Introduction and Proliferation of Multidrug-Resistant \textit{Streptococcus pneumoniae} Serotype 19A Clones That Cause Acute Otitis Media in an Unvaccinated Population

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(See the editorial commentary by Moore, on pages 771–3.)

**Background.** Introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in the United States in 2000 was temporally associated with an increase in the incidence of disease caused by \textit{Streptococcus pneumoniae} serotype 19A (Sp19A) and with increasing drug resistance within this serotype. A causative role of PCV7 was speculated. We prospectively studied the dynamics of acute otitis media (AOM) caused by Sp19A in southern Israel before the introduction of PCV7.

**Methods.** AOM in children <5 years old undergoing tympanocentesis during 1999–2006 was studied. Antibiotic prescriptions for ~20% of children <5 years old were recorded. Sp19A isolates were studied for antibiotic-resistance and pulsed-field gel electrophoresis patterns; multilocus sequence typing of representative isolates was compared with that of international clones.

**Results.** Sp19A caused 438 (9.8%) of 4449 pneumococcal AOM episodes, increasing by 63.1% from 1999–2001 (mean ± SD, 8.4% ± 0.8%) to 2004–2006 (mean ± SD, 13.7% ± 0.9%) among Bedouin children, who were characterized by overcrowding and high antibiotic use. Penicillin, erythromycin, and multidrug resistance increased from <10% to 78.6%, 50.0%, and 50.0%, respectively ($P < .001$), and was associated with the introduction and proliferation of 2 multidrug-resistant clones that were not previously associated with multidrug resistance. This was temporally associated with the introduction of and rapid increase in azithromycin use and the frequent use of oral cephalosporins.

**Conclusions.** The introduction and proliferation of multidrug-resistant Sp19A occurred before the introduction of PCV7. The increasing proportion of antibiotic-resistant Sp19A suggests that antibiotic use plays an important role in the community.

The 7-valent pneumococcal conjugate vaccine (PCV7) was licensed for children <5 years old in the United States in 2000 [1]. With universal PCV7 immunization, the frequencies of nasopharyngeal carriage, invasive pneumococcal disease (IPD), and acute otitis media (AOM) due to vaccine serotypes (VSTs) have decreased significantly, but rates of carriage and, to some extent, disease due to nonvaccine serotypes (NVSTs) have increased [2–6]. The decline in VSTs coincided with the proliferation of one of the most prevalent NVSTs during the prevaccine era, \textit{Streptococcus pneumoniae} serotype 19A (Sp19A), which became a leading serotype in nasopharyngeal colonization, IPD, and AOM [2, 4, 7–11]. In addition, Sp19A has become increasingly resistant to antibiotics [2, 4, 7–13].

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The mechanism by which the frequency of Sp19A is increasing in the United States is not clear, although in theory it could be facilitated by the reduction in the frequency of VSTs by PCV7 (replacement phenomenon). However, increased rates of Sp19A have not been universally observed after the introduction of PCV7. For example, among non–Alaska Native [7] and Navajo [14] children in the United States and among Australian children [15], Sp19A IPD did not significantly increase after the introduction of PCV7. Furthermore, the frequency of Sp19A IPD significantly increased in Korea [16] and Spain [17] even before PCV7 was introduced. An additional important mechanism could be the promotion of drug-resistant clones by frequent antibiotic use. This is supported by the finding that most of the post-PCV7 Sp19A proliferation has been due to antibiotic-resistant and multidrug-resistant clones.

In view of the controversy over the role played by PCV7 in the increasing frequency of Sp19A, information on Sp19A prevalence and dynamics in unvaccinated populations is important. The increase in Sp19A IPD in Korea before the introduction of PCV7 was mostly due to a single multidrug-resistant clone [16]. However, the dynamics of Sp19A disease are essentially unknown in other unvaccinated populations.

In southern Israel, a prospective epidemiologic study was initiated in 1999 to determine the dynamics, relative importance of VSTs and NVSTs, and antibiotic-resistance patterns of S. pneumoniae that cause AOM in 2 distinct populations of children differing in lifestyle and having minimal contact with each other. This study enabled us to prospectively follow Sp19A AOM dynamics for 8 years before the introduction of PCV7.

**METHODS**

**Setting.** In southern Israel (the Negev region), the Jewish and Bedouin populations, who differ in socioeconomic conditions and lifestyle, live side by side. However, both have access to the same medical services. The Jewish population is mainly urban, whereas the Bedouin population (formerly desert nomads) is transitioning to a Western lifestyle and lives in scattered clusters [18]. Contact between children of the 2 populations is rare. Children of the 2 populations do not frequent the same day-care facilities or schools and do not have a common social life. The Bedouin population, compared with the Jewish population, is characterized by overcrowding, lower education level, larger family size, and lower income [18]. In 2004, the crude birth rate was 55.3 and 21.0 births per 1000 persons in the Bedouin and Jewish populations [19], respectively, and the mean ± SD family size among the Bedouin population was 8.2 ± 0.9 persons, compared with only 3.2 ± 0.1 among the Jewish population [20]. The average monthly family income was 2-fold higher among the Jewish population [19]. Hospitalization rates for respiratory and other infectious diseases were higher among the Bedouin population [21].

All children in the area are born in one hospital, where they also receive all emergency and inpatient services. All children in Israel are entitled to medical insurance free of charge. No change in the recommendations concerning tympanocentesis in children with AOM occurred during the study period. All antibiotic drugs for children are given free of charge.

During the study period, PCV7 had not yet been introduced in Israel, and <3% of the population had participated in clinical trials with one of the experimental pneumococcal conjugate vaccines.

**Measurement of antibiotic prescription rates.** Data were gathered from 7 large pediatric primary-care clinics belonging to the General Health Insurance Plan (covering >60% of the Negev’s children), for which all prescriptions are computerized; 5 clinics in urban Jewish centers, caring for a yearly mean of 5780 (range, 5687–5917) children <5 years old; and 2 clinics in Bedouin townships, caring for a yearly mean of 6683 (range, 5655–7104) children <5 years old. This represents ~20% of the region’s children <5 years old. All antibiotic prescriptions for these children were recorded from 1998 through 2005. Oral antibiotics were grouped as (1) amoxicillin and amoxicillin-clavulanate, (2) oral cephalosporins (cefaclor, cepalexin-monohydrate, and cefuroxime-axetil), and (3) azithromycin. The use of penoxymethyl-penicillin and erythromycin were negligible during the study period and, thus, were excluded from the analysis when performed by drug group.

**Bacterial isolates and patient information.** Prospective surveillance was undertaken to include all middle ear fluid (MEF) specimens obtained from patients with AOM treated at the hospital and >90% of those obtained in the community. Approximately 85% of MEF specimens were obtained by tympanocentesis, and ~15% were obtained by draining ears. Specimens were cultured at the Clinical Microbiology Laboratory of the Soroka University Medical Center. All MEF pneumococcal isolates obtained from 1999 through 2006 were included. Diagnosis of AOM was made by a pediatrician, a family physician, or an otolaryngologist. The following information was prospectively obtained for each culture-positive episode: age, ethnicity, previous AOM episodes, and any antibiotic use in the preceding month. Data were obtained from medical records and the child’s physician or parents, as appropriate.

**Bacteriology.** Swabs of MEF aspirates were placed in MW173 Amies transport medium (TransSwab; Medical Wire and Equipment), plated immediately on trypticase agar containing 5% sheep blood and 5 μg/mL gentamicin and on chocolate agar, and incubated at 35°C for 48 h in a 5%-enriched CO₂ atmosphere. Identification, serotyping, and testing of antimicrobial susceptibility to penicillin, erythromycin, clindamycin, chloramphenicol, trimethoprim/sulfamethoxazole, and tetracycline were performed as described elsewhere [22]. Isolates with a penicillin MIC of ≥0.1 μg/mL were considered to be penicillin-nonsusceptible S. pneumoniae. Isolates with a penicillin MIC of
Bedouin children but increased slightly (from 30.1% to 38.7%) among Jewish children (P < .001). A total of 438 isolates (9.8% of all pneumococcal isolates) were Sp19A—187 (10.0%) and 251 (9.7%) among Jewish and Bedouin children, respectively.

Among Jewish children, the yearly proportion of Sp19A ranged from 8.0% to 14.0%, with no significant increase during the study period (figure 1). Among this population, the proportion of Sp19A isolates that were penicillin resistant increased sharply from 1999—2001 (yearly mean ± SD, 17.7% ± 6.3%) to 2002 and has remained steady since then (mean ± SD during 2002–2006, 37.6% ± 34% (P = .002) (figure 2). The proportion of macrolide- and multidrug-resistant Sp19A was low throughout the study period. Among Bedouin children, the proportion of Sp19A increased from a mean ± SD of 8.4% ± 0.8% during 1999–2001 to 13.7% ± 0.9% during 2004–2006 (a 63.1% increase) (P = .003). At the same time, the proportion of penicillin-, erythromycin-, and multidrug-resistant Sp19A rose sharply, from <10% for all 3 categories to 78.6%, 50.0%, and 50.0%, respectively, in 2006 (P < .001).

Of 438 Sp19A isolates obtained during the study period, 394 (90.0%) were available for PFGE, as were an additional 22 isolates from 1998 (before the initiation of the prospective study), for a total of 416. Six main PFGE clusters accounted for 98.3% of all Sp19A isolates (figure 3 and table 1). Five additional PFGE patterns were found in smaller numbers. Two representative strains from each of the common PFGE clusters were further characterized by MLST. For clusters with more diverse PFGE patterns (clusters A and E), MLST was done on 3 isolates showing the most diverse patterns. MLST was also performed on 5 additional isolates with PFGE patterns different from the 6 major ones. The 6 main PFGE patterns showed 6 distinct STs: ST-172, ST-2927, ST-276, ST-2928, ST-2929, and ST-1756. The clones of the 5 additional PFGE patterns were ST-156, ST-847, ST-2930, ST-2931, and ST-199.

The dynamics of the clonal distribution by year differed significantly between Jewish and Bedouin children (figure 4). Among Jewish children, a single, mainly penicillin-resistant clone (ST-172) constituted 69.4% of all tested Sp19A isolates and was dominant throughout the study period. The second most frequent clone (ST-2927), which was mainly penicillin intermediate, was first found in 1998 and has been present ever since, accounting for 12.0% of all Sp19A. A third clone (ST-2929) that was penicillin intermediate constituted 10.9% of all Sp19A isolates among Jewish children and was present during the entire study period.

Among Bedouin children, the mainly penicillin–intermediate clone ST-2927 was dominant until 2003, but its proportion significantly decreased during the study period (P = .005). In contrast to its proportion among Jewish children, the penicillin-resistant clone ST-172 accounted for only 33.9% of the isolates among Bedouin children (P < .001 vs. its proportion among Jewish children). Two multidrug-resistant clones, ST-276 and ST-2928, proliferated rapidly after 2001 and 2002, respectively.
The most prevalent one, ST-276, was first seen in 2001 and became the predominant clone among Bedouin children in 2005–2006 (P/H11021). This clone was observed in the Jewish population for 1 episode only. The second multidrug-resistant clone, ST-2928, was first seen in a Bedouin child in 1999, reappeared in 2002, and was present yearly thereafter.

Overall, antibiotic prescription rates were significantly higher among Bedouin children than Jewish children throughout the

Figure 1. Proportion of all pneumococcal acute otitis media (AOM) episodes that were caused by Streptococcus pneumoniae serotype 19A (Sp19A) among Jewish and Bedouin children <5 years old undergoing tympanocentesis in southern Israel, 1999–2006. The total no. of pneumococcal AOM isolates obtained from Jewish children each year was as follows: for 1999, n = 242; for 2000, n = 258; for 2001, n = 238; for 2002, n = 274; for 2003, n = 326; for 2004, n = 190; for 2005, n = 160; and for 2006, n = 158. The total no. of pneumococcal AOM isolates obtained from Bedouin children each year was as follows: for 1999, n = 433; for 2000, n = 468; for 2001, n = 442; for 2002, n = 411; for 2003, n = 371; for 2004, n = 201; for 2005, n = 125; and for 2006, n = 111. P values were derived from logistic regression analysis, which corrected for age and antibiotic use (any antibiotic treatment in last month, including on day of culture).

Figure 2. Proportion of all acute otitis media (AOM)–causing Streptococcus pneumoniae serotype 19A (Sp19A) isolates that were penicillin resistant, erythromycin resistant, and multidrug resistant. Sample sizes for total yearly pneumococcal AOM isolates are as shown in the legend for figure 1. P values were derived from logistic regression analysis, which corrected for age and antibiotic use (any antibiotic treatment in the last month, including on day of culture).
study ($P < .001$) (figure 5). This was also true for amoxicillin with or without clavulinate and oral cephalosporins ($P < .001$ for each). In both populations, a marked reduction in rates of prescriptions for amoxicillin with or without clavulinate and cephalosporin was observed ($P < .001$ for each antibiotic group per each population). In contrast, rates for azithromycin increased by 222% and 295% in Jewish and Bedouin children, respectively, between 1998–2000 and 2004–2005 ($P < .001$). Thus, the increased proportions of penicillin-, erythromycin-, and multidrug-resistant Sp19A isolates among Bedouin children correlated with the increased azithromycin prescription rates after 2001 but contrasted with the trends for all other antibiotic prescriptions.

Of the 6 major Sp19A clones, 5 belonged to an exclusive MLST group, whereas clone ST-2929 was a singleton (figure 6). The largest clone, ST-172, was previously associated with serotypes 6A, 15B, 19F, and 19A. The second most common clone, ST-2927 (reported first in the present study), is a single-locus variant (SLV) of ST-193, which was previously associated mainly with serotype 21 and 19A. ST-276, the most prevalent multidrug-

![Figure 3. Main pulsed-field gel electrophoresis patterns and clonal distribution of Streptococcus pneumoniae serotype 19A among children with acute otitis media in southern Israel.](image)

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<th>Table 1. Susceptibility patterns of the Streptococcus pneumoniae serotype 19A clones.</th>
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**NOTE.** PFGE, pulsed-field gel electrophoresis; R, resistant; S, susceptible; S/R, both susceptible and resistant isolates were prevalent; TMP/SMX, trimethoprim/sulfamethoxazole.
resistant clone, was formerly associated with Sp19A only. The second multidrug-resistant clone, ST-2928 (reported first in the present study), is a SLV of ST-63, which was previously associated mainly with serotype 15A. The penicillin-intermediate clone ST-2929 (reported first in the present study) is a singleton. The only penicillin-susceptible clone, ST-1756, was previously associated only with Sp19A. ST-2930 is a SLV of the previously reported ST-2218 or ST-2345, and ST-2931 is a double-locus variant of the previously reported ST-1730 or ST-2483.

**DISCUSSION**

Our study, conducted in a country in which PCV7 had not yet been introduced, has shown that Sp19A is important in AOM and that a significant increase in the frequency of Sp19A can occur in the absence of PCV7. In the crowded Bedouin population, among whom antibiotics were used more frequently than among the Jewish population, 2 multidrug-resistant Sp19A clones were introduced and have spread rapidly since 2001. This was temporally associated with an abrupt increase in azithromycin use in the community and with greater use of cephalosporins than among the Jewish population. Our findings are of importance in view of the emergence of Sp19A as the most common serotype in pneumococcal carriage and disease after the introduction of PCV7 in the United States. Despite the relatively modest role of the Sp19A increase compared with the overall decline in IPD in both children and adults, the >2.5-fold increase in the incidence of IPD caused by Sp19A has caused great concern [6, 11, 26].

Two main plausible explanations can be provided for the increase in Sp19A after the introduction of PCV7 in the United States. First, Sp19A appears to be equally capable of causing IPD, AOM, and nasopharyngeal colonization [11, 27–29]. Before the introduction of PCV7, Sp19A was the ninth most common serotype that caused IPD in children <5 years old in the United States, preceded only by the PCV7 serotypes and the related serotype 6A [30]. In France, children with AOM had an 8-fold greater risk of carrying Sp19A than healthy children, and it was the single most commonly carried *S. pneumoniae* serotype during AOM [31]. In southern Israel, Sp19A caused 17% of all IPD episodes in children <3 years old and ranked fourth after serotypes 1, 5, and 19F [27]. In the present study, Sp19A caused 10% of all pneumococcal AOM episodes among children <5 years old undergoing tympanocentesis. However, despite the hope that PCV7, which contains the related serotype 19F, could reduce the frequency of Sp19A, the vaccine was not effective against this serotype [32]. Because Sp19A was a common inhabitant of the nasopharynx even before the introduction of PCV7 [33], reduced carriage of serotypes affected by PCV7 could have potentially facilitated its spread by filling the niche cleared by PCV7 serotypes [7, 33, 34].
Second and perhaps most importantly, the increased Sp19A IPD incidence in the United States paralleled the increasing proportion of penicillin, macrolide, and multidrug resistance within this serotype [2, 10, 12, 13, 34, 35]. At least 2 plausible mechanisms can be theorized to associate increased antibiotic resistance with increased disease incidence. One is that antibiotics promote the carriage of drug-resistant S. pneumoniae, which in turn can increase its spread, resulting in increased disease rate. The magnitude of such an event is expected to be proportional to antibiotic prescription rates in the community [22, 34, 36]. The second is that blood and cerebrospinal fluid cultures or tympanocentesis are likely to be done for patients not responding to antibiotics. In a population in which use of antibiotics is widespread, it is expected that this selective culturing practice will favor isolation of serotypes with prevalent antibiotic resistance. This will increase the relative incidence of resistant serotypes compared with susceptible ones, overemphasizing their importance.

How did expansion of resistance within Sp19A occur? In theory, it could have occurred through several mechanisms: (1) proliferation of ≥1 preexisting clone; (2) introduction of new clones into the community; (3) capsular-switch events, in which clones previously identified as other serotypes switch their polysaccharides to become Sp19A; and (4) acquisition of new resistance mechanisms, either through mutation within the clone or acquisition from other bacteria. Studies conducted in the United States after the introduction of PCV7 have shown that the most common mechanism was proliferation of previously observed antibiotic-resistant clones, although introduction of new clones and capsular switch also did occur [2, 11, 35]. The lack of systematic molecular epidemiologic surveillance during the pre-PCV7 era does not permit determination of the causative role of PCV7. Therefore, examination of Sp19A clonal dynamics in regions in which PCV7 has not yet been introduced is important.

In Korea, although PCV7 was introduced only in late 2004 and its use was sparse until 2006, the proportion of Sp19A that caused IPD among children <5 years old increased from 0% in 1993–1994 to 8% and 20%–26% in 1995–1997 and 2001–2006, respectively (P < .005). All Sp19A were multidrug resistant [11]. Serotype 19F proportions decreased in parallel, from >30% of IPD isolates in 1991–1994 to <8% in 2001–2006 [16]. Furthermore, 2 studies from Korea demonstrated increased carriage of Sp19A among day-care attendees [37, 38].

Our study provides several important insights with regard to the potential causes of Sp19A proliferation. First, this study in an unvaccinated population clearly shows that a marked and rapid proliferation of Sp19A can occur as an event fully independent from the introduction of PCV7. Second, it confirms previous findings from vaccinated and unvaccinated populations in which the frequency of Sp19A increased mainly because of proliferation of existing clones or introduction of new clones. We did not observe new resistance patterns within any clone. Third, capsular-switch events could not be demonstrated, because we did not test MLST patterns in non-Sp19A serotypes. However,
the founding genotypes of the 2 multidrug-resistant clones ST-276 and ST-2928 (ST-230 and ST-63, respectively) have only rarely been associated with Sp19A, indicating that these clones resulted, most probably, from capsular-switch events. In particular, ST-63, the founder of the new multidrug-resistant clone ST-2928, has been associated mainly with multidrug-resistant 15A strains, with a resistance pattern similar to the new multidrug-resistant clone (http://www.mlst.net). Fourth, the introduction and proliferation of the 2 multidrug-resistant clones were temporally associated with the introduction and increase in azithromycin use in a community characterized by extensive antibiotic use and occurred despite a significant reduction in the rate for all other antibiotics. To date, proliferation of Sp19A has been reported only in countries with extensive use of antibiotics, including oral cephalosporins and/or azithromycin [39], such as the United States [2, 7–9, 12], Canada [40, 41], France [31], Spain [17, 42], Korea [16, 43], and Israel.

Recent data, including those from our region, suggest that use of oral cephalosporins and azithromycin in children is a major promoter of macrolide-, penicillin-, and multidrug-resistant pneumococci, especially if introduced when dual penicillin/macrolide resistance is already prevalent in the community [22, 44–46]. The proliferation of the 2 multidrug-resistant clones among the Bedouin population rather than the Jewish population could have been facilitated by more frequent use of antibiotics (especially oral cephalosporins) and greater crowding. Population density together with frequent antibiotic use are major determinants of drug- and multidrug-resistant pneumococci in the community [47], and it could also be speculated that these factors facilitated the introduction of Sp19A among Alaska Natives [7].

Of all pneumococcal serotypes that are both frequently drug resistant and associated with diseases (serotypes 6A, 6B, 9V, 14, 19A, 19F, and 23F), Sp19A is the only one against which PCV7 does not have demonstrated effectiveness. This might be the main reason why, in the United States, proliferation of Sp19A stands in sharp contrast to the reduction in frequency of all other highly resistant serotypes. However, in Israel [48] and Korea [16] before the introduction of PCV7, the population of serotype 6A had also been expanding with increasing multidrug resistance.

The present study has 2 important limitations. The first is that it was impossible to relate our findings to AOM incidence—we
could only relate them to the relative importance of Sp19A compared with other serotypes that cause AOM. However, that the multidrug-resistant Sp19A clones were absent before 2001 suggests a true increase in antibiotic-resistant Sp19A rather than just a change in the proportions of serotypes. The second limitation is that the change in reimbursement policy that occurred during the last 3 years of the study resulted in a decrease in the number of visits to the emergency department and could have led to a higher proportion of visits being made by persons with more severe cases, including antibiotic failure, although we adjusted our statistical calculations for age and previous antibiotic treatment.

It is impossible to predict the role that PCV7 (planned to be incorporated in the national schedule in 2009) may play in Israel, but future use of a PCV containing Sp19A, such as the investigational 13-valent vaccine [49, 50], may be a potential solution. We also believe that antibiotic use, especially antibiotic resistance-promoting drugs (such as oral cephalosporins and azithromycin), may reduce the spread of antibiotic-resistant clones, such as Sp19A. The recent introduction of PCV7 in countries in which antibiotic use and, hence, antibiotic resistance is less extensive (i.e., the United Kingdom, the Netherlands, and Scandinavia) may reveal the extent of the role that PCV7 plays in Sp19A proliferation when antibiotic pressure is low.

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