Spread of *Streptococcus pneumoniae* and Antibiotic-Resistant *S. pneumoniae* from Day-Care Center Attendees to Their Younger Siblings

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A prospective study was conducted to determine the association between pneumococcal carriage among 36 infants and young toddlers cared for at home and carriage among their older siblings who attended 8 day-care centers (DCCs); 71 pneumococcal strains acquired by the younger siblings were compared with those present in the DCCs for 6 months. In 76% of cases, ⩾1 strain identical by serotype and antibiogram was isolated in the older siblings' DCC versus 32%–63% in all other DCCs ($P < .001$). When phenotypically identical strains were compared by pulsed-field gel electrophoresis, the similarity between strains from older siblings' DCCs and the younger siblings' isolates was striking. This was not found when isolates from other DCCs were compared. Vaccinating DCC attendees with a 7-valent pneumococcal conjugate vaccine may play a key role in controlling the spread of antibiotic-resistant pneumococci, because the most resistant serotypes are included in the vaccine.

Children attending day-care centers (DCCs) are at higher risk of carriage of *Streptococcus pneumoniae* in general, and antibiotic-resistant *S. pneumoniae* in particular, than are children who are cared for at home [1–10]. Extensive carriage of the organisms in DCC attendees has been well documented [2, 3, 11–14]. Young children who have older siblings attending DCCs tend to have more respiratory diseases than do those who do not have older siblings in DCCs [15, 16]. They also have a higher carriage rate of antibiotic-resistant *S. pneumoniae* [2]. However, the role of DCCs in the spread of *S. pneumoniae* to the family, particularly to younger siblings of DCC attendees, has not been studied systematically. The present prospective study was conducted to determine the role of DCCs in the spread of *S. pneumoniae* to the community in general, and antibiotic-resistant *S. pneumoniae* in particular, by examining the association between carriage among infants and young toddlers being cared for at home and carriage in the DCCs attended by their older siblings.

Subjects, Materials, and Methods

Subjects. Healthy toddlers (both boys and girls; 12–35 months old) who attended DCCs and whose parents agreed to have their children monitored for 2 years were recruited in 8 DCCs in the city of Beer-Sheva, located in southern Israel. All 8 DCCs were located within a 2.5-mile radius in 6 different neighborhoods. The younger siblings of DCC attendees were recruited to the study if they were 0–18 months old at recruitment and were not attending any out-of-home facility. When a younger sibling reached age 18 months or started attending any out-of-home facility, he or she was excluded from further follow up. Some of these younger siblings were born during the follow-up period of the older siblings. None of the young siblings received any pneumococcal conjugate vaccine.

The present investigation was part of a randomized double-blinded study to evaluate the effect of a 9-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of *S. pneumoniae* and respiratory morbidity in toddlers attending DCCs [17, 18]. For the DCC attendees, 18 encounters were planned for each child during the 2-year follow-up period: monthly visits were planned for the first year of follow-up, and visits were planned every 2 months during the second year of follow-up. For the younger siblings, monthly visits were planned until they were 18 months old or started to attend any out-of-home facility.

Because our primary objective was to determine the influence of the older sibling's DCC on the nasopharyngeal carriage of *S. pneumoniae* in the younger sibling, all cultures obtained from DCC attendees after they left the original DCC were excluded from analysis. Similarly, all cultures obtained from younger siblings after the older sibling left the original DCC were excluded from the analysis.

Nasopharyngeal cultures. Nasopharyngeal samples for culture of *S. pneumoniae* were obtained at each visit by use of a flexible dacron-tipped swab, which was introduced into the nostrils and advanced until resistance was found. These swabs were inoculated in modified Stewart transport medium (Medical Wire & Equipment) 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 incubated aerobically at 35°C in a CO_2-enriched atmosphere for 48 h. This method was used in our pre-

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The study was approved by the Committee of Human Studies of the Soroka University Medical Center and the National Ethics Committee. Written informed consent was obtained from the parents of all the children before immunization.

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vious studies and provided a high rate of positive cultures [1–3, 19, 20].

Presumptive identification of *S. pneumoniae* was based on the presence of α-hemolysis and inhibition by optochin and was confirmed by a positive-slide agglutination test (Phadebact; Pharmacia Diagnostics). One *S. pneumoniae* colony per plate was then subcultured, harvested, and kept frozen at −70°C for further testing.

Serogrouping and serotyping. Serogrouping and serotyping of *S. pneumoniae* were performed by the quellung reaction with serum samples produced by the Statens Seruminstitut [21]. Isolates that tested negative by all pooled serum samples and by omni serum were defined as nontypeable. The vaccines that are under development or licensed contain 7 serotypes (7-valent vaccine containing serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F), 9 serotypes (9-valent vaccine containing the serotypes in the 7-valent vaccines plus serotypes 1 and 5), and 11 serotypes (11-valent vaccine, containing the serotype in the 9-valent vaccine plus serotypes 3 and 7). In the present study, the serotypes included in the 9-valent vaccine (1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F) are defined as vaccine-type (VT) pneumococci.

Antibiotic susceptibility testing. Susceptibility of isolates to oxacillin, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole (TMP/SMX), and chloramphenicol was performed by the disk-diffusion method of Bauer and Kirby, according to National Committee for Clinical Laboratory Standards recommendations [22]. Isolates exhibiting an inhibition zone ⩽19 mm around a 1-μg oxacillin disc were further tested by the E-test (PDM Epsilometer; AB Biodisk), according to the manufacturer's instructions [23], to determine the penicillin MIC. Susceptibility of isolates to penicillin was determined as follows: if penicillin MICs were <0.1 μg/mL, then the organism was defined as susceptible. All organisms with penicillin MICs ⩾0.1 μg/mL were designated as penicillin nonsusceptible.

Pulsed-field gel electrophoresis (PFGE). Chromosomal DNA fragments, generated by *Sma*I digestion, were prepared and analyzed as described elsewhere [24]. 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, ramped with initial forward time of 5 s and final forward time of 35 s. Gels were stained with ethidium bromide and photographed. The interpretation of strain relatedness on the basis of PFGE pattern was based on current consensus [25].

Data management and statistical analysis. Contingency-table analysis was performed using the χ^2 test or Fisher's exact test, as appropriate. The Mantel-Haenszel relative risk (RR_{MH}) and its 95% confidence intervals (CIs) were computed for comparison of matching between strains from older and younger siblings and matching between them and those from other DCCs.

We attempted to determine the relative contribution of the older sibling's DCC, compared with the rest of the community, by matching each strain newly acquired by the younger siblings to those isolated during the previous 6 months in the older sibling's DCC versus all other DCCs. The rank of the older sibling's DCC was determined according to the number of strains matching with the new strains acquired by the young siblings. The DCC with the highest proportion of matching strains was ranked "1," the next was ranked "2," and so forth. This calculation did not take into account the size of the DCC. For instance, if the number of matching strains was the highest, the rank of the DCC was 1. The comparison was performed by the goodness-of-fit χ^2 analysis. The probability that in 8 DCCs the rank would be ⩽3 was examined.

Results

The study was conducted from October 1996 through February 1999. A total of 262 DCC attendees and 46 younger siblings were recruited. In total, 4580 cultures were obtained: 4237 from DCC attendees and 343 from their younger siblings. During the 2-year follow-up period, a few children left their original DCC, and child-months after the move were excluded from the analysis. Therefore, the analysis was performed on 3400 cultures from DCC attendees and 219 cultures from younger siblings (262 and 36 subjects, respectively).

The mean age (±SD) of the 262 DCC attendees and 36 younger siblings at enrollment was 26.7 ± 6.8 and 5.9 ± 5.0 months, respectively. The age distribution at the time of nasopharyngeal culture for *S. pneumoniae* in the study subjects is presented in table 1. The mean number of cultures (±SD) obtained was 13.0 ± 3.9 for DCC attendees and 6.1 ± 3.8 for the younger siblings. The mean follow-up period (±SD) was 17.0 ± 7.1 months for the DCC attendees and 6.5 ± 4.1 months for the younger siblings.

Of the 3400 cultures obtained from the DCC attendees and 219 cultures obtained from younger siblings, 2320 (68%) and 113 (52%), respectively, were positive for *S. pneumoniae.* An antibiogram was not performed on only 1 strain in each group. Thus, the final number of strains available for analysis was 2319 for the DCC attendee group and 112 for their younger siblings. Resistance to at least 1 antibiotic drug was found in 34% of strains in both the DCC attendee and the younger sibling groups, whereas resistance to penicillin was found in 28% and 31% of strains in each respective group (table 2). The serotype distribution is shown in figure 1.

The serotypes included in a 7-, 9-, and 11-valent vaccine grew in 20%, 21%, and 22%, respectively, of all cultures from the DCC attendees and in 31%, 31%, and 32%, respectively of

Table 1. Age distribution at the time of nasopharyngeal culture for *Streptococcus pneumoniae* in 262 toddlers attending day-care centers (DCCs) and their 36 younger siblings.

Age at the time of culture, months	DCC attendees	Younger siblings
0–5	0	69 (31.5)
6–11	0	88 (40.2)
12–17	118 (3.5)	62 (28.3)
18–23	343 (10.1)	0
24–39	626 (18.4)	0
30–35	900 (26.5)	0
36–41	858 (25.2)	0
42–47	404 (11.9)	0
48–53	133 (3.9)	0
54–60	18 (0.5)	0
Total	3400 (100)	219 (100)

NOTE. Data are no. (%) of cultures.

Table 2. Carriage rate of *Streptococcus pneumoniae* and antibiotic-resistant *S. pneumoniae* among 262 toddlers attending day-care centers (DCCs) and their 36 younger siblings.

Child group	Total no. of cultures	Positive cultures	Resistance to ⩾1 antibiotic	Resistance to ⩾2 antibiotics	Resistance to ⩾3 antibiotics	Resistance to penicillin
DCC attendees	3400	2319 (68)	1169 (34)	782 (23)	196 (8)	962 (28)
Younger siblings	219	112 (51)	74 (34)	59 (27)	13 (6)	68 (31)

NOTE. Data are no. (%) of cultures.

cultures from the younger siblings. The rate of carriage of VT *S. pneumoniae* among the younger siblings was significantly higher than that among the DCC attendees ($P < .001$). For vaccine-related serotypes (serotypes other than the 7-valent vaccine but belonging to the serogroups in the 7-valent vaccine [6A, 9A/L/N, 18A/B, 19 A/B/C, and 23A/23B]), the proportion was 17% in the DCC attendees and 11% in the younger siblings. For serotype 6A, the respective proportions were 10% versus 5%, and, for serotype 9A, the respective proportions were 3% versus 3%.

Among the DCC attendees, a total of 1169, 196, and 962 cultures were positive for a strain that was resistant to ⩾1 antibiotics, ⩾3 antibiotics, and penicillin, respectively (table 2). Serotypes included in the 7-valent vaccine constituted 47% (550/1169) of the isolates resistant to ⩾1 antibiotics, 73% (144/196) of the isolates resistant to ⩾3 antibiotics, and 49% (476/962) of the isolates not susceptible to penicillin. The respective percentages for the isolates from younger siblings were 81% (60/74), 100% (13/13), and 82% (56/68). The respective percentages for serotype 6A among the DCC attendees were 16% (192/1169), 2% (3/196), and 19% (186/962). Among the younger siblings, the respective percentages were 9% (7/74), 0% (0/13), and 10% (7/68).

To examine the association between a positive nasopharyngeal culture in a younger sibling and the older sibling's DCC, we first took the phenotypic approach, characterizing each strain by the serotype and antibiogram patterns. A total of 71 new acquisitions of *S. pneumoniae* occurred in the 36 younger siblings during the follow-up period. Each time a new strain of *S. pneumoniae* was acquired by a younger sibling (defined as the first detection of a serotype), we investigated the presence of such a phenotype among the older siblings and in the older siblings' DCC during the preceding 6 months (including the month in which the new acquisition occurred). If a child carried the above strain more than once during the preceding 6 months, we chose only 1 isolate randomly. Thus, we excluded repeated isolates of the same strain.

Of the 71 newly acquired pneumococcal strains in the younger siblings, a phenotypically identical strain was isolated at least once from their older sibling on 27 (38%) occasions within the last 6-month period. This figure was significantly higher (54 [76%]) when the newly acquired strain was compared with the strains isolated from all cultures in the older sibling's DCC ($P < .001$). A strain phenotypically identical to the newly acquired strain by a younger sibling was isolated at least once from any of the DCCs not attended by the older sibling on 32%–63% of the occasions, compared with 76% occasions from the DCC attended by the older sibling (RR_{MH}, 1.47; 95% CI, 1.34–1.62; $P < .001$). Furthermore, when comparing the number of matching cultures (i.e., how many times at least 1 isolate of phenotypes acquired by the young siblings could be found in the previous 6 months among the older sibling's own DCC versus each of the 7 other DCCs), the older sibling's own DCC ranked always first or second, demonstrating that there was a significant matching of the newly acquired *S. pneumoniae* strains with the older sibling's own DCC ($P = .0256$) (table 3).

For the next step, representative *S. pneumoniae* strains newly acquired by the younger siblings that were relatively common in several DCCs were chosen to be studied on a genotypic basis—namely, comparing strains identical phenotypically by PFGE. This approach was taken to investigate whether the genotype of the younger sibling's newly acquired strains was more often similar to that of the older sibling's own DCC than to strains bearing the same phenotype (same serotype and same antibiogram patterns) isolated from attendees of other DCCs. We chose 3 strains (23F, resistant to penicillin and TMP/SMX; 19F, susceptible to all tested antibiotic drugs; and 6A, susceptible to all tested antibiotic drugs). For each of these 3 strains,

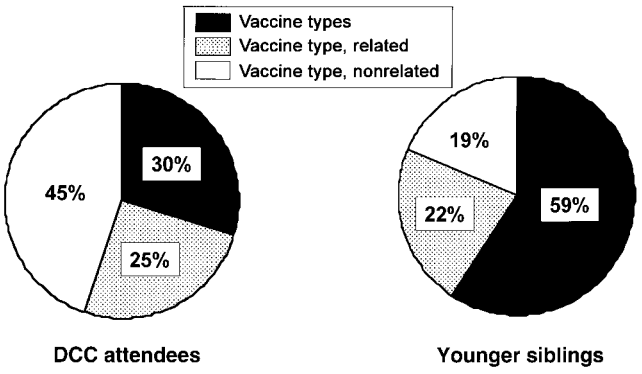

Figure 1. Serotype distribution of pneumococcal strains isolated from day-care center (DCC) attendees and younger siblings. Vaccine serotypes refer to the 7-valent pneumococcal conjugate vaccine (4, 6B, 9V, 14, 18C, 19F, and 23F), vaccine-related serotypes (6A, 9A/L/N, 18A/B, 19A/B/C, and 23A/B), and serotypes that are not vaccine related (all others).

Table 3. No. of matching strains between younger siblings' cultures and cultures obtained in the older siblings' own day-care center (DCC) vs. isolates from the other 7 DCCs in the past 6 months.

DCC	No. of new acquisitions in younger siblings of attendees	No. of matching strains in older siblings' DCC during past 6 months, by DCC								Rank of no. of matching strains
		1	2	3	4	5	6	7	8	
1	8	**6**	5	5	7	6	5	2	5	2
2	8	2	**4**	3	4	3	3	4	1	1
3	11	6	8	**7**	7	5	6	4	4	2
4	10	6	3	3	**7**	5	5	3	3	1
5	16	8	9	6	11	**14**	8	6	6	1
6	12	6	8	7	10	7	**10**	9	4	1
7	4	3	4	2	4	4	2	**4**	0	1
8	2	2	1	2	2	1	0	1	**2**	2

NOTE. Bold type indicates the older sibling's own DCC.

we compared the isolate of the younger sibling with all those phenotypically identical that were isolated in the 8 DCCs in the past 6 months.

Two younger siblings and their older siblings attending DCC 6 had a serotype 23F that was resistant to penicillin and TMP/SMX (figure 2). The 2 strains were identical by PFGE. Of the 13 phenotypically identical isolates from DCC 6, 11 (85%) had the same PFGE pattern. This PFGE pattern was found only in 2 (3%) of 75 of all other phenotypically identical isolates from the 7 other DCCs ($P < .001$). The pattern was found in 0 of 44 isolates from DCCs 1, 2, 3, 7, and 8; in 1 (7%) of 14 isolates in DCC 5; and in 1 (6%) of 17 isolates in DCC 4 ($P < .001$).

For strain 19F, which is susceptible to all drugs, 4 of 5 isolated from older siblings' DCCs were identical by PFGE, but none of the 23 phenotypically identical strains from the other DCCs were identical by PFGE ($P < .001$). Similarly, for serotype 6A, which is susceptible to all drugs, 3 of 7 phenotypically identical isolates from the older siblings' DCCs were identical by PFGE, versus 0 of 42 in phenotypically identical strains from all other DCCs ($P = .002$) (figure 3).

Discussion

In examining the spread of *S. pneumoniae* from DCC attendees to family members, we focused our study on the younger siblings of the toddler attendees because they are the most vulnerable to both pneumococcal carriage and pneumococcal disease, compared with other family members. Because we specifically excluded the younger siblings who were attending any out-of-home facility, we believe that the main source for acquisition of *S. pneumoniae* in general, and antibiotic-resistant *S. pneumoniae* in particular, is the older sibling's DCC. Indeed, the present study, which, to the best of our knowledge is the first study to have examined prospectively and directly the spread of *S. pneumoniae* from DCC attendees to their siblings, clearly demonstrates the important role of DCCs in the spread of these organisms to the community. The use of molecular techniques has enhanced the ability to demonstrate the probable source of *S. pneumoniae* newly acquired by the younger siblings.

We found high pneumococcal carriage rates and high antibiotic resistance among both the toddlers attending DCCs and their young siblings. The patterns of resistance were similar in the 2 groups. However, the carriage of the serotypes that are included or immunologically related to the 7-valent licensed pneumococcal conjugate vaccines (serotypes 4, 6B, 9V, 14, 18C,

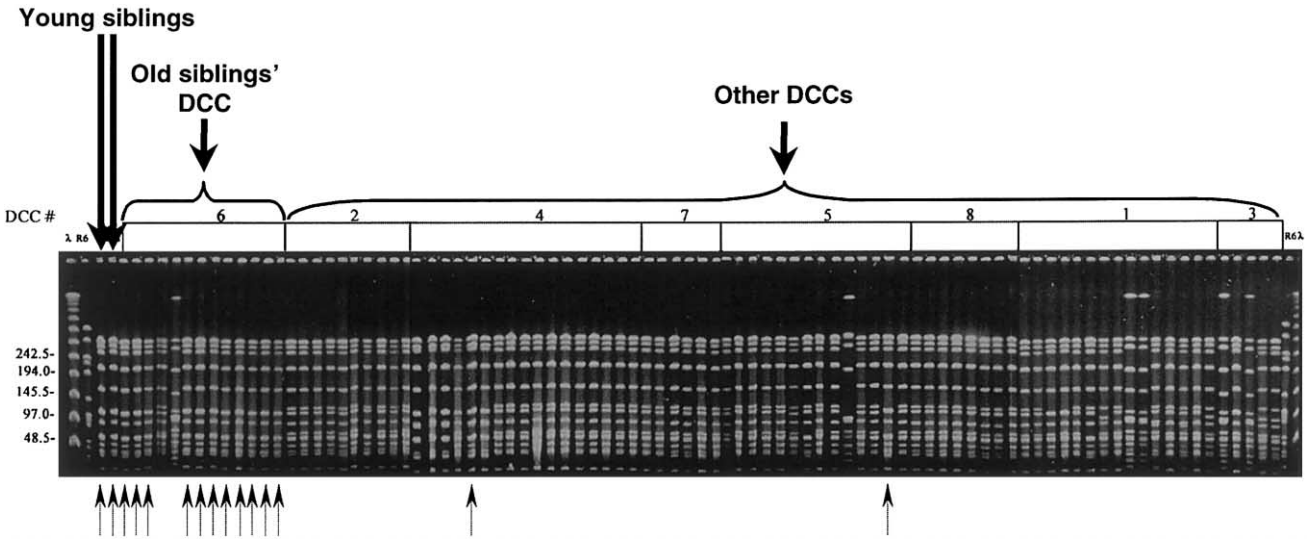

Figure 2. Pulsed-field gel electrophoresis patterns of *Streptococcus pneumoniae* serotype 23F, resistant to penicillin and trimethoprim-sulfamethoxazole, in the older sibling's day-care center (DCC) and in the other DCCs. DNA was digested by *Sma*I. The arrows at the bottom point to all isolates that were identical to those of the younger siblings.

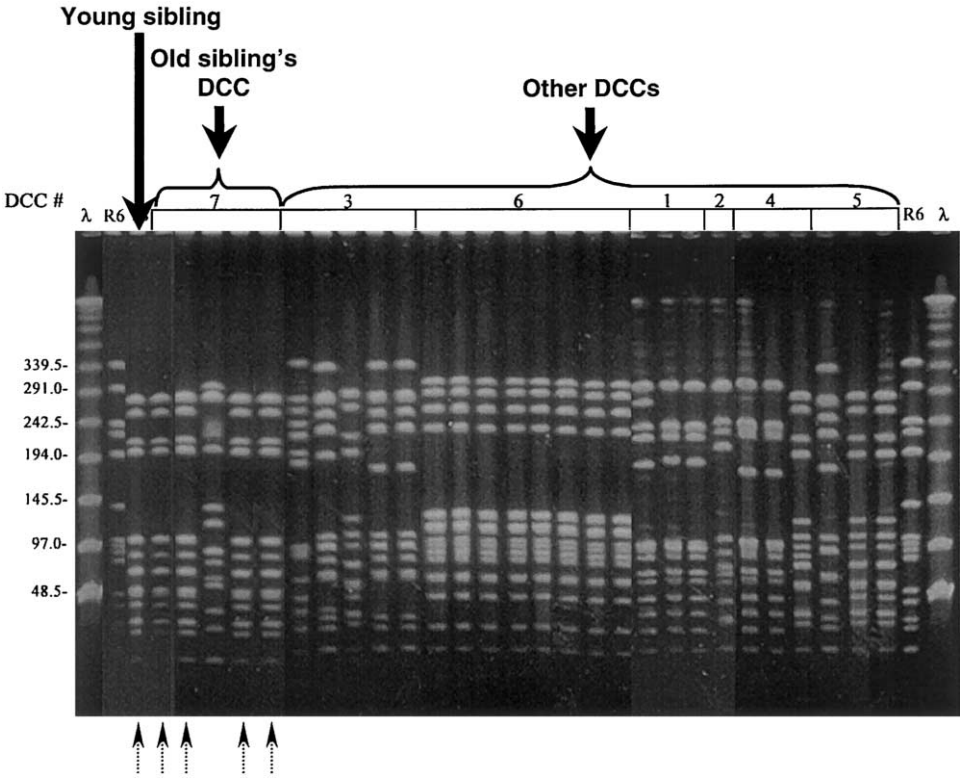

Figure 3. Pulsed-field gel electrophoresis patterns of *Streptococcus pneumoniae* serotype 6A, which is susceptible to penicillin, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, and chloramphenicol, in the old sibling's day-care center (DCC) and in the other DCCs. DNA was digested by *Sma*I. The arrows at the bottom point to all isolates that were identical to those of the younger siblings.

19F, and 23F) was significantly higher among the younger siblings. The higher carriage rate of the vaccine-related serotypes among the younger siblings was expected, because carriage of the vaccine-related serotypes (especially 6A, 6B, 9V, 14, 19F, and 23F) is prevalent during the first 24 months of life and tends to decline at a later age, to be partially replaced by other serotypes [26, 27].

The experience gained so far with the various pneumococcal conjugate vaccines shows that they are able to reduce invasive disease (i.e., bacteremia and meningitis) [28, 29] and mucosal disease (i.e., otitis media) [17, 28, 30, 31] caused by the serotypes included in the vaccines. Furthermore, protection against disease caused by the vaccine-related serotypes 6A and 19A was demonstrated by the conjugate vaccine–containing polysaccharide antigens of serotypes 6B and 19F [30, 32], although the protection was of a lesser degree, compared with that afforded against serotypes 6B and 19F. In addition to protection against disease, the conjugate vaccines can reduce carriage of the serotypes included in the vaccine [18, 33–37]. Here again, a reduction of carriage of the vaccine-related serotype 6A was achieved by vaccines containing serotype 6B antigen [18].

The ability of the conjugate pneumococcal vaccines to reduce carriage of the vaccine serotypes and vaccine-related serotypes (such as serotype 6A) suggests that immunizing a large proportion of the population can induce herd immunity and thus reduce the carriage of vaccine serotypes and vaccine-related serotypes among contacts of vaccinated children. This was indeed demonstrated in a recent study performed with Native American infants. In communities where extensive vaccination took place, the carriage of VT pneumococci was reduced not only among vaccinees but also among unvaccinated individuals living in the same community [38]. Furthermore, reduction in disease within a short time after initiation of widespread vaccination program among nonvaccinated children and adults was suggested recently in the United States [39, 40], although additional data are still needed before this is confirmed.

It is well established that the highest rate of antibiotic resistance and multiresistance is found among the serotypes included in the conjugate vaccines (mainly serotypes 6B, 9V, 14, 19F, and 23F) or serotypes antigenically related to them (mainly serotypes 6A and 19A). Therefore, by reducing carriage and transmission of disease caused by *S. pneumoniae,* a reduction of disease caused by antibiotic-resistant *S. pneumoniae* may occur not only in vaccinated children but also among those who come in close contact with the vaccinated subjects.

The fact that, among the younger siblings in our study, 58%

of all carried pneumococci were of VT serotypes and an additional 19% were of vaccine-related serotypes (figure 1) suggests that vaccination of DCC attendees may have a beneficial effect on their younger siblings by reducing the carriage of *S. pneumoniae* in general and that of antibiotic-resistant strains in particular. This could constitute a potential added benefit of a widespread vaccination program of infants and toddlers with conjugate pneumococcal vaccines.

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