeal colonization in their population of healthy adults may differ from that in ours of children with chronic suppurative otitis media. The use of different culture swabs may also have played a role. Chi and colleagues used a culture swab with a 60° bent metal shaft to swab the nasopharynx transorally. We used the flexibility of the shaft to curve the swab from the posterior oropharyngeal wall into the nasopharynx. This method is preferred in children, since their smaller dimensions of the oral and pharyngeal cavity do not allow easy passage of a bent shaft into the nasopharynx. Furthermore, our method can be performed quicker, which is advantageous in children, who are usually less cooperative than adults. On the other hand, it is possible that the samples we obtained transorally intentionally reflect the oropharyngeal rather than the nasopharyngeal flora. Previous studies comparing the culture results of different sampling sites in the nasopharynx and oropharynx support this explanation: higher detection rates of *S. pneumoniae* are found at nasopharyngeal sites than at oral or oropharyngeal sites. For *H. influenzae*, reported carriage rates varied across studies, and similar to our findings, differences in yield between nasopharyngeal and oropharyngeal samples were relatively small. In clinical practice, this contamination is inevitable and in fact preferable in view of a better detection of potential upper respiratory tract pathogens.

It may be important to know whether the strains isolated by either approach differ genetically. Although the isolates obtained in this study were not characterized, other researchers performed serotyping for *S. pneumoniae* isolates and found no important differences between serotypes cultured from the nose, nasopharynx, or oropharynx.

Our VAS scores indicate that children prefer the transoral approach over the transnasal approach. Although Hendley et al. previously suggested that transnasal sampling is not well tolerated by children, this is the first study, to our knowledge, to give evidence that the transoral sampling technique is tolerated best.

In conclusion, although the transoral approach is better tolerated in children, the isolation rate of the transoral approach is higher, especially for *S. pneumoniae*. The transnasal sampling technique should therefore be the preferred approach for detection of potential pathogens in the nasopharynx in children.

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### REFERENCES


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