Antibiotic Treatment in Acute Otitis Media Promotes Superinfection with Resistant *Streptococcus pneumoniae* Carried before Initiation of Treatment

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Antibiotic-resistant pneumococci are difficult to eradicate from middle ear fluid (MEF) and the nasopharynx (NP). Bacteriologic eradication from the NP and MEF during acute otitis media (AOM) by 3 common antibiotic drugs was prospectively evaluated. In 19 (16%) of 119 MEF culture-positive patients, an organism susceptible to the treatment drug (*Haemophilus influenzae*, *Streptococcus pneumoniae*, or both) was isolated from the initial MEF, whereas resistant *S. pneumoniae* was present in the NP; in 9 (47%) patients, the initial resistant NP organism (identified by serotyping, resistance to the administered drug, and pulsed-field gel electrophoresis) replaced the susceptible MEF organism within only a few days after initiation of treatment. In regions where resistant pneumococci are prevalent, antibiotics may not only fail to eradicate the organisms, but they may often induce MEF superinfection with resistant pneumococci initially carried in the NP. This is an important mechanism by which, in recently treated patients, AOM infections often become refractory to treatment.

When an antibiotic is administered for acute otitis media (AOM), it is targeted to penetrate into the middle ear fluid (MEF). However, the drug is absorbed and distributed to all compartments of the body, including areas that harbor normal flora, such as the nasopharynx (NP), oropharynx, mouth, skin, gut, and genital tract. Once the antibiotic drug reaches these areas, it rapidly tends to reduce the concentration of or eliminate the organisms that are susceptible to the drug. A rapid replacement occurs, either with the already existing organisms that are more resistant to the drug or with newly acquired resistant organisms. The promotion of NP carriage of pneumococci that are nonsusceptible to the administered antibiotic was clearly demonstrated in several studies [1–8].

Clinical experience indicates that AOM usually occurs concurrently with or immediately after a viral upper respiratory infection, usually within 10 days after onset of viral infection symptoms and peaking 3 or 4 days after onset [9–11]. During acute viral infection, NP carriage rate of *Streptococcus pneumoniae* and other otitis pathogens increases. Epidemiologic [12–16] and animal studies [17] suggest that this colonization predisposes to the development of AOM. It is, therefore, biologically plausible that, in some cases, the rapid sterilization of the MEF by antibiotics is accompanied by the promotion of growth of preexisting nonsusceptible NP organisms and may predispose to rapid superinfection of the middle ear if the predisposing condition (e.g., the prior viral infection) is still present. The replacement of the old pathogen with the newly acquired one in the MEF may contribute to the continuing disease and may not be differentiated clinically from persistent or nonresponsive AOM. This is expected to be more prevalent in regions in which antibiotic resistance is common.

In southern Israel, antibiotic resistance among *S. pneumoniae* carried by healthy children [18, 19] or isolated from MEF during AOM [20–23] is common. The present study was conducted to determine whether some clinically nonresponsive otitis media (OM) cases are, in fact, newly acquired infections (superinfections) caused by an antibiotic-nonsusceptible *S. pneumoniae* strain that was present in the NP before initiation of treatment. The patients were participants in studies designed to determine the bacteriologic outcome of azithromycin, amoxicillin/clavulanate, and trimethoprim/sulfamethoxazole (TMP-SMZ) in AOM and the effect of these drugs on the NP carriage of *S. pneumoniae*.

**Patients and Methods**

**Study design.** Children 3–48 months old seen at the Pediatric Emergency Room of Soroka University Medical Center (SUMC; Beer-Sheva, Israel) from January 1998 through February 1999 were included in the study if they met the following 5 criteria: symptoms and physical findings consistent with OM (e.g., fever, irritability, tugging of the ear, and signs of redness and bulging of the tympanic membrane, with blurring of tympanic membrane anatomic landmarks); acute illness for <7 days; no spontaneous perforation and no tympanostomy tubes; follow-up for >4–6 days (second study visit) with a second culture done on visit 2 (72–120 h after initiation.
of the antibiotic treatment); and ≥1 NP culture for *S. pneumoniae* obtained before initiation of antibiotic treatment. Patients whose MEF culture yielded ≥1 pathogen were included in the analysis. Children with initial culture-negative MEF were excluded from the analysis. Patients were assigned to receive 1 of the following 3 regimens: (1) oral amoxicillin/clavulanate 22.5/3.2 mg/kg, twice daily for 10 days; (2) oral azithromycin 10 mg/kg/day, 1 dose on the first day, followed by 4 days of 5 mg/kg once daily; and (3) TMP-SMZ 4/20 mg/kg, twice daily for 10 days.

Typanocentesis was done at enrollment by an otolaryngologist, as described elsewhere [24, 25]. By definition, the initial otologic findings always included the presence of purulent, mucopurulent, or seropurulent MEF. NP cultures for *S. pneumoniae* were obtained from all patients at enrollment, as described elsewhere [18].

At enrollment and on days 4–6, samples for MEF culture by typanocentesis and for NP culture were obtained from all children. The children were followed up until days 10–14. If a clinical relapse occurred, a third typanocentesis was done, with simultaneous NP culture. All patients with a second or third tympanocentesis positive for *S. pneumoniae* were grouped as having a second positive MEF.

**Microbiology.** Transport swabs of MEF aspirates and NP cultures were sent to the Clinical Microbiology Laboratory of the SUMC and were processed within 16 h, as described elsewhere [18, 24, 25]. Typing of recovered isolates was done by the Quellung reaction, according to established procedures [26]. MICs of amoxicillin/clavulanate and TMP were determined by the E-test (PDM epsimeter; AB Biodisk). The cutoff MICs were defined for the 2 drugs, according to National Committee of Clinical Laboratory Standards criteria [27]: amoxicillin/clavulanate MICs for pneumococci of ≤0.5–25 μg/mL were defined as susceptible, MICs of 1.0–0.5 μg/mL as intermediate, and MICs of ≥2.0–1.0 as resistant. For TMP-SMZ, TMP MICs for pneumococci were as follows: ≤0.5 μg/mL, susceptible; 1.0–2.0 μg/mL, intermediate; and ≥4.0 μg/mL, resistant. Because we encountered technical problems with the E-test for testing the susceptibility of *S. pneumoniae* to azithromycin [28], the testing was done by microdilution methods (M. Jacobs, Clinical Microbiology Laboratory, Case Western Reserve University, Cleveland) [27]. For this study, we considered both intermediate and fully resistant strains to be resistant. Identity between pneumococcal isolates was defined by same serotype with ≤1 band difference in the pulse-field gel electrophoresis (PFGE) pattern, done as described elsewhere [29].

**Statistical analysis.** The difference in proportion was tested by χ² or Fisher’s exact tests, as appropriate. *P < .05* was considered significant.

### Results

In total, 192 patients were enrolled to receive 1 of the 3 drugs: 59, 60, and 73 patients received amoxicillin/clavulanate, azithromycin, and TMP-SMZ, respectively. The initial MEF culture was positive in 162 (84%) patients: 48, 49, and 65 patients in the amoxicillin/clavulanate, azithromycin, and TMP-SMZ groups, respectively. Of those, 119 (73%) were followed up until at least days 4–6 and had a second typanocentesis: 29, 41, and 49 patients in the amoxicillin/clavulanate, azithromycin, and TMP-SMZ groups, respectively. These 119 patients constituted the study group. The patients were young (over half were <12 months old), most were boys, a considerable proportion had ≥3 episodes of AOM before the study episode, and many had received antibiotics in the preceding month (table 1).

A total of 151 organisms was isolated initially from the 119 patients. *Haemophilus influenzae* was the most common (88 [58%] patients), followed by *S. pneumoniae* (54 [36%] patients) and others (8 [6%] patients). In 28 (24%) of 119 patients, both *H. influenzae* and *S. pneumoniae* were isolated from the initial MEF culture.

In general, in each study group, the MEF and NP initial isolates did not differ significantly in their MIC distribution (table 2). A correlation was found between the NP and MEF pretreatment culture positivity rate for *S. pneumoniae* (table 3). In only 5 (17%) of 30 children with *S. pneumoniae*–negative NP, MEF yielded *S. pneumoniae*, in contrast with 49 (55%) of 89 children with positive NP culture (*P = .011*). A resistant *S. pneumoniae* strain was isolated from the MEF in 13 (41%) of 32 children with NP-resistant *S. pneumoniae*, versus only 3 (5%) of 57 and 3 (10%) of 30 of those with NP-susceptible *S. pneumoniae* and negative NP cultures, respectively (*P < .001*).

All 119 patients had a second typanocentesis on days 4–6.
Table 3. Rate of *Streptococcus pneumoniae* (SP)-positive middle ear fluid (MEF) at the second or third tympanocentesis in relation to pretreatment nasopharyngeal (NP) and MEF presence and susceptibility of *S. pneumoniae* in 119 children with acute otitis media (AOM) treated with amoxicillin/clavulanate, azithromycin, or trimethoprim-sulfamethoxazole (TMP-SMZ).

<table>
<thead>
<tr>
<th>NP susceptibility, treatment group</th>
<th>MEF susceptibility</th>
<th>Total (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-SP</td>
<td>Non-SP</td>
<td>0/25 (4)</td>
</tr>
<tr>
<td>Amoxicillin/clavulanate</td>
<td>11</td>
<td>0/11</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>7</td>
<td>0/7</td>
</tr>
<tr>
<td>TMP-SMZ</td>
<td>12</td>
<td>0/12</td>
</tr>
<tr>
<td>Total (%)</td>
<td>30</td>
<td>2/30 (7)</td>
</tr>
<tr>
<td>S-SP</td>
<td>1/2</td>
<td>2/3</td>
</tr>
<tr>
<td>Amoxicillin/clavulanate</td>
<td>9</td>
<td>2/9</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>29</td>
<td>2/29</td>
</tr>
<tr>
<td>TMP-SMZ</td>
<td>19</td>
<td>1/14 (9)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>57</td>
<td>8/57 (14)</td>
</tr>
<tr>
<td>R-SP</td>
<td>1/6</td>
<td>7/16 (44)</td>
</tr>
<tr>
<td>Amoxicillin/clavulanate</td>
<td>9</td>
<td>2/9</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>5</td>
<td>1/5</td>
</tr>
<tr>
<td>TMP-SMZ</td>
<td>18</td>
<td>10/10 (55)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>32</td>
<td>15/18 (47)</td>
</tr>
<tr>
<td>NP susceptibility, treatment group</td>
<td>Non-SP</td>
<td>0/23 (1)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>11</td>
<td>21/32 (66)</td>
</tr>
</tbody>
</table>

NOTE: Data are no. MEF samples positive/no. tested (%), except where noted. Non-SP, no *S. pneumoniae* isolated; R-SP, resistant *S. pneumoniae*; S-SP, susceptible *S. pneumoniae*.

and 1 child had a third tympanocentesis because of clinical failure on day 10. The MEF-positivity rate for *S. pneumoniae* during the second or third tympanocentesis differed significantly according to the pretreatment NP culture: 2 (7%) of 30 among the children for whom the pretreatment NP culture yielded *S. pneumoniae* susceptible to the treatment drug and 8 (14%) of 57 among children for whom the pretreatment NP culture did not yield *S. pneumoniae* versus 21 (66%) of 32 of those with pretreatment NP culture that yielded a resistant *S. pneumoniae* (*P < .001*). The highest positivity rate of the second or third tympanocentesis was seen when both NP and MEF cultures were positive for resistant *S. pneumoniae* (92%); the lowest was when no resistant *S. pneumoniae* was isolated from either the NP or MEF (9%; figure 1). When NP only or MEF only yielded resistant *S. pneumoniae*, intermediate positivity rates at the time of the second or third tympanocentesis were observed.

Of the 14 children for whom pretreatment MEF culture yielded resistant *S. pneumoniae* and who remained positive during the second tympanocentesis, in 13 (93%) the isolate from the second tympanocentesis was identical to the initial MEF isolate by both serotype and antibiogram. Therefore, these children had true treatment failure (figure 1). In contrast, none of the 9 strains isolated from the second or third tympanocentesis of the children with pretreatment-resistant *S. pneumoniae* isolated from NP only were identical to the original MEF isolate (figure 1 and table 4). Even in the 2 cases in which both first and second tympanocentesis yielded *S. pneumoniae*, the organism differed by serotype, antibiogram, and PFGE analysis. However, all 9 isolates were identical by serotype, antibiogram, and PFGE analysis to the pretreatment NP isolate, thus representing superinfection of the middle ear by an isolate preexisting in the NP but not in MEF before treatment. All 9 isolates were resistant to the drug that the children were receiving.

Figure 2 shows 2 representative examples of superinfection by resistant *S. pneumoniae* preexisting in the NP before treatment (cases 5 and 7; table 4). In both cases, the children were treated with TMP-SMZ. In 1 case (figure 2A), the original NP culture yielded 2 *S. pneumoniae* strains—1 susceptible to TMP-SMZ (serotype 18C; MIC 0.25 mg/mL) and 1 resistant to TMP-SMZ (serotype 23F; MIC 2.0 mg/mL)—whereas the MEF isolate was susceptible to TMP-SMZ. On day 4, the child did not respond clinically to antibiotic treatment and was still febrile with bulging eardrums. A second tympanocentesis showed that the original MEF-susceptible *S. pneumoniae* had been replaced.
by the resistant \textit{S. pneumoniae} that persisted in the NP. In the second case (figure 2B), the original NP \textit{S. pneumoniae} isolate was highly resistant to TMP-SMZ (serotype 9V; MIC 8.0 \text{µg/mL}), but the MEF isolate was \textit{H. influenzae} susceptible to TMP-SMZ. On day 5, the child was well, and his MEF culture was sterile; however, the NP carriage of the original TMP-SMZ–resistant 9V persisted. When, on day 10, the child had a clinical relapse, the original TMP-SMZ–resistant \textit{S. pneumoniae} grew from both right and left MEF samples obtained by tympanocentesis and from the NP.

**Discussion**

In this prospective study, we clearly demonstrated that antibiotic-resistant \textit{S. pneumoniae} in the NP can interfere with clinical success in the treatment of AOM by rapidly causing superinfection of the middle ear. In these circumstances, cases that appear as nonresponsive AOM may, in reality, represent new infections that occurred during treatment. In regions with high prevalence of antibiotic-resistant \textit{S. pneumoniae}, this phenomenon is probably not rare, since, in this study, we found that it can occur in \textasciitilde50\% of cases when NP \textit{S. pneumoniae} is resistant to the administered drug—even if the initial MEF organism is susceptible to the drug.

In clinical terms, our findings are important, because they show that the presence of NP \textit{S. pneumoniae} resistant to the administered drug is highly predictive (66\%) of bacteriologic failure either by being associated with the presence of pretreatment MEF-resistant \textit{S. pneumoniae} (when pneumococcal positivity after \textasciitilde3 days of treatment was 92\%) or by being responsible for superinfection (when pneumococcal positivity was 47\%). In contrast, when NP culture did not yield \textit{S. pneumoniae} or yielded antibiotic-susceptible \textit{S. pneumoniae}, the positivity rate after 3 days of treatment was only 11\%.

Because AOM usually occurs secondary to acute viral infections, rapid initiation of antibiotic treatment may result in eradication or reduction of the susceptible organisms in both the MEF and the NP, permitting the overgrowth of the NP flora organisms that are not susceptible to the drug. Since the predisposing condition (the viral infection) may still be present, a new infection of the middle ear may then take place with the newly selected resistant pathogen. This seems to be the only plausible explanation, since we could demonstrate the preexistence of the pathogen causing the superinfection in the NP before initiation of treatment; serotyping and PFGE showed that the MEF superinfection did not occur because of an alteration of the initial organism in the MEF, since the pathogens were identical to the preexisting NP pathogens and differed from the initial MEF isolate.

As a rule, high carriage of antibiotic-resistant \textit{S. pneumoniae} in the community is associated with high prevalence of disease caused by this resistant organism [21]. Antibiotic administration selects for carriage of resistant \textit{S. pneumoniae}, [1–8, 18, 23, 30–36], which, in turn, predisposes to AOM with resistant pneumococci [24]. It is therefore understandable that the prevalence of antibiotic-resistant \textit{S. pneumoniae} among MEF isolates of children previously treated with antibiotics is higher than that among those who did not receive antibiotics recently [22, 25, 37, 38]. In a recent study [22], we showed that penicillin-non-susceptible isolates were present in the MEF of 44\% of children who did not receive antibiotics in the 3 months preceding tympanocentesis versus 64\% of children who received antibiotics during this period and 81\% of children who received antibiotics at the time of tympanocentesis (\(P < .001\)). The respective numbers for fully penicillin-resistant isolates (MIC \textasciitilde 1.0 \text{µg/mL}) were 6\%, 16\%, and 25\% (\(P < .001\)). Thus, it is clear that antibiotic treatment promotes subsequent episodes of antibiotic-resistant pneumococcal AOM. The present study clarifies an

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**Table 4.** Culture results on day 1 (before treatment) and days 4–6 (during treatment) for 9 patients with pneumococcal middle ear fluid (MEF) superinfection caused by preexisting resistant nasopharyngeal (NP) \textit{Streptococcus pneumoniae} (SP).

<table>
<thead>
<tr>
<th>Case</th>
<th>Treatment drug</th>
<th>NP-Sp</th>
<th>MEF isolate</th>
<th>Before treatment</th>
<th>During treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Azithromycin</td>
<td>SP type 19F (≥64.0)</td>
<td>\textit{H. influenzae}</td>
<td>SP type 19F (≥64.0)</td>
<td>SP type 19F (≥64.0)</td>
</tr>
<tr>
<td>2</td>
<td>Azithromycin</td>
<td>SP type 6B (≥64.0) + SP type 12 (0.25)</td>
<td>\textit{H. influenzae}</td>
<td>SP type 6B (≥64.0)</td>
<td>SP type 6B (≥64.0)</td>
</tr>
<tr>
<td>3</td>
<td>Amoxicillin/clavulanate</td>
<td>SP type 23F (1.0)</td>
<td>\textit{H. influenzae} + SP type 3 (0.016)</td>
<td>SP type 23F (1.0)</td>
<td>SP type 23F (1.0)</td>
</tr>
<tr>
<td>4</td>
<td>Amoxicillin/clavulanate</td>
<td>SP type 23F (1.0)</td>
<td>\textit{H. influenzae}</td>
<td>SP type 23F (1.0)</td>
<td>SP type 23F (1.0)</td>
</tr>
<tr>
<td>5</td>
<td>TMP-SMZ</td>
<td>SP type 23F (2.0) + SP type 18C (0.25)</td>
<td>\textit{H. influenzae}</td>
<td>SP type 23F (2.0)</td>
<td>SP type 23F (2.0)</td>
</tr>
<tr>
<td>6</td>
<td>TMP-SMZ</td>
<td>SP type 35 (8.0)</td>
<td>\textit{H. influenzae}</td>
<td>SP type 35 (8.0)</td>
<td>SP type 35 (8.0)</td>
</tr>
<tr>
<td>7</td>
<td>TMP-SMZ</td>
<td>SP type 9V (8.0)</td>
<td>\textit{H. influenzae}</td>
<td>SP type 9V (8.0)</td>
<td>SP type 9V (8.0)</td>
</tr>
<tr>
<td>8</td>
<td>TMP-SMZ</td>
<td>SP type 19F (1.0)</td>
<td>\textit{H. influenzae}</td>
<td>SP type 19F (1.0)</td>
<td>SP type 19F (1.0)</td>
</tr>
<tr>
<td>9</td>
<td>TMP-SMZ</td>
<td>SP type 35 (8.0)</td>
<td>\textit{H. influenzae}</td>
<td>SP type 35 (8.0)</td>
<td>SP type 35 (8.0)</td>
</tr>
</tbody>
</table>

**NOTE.** For each child, all isolates with same serotypes had identical pulsed-field get electrophoresis (PFGE) pattern; different serotypes, if present, had different PFGE patterns. Data in parentheses are MIC (\text{µg/mL}) to treatment drug. \textit{H. influenzae}, \textit{Haemophilus influenzae}; TMP-SMZ, trimethoprim-sulfamethoxazole.

\* In this case, second tympanocentesis on day 4 was negative, but SP type 9V appeared on third tympanocentesis, 1 day after treatment (figure 2).
Figure 2. Pulsed-field gel electrophoresis patterns of nasopharynx (NP) and middle ear fluid (MEF) *Streptococcus pneumoniae* isolates from 2 children with acute otitis media treated with trimethoprim/sulfamethoxazole (TMP-SMZ). Day 1 samples were obtained before treatment. Hi, *Haemophilus influenzae*; L, left ear; neg, culture negative; R, right ear. Arrows, passage of original TMP-SMZ-resistant isolate to MEF.

important mechanism by which this phenomenon occurs and the rapidity of the process.

A series of recent studies revealed some of the early processes that occur in the NP during and in the immediate posttreatment period in cases of AOM, with regard to antibiotic-resistant *S. pneumoniae*. Three studies [1, 4–8, 39] show that, when treating AOM with antibiotics, most NP *S. pneumoniae* isolates that are susceptible to the treatment drug disappear rapidly, whereas those not susceptible to the drug tend to persist. Recent evidence suggests that the magnitude of this effect differs with antibiotic class. Although β-lactam antibiotics do not significantly decrease the NP carriage of penicillin-nonsusceptible pneumococci, this does not usually result in a net increase in carriage of these resistant organisms [4–8]. In contrast, the administration of antibiotics of other classes, such as macrolides and TMP-SMZ, may result in a net increase in NP carriage of *S. pneumoniae* resistant to those drugs [1–3, 8, 34]. Furthermore, because of frequent association between penicillin nonsusceptibility and resistance to other classes, 1 antibiotic (e.g., TMP-SMZ or a macrolide) can even promote NP carriage of penicillin-nonsusceptible pneumococci [8, 34]. In the presence of such rapidly occurring promotion and selection of resistance, the only 2 conceivable solutions are judicious use of antibiotics, with the aim of reducing antibiotic pressure, and vaccinating against pneumococcal serotypes that carry the highest antibiotic resistance rate.

An initiative to limit the use of antibiotics to appropriate indications was launched recently by a number of pediatricians and infectious diseases organizations [40]. A joint committee of the Centers for Disease Control and Prevention and the American Academy of Pediatrics reviewed judicious uses of antimicrobial agents for common infections and developed guidelines for various infections, including AOM [41]. Those guidelines primarily stress limiting the use of antimicrobial agents. If the guidelines gain acceptance, the selective pressure by the widespread use of antibiotic may be reduced.

Worldwide, most antibiotic-resistant *S. pneumoniae* belong to a limited number of serotypes, namely 6B, 9V, 14, 19F, and 23F [21, 22]. These 5 serotypes are included in all candidate pneumococcal conjugate vaccines designed for use in infants and children [21, 22]. We recently reported the antibiotic coverage of 1,800 successive pneumococcal MEF isolates from AOM [22]. We found that coverage of the antibiotic-susceptible *S. pneumoniae* isolates by the 7-, 9-, and 11-valent conjugate vaccines were 24%, 30%, and 45%, respectively. For resistant *S. pneumoniae* strains, coverage was as follows: penicillin-intermediate strains, 68%, 69%, and 69%, respectively; fully penicillin-resistant strains, 92%, 93%, and 93%, respectively; and multiresistant strains (resistant to >3 antibiotic classes), 87%, 87%, and 88%, respectively [22].

Because recent studies show that the conjugate pneumococcal vaccine not only protects against pneumococcal AOM [42, 43] but may also bring about a marked reduction of carriage of the *S. pneumoniae* serotypes included in the vaccine [21], we hope that widespread vaccination coupled with a judicious approach aimed at limiting antibiotic use will contribute to the
reduction of the alarming phenomenon described in the present study.

References


