Four Antibiotic-Resistant *Streptococcus pneumoniae* Clones Unrelated to the Pneumococcal Conjugate Vaccine Serotypes, Including 2 New Serotypes, Causing Acute Otitis Media in Southern Israel

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This study examined the prevalence of antibiotic-resistant clones that belong to serotypes not included in the pneumococcal conjugate vaccines and that cause a significant percentage of acute otitis media (AOM) in children in southern Israel. During 1998–2001, 2467 pneumococcal isolates, obtained from middle-ear fluid of children <3 years old with AOM, were characterized by antimicrobial susceptibility testing, serotype testing, and pulsed-field gel electrophoresis. Non–vaccine type (NVT) strains constituted 477 (19%) of the 2467 isolates, of which 173 (36%) belonged to only 4 serotypes: 35B, 33F, 21, and 15B/C. For serotype 35B, 47 (96%) of 49 strains were penicillin nonsusceptible, and 93% constituted a single clone; for serotype 33F, 31 (82%) of 38 strains were penicillin nonsusceptible, and 95% constituted a single clone; for serotype 21, 38 (93%) of 41 strains were penicillin nonsusceptible, and 93% constituted a single clone; for serotype 15B/C, 22 (49%) of 45 strains were penicillin nonsusceptible, and 42% constituted a single clone. Two of these clones have not been described elsewhere. The high prevalence of NVT clones should increase the awareness of the potential for replacement of the vaccine strains with these NVT antibiotic-resistant strains.

*Streptococcus pneumoniae* is the most commonly reported bacterial cause of acute otitis media (AOM), accounting for 28%–55% of cases [1]. Of the 90 pneumococcal serotypes that have been identified so far, the most common serotypes associated with AOM are 6A, 6B, 9V, 14, 19F, 19A, and 23F [2]. These serotypes also carry the highest rates of resistance to penicillin and other antibiotics, leading to increased failure rates for treatment with many antimicrobial agents [3–6].

Vaccination may offer the simplest and most effective approach to controlling drug-resistant pneumococci. The most promising approach has been the development of a protein-polysaccharide conjugate vaccine for selected serotypes [2, 7]. Vaccine formulations have included at least 7 serotypes, (4, 6B, 9V, 14, 18C, 19F, and 23F), of which 5 (6B, 9V, 14, 19F, and 23F), as well as other serotypes in these serogroups, frequently carry resistance to antibiotics. New vaccine formulations have expanded lately to include 9 (addition of serotypes 1 and 5) and 11 (further addition of serotypes 3 and 7F) capsular types that are considered to be the most important types in pneumococcal disease in children.

Several studies have reported that pneumococcal conjugate vaccines decrease pneumococcal nasopharyngeal carriage of serotypes included in the vaccine, as well as that of some immunologically related ones, especially serotype 6A [2, 8–12]. However, replacement of these serotypes with serotypes not immunologically related to the vaccine serotypes found in the nasopharynx was documented in most of the studies [2, 9–12]. Efficacy studies have already demonstrated that the use of the vaccine was associated with an increase in the rate of AOM caused by pneumococcal serotypes
immunologically unrelated to the vaccine serotypes [1, 13]. This replacement phenomenon has brought up some serious concerns regarding the possibility that resistance to antimicrobial agents will spread to or develop in non–vaccine type (NVT) strains, making them the future candidates to cause morbidity and mortality among children.

In an era of widespread vaccination programs, the increased prevalence of NVT strains causing AOM may constitute a significant problem. This risk has gained more focus since some of the NVT isolates have developed resistance to penicillin and other antimicrobial agents [14, 15]. Therefore, we conducted a study to determine the prevalence of antibiotic-resistant AOM-causing clones that belong to serotypes not included in the 11-valent conjugate vaccine. If such antibiotic-resistant NVT clones are prevalent, they present a threat to cause antibiotic-resistant replacement disease.

SUBJECTS, MATERIALS, AND METHODS

Population. This 4-year prospective study was conducted in the Negev area in southern Israel during 1998–2001. The study population in this area consists of Jews with a lifestyle resembling that in developed populations and Muslim bedouins with standards of living resembling those in developing populations. The 2 pediatric populations differ in disease patterns and rates. Hospitalization rates for respiratory and other infectious diseases are higher among bedouin infants, and the infectious agents responsible for these clinical manifestations are also distributed differently [16]. The average population during 1998–2001 was 475,600 persons/year; this included 39,500 children <3 years old, of which 20,800 (53%) were Jewish and 18,700 (47%) were bedouin.

Study design and microbiology. The study included >95% of pneumococcal isolates obtained from middle-ear fluid (MEF) of children <3 years old with AOM in the Negev area, from January 1998 to December 2001. Approximately 10% of the patients were enrolled in various antibiotic studies, and the rest of the specimens were obtained for clinical indications. The diagnosis for AOM was made by either study physicians, pediatricians, or ear-nose-throat specialists at the clinical facility. Information regarding ethnic origin (Jewish or bedouin), age, sex, previous number of AOM episodes, and antibiotic treatment during the previous 3 months was obtained from the patient’s medical chart and was completed by the parents when needed. Pneumococcal isolates were characterized by inhibition with optochin and a positive slide agglutination test (Phadebact; Pharmacia Diagnostics). One S. pneumoniae colony per culture and per episode of AOM was subcultured, harvested, and kept frozen at −70°C for further testing. In cases in which several cultures were obtained from the same child, resulting in the same serotype test result, only 1 representative isolate/child within a period of 30 days was included in the study.

Antibiotic susceptibility testing. Susceptibility of isolates to penicillin, erythromycin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMZ) was performed by the disk-diffusion method of Bauer and Kirby, in accordance with the NCCLS recommendations [17]. Isolates exhibiting an inhibition zone with a diameter <19 mm around a 1-μg oxacillin disk were further tested for susceptibility to penicillin by means of the E-test (AB biodisk; Solna), in accordance with the manufacturer’s instructions, and was confirmed by reference NCCLS microdilution [18] for 310 representative strains. E-test MICs between 2 standard doubling dilution values were adjusted up to the next highest standard doubling dilution value. Correlation between penicillin MICs determined by E-test and microdilution for the 310 strains was excellent, with 95% of E-test results within 1 doubling dilution of microdilution results and 99% within 2 doubling dilutions. Resistance to other agents that was detected by disk diffusion was also confirmed by microdilution for 310 representative strains and showed agreement between susceptibility categories for both methods. Isolates with a penicillin MIC <0.1 μg/mL were considered to be susceptible to penicillin, and those with an MIC ≥0.1 μg/mL were considered to be nonsusceptible (MICs of 0.1–1 μg/mL were considered to be intermediately resistant, and MICs of ≥2 were considered to be resistant). Isolates with resistance to ≥3 antibiotic classes were considered to be multidrug resistant (MDR).

Serogroup and serotype testing. Serogroup and serotype testing of S. pneumoniae was done by use of the quellung reaction, using antisera from Statens Serum Institute of Copenhagen, Denmark [19].

Pulsed-field gel electrophoresis (PFGE). Chromosomal DNA fragments, generated by SmaI digestion, were prepared and analyzed as described elsewhere [20]. A CHEF-DRIII apparatus (Bio-Rad Laboratories) was used for running the gels. Running conditions were 23 h at 11.3°C at 200 V, with initial forward time of 5 s and final forward time of 35 s. Gels were stained with ethidium bromide and photographed. Interpretation of strain relatedness, on the basis of PFGE pattern, was performed according to current consensus criteria [21].

Statistical analysis. Statistical analysis was conducted by use of the Epi-Info 2000 package (Centers for Disease Control and Prevention). Contingency table analysis was conducted by use of the χ² test. Goodness-of-fit χ² analysis was used for proportions of cultures between the 2 ethnic groups.

RESULTS

Serotype distribution among MEF isolates. A total of 2523 MEF pneumococcal isolates obtained from children <3 years
old with AOM were included in the present study. Serotype testing was performed on 2467 isolates (98%); 1542 isolates (63%) were found to belong to serotypes included in the 11-valent conjugate vaccine (vaccine type [VT]), and 448 isolates (18%) had serotypes that were immunologically related to the VT isolates (vaccine type related [VTR]). The VTR isolates consisted mainly of serotypes 6A and 19A, as well as serotypes 7A/B/C, 9A/L/N, 18A/B/F, and 23A/B. However, 477 isolates (19%) belonged to serotypes that were not included in and that were not immunologically related to the VT isolates. These were considered to be NVT strains (figure 1). The characteristics of the pediatric patients with pneumococcal AOM are summarized in table 1. Sixty percent of all isolates were obtained from boys. Most of the strains (84.6%) were recovered during the first 2 years of life: 38.6% during the first year, and only 15.3% during the third year. The proportion of all isolates obtained from bedouins was higher than that from Jews: 62.6% versus 37.4%, respectively (P < .001). This difference was most pronounced among the NVT strains, of which 76.3% were obtained from bedouins and only 23.7% from Jews (P < .001).

**Antimicrobial susceptibility.** Antibiotic susceptibility testing was performed on the 2467 isolates for which serotypes were determined. The highest prevalence of resistance to any drug class was recorded in the VT group, followed by the VTR group. Of the 1542 VT isolates, 1102 (71.5%) were penicillin nonsusceptible, and 215 (19.9%) of those had a penicillin MIC $\geq$ 2.0 $\mu$g/mL; 1243 (80.6%) were resistant to $\geq$ 1 antimicrobial class, and 367 (23.8%) were MDR. Of the 448 VTR isolates, 330 (73.7%) were penicillin nonsusceptible, and 5 (1.1%) of those had a penicillin MIC $\geq$ 2.0 $\mu$g/mL; 346 (77.2%) were resistant to $\geq$ 1 antimicrobial class, and 29 (6.5%) were MDR. Of the 477 NVT strains, 190 (39.8%) were penicillin nonsusceptible, and 6 (3.2%) of those had a penicillin MIC $\geq$ 2.0 $\mu$g/mL; 245 (51.4%) were resistant to $\geq$ 1 antibiotic class, and 19 (4%) were MDR.

Of the 477 NVT isolates, 173 (36%) belonged to 4 serotypes: 35B ($n = 49$ [28%]), 33F ($n = 38$ [22%]), 21 ($n = 41$ [24%]), and 15B/C ($n = 45$ [26%]). The remaining 304 isolates belonged to 31 serotypes. Among the 4 predominant serotypes, 138 (80%) of 173 isolates were penicillin nonsusceptible (table 2). Of the 49 serotype 35B isolates, 47 (96%) were penicillin nonsusceptible, whereas 31 (82%) of the 38 serotype 33F isolates, 38 (93%) of the 41 serotype 21 isolates, and 22 (49%) of the 45 serotype 15B/C isolates were penicillin nonsusceptible. Virtually all penicillin-nonsusceptible isolates from these 4 serotypes were immediately resistant to penicillin, with MIC$_{\text{in}}$ and MIC$_{\text{co}}$ values of 0.12–0.25 $\mu$g/mL (table 2). All these isolates were susceptible to amoxicillin, ceftriaxone, erythromycin, clindamycin, tetracycline, and chloramphenicol, whereas susceptibility to TMP-SMZ was variable: 45%, 81%, 95%, and 82% for serotypes 35B, 33F, 21, and 15B/C, respectively. In contrast, 19.5% of the penicillin-nonsusceptible VT isolates were resistant, rather than intermediate resistant, to penicillin, and 23.8% of the VT and 6.5% of the VTR isolates were resistant to $\geq$ 3 drug classes.

**Molecular typing.** PFGE was performed on 245 (51.4%) of the 477 NVT strains that were resistant to $\geq$ 1 antibiotic class; 168 of 173 isolates belonging to serotypes 35B ($n = 46$), 33F ($n = 37$), 21 ($n = 40$), and 15B/C ($n = 45$) were available for serotype testing. For serotype 35B (figure 2A), 43 (93%) of 46 isolates belonged to the same clone, of which 42 (98%) were penicillin nonsusceptible. The PFGE pattern of this clone, generated by SmaI digestion, resembled that of the penicillin-nonsusceptible serotype 35B clone, described recently by Beall et al. [14].

For serotype 33F (figure 2B), 35 (95%) of 37 isolates belonged to the same clone, of which 31 (89%) were penicillin nonsusceptible. For serotype 21 (figure 2C), 37 (93%) of 40 isolates belonged to the same clone, of which 34 (92%) were penicillin nonsusceptible. The PFGE pattern of this clone resembled that of the penicillin-nonsusceptible serotype 21 clone, described elsewhere [15]. The 3 other serotype 21 isolates each had a unique PFGE pattern. For serotype 15B/C (figure 3A), 19 (42%) of the 45 strains belonged to the same clone, all of which were penicillin nonsusceptible. This included 12 isolates of serotype 15B, 6 isolates of serotype 15C, and 1 isolate with a mixture of serotypes 15B and 15C. In contrast, the other 26 serotype 15B/C isolates that were susceptible to penicillin...
showed 8 different PFGE patterns (figure 3B), none of which was similar to that of the penicillin-nonsusceptible 15B/C clone.

**Previous antibiotic treatment and recurrent AOM.** We compared the resistance rates of the 4 major NVT clones with those of all other serotypes found among MEF isolates. The strains were divided into 3 groups: group 1, strains belonging to serotypes included in the 11-valent conjugate vaccine (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F); group 2, strains belonging to the 4 predominant NVT clones (serotypes 35B, 33F, 21, and 15B/C); group 3, NVT strains not included in group 2 (table 3). Rates of nonsusceptibility to penicillin for the 4 clones (group 2) resembled those for the internationally recognized, highly resistant VT and VTR serotypes (group 1), but not those for the NVT strains (group 3): 96% and 64% vs. 18%, respectively ($P < .001$, for group 2 vs. group 3).

In the 3 groups mentioned above, an association was found between nonsusceptibility to penicillin and the clinical presentation of AOM. In groups 1 and 2, we found high rates of antibiotic treatment during the previous 3 months (72% and 74%) and recurrent AOM (64% and 63%), whereas, in group 3, these rates were significantly lower: 50% for previous antibiotic treatment during the previous 3 months and recurrent AOM were associated with all of the 4 NVT clones: 78%, 61%, 69%, and 67% of the children with serotypes 15B/C, 33F, 21, and 15B/C, respectively, received antibiotic treatment during the previous 3 months. The respective figures for >1 previous AOM episodes were 73%, 41%, 68%, and 68%.

**DISCUSSION**

The conjugate pneumococcal vaccines have been shown to have a dramatic effect on the carriage of antibiotic-resistant *S. pneumoniae* [2, 8–12]. The strong mucosal effect of these vaccines is promising and suggests that protection against AOM might occur as well. In the first efficacy trial conducted in northern California [22], the heptavalent conjugate vaccine appeared to be highly effective in preventing invasive disease in young children and to have a significant effect on otitis media. However, 2 recent studies conducted in Finland have shown that the rates of AOM were only slightly reduced by the vaccines, if at all: the overall protective efficacies of the 7-valent–CRM197 and 7-valent–meningococcal outer membrane protein complex conjugate vaccines were 6% and 1%, respectively (both not statistically significant) [1, 13]. Although there was a significant reduction in the rate of AOM caused by VT, and even in that caused by 1 VTR serotype, administration of the vaccines was associated with an increase in the rate of otitis media caused by NVT strains. A major concern was raised with regard to the possibility that replacement of VT strains can lead to disease modification expressed by a higher prevalence of antibiotic-resistant NVT strains, instead of the desired overall decrease in the rates of antibiotic resistance, if NVT strains have or develop significant resistance to antibiotics.

**Table 1. Characteristics of pediatric patients with pneumococcal acute otitis media in southern Israel during 1998–2001.**

<table>
<thead>
<tr>
<th>Strain type</th>
<th>No. of subjects</th>
<th>Sex</th>
<th>Ethnic group</th>
<th>Age range, years</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT</td>
<td>1542</td>
<td></td>
<td>Male</td>
<td>1–2</td>
</tr>
<tr>
<td>VTR</td>
<td>448</td>
<td></td>
<td>Female</td>
<td>1–2</td>
</tr>
<tr>
<td>NVT</td>
<td>477</td>
<td></td>
<td>ND</td>
<td>1–2</td>
</tr>
<tr>
<td>Total</td>
<td>2467</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain type</th>
<th>No. of subjects</th>
<th>Male</th>
<th>Female</th>
<th>ND</th>
<th>Jewish</th>
<th>Bedouin</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT</td>
<td>1542</td>
<td>924</td>
<td>616</td>
<td>40.0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>VTR</td>
<td>448</td>
<td>269</td>
<td>178</td>
<td>39.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>NVT</td>
<td>477</td>
<td>283</td>
<td>192</td>
<td>40.4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2467</td>
<td>1476</td>
<td>986</td>
<td>40.0</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of subjects. ND, no data; NVT, non-vaccine type (serotypes not included and not immunologically related to the 11-valent conjugate vaccine); VT, vaccine type (serotypes included in the 11-valent conjugate vaccine: 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F); VTR, vaccine type related (serotypes immunologically related to the 11-valent conjugate vaccine: 6A, 7A/B/C, 9A/L/N, 18AB/F, 19A, and 23A/B).

**Table 2. Antimicrobial susceptibility of penicillin-nonsusceptible isolates of serotypes 15B/C, 21, 33F, and 35B.**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>15B/C</th>
<th>21</th>
<th>33F</th>
<th>35B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin, MIC range</td>
<td>0.06–0.5</td>
<td>0.12–2.0</td>
<td>0.12–0.25</td>
<td>0.12–1.0</td>
</tr>
<tr>
<td>Penicillin, MIC (µg/mL)</td>
<td>0.25/0.25</td>
<td>0.25/0.25</td>
<td>0.12/0.12</td>
<td>0.12/0.25</td>
</tr>
<tr>
<td>Amoxicillin, MIC (µg/mL)</td>
<td>0.25/0.25</td>
<td>0.25/0.25</td>
<td>0.12/0.12</td>
<td>0.12/0.25</td>
</tr>
<tr>
<td>Ceftriaxone, MIC (µg/mL)</td>
<td>0.12/0.12</td>
<td>0.12/0.12</td>
<td>0.03/0.03</td>
<td>0.06/0.25</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>TMP-SMZ</td>
<td>82</td>
<td>95</td>
<td>81</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>22 (13)</td>
<td>38 (27)</td>
<td>31 (23)</td>
<td>47 (28)</td>
</tr>
</tbody>
</table>

**NOTE.** MICs are presented in micrograms per milliliter, and all other data, unless otherwise noted, are percentage susceptible. MICs of β-lactams are based on microdilution MICs performed on a subset of strains of each serotype, and susceptibility to other drug classes was performed by disc diffusion on all penicillin-nonsusceptible isolates. TMP-SMZ, trimethoprim-sulfamethoxazole.

a Two isolates with MICs of 2 µg/mL.

b One isolate with an MIC of 1 µg/mL.

c No. of isolates tested, with subset used for reference MIC determinations in parentheses.
Figure 2. Pulsed-field gel electrophoresis patterns generated by SmaI digestion of selected non–vaccine type (NVT) Streptococcus pneumoniae isolates recovered from middle-ear fluid of children in southern Israel during 1998–2001. A, Lanes 1–43 contain the serotype 35B major clone, with all isolates except for 1 (lane 42) being penicillin nonsusceptible; lanes 44–46 contain 2 different patterns of penicillin-susceptible serotype 35B isolates. B, Lanes 1–35 contain the serotype 33F major clone, with all isolates except for 4 (lanes 1, 3, 10, and 15) being penicillin nonsusceptible; lanes 36 and 37 contain different patterns of penicillin-susceptible serotype 33F isolates. C, Lanes 1–37 contain the serotype 21 major clone, with all isolates except for 3 (lanes 8, 9, and 14) being penicillin nonsusceptible; lanes 38–40 contain 2 different patterns of penicillin-susceptible serotype 21 isolates. λ, lambda ladder; R6, S. pneumoniae reference strain used as a molecular weight marker.
Figure 3. Pulsed-field gel electrophoresis patterns generated by Smal digestion of selected non–vaccine type (NVT) Streptococcus pneumoniae isolates recovered from middle-ear fluid of children in southern Israel during 1998–2001. A, Lanes 1–19 contain the serotype 15B/C major penicillin-nonsusceptible clone; lanes 1, 2, 4–7, 9, 11, 14–16, and 18 contain serotype 15B; lanes 3, 10, 12, 13, 17, and 19 contain serotype 15C; and lane 8 contains both 15B and 15C. B, Lanes 1–26 contain 8 different patterns of penicillin-susceptible serotype 15B/C isolates; lanes 1, 2, 6, 7, 10, 12–16, 18, 21, and 23–26 contain serotype 15B; lanes 3–5, 8, 9, 17, 19, 20, and 22 contain serotype 15C; lane 11 contains both 15B and 15C. λ, lambda ladder; R6, S. pneumoniae reference strain used as a molecular weight marker.
for this hypothesis is provided by studies in which pneumococci exposed to β-lactams in vitro have only been able to become intermediately resistant to penicillin [24–26].

It is also likely that resistance in 3 of these clones (serotypes 21, 33F, and 35B) arose independently in each clone, since clonally related or identical penicillin-susceptible variants of these clones were also found. Since no clonally related penicillin-susceptible strains of the nonsusceptible serotype 15B/C clone were found, this clone may have arisen spontaneously or may have derived its changes in penicillin-binding protein genes by transformation, with one of the other clones described here as the DNA source. It is also possible that these penicillin-nonsusceptible clones switched capsular types, as has been described for some VT strains [27], but this is unlikely since no common PFGE patterns were found among these clones. It is also likely that resistance to TMP-SMZ, which was present in one-fourth to one-half of the isolates of serotype 35B, 33F, 21, and 15B/C, also arose by mutation in each clone, since a single amino acid substitution in the chromosomal dihydrofolate reductase gene resistance results in resistance to trimethoprim, and repetitions of 1 or 2 amino acids in the chromosomal dihydropteroate synthase gene results in resistance to sulfonamide [28]. It is also possible that resistance to TMP-SMZ was lost or was not expressed by some isolates.

Serotypes 15B and 15C are known to interconvert in vitro and in vivo by the addition of an acetyl group to the capsular polysaccharide of serotype 15C to form an O-acetylated 15B variant [29–30]. In the present study, 19 penicillin-nonsusceptible strains shared the same clone and serogroup but differed in their capsular subtype. We therefore regarded the difference in serogroup 15 capsular polysaccharides to reflect this chemical interconversion of the acetyl group, rather than genetic recombination at the capsular locus. The control mechanism for these chemical elimination/addition processes is yet unknown.

AOM caused by these clones was associated with a high rate of previous antibiotic treatment. Similarly, these clones were cultured more often from children with recurrent AOM than from children with AOM caused by other NVT strains. These findings indicate that the 4 NVT clones described in the present study are associated with complicated AOM and constitute a challenge for the treating physicians.

It is already known from many epidemiological studies [23] that resistant clones persist for long periods of time and spread worldwide. The high prevalence of the penicillin-nonsusceptible NVT clones observed during the last 4 years in southern Israel, even before the introduction of pneumococcal conjugate vaccines, should focus our attention on these clones as potential candidates for replacement disease. One of the serious problems among the prevalent pneumococcal strains is the emerging resistance to >1 antibiotic drug class [31]. Thus, the widespread use of antibiotics for AOM may result in the development of

Table 3. Comparison of nonsusceptibility rates and clinical presentation of the 4 non–vaccine type (NVT) clones belonging to serotypes 35B, 33F, 21, and 15B/C, to all other serotypes found among acute otitis media (AOM) isolates.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1a</th>
<th>Group 2b</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of isolates</td>
<td>1921</td>
<td>134</td>
<td>412</td>
</tr>
<tr>
<td>Penicillin nonsusceptible</td>
<td>$1227/1921$ (64)</td>
<td>$129/134$ (96)</td>
<td>$73/412$ (18)</td>
</tr>
<tr>
<td>Previous antibiotic treatment</td>
<td>$1344/1872$ (72)</td>
<td>$95/128$ (74)</td>
<td>$190/378$ (50)</td>
</tr>
<tr>
<td>&gt;1 AOM episode during the previous 3 months</td>
<td>$1201/1882$ (64)</td>
<td>$80/128$ (63)</td>
<td>$169/382$ (44)</td>
</tr>
</tbody>
</table>

### NOTE.

- Data are no. of isolates/total no. (%) of isolates, unless otherwise noted. *P* < .001 for groups 2 versus groups 3, for previous antibiotic treatment and >1 AOM episode during the previous 3 months. Group 1, vaccine type and vaccine type–related (VTR) clones, internationally recognized as antibiotic resistant and multi-drug resistant; Group 2, NVT clones 35B, 33F, 21, 15B/C; Group 3, NVT (other than the 4 clones in group 2 and the VTR [6A and 19A] in group 1).

- a Serotypes included in the 11-valent conjugate vaccine (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) plus 2 VTR serotypes (6A and 19A).

- b Includes only the major clone of each of these serotypes, which includes all penicillin-nonsusceptible isolates of these serotypes.

We undertook the present study to evaluate the potential role that antibiotic-resistant, NVT strains play in AOM in southern Israel, before the introduction of any conjugated pneumococcal vaccine. We observed that ~20% of all AOM episodes were caused by serotypes not included and not immunologically related to the 11-valent conjugate vaccine. Of these episodes, more than a third (36%) belonged to only 4 serotypes: 35B, 33F, 21, and 15B/C. It is an additional concern that strains belonging to these serotypes are of clonal origin and that most of the isolates of the predominant serotypes 21, 33F, and 35B clones and all the isolates of the serotype 15B/C clone were penicillin nonsusceptible.

Two of the NVT clones detected have been described elsewhere [9, 10]. The antimicrobial-susceptibility profile and PFGE patterns of the penicillin-nonsusceptible serotype 35B clone resembles the invasive, penicillin-nonsusceptible serotype 21, 33F, and 35B clones and all the isolates of the serotype 15B/C clone were penicillin nonsusceptible.

Another lineage that has already been published in the literature is the penicillin-nonsusceptible serotype 21 clone, which was present among day-care center attendees in Greece [15]. The other 2 clones show totally novel PFGE patterns that are not related to other resistant clones that have been described elsewhere or characterized in the international data library [23]. The major concern with regard to all of these clones is their high rates of nonsusceptibility to penicillin. However, it is of interest to note that virtually all of the isolates belonging to these clones are intermediate resistant to penicillin and are not MDR, which is so frequently the case with drug-resistant VT and VTR isolates. This would suggest that resistance to penicillin in these clones has arisen by mutation rather than by incorporation of foreign DNA by transformation. Support...
further drug resistance among these penicillin-nonsusceptible NVT clones.

References