Recovery of Potential Pathogens and Interfering Bacteria in the Nasopharynx of Otitis Media–Prone Children and Their Smoking and Nonsmoking Parents

Itzhak Brook, MD, MSc; Alan E. Gober, MD

Objective: To study the frequency of isolation of potential pathogens and interfering bacteria in the posterior nasopharynx of otitis media–prone (OMP) children and their smoking and nonsmoking parents to possibly explain why active and passive exposure to smoking is associated with carriage of potentially pathogenic bacteria and an increased risk of respiratory tract infection in both adults and children.

Setting: Outpatient clinic.

Participants: Twenty OMP children and their smoking parents (smoking group) and 20 OMP children and their nonsmoking parents (nonsmoking group).

Interventions: Posterior nasopharynx cultures were taken from 20 OMP children and their smoking parents and 20 OMP children and their nonsmoking parents.

Main Outcome Measure: Potential pathogens and aerobic and anaerobic bacteria with interfering capabilities against these organisms were identified.

Results: Fourteen potential pathogens were isolated from smoking parents, and 17 were recovered from their children. Concordance in isolation of a pathogen between a parent and child was noted in 11 instances. Three potential pathogens were isolated from nonsmoking parents (P<.001 compared with the parents and children in the smoking group and children in the nonsmoking group), and 16 were recovered from their children. Bacterial interference by normal flora isolates against potential pathogens was noted in 58 instances in smoking parents and in 55 instances in their children (P<.05). Bacterial interference was noted in 129 instances in nonsmoking parents (P<.05 compared with the parents and children in the smoking group and children in the nonsmoking group) and in 55 instances in their children.

Conclusions: A high recovery rate of potential pathogens and a low number of interfering organisms were observed in OMP children. This was not related to their parents’ smoking habits. The posterior nasopharynx flora of smoking parents contained more potential pathogens similar to the ones recovered from OMP children and fewer interfering organisms than nonsmoking parents.


Methods

Patients

Included in the study were 40 OMP children consecutively seen for their annual physical ex-
amination in an outpatient clinic and 1 of each of their parents. Twenty of the parents were smokers (defined as smoking at least 10 cigarettes a day for the past 5 years), and 20 were nonsmokers. None of the parents had any chronic illness, received antimicrobial therapy, or had a respiratory tract infection in the 3 months prior to study enrollment. Parents varied in age from 25 to 44 years (average age, 33 years), and 22 were women. Age and sex distribution was similar in both groups.

The children’s ages ranged from 20 to 66 months (average age, 38 months), and 26 were boys. Age and sex distribution was similar in both groups. The OMP patients were defined as having at least 6 episodes of acute suppurative otitis media in the previous 2 years. None of the children had prior adenoidectomy or tonsillectomy, perforation of the tympanic membranes, treatment with antimicrobials or other medications, or any infections in the preceding 4 weeks, and none had chronic medical problems. The protocol was approved by the institutional review board.

Cultures were obtained using sterile calcium alginate swabs. Specimens were collected from the posterior nasopharynx (through the mouth) and were immediately plated into media supportive of the growth of aerobic and anaerobic bacteria.

**MICROBIOLOGIC EXAMINATION**

Sheep’s blood (5%), chocolate, and MacConkey agar plates were inoculated for the isolation of aerobic organisms. The culture plates were incubated aerobically at 37°C (MacConkey agar) and under 5% carbon dioxide (blood and chocolate agar), and they were examined at 24 and 48 hours. For the recovery of anaerobic bacteria, the specimens were inoculated onto prerduced Brucella blood agar enriched with phytonadione (vitamin K1), blood agar that contained kanamycin and vancomycin, and an aerobic blood plate that contained phenylethyl alcohol and enriched thioglycolate broth. These media were immediately incubated in BBL Microbiology GasPack jars (Becton, Dickinson and Company, Franklin Lakes, NJ) at 37°C and examined after 48 and 96 hours of incubation at 37°C. All types of colonies on each plate were isolated. The total number of aerobic and anaerobic bacterial isolates processed from each individual varied from 4 to 17 (average, 7.8). Aerobic and anaerobic bacteria were identified by previously described methods.9,10

**TESTING FOR INTERFERENCE**

The inhibitory activity was tested in a blind fashion against 1 strain each of a recent clinical isolate of Streptococcus pneumoniae, Haemophilus influenzae (non–type b), Moraxella catarrhalis, and S pyogenes. Inhibitory activity of 3 separate colonies of all aerobic and anaerobic isolates was evaluated. The inhibitory activity of each isolate was individually tested against the test organisms, using the Steer steel pin replicator as previously described.11 In brief, minidrops of log-phase broth cultures of the isolates were transferred with the pin replicator to vitamin K1–enriched Brucella blood or chocolate (for H influenzae) agar plates and allowed to dry for 15 minutes at room temperature. A sample of a log-phase broth culture of the target strain was applied adjacent to each of the isolated strains, and the plates were incubated in 5% carbon dioxide or anaerobically at 37°C for 48 hours. Bacterial interference was defined as any reproducible inhibition of growth. Degrees of inhibition varied from complete absence of growth to a narrow zone of poor growth along the proximal area of the colony. Statistical significance was calculated using the $\chi^2$ test with Yates correction.

**RESULTS**

**RECOVERY OF PATHOGENS**

In the smoking group, 14 potential pathogens were isolated from 12 parents (0.7 per parent), and 17 potential pathogens were recovered from 15 of their children (0.85 per child). Concordance in isolation of a pathogen between a parent and child was noted in 11 instances (Table 1). In the nonsmoking group, 3 potential pathogens were isolated from 3 parents (0.15 per parent; $P<.001$ compared with the parents and children in the smoking group and children in the nonsmoking group), and 16 potential pathogens were recovered from 14 of their children (0.8 per child). Concordance in isolation of a pathogen between a parent and child was noted in 2 instances (Table 1).

**BACTERIAL INTERFERENCE**

Bacterial interference between 2 aerobic (alpha and non-hemolytic streptococci) and 2 anaerobic species (Prevotella and Peptostreptococcus species) and 4 potential pathogens (S pneumoniae, H influenzae, M catarrhalis, and S pyogenes) was observed. In the smoking group, bacterial interference was noted in 58 instances against the 4 potential pathogens by 21 normal flora isolates that were recovered from the parents and in 55 instances by 18 isolates from the children (Table 2). In the nonsmoking group, bacterial interference was noted in 129 instances against the 4 potential pathogens by 44 normal flora isolates that were recovered from the parents ($P<.05$ com-
pared with the parents and children in the smoking group and children in the nonsmoking group) and in 55 instances by 20 isolates from the children (Table 3).

**COMMENT**

We observed a high recovery rate of potential pathogens and a low number of interfering organisms in OMP children. This was not related to their parents' smoking habits. The prevalence of potentially pathogenic bacteria and interfering organisms in the respiratory tract of OMP children did not depend on whether the parents smoked. The study also illustrates that the posterior nasopharyngeal flora of smoking parents was similar to that recovered from their OMP children and contained more potential pathogens and fewer interfering organisms than in the nonsmoking parents. These findings confirm previous observations in smokers.6-8

The association between passive exposure to smoking and recurrent otitis media is well established.4 Since smoking parents harbor more potential pathogens and fewer interfering organisms, they may serve as a source of pathogens that can colonize and/or infect their children. Conversely, organisms that originated from their own children could have colonized these parents. It is possible that the exposure to second-hand smoke also contributes to better adherence of pathogens to the nasopharyngeal mucosa. Furthermore, children of smoking parents are exposed to more pathogenic bacteria that colonize their parents. It is also possible that pathogens may be shared between smoking parents and their children, since horizontal spread of organisms in the family setting can occur.12 A cycle can therefore exist in families of OMP children whose parents smoke such that potential microbial pathogens circulate between parents and children. Support for this explanation will come if future studies of smoking parents of non-OMP children show the presence of similar patterns of colonization in them as was observed in smoking parents of OMP children. Of interest is that such a situation does not seem to exist in families where the parents do not smoke, since pathogenic bacteria do not colonize the parents. However, without confirmation by a serologic or nucleic-acid typing procedure, it cannot be ruled out that the concordance of organisms in a parent and a child represents unrelated serotypes.

Previous studies have demonstrated a high recovery rate of potential pathogens (H influenzae, S pneumoniae, and M catarrhals) and a lower number of interfering organisms in OMP children than in non-OMP children.13-16 Bernstein et al13 and Fujimori et al15 documented a higher recovery of interfering alpha-hemolytic streptococci in non-OMP than in OMP children. Brook and Gober16 illustrated that the nasopharyngeal flora of non-OMP children contains more aerobic and anaerobic organisms with interfering capability and fewer potential pathogens than that of OMP children.

### Table 2. Isolates With Interfering Capabilities Recovered in the Retropharynx of Smoking Parents and Their Children

<table>
<thead>
<tr>
<th>Target Organism</th>
<th>Parents, No. (%) (n = 20)</th>
<th>Children, No. (%) (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP</td>
<td>HI</td>
</tr>
<tr>
<td>Alpha-hemolytic streptococci</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Nonhemolytic streptococci</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Prevotella species</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Peptostreptococcus species</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Subtotal</td>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>

Total instances of bacterial interference/No. interfering isolates: 58/21

### Table 3. Isolates With Interfering Capabilities Recovered in the Retropharynx of Nonsmoking Parents and Their Children

<table>
<thead>
<tr>
<th>Target Organism</th>
<th>Parents, No. (%) (n = 20)</th>
<th>Children, No. (%) (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP</td>
<td>HI</td>
</tr>
<tr>
<td>Alpha-hemolytic streptococci</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Nonhemolytic streptococci</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td>Prevotella species</td>
<td>9</td>
<td>465</td>
</tr>
<tr>
<td>Peptostreptococcus species</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>Subtotal</td>
<td>31</td>
<td>32</td>
</tr>
</tbody>
</table>

Total instances of bacterial interference/No. interfering isolates: 129/44

Abbreviations: GAS, group A beta-hemolytic streptococcus (Streptococcus pyogenes); HI, Haemophilus influenzae; MC, Moraxella catarrhals; SP, Streptococcus pneumoniae.
The presence of organisms with interfering potential may play a role in the prevention of upper respiratory tract infection. It is possible that the prior frequent use of antibiotics in infection-prone individuals may have reduced the number of organisms inhibitory to the growth of pathogens. However, since we studied only children who had not received antibiotics in the past month and adults who did not get antibiotics in the past 3 months, prior antibiotic use does not explain the bacterial discrepancies.

The ability of indigenous normal nasopharyngeal flora to inhibit colonization with potential pathogens has been studied in several types of upper respiratory tract infection.17-22 Alpha-hemolytic streptococci were found to inhibit the colonization in patients and in vitro growth of a variety of pathogenic bacteria. These include S pneumoniae, S pyogenes, and Staphylococcus aureus.17-25 The production of bacteriocin and other inhibitory substances that suppresses some bacterial growth or the use of nutrients in the nasopharyngeal environment essential for the potential pathogens may explain this phenomenon.23

Therapeutic colonization of the nasopharynx with interfering bacteria was recently studied by Roos et al,22 who inoculated children with repeated tonsillitis to either alpha-hemolytic streptococci or placebo spray. Clinical recurrences occurred in 2% of the alpha-hemolytic streptococci group (1/51) and 23% of the placebo-treated group (14/61). Similarly, these investigators showed that re-colonization with alpha streptococci with the ability to inhibit the growth of pathogens reduced the recurrence of acute otitis media and the frequency of otitis media with effusion in susceptible children.23 Three months after colonization with alpha-hemolytic streptococci, 22 (42%) of the children given the streptococcal spray were otitis media free and had normal tympanic membranes compared with 12 (22%) of those given placebo.

Further studies are warranted to investigate the colonization patterns in nonsmoking parents and whether colonization of the posterior nasopharynx with interfering organisms and/or cessation of smoking by parents would be beneficial to them as well as their children in allowing for the return of the normal inhibitory flora and the reduction in the number of pathogens and subsequent infections. This would be beneficial if the smoking parents were the source of the potential pathogens in the household setting.

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REFERENCES