Marked Differences in Pneumococcal Carriage and Resistance Patterns between Day Care Centers Located within a Small Area

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Carriage rates of Streptococcus pneumoniae and their antibiotic resistance, capsular types, and genetic patterns were studied among 264 children aged 12–35 months attending 8 day care centers located within a 2.5-mile radius in the same city. Nasopharyngeal cultures were obtained within a 2-month interval from all 264 children. Significant differences in each of the studied characteristics were found between day care centers, and each day care center had a unique pattern of the carried pneumococci. Our findings show that day care centers are independent microenvironments and emphasize their role in the transmission and augmentation of antibiotic-resistant S. pneumoniae in the community.

Streptococcus pneumoniae is an important cause of morbidity and mortality in all societies [1]. The spectrum of illness caused by the pneumococcus is a reflection of its ability to survive in various ecological niches in the human body. S. pneumoniae resides in the nasopharynx, most commonly without adverse effects to the host, but may spread locally to cause upper or lower respiratory tract infection. In some circumstances, the organisms are able to enter the bloodstream from the nasopharynx via the cervical lymphatics, leading to bacteremia and, occasionally, infection of other organ systems [2–4]. Asymptomatic nasopharyngeal carriage of pneumococci is very widely prevalent among young children in both developed and developing countries [2, 4–10]. Pneumococcal nasopharyngeal carriage is important, since it is related both to development of disease and to spread of the pathogen [11, 12].

Pneumococcal resistance to antibiotic drugs, first reported in the mid-1960s [13, 14], is increasing worldwide [15–23]. Antibiotic-resistant strains are more often carried by infants and young children than by adults [24–26]. Most of the resistant strains belong to a limited number of serotypes that are also among the most common serotypes causing pediatric infections [14, 27–30].

Most of the reported outbreaks of resistant pneumococcal disease in the United States have involved child care facilities, usually day care centers (DCCs) [4, 22]. In DCCs, 3 conditions exist that favor the development and transmission of resistant pneumococci [16]: a large number of children, frequent close person-to-person contacts, and intensive antimicrobial use, which seems to be particularly important in the selection of antibiotic-resistant strains [24, 31]. Several studies have reported significantly greater antimicrobial use by children who attend DCCs than by children who attend day care homes or remain at home. It seems likely that intensive antimicrobial usage provides selective pressure that favors the appearance of resistant pneumococci, whereas the day care environment fosters the transmission of these strains [26, 28].

In a previous prospective study that we conducted in 1 DCC in southern Israel, we demonstrated extensive person-to-person spread of pneumococci in general and antibiotic-resistant pneumococci in particular [32], which suggests that the DCC may serve as a reservoir of antibiotic-resistant pneumococci and so augments their carriage in the community.

In the present study, we examined children attending 8 DCCs located in the same city within a 2.5-mile radius in 6 different neighborhoods, to determine whether each DCC acts as an independent microenvironment as far as S. pneumoniae carriage and amplification are concerned. Such findings will enable further definition of the role of the DCC as a site of amplification and spread of pneumococci in general and resistant pneumococci in particular. We included children during their second and third year of life, since the carriage rate is usually maximal in this age group.

Patients and Methods

Population and study design. Children attending 8 DCCs in the city of Beer-Sheva in southern Israel were recruited. All 8 DCCs were within a 2.5-mile radius, in 6 different neighborhoods. DCCs 1 and 5 were in the same neighborhood, as were DCCs 2 and 4. The study was proposed to that parents of children attending DCCs if they were 12–35 months of age at enrollment. The parents of
about two-thirds of the children in each DCC agreed to enroll their children in the study. A total of 264 eligible subjects were enrolled. Nasopharyngeal cultures were obtained 3x from each child, 1 month apart, from October 1996 through February 1997.

**Nasopharyngeal cultures.** Nasopharyngeal samples were obtained by use of a flexible cotton-tipped swab, which was introduced into the nostrils and advanced until resistance was found. Swabs were inoculated into modified Stewart transport medium (Medical Wire & Equipment, Corsham, Wiltshire, U.K.) and were processed within 1 h at the Clinical Microbiology Laboratory of the Soroka University Medical Center, Beer-Sheva. Swabs were plated on Columbia agar with 5% sheep blood and 5.0 µg/mL gentamicin and were incubated aerobically at 35°C for 48 h. Presumptive identification of *S. pneumoniae* was based on the presence of α-hemolysis and inhibition by optochin and confirmed by a positive slide agglutination test (Phadebact, Pharmacia Diagnostics, Uppsala, Sweden). One *S. pneumoniae* colony per plate was then subcultured, harvested, and kept frozen at −70°C for further testing.

**Antibiotic susceptibility testing** Susceptibility of isolates to oxacillin, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, and chloramphenicol was determined by the disk-diffusion method of Bauer and Kirby according to the National Committee for Clinical Laboratory Standards recommendations [33]. Isolates exhibiting an inhibition zone of <19 mm around a 1-µg oxacillin disc were further tested by the Etest (PDM Epsilometer; AB Biodisk, Solna, Sweden), following the manufacturer’s instructions [34], to determine the penicillin MIC. Susceptibility of isolates to penicillin was determined as follows: if penicillin MICs were <0.1 µg/mL, the organism was defined as susceptible; if they were 0.1–1.0 µg/mL, the organism was defined as intermediate; and if they were >1.0 µg/mL, the organism was defined as fully resistant to penicillin. All organisms with penicillin MICs of >0.1 µg/mL were designated penicillin-resistant.

**Serogrouping and serotyping.** Serogrouping and serotyping of *S. pneumoniae* were done by the quelling reaction with sera produced by the Statens Seruminstitut (Copenhagen) [35]. Isolates negative by all pooled sera and to omnisera were defined as nontypeable.

**Ribotyping.** The DNA of *S. pneumoniae* strains was analyzed by restriction fragment length polymorphism of ribosomal RNA genes. Isolates were grown overnight in 20 mL of brain-heart infusion broth (Difco, Detroit) containing 5% horse serum in a 5% CO₂-enriched atmosphere at 37°C. Cells were harvested by centrifugation at 5000 g for 15 minutes. Genomic DNA was extracted by the cetlytrimethylammonium bromide (CTAB) method [36]. The restriction enzymes *Pst*I and *Bsu*151 were used to digest the CTAB-purified chromosomal DNA samples. The resulting restriction fragments were separated electrophoretically on a 0.8% agarose gel, denatured, and transferred to Gene-Screen plus membranes (NEN, Boston, MA). λ phage DNA digested with *Hind*III was used as a molecular weight marker in each gel. The membranes were incubated in a prehybridization solution for 4 h at 65°C. Hybridization and detection were done with plasmid pKK223 carrying the 5S, 16S, and part of the 23S ribosomal RNA genes of *Escherichia coli*, was used [37]. A 2.5-kb EcoRI-*Hind*III restriction fragment, encoding for the 16S rRNA and part of the 23S, was used as a probe. We radiolabeled the probe with [α-³²P]dCTP (3000 Ci/mmol) using the Rediprime DNA Labeling System (Amersham International, Amersham, UK) according to the manufacturer’s recommendations. In a former study carried out by our group [32], this method proved to be informative as well as discriminative in showing the homogeneity of the strains carried by children at 1 DCC as opposed to the heterogeneity of the strains carried in the community.

**Statistical analysis.** Contingency table analysis was performed by use of the χ² test or Fisher’s exact test, as appropriate. Relative risk was computed across DCCs by use of the Mantel-Haenszel procedure with EpiInfo version 6 (Centers for Disease Control and Prevention, Atlanta), and 95% confidence intervals were also calculated. A confidence interval that does not include the value 1.0 shows that the relative risk is significantly different from 1.0 at *P*<.05.

**Results**

A total of 772 cultures (representing 772 child-months of observation) were obtained from all 264 children during a 2-month interval (3 cultures were taken from 252 children and 1 or 2 cultures from the remaining 12). The mean duration ± SD between the first and the second cultures was 31.0 ± 4.2 days (median, 30 days) and between the second and the third cultures was 35.2 ± 5.3 days (median, 32 days). The number

<table>
<thead>
<tr>
<th>DCC</th>
<th>No. of child-months</th>
<th>No. of child-months with carriage</th>
<th>Penicillin resistance</th>
<th>Any resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>5 (83)</td>
<td>4 (67)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>36 (82)</td>
<td>23 (52)</td>
<td>27 (61)</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>30 (71)</td>
<td>22 (52)</td>
<td>23 (55)</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>27 (79)</td>
<td>14 (41)</td>
<td>16 (47)</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>32 (84)</td>
<td>19 (50)</td>
<td>22 (58)</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>31 (91)</td>
<td>18 (53)</td>
<td>20 (59)</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>7 (35)</td>
<td>2 (10)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>14 (93)</td>
<td>9 (60)</td>
<td>10 (67)</td>
</tr>
<tr>
<td>Total</td>
<td>233</td>
<td>182 (78)</td>
<td>111 (48)</td>
<td>124 (53)</td>
</tr>
</tbody>
</table>

NOTE. Parentheses indicate rate per 100 child-months of follow-up.
of children enrolled in each DCC ranged from 16–54 (median, 32).

Carriage of S. pneumoniae. Of the 772 cultures, S. pneumoniae was detected in 594 (77%). The carriage rate in each DCC ranged from 64–82 per 100 child-months. The carriage rate among children aged <2 years was similar to that of children aged ≥2 years (table 1). There was no significant difference in total carriage rates between the 8 DCCs.

Antibiotic resistance of S. pneumoniae. Of the 594 S. pneumoniae isolates, 256 (43%) were nonsusceptible to penicillin. Of these 256, 253 (99%) were intermediate and 3 (1%) were fully resistant to penicillin. Resistance to ≥3 antibiotic classes (defined as multidrug resistance) was found in 65 isolates (11%); 334 isolates (56%) were resistant to at least 1 antibiotic drug. Significant differences in the carriage rates of resistant S. pneumoniae were observed between children aged <2 years and those aged ≥2 years: 48 per 100 child-months in toddlers aged <2 years versus 27 per 100 child-months in toddlers aged ≥2 years (the RR in older vs. younger toddlers computed across all DCCs was 1.72; 95% CI, 1.41–2.11). The carriage rate of S. pneumoniae resistant to at least 1 antibiotic class among toddlers aged <2 years was 53, versus 39 per 100 child-months among older toddlers (the RR computed across all DCCs was 1.32; 95% CI, 1.12–1.56).

On the basis of these results, data analysis with regard to the differences between the DCCs was carried out separately for each age group. Carriage rates of penicillin-resistant S. pneumoniae were significantly different among the 8 DCCs for the older toddlers (P = .019) and for the young age group (P = .037). Analysis of S. pneumoniae isolates resistant to at least 1 of the antibiotics tested resulted in significant differences among the various DCCs for the young age group (P = .008), whereas no difference could be found for the older group (table 1).

Serologic typing of S. pneumoniae. Six serotypes (6A, 6B, 14, 15, 19F, and 23F) constituted 66% of all 594 isolates. Serotypes 23F, 6A, and 15 were found in all 8 DCCs, and serotypes 19F, 6B, and 14 were found in 7 of the 8 (table 2). Significant differences were found among the 8 DCCs in the

Table 2. Streptococcus pneumoniae serotypes detected at least once among children attending 8 day care centers (isolates detected in >1 repeat positive culture with the same serotype were not counted).

<table>
<thead>
<tr>
<th>Serotype</th>
<th>1 (n = 16)</th>
<th>2 (n = 33)</th>
<th>3 (n = 36)</th>
<th>4 (n = 25)</th>
<th>5 (n = 32)</th>
<th>6 (n = 54)</th>
<th>7 (n = 30)</th>
<th>8 (n = 38)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>6A</td>
<td>6 (38)</td>
<td>16 (48)</td>
<td>5 (14)</td>
<td>5 (20)</td>
<td>12 (38)</td>
<td>16 (30)</td>
<td>6 (20)</td>
<td>7 (18)</td>
<td>.023</td>
</tr>
<tr>
<td>23F</td>
<td>8 (50)</td>
<td>8 (24)</td>
<td>9 (25)</td>
<td>7 (28)</td>
<td>9 (28)</td>
<td>9 (17)</td>
<td>10 (33)</td>
<td>6 (16)</td>
<td>.9</td>
</tr>
<tr>
<td>19F</td>
<td>7 (21)</td>
<td>3 (8)</td>
<td>7 (28)</td>
<td>8 (25)</td>
<td>14 (26)</td>
<td>3 (10)</td>
<td>6 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>5 (31)</td>
<td>1 (3)</td>
<td>16 (44)</td>
<td>1 (4)</td>
<td>1 (3)</td>
<td>7 (13)</td>
<td>5 (17)</td>
<td>7 (18)</td>
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</tr>
<tr>
<td>14</td>
<td>1 (3)</td>
<td>6 (3)</td>
<td>2 (8)</td>
<td>7 (22)</td>
<td>9 (17)</td>
<td>6 (20)</td>
<td>2 (5)</td>
<td></td>
<td>.08</td>
</tr>
<tr>
<td>6B</td>
<td>2 (13)</td>
<td></td>
<td>5 (14)</td>
<td>5 (20)</td>
<td>1 (3)</td>
<td>2 (4)</td>
<td>1 (3)</td>
<td>5 (13)</td>
<td>.4</td>
</tr>
<tr>
<td>19A</td>
<td>2 (6)</td>
<td>1 (3)</td>
<td></td>
<td>4 (7)</td>
<td>1 (3)</td>
<td>3 (8)</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>9V</td>
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<td></td>
<td>1 (4)</td>
<td>1 (3)</td>
<td>7 (13)</td>
<td></td>
<td>4 (11)</td>
<td>.09</td>
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</tr>
<tr>
<td>23A</td>
<td></td>
<td>1 (3)</td>
<td></td>
<td>3 (9)</td>
<td>1 (2)</td>
<td></td>
<td>8 (21)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>2 (8)</td>
<td>7 (22)</td>
<td>1 (3)</td>
<td></td>
<td></td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>23B</td>
<td></td>
<td></td>
<td></td>
<td>4 (16)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>18C</td>
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<td>1 (3)</td>
<td></td>
<td></td>
<td>1 (2)</td>
<td>1 (3)</td>
<td></td>
<td>≥1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3 (9)</td>
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<td></td>
<td></td>
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<td>4</td>
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<td></td>
<td></td>
<td></td>
<td>1 (2)</td>
<td></td>
<td></td>
<td>≥1</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data are no. of children with serotype (% in each day care center carrying isolate at least once). −, none.

* Calculated for each serotype separately, comparing all 8 day care centers.

Table 3. Distribution of unique Streptococcus pneumoniae strains, characterized by serology and resistance pattern, in the 8 day care centers.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Resistance</th>
<th>1 (n = 16)</th>
<th>2 (n = 33)</th>
<th>3 (n = 36)</th>
<th>4 (n = 25)</th>
<th>5 (n = 32)</th>
<th>6 (n = 54)</th>
<th>7 (n = 30)</th>
<th>8 (n = 38)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>6A</td>
<td>Pen, Em</td>
<td>15 (45)</td>
<td></td>
<td>2 (8)</td>
<td>6 (19)</td>
<td>5 (9)</td>
<td></td>
<td>1 (3)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>S</td>
<td>5 (31)</td>
<td>3 (8)</td>
<td></td>
<td></td>
<td></td>
<td>4 (13)</td>
<td>2 (5)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>19F</td>
<td>Pen</td>
<td>1 (3)</td>
<td>10 (28)</td>
<td></td>
<td>1 (3)</td>
<td></td>
<td>1 (3)</td>
<td></td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>19F</td>
<td>Pen, Em, TMP-SMZ, Tet</td>
<td>8 (15)</td>
<td></td>
<td>7 (22)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>23A</td>
<td>S</td>
<td>1 (3)</td>
<td></td>
<td>3 (9)</td>
<td></td>
<td>8 (21)</td>
<td></td>
<td></td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>23B</td>
<td>S</td>
<td></td>
<td></td>
<td>4 (16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data are no. of children with serotype (% in each day care center carrying isolate at least once.) Em, erythromycin; Pen, penicillin; S, susceptible to all tested antibiotics; Tet, tetracycline; TMP-SMZ, trimethoprim-sulfamethoxazole; −−, none.
carriage rates of 7 of 14 strains; 5, 6A, 6B, 11, 15, 23A, and 23B. Some of the less common serotypes in this population, such as 4, 5, and 23B, were found in only 1 or 2 DCCs. The differences are further emphasized in table 3, which shows the distribution in the DCCs of strains characterized by their serotype and by their resistance pattern.

*Genetic typing of S. pneumoniae strains.* Three of the most common serotypes with an identical antibiotic resistance pattern were also typed genetically by the ribotyping method. Strain 23F, which was resistant to penicillin and trimethoprim-sulfamethoxazole, was the most common strain and was found in all of the DCCs. The ribotyping patterns of the Bsu15I-digested DNA were identical for all of the isolates except for 2 of 7 isolates from DCC 2 and all 3 isolates from DCC 8 (data not shown). However, after digestion with PvuII, the ribotyping showed 4 different patterns, with only 1 dominant pattern for each DCC (figure 1). One pattern was found in DCCs 2 and 6, another 1 in DCCs 5, 4, 3, and 7, 1 in DCC 1, and 1 in DCC 8.

Another common strain was 19F, which was susceptible to all of the tested antibiotics. Although the Bsu15I-digested DNA gave the same restriction pattern for most strains (32 of 36; data not shown), the PvuII-digested DNA showed different patterns in the various DCCs (figure 2). For most DCCs, 1 dominant pattern could be demonstrated, which differed from the patterns in the others. All samples were identical within each DCC (2 and 3), but differed between the 2 DCCs. In DCC 4, 6 of 7 isolates gave indistinguishable patterns, all 7 isolates differing from the patterns in DCCs 2 and 3.

Strain 6A also expressed a different dominant restriction pattern in most of the DCCs. For example, for 4 DCCs, 69%–100% of the isolates had identical ribotyping patterns within each

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**Figure 1.** Ribotyping patterns of *Streptococcus pneumoniae* serotype 23F, which is resistant to penicillin and trimethoprim-sulfamethoxazole. DNA was digested by *Pvu*II. Numbers at top indicate which day care centers the samples came from.

**Figure 2.** Selected samples of ribotyping patterns of *Streptococcus pneumoniae* serotype 19F, which is susceptible to penicillin, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, and chloramphenicol. DNA was digested by *Pvu*II. Numbers at top indicate which day care centers the samples came from.
DCC but differed from 1 DCC to another (selected samples are shown in figure 3).

Discussion

Our hypothesis, that DCCs are independent microenvironments responsible for the amplification and spread of pneumococci in general and resistant pneumococci in particular, was supported by the following findings. First, pneumococcal carriage in the DCCs was highly prevalent, ranging from 64%–82%. In addition, a high proportion of the carried pneumococci were resistant to ≥1 antibiotics: The prevalence of children carrying resistant *S. pneumoniae* ranged from 28%–58%. Finally, each DCC had a unique pattern of pneumococcal clones, which indicates frequent spread within the DCC.

The unique distribution of pneumococcal strains in each DCC was seen at 3 different levels. First, the phenotypic distribution by serotypes was examined; we found significant differences among the 8 DCCs regarding most of the serotypes, and in some cases, a few serotypes (e.g., 11 and 23B) were found in only a minority of the DCCs. Second, differences in the antibiotic resistance patterns between the various DCCs were common even for the same serotype. Third, even isolates with the same serotype and antibiotic resistance pattern nearly always yielded different genotypic patterns for each DCC, despite being homogenous within each DCC. Our results show clearly that each DCC is a microenvironment with unique features.

It is possible that the results of the present study do not support the hypothesis that the DCCs are responsible for the amplification and spread of pneumococci; it could be that the DCCs just reflect the situation in the surrounding neighborhood. However, the fact that 2 pairs of DCCs (1 and 5, and 2 and 4) were located in the same neighborhoods but were substantially different in their circulating *S. pneumoniae* strains makes this speculation unlikely.

Both pneumococcal carriage and morbidity are found mainly in young pediatric populations [2–4]. For these populations, attending a DCC is an important risk factor because of the frequent close contacts between susceptible hosts and the difficulty of maintaining strict hygienic conditions [16]. The serotypes most frequently carried in infants and young children are also those frequently causing disease. In the case of otitis media, we found that 90% of the strains cultured from the middle ear fluids of children with this infection are those carried in the nasopharynx and are frequently antibiotic-resistant (unpublished data). Therefore, efforts to prevent pneumococcal diseases in communities with high DCC attendance should focus also on preventing the of carriage of resistant pneumococci by DCC attendees, among whom resistant pneumococci are extremely prevalent.

Carriage of resistant pneumococci in various areas of the world is well documented in the literature, and recommendations for antibiotic treatment are often based on national or regional surveys [23, 38–40–]. The purpose of the present study was not to document another instance of such high carriage per se, but rather to demonstrate that, in a small city in southern Israel, each of several DCCs located not far apart is a microenvironment with unique characteristics that harbors a unique pattern of carriage of and resistance to resistant pneumococci. Our study shows that treatment recommendations based on surveillance of wide areas may not be adequate. As a result, treatment of pneumococcal disease should not be extrapolated from the general resistance patterns reported from the entire region.

Vaccination strategies offer the simplest and most effective approach to controlling drug-resistant *S. pneumoniae* [15, 22]. Conjugate vaccines aimed at the pediatric serotypes may provide a useful tool to reduce nasopharyngeal carriage and limit
the spread of resistant pneumococci. Previous studies by our group showed reduction in nasopharyngeal carriage following vaccination with such vaccines [20, 41]. Most of the resistant strains belonged to serogroups 6, 9, 14, 19, and 23. This distribution of resistance suggests that the conjugate pneumococcal vaccines that have been developed to date, which include serotypes representing the above-listed serogroups, may be helpful in preventing the carriage and spread of resistant S. pneumoniae in DCCs. Further studies on this role of vaccination are needed.

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References


