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Alternative Sampling Methods for Detecting Bacterial Pathogens in Children with Upper Respiratory Tract Infections

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Nasopharyngeal sampling is used for detecting bacteria commonly involved in upper respiratory tract infections, but it requires training and may not always be well tolerated. We sampled children (n = 66) of ages 0 to 4 years, with rhinorrhea, by using a nasopharyngeal swab, a nasal swab, and nose blowing/wiping into a paper tissue. Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Staphylococcus aureus were cultured at similar rates across methods with high concordance (80 to 97%), indicating that they are reliably detected by alternative means.

Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Staphylococcus aureus are bacterial pathogens commonly involved in upper respiratory tract infections in young children. The recommended sampling site for S. pneumoniae and M. catarrhalis is the nasopharynx (10, 11), while for H. influenzae and S. aureus this is the naso- and oropharynx (4) and the nasal vestibule (17), respectively. However, sampling these sites requires training and may not always be well tolerated by the child, especially when conducted repeatedly.

At the time of an upper respiratory tract infection, secretions from the nasopharynx, paranasal sinuses, and nasal cavity accumulate and discharge spontaneously from the nose. Theoretically, bacteria residing at various niches should be detectable in these nasal secretions.

We compared conventional culture results of nasal secretions collected by blowing or wiping the nose into a paper tissue to the current standards for detection of S. pneumoniae, H. influenzae, H. catarrhalis, and S. aureus in a group of children with rhinorrhea as a symptom of an upper respiratory tract infection.

Children (n = 66) of ages 0 to 4 years with nasal secretions as symptom of an upper respiratory tract infection were eligible for the present cross-sectional study. Children were recruited from the outpatient clinic of the ear-nose-throat department of the Wilhelmina Children’s Hospital, University Medical Center Utrecht, the Netherlands, from two local day care centers, and from a population of children who had previously participated in a randomized trial (14). Children with craniofacial abnormalities were excluded, as were children who had received antimicrobial therapy in the previous 2 weeks.

The study was approved by the Institutional Review Board of the University Medical Center Utrecht (available at http://www.umcutrecht.nl/metc) and undertaken in accordance with the European Guidelines for Good Clinical Practice, which incorporate the provisions of the Declaration of Helsinki. Written informed consent was obtained from both parents.

The sequence of sampling was randomized. Pediatric swabs with a flocked nylon fiber tip were used (Eswab 482CE; Copan, Brescia, Italy). A sample from the nasal vestibule (nasal swab) was taken by inserting a swab 1 cm into the nostril and rotating it three times. A nasopharyngeal sample was obtained according to World Health Organization guidelines (11). Nasal secretions were collected with a paper tissue as described by Leach and colleagues (9) by blowing or wiping the nose. The first few paper tissues were discarded, and disposable (nonsterile) gloves were worn during and hands disinfected between procedures. After swabbing visible discharge, the paper tissue was placed as a whole in a container with 10 ml of phosphate-buffered saline (PBS). All swabs were immediately inoculated in 1 ml of modified liquid Amies transport medium, stored at room temperature, and transferred to the laboratory. All samples were cultured within 24 h on Trypticase soy agar supplemented with 5% defibrinated sheep blood with and without 5 mg/liter gentamicin, Hektoen enteric agar 2, and mannitol salt agar by dipping the swab into the medium for each new plate separately. For the paper tissue samples, a cotton swab was used to inoculate the plates. Identification of S. pneumoniae, H. influenzae, M. catarrhalis, and S. aureus was based on colony morphology and conventional methods of determination (16). Growth was measured semiquantitatively.

To detect a concordance rate for each bacterium of at least 80% with 90% power and a two-sided alpha of 5%, a sample size of 66 subjects was required for this study. Concordance rates between sampling methods and the specificities and sensitivities of the alternative methods were calculated. Cohen’s kappa statistic was computed to take chance agreement between pairs (alternative versus standard sampling method) into account. A value of 1 indicates full agreement, while a value of 0 indicates merely chance. A P value of <0.05 was considered statistically significant. Data were analyzed with the statistical software package SPSS, version 18.0, and Episheet (13).

The study was conducted between April 2010 and November 2011.
Staphylococcus aureus
Moraxella catarrhalis
Haemophilus influenzae
collected by blowing the nose into a paper tissue was highly sen-
pared this with culture of a paper tissue directly. While Leach et al.
was cultured even more frequently from a paper tis-
sue directly than from a nasopharyngeal swab (7). Like-
wise, the detection rate for S. aureus ranged from good (kappa statistic, 0.57) to excellent (0.93). S. pneumoniae was cultured even more frequently from a paper tis-
sue directly than from a nasopharyngeal swab (P = 0.039). Like-
ewise, the detection rate for S. aureus was highest for culture of a paper tissue (Table 1). H. influenzae and M. catarrhalis were detected at comparable rates across sampling methods. The density of pneumococci was highest in cultures of a paper tissue directly compared to each of the other sampling methods (Fig. 1).

This is the first study comparing culture of nasal secretions, collected by wiping or blowing the nose into a paper tissue, with the current standards for detecting bacterial pathogens in children with an upper respiratory tract infection. Bacteria were generally detected at similar rates across sampling methods. Most import-
antly, concordance between sampling methods was high, indicat-
that in children with rhinorrhea, culture of a paper tissue col-
lected by simply blowing or wiping the nose reliably detects four common bacterial pathogens. This may have major implications since it is a familiar and, especially for older children, a less un-
pleasant procedure than nasopharyngeal sampling. Moreover, it does not require training.

Nasopharyngeal sampling has been previously compared with other sampling techniques (1, 3, 4, 6, 12, 15), but no studies com-
pared this with culture of a paper tissue directly. While Leach et al. found that detecting pneumococci in a swab from nasal secretions collected by blowing the nose into a paper tissue was highly sen-
sitive for children with visible secretions, this was not compared either with a nasopharyngeal sample or with culture of the paper tissue as a whole (9). The latter is attractive since it does not re-
quire expensive swabs and is even simpler to perform.

We used PBS to transport the paper tissue. PBS is a water-based salt solution commonly used in research, but contrary to the case with modified liquid Amies, it is not specifically formulated to sustain viability of microorganisms. We postulate that the higher pneumococcal detection rate is related to the (abundant) presence of mucus in samples collected by a paper tissue. The physiologic composition of mucus produced during an upper respiratory tract infection may provide a good substrate for bacteria to abide in (2, 8). Our results support the concept that in children with an upper respiratory tract infection, nasal secretions contain bacteria whose detection during asymptomatic episodes would be more con-
strained to a specific ecologic niche. Moreover, pneumococci re-
side in the nasopharynx, an anatomical site that normally prevents easy dispersion. Changes in the upper respiratory tract during infection may therefore be linked to enhanced bacterial transmis-
sion.

Our study has some limitations. First, the specific criterion for rhinorrhea to be present limits the generalizability of our findings. Importantl,
our results cannot be extrapolated to assessment of colonization in asymptomatic children (11). Second, mild respira-
atory infections are mostly attributed to a viral infection (5). The presence of bacterial pathogens may change during such an infec-
tion (7).

Our data show that culturing nasal secretions from a paper
tissue reliably detects bacterial pathogens in children with rhinorhea. This sampling method provides valuable information for studies of bacterial transmission or surveillance in children with upper respiratory tract infections.

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