Nasopharyngeal Carriage of *Streptococcus pneumoniae* at the Completion of Successful Antibiotic Treatment of Acute Otitis Media Predisposes to Early Clinical Recurrence

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(See the editorial commentary by Klugman and Welsh, on pages 1790–2.)

**Objective.** We sought to investigate the role of *Streptococcus pneumoniae* (SP) nasopharyngeal (NP) colonization after successful antibiotic treatment (Rx) of acute otitis media (AOM) in recurrent AOM (RAOM).

**Patients and methods.** NP cultures were obtained from 494 (93%) of 530 patients at the end of antibiotic treatment (EOT).

**Results.** At enrollment, middle ear fluid (MEF) cultures in 418 (79%) of 530 patients were positive for pathogens. At EOT, NP cultures in 208 (42%) of 494 patients were positive for SP. RAOM was found in 130 (26%) of 494 patients: 66 (32%) of 208 with SP-positive NP and 64 of 286 (22%) without SP-positive NP at EOT (P = .026). MEF was positive for SP during RAOM in 34 (61%) of 56 patients with SP-positive NP and 17 (36%) of 47 patients without SP-positive NP at EOT (P = .022). The same serotype was identified in 24 (80%) of 30 SP pairs; complete identity was found between isolates in 22 (96%) of 23 SP pairs.

**Conclusions.** Early RAOM was more commonly caused by SP if the organism was present in NP at EOT during the initial AOM episode. Most SP-RAOM episodes were caused by SP isolates present in NP at EOT during the previous AOM episode.

Recurrent acute otitis media (AOM) is encountered in 5%–30% of all children with AOM. These AOM-prone children may experience ≥4 episodes of AOM during their first year of life [1–3]. An infection with a new pathogen was reported in the majority of the patients with clinical recurrence of AOM [4–8]. However, eradication of the pathogen that caused the initial episode of AOM was not demonstrated in the majority of these patients. Leibovitz et al. [9] recently described 108 cases of clinical recurrence of AOM found during a 3–4-week follow-up of 1077 children who were enrolled in various double-tympanocentesis antibiotic efficacy studies. These patients developed clinical recurrence of AOM after achieving bacteriologic eradication of the pathogen that caused the initial episode of AOM, which therefore excluded the possibility that the recurrences represented persistent infections. These authors found that (1) true bacteriologic relapses were found in only 28% of the patients, (2) the absolute numbers and proportions of true bacteriologic relapses decreased as time elapsed after the completion of successful antibiotic treatment, and (3) most early true bacteriologic relapses were caused by *Streptococcus pneumoniae*.

Previous studies have shown that children with clinical recurrence of AOM carry a variety of potential AOM-causing pathogens in the nasopharynx both during and between episodes of upper respiratory tract infections [10, 11]. Although low positive predictive values were uniformly reported for the use of nasopharyngeal cultures in the assessment of pneumococcal AOM, the absence of *S. pneumoniae* in the nasopharynx
has a higher negative predictive value for its recovery in the middle ear fluid (MEF) of patients with AOM [12, 13].

The relationship between nasopharyngeal colonization with S. pneumoniae during AOM and the etiology of subsequent episodes of AOM has not yet been investigated. This relationship is of importance, because if the presence of S. pneumoniae at the completion of successful antibiotic treatment of AOM is associated with an increased risk of clinical recurrence of AOM, nasopharyngeal cultures should be used in the evaluation of the efficacy of the antimicrobial agents used in the treatment of this disease. Such a relationship, if found, could also explain the selective role that previous antibiotic treatments play in increasing the antibiotic resistance of the pathogens isolated in subsequent episodes of AOM. Therefore, we conducted this study to investigate the relationship between nasopharyngeal colonization with S. pneumoniae at the completion of successful antibiotic treatment of AOM and the etiology of a subsequent episode of AOM occurring within 3 weeks.

**PATIENTS AND METHODS**

**Patients and study procedures.** The initial baseline population included all patients 3–36 months old with AOM who presented at the Pediatric Emergency Room of the Soroka University Medical Center from 1 January 1996 through 31 December 2002, were enrolled in various double-tympanocentesis antibiotic efficacy studies conducted by the Pediatric Infectious Disease Unit, and were followed for at least 3 weeks after the completion of antibiotic treatment [14–21]. All patients who had clinical recurrence of AOM within 3 weeks of the completion of antibiotic treatment (day 10). For those patients who had positive MEF cultures on day 1, completion of successful antibiotic treatment for the initial episode of AOM was defined as clinical improvement or cure at the completion of antibiotic treatment (day 10).

Clinical recurrence of AOM was defined as the occurrence of an episode of AOM during a 3-week follow-up period after the completion of antibiotic treatment. Tympanocentesis was performed in those patients who developed clinical recurrence of AOM and were available for a follow-up examination.

**Microbiological assessment.** MEF and nasopharyngeal aspirate samples were sent to the Clinical Microbiology Laboratory of the Soroka University Medical Center and were processed within 16 h, as described elsewhere [15]. Swabs of MEF and nasopharyngeal aspirate were plated on trypticase agar medium that contained 5% sheep blood and chocolate agar. The plates were incubated aerobically for 48 h at 35°C. Presumptive identification of S. pneumoniae was based on the presence of α-hemolysis and inhibition of optochin by a positive slide agglutination test (Phadebact; Pharmacia Diagnostics). S. pneumoniae isolates were serotyped by the quelling reaction, in accordance with established procedures [22].

When comparisons were made between S. pneumoniae isolates from the nasopharyngeal aspirate obtained at the completion of antibiotic treatment and from the MEF obtained during the subsequent episode of AOM, similarity (or dissimilarity) between the isolates was defined in accordance with phenotypic (serotyping) and genotypic (pulsed-field gel electrophoresis [PFGE]) patterns.

**PFGE.** PFGE was performed on paired S. pneumoniae isolates in which the isolate recovered from the MEF at the time of clinical recurrence of AOM was phenotypically identical to (i.e., was the same serotype as) the isolate recovered from the nasopharyngeal aspirate obtained at the completion of antibiotic treatment. Chromosomal DNA fragments were generated by SmaI digestion, prepared, and analyzed as described elsewhere [23]. Samples were analyzed on a CHEF-DRIII apparatus (BioRad Laboratories). Settings for the PFGE were as follows:
initial forward time, 5 s; final forward time, 35 s; current, 200 V; run time and temperature, 23 h at 11.3°C. Gels were stained with ethidium bromide and were photographed. Interpretation of the relatedness of strains on the basis of the PFGE pattern was made in accordance with present consensus [23].

**Statistical analysis.** The significance of the differences in nasopharyngeal *S. pneumoniae* carriage rates at the completion of antibiotic treatment between the different groups of patients was calculated using the χ² test. *P < .05* was considered to be significant.

**RESULTS**

A total of 932 patients who had AOM were initially enrolled during the study period and were followed for at least 3 weeks after the completion of antibiotic treatment. Of these, 530 (57%) had successful completion of the antibiotic treatment. Of these, 530 (57%) had successful completion of the antibiotic treatment (figure 1). The MEF culture in 418 (79%) of 530 patients tested positive for the presence of pathogens at enrollment, and 517 pathogens were recovered: 303 (59%) *Haemophilus influenzae* isolates, 191 (37%) *S. pneumoniae* isolates, 21 (4%) *Moraxella catarrhalis* isolates, and 2 (0.4%) *S. pyogenes* isolates (table 1).

At the completion of successful antibiotic treatment, nasopharyngeal aspirate samples were obtained from 494 (93%) of 530 patients, and 208 (42%) cultures tested positive for *S. pneumoniae*. Eighty-six (41%) of the 208 patients who had a nasopharyngeal culture positive for *S. pneumoniae* at the successful completion of antibiotic treatment (day 10) also had a nasopharyngeal culture positive for *S. pneumoniae* at enrollment (day 1). Of the 86 *S. pneumoniae* isolates found, 59 (69%) represented persistence of the initial nasopharyngeal isolate (same serotype), whereas the other 27 (31%) represented new pneumococcal nasopharyngeal acquisitions. Table 2 presents the various types of antibiotic treatment received by the 208 patients who had nasopharyngeal cultures positive for *S. pneumoniae* at the completion of successful antibiotic treatment (day 10), compared with the 286 patients who had nasopha-
Table 1. Distribution of pathogens in initial and recurrent episodes of acute otitis media (AOM).

<table>
<thead>
<tr>
<th>Pathogen(s)</th>
<th>Episode of AOM</th>
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<tr>
<td></td>
<td>Initial (n = 418)</td>
<td>Recurrent (n = 83)</td>
<td></td>
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<tr>
<td>Haemophilus influenzae</td>
<td>209 (50)</td>
<td>30 (36)</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>98 (23)</td>
<td>34 (40)</td>
<td></td>
</tr>
<tr>
<td>H. influenzae and S. pneumoniae</td>
<td>87 (21)</td>
<td>17 (21)</td>
<td></td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>8 (2)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>4 (1)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>12 (3)</td>
<td>...</td>
<td></td>
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</table>

NOTE. Data are no. (%) of patients. In the initial AOM episodes, 517 pathogens were isolated from middle ear fluid; in the recurrent AOM episodes, 101 pathogens were isolated from middle ear fluid.

ryngal cultures negative for S. pneumoniae at the completion of successful antibiotic treatment.

Clinical recurrence of AOM during the follow-up period was observed in 130 (26%) of 494 patients in whom a nasopharyngeal culture was performed at the completion of successful antibiotic treatment. Of the 208 patients who had a nasopharyngeal culture positive for S. pneumoniae at the completion of successful antibiotic treatment, 66 (32%) had a subsequent episode of AOM within 3 weeks, compared with 64 (22%) of 286 of those who had a nasopharyngeal culture negative for S. pneumoniae at the completion of successful antibiotic treatment (P = .026) (figure 1). There were no significant differences between the patients who developed clinical recurrence of AOM and those who did not develop clinical recurrence of AOM. There were also no significant differences in terms of age, sex, ethnic origin, and history of AOM between the patients colonized with S. pneumoniae and those not colonized with S. pneumoniae at the completion of successful antibiotic treatment.

In 103 (79%) of 130 patients, a tympanocentesis was performed at the time of clinical recurrence of AOM. The MEF culture at the time of clinical recurrence of AOM tested positive for the presence of pathogens in 83 (81%) of 103 patients, and 101 pathogens were recovered: 51 (50%) S. pneumoniae isolates, 47 (47%) H. influenzae isolates, and 3 (3%) M. catarrhalis isolates (table 1).

At the time of clinical recurrence of AOM, the MEF tested positive for S. pneumoniae in 34 (61%) of 56 patients who had a nasopharyngeal culture positive for S. pneumoniae at the completion of successful antibiotic treatment, compared with 17 (36%) of 47 patients who had a nasopharyngeal culture negative for S. pneumoniae at the completion of successful antibiotic treatment (P = .022) (figure 1).

The S. pneumoniae isolates from nasopharyngeal aspirate samples obtained at the completion of successful antibiotic treatment were compared with those from the MEF obtained at the time of the diagnosis of the subsequent episodes of AOM. Of these 34 paired S. pneumoniae isolates, 30 were available for evaluation, and 6 represented infections with new S. pneumoniae serotypes. In 24 (80%) of 30 evaluable paired isolates, the pneumococcal serotypes were identical: serotypes 14 (10 isolates); 19F and 23F (3 isolates of each); 19A (2 isolates); and 6B, 7F, 9V, 13, 15B/C, and 35B (1 isolate of each). PFGE was performed in 23 (96%) of 24 available paired S. pneumoniae isolates that had identical serotypes, and complete genotypic identity was found in 22 (96%) of 23 paired isolates (figure 2).

DISCUSSION

Significant changes in pneumococcal nasopharyngeal carriage have been shown to occur early during antibiotic treatment for AOM and to vary as a function of the different antibiotic drugs used. Major differences between various β-lactam and macrolide drugs in their effect on nasopharyngeal colonization during treatment have been shown elsewhere [24–28]. Haiman et al. [29] analyzed the effects of 2 dosages (50 mg/kg/day for either 1 or 3 days) of intramuscular ceftriaxone during and at the completion of antibiotic treatment for AOM and demonstrated a major reduction (to 48% and 20%, respectively, of the initial colonization rates) in nasopharyngeal carriage of S. pneumoniae on days 4–5 of treatment with both regimens. However, this reduction was short-lived, and 2–3 weeks after initiation of the ceftriaxone therapy, the carriage rate returned to that observed before therapy. Dagan et al. [24, 30] demonstrated that antibiotic treatment with azithromycin or trimethoprim-sulfamethoxazole increases the nasopharyngeal carriage rate of antibiotic-resistant S. pneumoniae during treatment and may also induce superinfection of the MEF within 3–5 days after initiation of antibiotic treatment if the nasopharynx already harbors S. pneumoniae strains resistant to the administered drug.

The events responsible for a true bacteriologic relapse of AOM are not fully elucidated. The commonly accepted pathogenetic mechanism attributes a major role to the persistence of pathogens in the MEF at the completion of antibiotic treatment. However, 2 other mechanisms may theoretically be involved in a true bacteriologic relapse: a reacquisition of the initial pathogen (acquired from carrier contacts) and persistent nasopharyngeal colonization in which the initial strain identified during antibiotic treatment is responsible for the subsequent episode. Nevertheless, because most subsequent episodes of AOM may be new infections rather than true bacteriologic relapses [9], we believe that such episodes are caused, in the majority of cases, by a new colonizing strain acquired during or after the initial antibiotic treatment.

In the present study, we investigated the relationship between nasopharyngeal colonization with S. pneumoniae at the completion of successful antibiotic treatment for an initial episode
Table 2. Antibiotic drugs used in the treatment of the initial episode of acute otitis media (AOM).

<table>
<thead>
<tr>
<th>Antibiotic drug, dose</th>
<th>Nasopharyngeal culture</th>
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<tr>
<td></td>
<td>Positive (n = 208)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
</tr>
<tr>
<td>50 mg/kg/day for 1 day</td>
<td>25 (12)</td>
</tr>
<tr>
<td>50 mg/kg/day for 3 days</td>
<td>33 (16)</td>
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<tr>
<td>Amoxicillin/clavulanate</td>
<td></td>
</tr>
<tr>
<td>45/6.4 mg/kg/day divided into 2 doses/day for 10 days</td>
<td>40 (19)</td>
</tr>
<tr>
<td>90/6.4 mg/kg/day divided into 2 doses/day for 10 days</td>
<td>17 (8)</td>
</tr>
<tr>
<td>Amoxicillin (80 mg/kg/day divided into 3 doses/day for 10 days)</td>
<td>9 (4)</td>
</tr>
<tr>
<td>Azithromycin (10 mg/kg for 3 days)</td>
<td>10 (5)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole (8 mg/kg/day divided into 2 doses/day for 10 days)</td>
<td>18 (9)</td>
</tr>
<tr>
<td>Gatifloxacin (10 mg/kg/day for 10 days)</td>
<td>56 (27)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients and may not add to 100% because of rounding.

of AOM and the etiology of a subsequent episode of AOM occurring within 3 weeks. We did not try to establish the true rate of clinical recurrence of AOM in the patients enrolled in this study, because some patients received antibiotic treatment for the clinical recurrence of AOM but there was no microbiological documentation of their etiology and also because episodes of AOM may have been underreported. In addition, we did not intend to determine the true rates of nasopharyngeal colonization with S. pneumoniae at the completion of successful antibiotic treatment in the patients enrolled in this study, because the patients received various antibiotic treatments that had different efficacies in the reduction of nasopharyngeal colonization with S. pneumoniae.

We have clearly demonstrated the important roles that persistence and new acquisition of nasopharyngeal S. pneumoniae during and after antibiotic treatment play in the clinical recurrence of AOM. We have now established that the presence of S. pneumoniae at the completion of successful antibiotic treatment for AOM constitutes a risk factor for the development of a subsequent early recurrence caused by the same strain. We did not
study this relationship with regard to the second most important AOM-causing pathogen—namely, nontypable *H. influenzae*—but we believe that the existence of a similar relationship between clinical recurrence of AOM and nasopharyngeal colonization with this pathogen is plausible.

This study is unique in its concept and probably is unrepeatable in its magnitude. We were able to enroll >900 patients who were analyzed by use of the double-tympanocentesis method to study the bacteriologic efficacy of various antibiotic drugs in the eradication of AOM-causing pathogens. The establishment of successful bacteriologic eradication was a prerequisite for the next step, which was—after exclusion of the possibility that the pathogen persisted in the MEF—to study the relationship between nasopharyngeal colonization with *S. pneumoniae* at the completion of successful antibiotic treatment and the subsequent episode of AOM.

Our findings showed that clinical recurrence of AOM caused by *S. pneumoniae* occurs in the vast majority of patients as a result of nasopharyngeal colonization with this pathogen, despite its eradication from the MEF, at the completion of successful antibiotic treatment for the initial episode of AOM. These findings underline the importance of the drug-pathogen interaction in the nasopharynx as well as in the middle ear. We believe that this relationship should be taken into consideration when the true efficacy of an antibiotic drug in the treatment of AOM is established. A drug that fails to reduce nasopharyngeal colonization with *S. pneumoniae* at the completion of successful antibiotic treatment of AOM will be associated with an increased rate of clinical recurrence of AOM (most instances of which are true bacteriologic relapses) caused by *S. pneumoniae*.

In conclusion, we have established the etiological role that nasopharyngeal colonization with *S. pneumoniae* at the completion of a successful antibiotic treatment of AOM plays in subsequent early clinical recurrence of AOM. We therefore believe that the concept of drug efficacy in the treatment of AOM may be broadened to include nasopharyngeal eradication of *S. pneumoniae*. Although it is likely that other nasopharyngeal pathogens play a role in the clinical recurrence of AOM, this is yet to be established.

References


