Nasopharyngeal Colonization in Southern Israel with Antibiotic-Resistant Pneumococci during the First 2 Years of Life: Relation to Serotypes Likely to Be Included in Pneumococcal Conjugate Vaccines

Ron Dagan, Rimma Melamed, Marie Muallem, Lolita Piglansky, and Pablo Yagupsky

Pediatric Infectious Disease Unit and Clinical Microbiology Laboratory, Soroka University Medical Center and Faculty of Health Services, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Nasopharyngeal carriage of Streptococcus pneumoniae was studied in 162 healthy infants at ages 2, 4, 6, 7, 12, and 13 months and in an additional 352 healthy children at ages 12, 15, 18, 21, and 24 months. Carriage was 26%, 39%, and 62% at 2, 12, and 24 months, respectively, and the respective resistance to ≥1 antibiotic was 11%, 19%, and 27%. The presence of an older sibling or antibiotic treatment during the month preceding the culture was associated with carriage of resistant pneumococci in infants, whereas attendance at large day care centers was associated with carriage during the second year of life. Antibiotic resistance was detected in all 7 serotypes included in the candidate pediatric conjugate vaccines and was significantly more prevalent among vaccine-type pneumococci than among non–vaccine-type pneumococci. The use of conjugate vaccines may reduce the spread of resistant pneumococci.

Streptococcus pneumoniae is an important cause of morbidity and mortality, and its main reservoir is the nasopharynx. Most children are colonized sometime during the first 2 years of life [1].

The prevalence of antibiotic-resistant (R) pneumococci is increasing worldwide with an enormous impact on clinicians, microbiologists, drug manufacturers, and public health authorities [2]. R-pneumococci are carried more often by young children than by adults and belong to only a limited number of serotypes that are also among the most common causes of pediatric infections [2-4]. Therefore, candidate pneumococcal conjugate vaccines containing the 7 most prevalent pediatric serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) may reduce the carriage of R-pneumococci [2, 5, 6]. This notion is supported by the success of Haemophilus influenzae type b (Hib) conjugate vaccines in reducing carriage [7].

The present study was conducted to determine the prevalence of pneumococcal nasopharyngeal colonization, particularly with R-pneumococci, in healthy children during the first 2 years of life, and the relation of antibiotic resistance to the pneumococcal serotypes likely to be included in candidate conjugate pneumococcal vaccines.

Patients and Methods

Population and study design. Two groups of children were examined. The first cohort comprised 162 healthy Jewish infants from seven maternal and child health centers in Beer-Sheva (southern Israel), who were enrolled from November 1993 through March 1994 in a comparative conjugate Hib vaccine study. They were examined at ages 2, 4, 6, 7, 12, and 13 months (±2 weeks). The second cohort consisted of 352 healthy Jewish children from the same neighborhoods and health centers, enrolled from February through June 1994, to receive various pneumococcal vaccines at ages 12, 15, 18, 21, or 24 months (range, -2 to +4 weeks of above ages).

To test the homogeneity of the two populations, we compared their demographic characteristics and colonization rates with the various pneumococcal strains (including R-pneumococci) at the overlapping age (12 months). Since there was no difference between the groups at this age, they were considered homogeneous and were combined into one study group.

Information regarding older siblings and antibiotic drug administration during the month preceding the visit was obtained at each visit for infants ages 2-13 months. Information on day care attendance (including size of the day care center) was obtained for children ages 12-24 months. A nasopharyngeal culture was obtained at each visit.

Bacteriology. Nasopharyngeal swabs were placed in MW 173 Amies medium (Transwab; Medical Wire and Equipment, Potley, UK) and were processed within 4 h at the Clinical Microbiology Laboratory, Soroka University Medical Center.

Swabs were plated immediately on trypticase agar containing 5% sheep blood and 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 was confirmed by slide agglutination (Phadebacl; Pharmacia Diagnostics, Uppsala, Sweden). Typing was by quellung reaction [8] using reagents from Statens Seruminstitut (Copenhagen). Susceptibility to trimethoprim-sulphamethoxazole, tetracycline, erythromycin, clindamycin, and chloramphenicol was determined by the disk-diffusion method.
method interpreted according to National Committee for Clinical Laboratory Standards (NCCLS). For all antibiotics except penicillin, only isolates with inhibition zones considered “resistant” by NCCLS were defined as resistant; those in the “susceptible” and “intermediate” ranges were defined as susceptible. Isolates exhibiting inhibition zones ≤19 mm with a 1-μg oxacillin disk were further tested by the E-Test to penicillin (PDM Epsilometer; AB Biodisk, Solna, Sweden) [9]. Isolates with a MIC of 0.1–1.0 μg/mL were considered intermediated resistant and those with MICs >1.0 μg/mL were considered highly resistant.

All laboratory work was done in a blinded manner. Susceptibility testing and typing were done by technicians unaware of the serotypes and of the susceptibility test results. Neither had information about the patients.

Statistical analysis. We used the Epi Info statistical package (version 6; USD, Stone Mountain, GA). Analysis of contingency data was done by two-tailed $\chi^2$ test. Linear trend was tested by $\chi^2$ test for linear trend in proportions. Relative risk (RR) and 95% confidence intervals (CIs) were calculated. $P < .05$ was considered significant.

Results

Of the 1324 cultures planned, we did 1141 (86%; 77%–100% per visit). The overall carriage rate rose gradually from 26% at age 2 months to 62% at age 2 years (figure 1; $P < .001$). Similarly, the carriage rate of R-pneumococci rose from 7% at 2 months to 16% at 2 years ($P = .04$). For strains resistant to ≥1 antibiotic, the carriage rate rose from 11% to 27% ($P < .01$); for strains resistant to ≥2 antibiotics it rose from 4% to 18% ($P < .001$).

During the first 6 months of life, infants in our region usually do not attend day care facilities. Therefore, because older siblings are the main potential source of pneumococcal infection, we examined the effect of the presence of siblings on carriage in general and on that of R-pneumococci in particular. We isolated pneumococci at least once from 57 (68%) of 85 infants with ≥1 sibling and from 18 (37%) of 49 with no siblings (RR, 1.9; CI, 1.2–2.7; $P < .001$). Similarly, R-pneumococci were isolated from 24 (28%) of 85 versus 7 (14%) of 49 infants with and without siblings, respectively (RR, 2.0; CI, 0.9–4.3; $P = .1$). Penicillin-resistant pneumococci were isolated from 15 (18%) of 85 and 2 (4%) of 49 infants with and without siblings, respectively (RR, 4.3; CI, 1.0–18.1; $P < .05$).

Information regarding the use of antibiotics during the month before the culture was available for children ages 2–13 months. Although the use of antibiotics was not associated with a different pneumococcal carriage rate, it was associated with a higher proportion of R-pneumococci: Pneumococci were recovered in 45 (37%) of 122 visits within a month after antibiotic administration versus 224 (32%) of 690 visits without any prior antibiotic treatment ($P > .05$). In contrast, 28 (64%) of 44 isolates recovered within 1 month of antibiotic administration were R-pneumococci versus only 68 (33%) of 207 isolates from children who received no antibiotics during the month preceding their visit (RR, 2.6; CI, 1.9–3.5; $P < .001$).

Since day care attendance in Israel generally starts during the second year of life, we examined the carriage rate in children ages 12–24 months attending day care facilities and of those staying at home. We also attempted to determine whether carriage of R-pneumococci increased with the size of the day care facility. Data on day care attendance, culture, and susceptibility were available for 469 children ages 12–24 months. Pneumococci were isolated from 52 (61%) of 85 children attending day care facilities with ≥6 children and from
Figure 2. Resistance patterns among 209 vaccine-type Streptococcus pneumoniae isolates and 192 non–vaccine-type isolates from healthy infants and children ages 2–24 months. PEN = penicillin, TMP/SMX = trimethoprim-sulfamethoxazole, TETRA = tetracycline, ERYTHRO = erythromycin, CLIND = clindamycin. No resistance to chloramphenicol was found in 401 isolates.

(41%) of 384 children attending facilities with ≤6 children or not attending day care (RR, 1.5; CI, 1.2–1.8; P < .005). Similar carriage of R-pneumococci was found in the respective groups: 27 (36%) of 74 versus 66 (18%) of 373 children (RR, 2.1; CI, 1.4–3.0; P < .001).

We attempted to determine both the proportion of serotypes most likely to be included in pneumococcal conjugate vaccines (4, 6B, 9V, 14, 18C, 19F, and 23F) among the nasopharyngeal isolates and their relationship to antibiotic-resistance. Of the 401 isolates typed, 209 (52%) were of the vaccine type. An additional 18 isolates (4%) were type 6A, which cross-reacts with type 6B. We included this type in the non–vaccine-type group. The most common vaccine-type strain was 23F (34% of vaccine-type isolates), followed by 6B (22%), 19F (15%), 14 (13%), 9V (9%), 18C (4%), and 4 (3%). Antibiotic resistance was found mainly among type 23F (99% of isolates), followed by types 14, 9V, 6B, 18C, 4, and 19F (62%, 58%, 46%, 25%, 14%, and 10% of isolates, respectively). Resistance to ≥2 antibiotics was more common among type 23F (81%), followed by types 14, 6B, 9V, 4, 19F, and 18C (27%, 24%, 21%, 14%, 3%, and 0% respectively).

In 401 isolates, the most common resistance was to penicillin (126 [31%]), followed by trimethoprim-sulfamethoxazole (118 [29%]), tetracycline (16 [4%]), erythromycin (17 [4%]), and clindamycin (4 [1%]). Only 3 isolates were highly resistant to penicillin. No resistance to chloramphenicol was found. Resistance to ≥1, ≥2, and ≥3 drugs was found in 174 isolates (43%), 82 (20%), and 14 (4%), respectively.

Resistance was significantly more prevalent among vaccine-type than among non–vaccine-type isolates (figure 2). This pattern was observed for each drug tested separately and for resistance to ≥1, ≥2, and ≥3 drugs. Vaccine-type pneumococci made up most of the resistant isolates: 68% of 174 isolates resistant to ≥1 antibiotic, 81% of 126 isolates resistant to penicillin, and 91% of 82 isolates resistant to ≥2 drugs.

Discussion

Our study confirms the high rate of pneumococcal nasopharyngeal colonization in healthy infants and children ≤2 years of age found in previous studies [2] and that colonization may be detected in early infancy but peaks toward the second year of life. Although we did not use the mouse inoculation method, which might have enhanced our ability to detect carriers, we found a high rate of pneumococcal carriage. Furthermore, other studies (also without the use of mice) have reported that the distribution of capsular types was not skewed [10]. We observed a high carriage rate of R-pneumococci as early as age 2 months. Since no systematic studies on R-pneumococci carriage among healthy infants and young children have been published, a direct comparison of our prevalence to that in other areas was impossible. However, in a recent US study [11], 44% of healthy infants ≤6 years old carried pneumococci and 37% of all isolates were penicillin-resistant, figures strikingly similar to those in our study. We believe that the alarming prevalence of R-pneumococci carriage is of utmost importance, since there is a clear relationship between antibiotic resistance in nasopharyngeal isolates and its presence in isolates from infected sites [12].

Nasopharyngeal colonization with R-pneumococci was strongly associated with day care attendance. This finding is consistent with other reports [2, 4]. However, we could not find
any study that compared the risk of carrying R-pneumococci in children attending day care facilities with that in children not attending day care.

The presence of older siblings at home was associated with a higher risk of pneumococcal carriage in general and that of R-pneumococci in particular during early infancy. Thereafter, this effect was less pronounced but was associated with increased contact with children in day care centers. The transmission of bacteria within households has been documented: When a new serotype is brought into a family, the introducer is generally a child [13], a situation which should also apply for R-pneumococci [2].

Antibiotic treatment during the month preceding culture in infants selected for resistant strains. A similar association was found in other studies [3, 4, 11]. Antimicrobial drugs may not only affect colonization with resistant organisms but may also increase the risk of subsequent disease caused by R-pneumococci in children and adults [14, 15].

Until recently, vaccine technology was unable to overcome the poor immunogenicity of native capsular polysaccharide antigens. The current approach of presenting the capsular polysaccharide as a protein-polysaccharide conjugate [2, 5] has the disadvantage of limiting the number of serotypes that may be included. Thus, calls have been made for careful selection of serotypes on the basis of evidence in an extensive epidemiologic database. Because 7 serotypes cause the majority of childhood infections in the developed world, these types are included in the various candidate conjugate vaccines [5]. It is of great importance that most reported resistance is found among the above-mentioned pediatric vaccine types, especially 6B, 9V, 14, 19F, and 23F. Furthermore, over 95% of the multidrug resistant strains belong to these 5 serotypes worldwide [2, 6, 5] (our experience).

In a recent study, we demonstrated the reduction of R-pneumococci carriage in children ages 12–18 months by the administration of a 7-valent conjugate pneumococcal vaccine [6]. We are not aware of other studies that have assessed the potential for reducing pneumococcal carriage by conjugate vaccine; however, based on data showing reduction of colonization after immunization with Hib conjugate vaccines [7], we suggest that conjugate pneumococcal vaccines might play an important role in preventing the spread of R-pneumococci by reducing pneumococcal acquisition or persistence.

References