Effect of Clonal and Serotype-Specific Properties on the Invasive Capacity of *Streptococcus pneumoniae*

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The present study compares the molecular epidemiology of *Streptococcus pneumoniae* causing invasive disease and carriage, respectively, in one geographic area (Stockholm, Sweden) during a specific point in time (the year 1997). A total of 273 invasive isolates (257 from adults and 16 from children) obtained from the 2 major hospitals in Stockholm, as well as 246 nasopharyngeal isolates recovered from children attending 16 day-care centers in the Stockholm area, were analyzed by serotyping, molecular typing (by pulsed-field gel electrophoresis and multilocus sequence typing), and antibiotic susceptibility testing. Of the 34 different serotypes plus nontypeable strains identified in the present study, 12 were never found among the 246 colonizing isolates, whereas only 3 were never found among the 273 invasive isolates. The isolates formed 2 major classes: 1 class that was found mainly among invasive isolates (type 1, 4, 7F, and 9V isolates) and was clonally highly related and 1 class that caused invasive disease but was also common in carriage (including type 6A, 6B, 14, and 19F isolates) and was genetically more diverse. Clones were found that belonged to the same serotype but had different abilities to cause invasive disease. Also, isolates belonging to the same clone were found, although they had different capsules because of serotype switch, and were found to have the same disease potential. Hence, properties associated with a particular clonal type, in addition to capsular serotype, are likely to be important for the potential of pneumococci to cause invasive disease.

*Streptococcus pneumoniae* is a major cause of community-acquired infections that range in severity from local otitis media to pneumonia with or without septicemia or meningitis [1–5]. When the bacteria are isolated from blood or cerebrospinal fluid, the infection is categorized as invasive. Groups at risk for the acquisition of pneumococcal infections are small children, elderly individuals, and immunocompromised individuals [6]. The normal habitat for pneumococci is the nasopharynx, and as many as 60% of small children who attend day-care centers have been found to be carriers of pneumococci [7]. In contrast, adults who work at the same day-care centers are only rarely colonized, presumably because of the presence of humoral immunity. It is generally believed that the nasopharynx constitutes the main reservoir for pneumococci in children.

Even though we have gained more knowledge of the basic aspects of the pathogenesis of pneumococcal disease, we do not know why certain pneumococcal clones frequently cause invasive disease. To distinguish between clones capable of causing invasive disease or carriage, it is necessary to perform epidemiological studies in which nasopharyngeal isolates and invasive isolates from the same geographic area and the same period are compared. Surprisingly, few such studies have been conducted [8–11]. Three studies have been published that have attempted to understand the relationship between serotypes that cause invasive disease and serotypes that circulate in the community during the same period [8–10]. Takala et al. [10] and Robinson et al. [8]...
studied 149 and 182 pneumococcal isolates, respectively, from patients with invasive disease or nasopharyngeal colonization, and they concluded that the overall diversity was higher among isolates carried than among isolates from patients with invasive disease. Brueggeman et al. [9] also provided some indication of the role of capsular serotype and genotype in the ability of pneumococcal clones to cause invasive disease, by comparing pneumococcal isolates from children in Oxford during 1995–2001, by use of multilocus sequence typing (MLST).

In the present study, we performed serotyping, molecular typing with pulsed-field gel electrophoresis (PFGE) and MLST, and antibiotic susceptibility testing of 2 collections of isolates, both of which were obtained from the Stockholm, Sweden, area during 1997. A collection of 273 invasive isolates from adults and children was compared with a collection of 246 nasopharyngeal isolates from children attending 16 day-care centers. We found support for our hypothesis that invasive disease is caused by 2 different groups of pneumococcal clones, one including highly virulent clones that appear primarily among invasive isolates and the other including clones that cause invasive disease because they are highly efficient colonizers and are spread in the community. Also, some clones were found only among carriers in the present study, a finding that suggests a low potential for disease.

**MATERIALS AND METHODS**

**Clinical isolates.** Carrier and invasive isolates were recovered from individuals in the Stockholm area during 1997. Nasopharyngeal swab specimens were obtained from 611 children who attended 16 day-care centers in the Stockholm area. Of these 611 children, 246 harbored pneumococci. The specimens were obtained from 12–72 children in each day-care center; the mean age of the children was 3 years (range, 1–6 years). The day-care centers were chosen because of the presence of index cases patients (i.e., children with cases of penicillin-nonsusceptible pneumococci in the nasopharynx that were reported to the Regional Center of Communicable Disease Control [Stockholm]), which motivated extensive procurement of specimens from all children attending the day-care centers.

A total of 273 invasive isolates were obtained from the laboratories at the 2 major hospitals in the Stockholm area: Karolinska Hospital (n = 113) and Huddinge Hospital (n = 160). These isolates were obtained from 16 children (mean age, 7 years) and 257 adults; 145 of the adults were >65 years of age. The ages of 2 patients were unknown. A total of 14 isolates were recovered from individuals with meningitis. Three isolates from the group of 16 children were recovered from cerebrospinal fluid. For patients who had isolates recovered from both the nasopharynx and blood, only the isolate from blood was included in the study.

**Antibiotic susceptibility testing.** Antibiotic susceptibility was determined using the disk diffusion method, according to the Swedish Reference Group for Antibiotics. Strains were inoculated onto Iso-Sensitest agar (Oxoid) supplemented according to recommendations, and antibiotic disks (Oxoid) were applied. Inhibition-zone diameters were read to the nearest millimeter and were interpreted according to SRGA guidelines (available at: http://www.srga.org).

For pneumococci with an inhibition-zone diameter of <18 mm for 1 µg of oxacillin, the MIC of benzylpenicillin was determined using the E-test (AB Biodisk). Penicillin-nonsusceptible pneumococcal strains were also analyzed for susceptibility to cefotaxime by use of the E-test.

**Serotyping of pneumococcal isolates.** Serotyping of all 519 pneumococcal isolates was performed, at the Swedish Institute for Infectious Disease Control (Solna, Sweden), by use of gel diffusion with 46 serotype or serogroup serum samples obtained from the World Health Organization (WHO) Collaborating Center for Reference and Research on Pneumococci, which is located at the Statens Seruminstitut in Copenhagen [12]. Isolates that belonged to a serogroup that included >1 serotype were examined by gel diffusion and/or the capsular reaction test (i.e., the Quellung test), with the use of type-specific serum samples [13, 14] from the WHO Collaborating Center for Reference and Research on Pneumococci.

**Molecular typing with PFGE.** One hundred seventy-four invasive isolates and 169 carrier isolates belonging to the most prevalent serotypes and, also, to some minor serotypes were analyzed by PFGE. These isolates were of types 1, 4, 6A, 6B, 7F, 9N, 9V, 14, 19A, 19F, 35B, and 35F, and all isolates that belonged to these types, with the exception of 1 isolate, were tested. The PFGE procedure was adapted from the procedure described by Hermans et al. [15]. Chromosomal restriction fragments that were obtained by cleavage with the restriction enzyme *ApaI* were embedded in agarose. The fragments then were separated on a 1% agarose gel, by use of PFGE performed for 22 h at 14°C at 6 V/cm in 0.5 mM Tris-borate EDTA, with pulse times of 2–30 s and an angle of 60 degrees. After electrophoresis, the gel was stained with 1 mg/mL of ethidium bromide for 30 min. This method has been shown to provide data consistent with the findings of MLST but with a slightly higher discriminatory power [15]. The PFGE patterns of the clinical isolates were compared with those of 15 internationally recognized major antimicrobial-resistant pneumococcal clones obtained from Dr. A. Tomasz and Dr. H. de Lencastre [16]. Analysis was done optically and by computer-assisted analysis of the banding pattern with the use of BioNumerics software (Applied Maths). Comparison of the banding patterns was performed by use of the Jaccard similarity coefficient applied to peaks, as well as by use of the unweighted pair group method with arithmetic means (UPGMA), with a position tolerance of

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2%. A “clone” was defined as isolates that differed by not >3 bands or 80%.

**Molecular typing with MLST.** Seventy-four isolates were analyzed by MLST (there were 9 type 1 isolates, 3 type 4 isolates, 4 type 6A isolates, 8 type 6B isolates, 3 type 7F isolates, 2 type 9N isolates, 11 type 9V isolates, 19 type 14 isolates, 3 type 19A isolates, 9 type 19F isolates, 2 type 35F isolates, and 1 type 35B isolate). The sequences of the internal fragments of the 7 housekeeping loci used in the pneumococcal MLST scheme were determined for each isolate, as described elsewhere [17]. The alleles at each locus were assigned, via the Internet, by use of software available at the MLST Web site (http://www.mlst.net), and the 7 allele numbers (in the following order: aroE, gdh, gki, recP, spi, xpt, and ddl) define the allelic profile or sequence type (ST). The allelic profiles of the isolates were compared with those in the pneumococcal database at the MLST Web site.

**Data analysis.** The statistical methods used were \( \chi^2 \)-analysis and Fisher’s exact test [8]. An empirical odds ratio (OR) was calculated as described by Brueggeman et al. [9], to compare the probability of invasive disease with serotypes or clones. The equation used for the calculation of the OR was \( \text{OR} = \frac{a \times d}{b \times c} \), where \( a \) was the number of invasive X serotypes or clones, where \( b \) was the number of carriage X serotypes or clones, where \( c \) was the number of invasive non-X serotypes or clones, and where \( d \) was the number of carriage non-X serotypes or clones. An OR of 1 indicated that the serotype or clone was equally likely to be recovered from invasive disease and carriage, whereas an OR >1 indicated an increased probability to cause invasive disease.

### RESULTS

**Many Serotypes Were Uniquely Associated with Invasive Disease**

There was a significant difference in the distribution of serotypes between invasive isolates and carrier isolates (table 1) in the Stockholm area. A total of 34 different serotypes were found. Among the invasive isolates, serotypes 14, 4, 7F, 1, 9V, 6B, 3, 23F, and 19A dominated (in descending order), accounting for 67% of the invasive isolates (figure 1), whereas, among carrier isolates, serotypes 6A, 19F, 6B, 23F, and 14 were most frequently found (in descending order), accounting for 68% of the carrier isolates. Invasive isolates expressed more variations of serotypes: only 3 of the 34 serotypes detected were absent from the strains. In contrast, as many as 12 of the 34 serotypes were undetectable among the strains carried. Types 1, 4, 8, and 12F and some minor serotypes were the serotypes most commonly found to uniquely cause invasive disease, whereas types 16, 21, and 35B were found only among carriers, with each accounting for just 1%–5% of the carrier isolates (figure 1). Of the serotypes associated with both invasive disease and carriage, types 14, 7F, 9V, 3, and 19A were more abundant among invasive isolates, whereas types 6A, 6B, 23F, and 19F were more common among isolates recovered from the nasopharynx.

Of the 273 invasive isolates, 16 were recovered from children. The serotypes found among these 16 isolates were types 6B (3 isolates), 7F (3 isolates), 23F (2 isolates), 11A (2 isolates), 19A (2 isolates), 14 (2 isolates), 4 (1 isolate), and 18C (1 isolate). The serotypes found among the 14 isolates recovered from individuals with meningitis were types 14 (4 isolates), 6B (2
Figure 1. Serotype distribution among invasive (A) and carrier (B) *Streptococcus pneumoniae* isolates from the Stockholm area during 1997. Serotypes found only among invasive isolates or carrier isolates, respectively, are shown in red, and serotypes found among both invasive isolates and carrier isolates are shown in blue.
Clonal Analysis of Serotypes 1, 4, 7F, and 9V, Serotypes Common in Invasive Isolates but Rarely Seen among Carrier Isolates

Serotypes 1, 4, 7F, and 9V were common among isolates from patients with invasive disease. However, these serotypes were rarely or never seen among isolates from carriers (OR, >1) (figure 2A–D).

**Serotype 1.** A total of 20 (7%) of all invasive isolates belonged to serotype 1, a serotype that is not included in the 7-valent conjugate vaccine. The 20 isolates, all of which were susceptible to penicillin, showed 6 different PFGE patterns. Fourteen of these isolates belonged to a clonal complex with ≥5 of 7 identical alleles, according to MLST; clone SWE1-1 (formerly, S1: 1 with ST306) [9], and clone SWE1-2 (with ST228) (figure 2A). The fact that the 2 loci differ at several sites argues that they have arisen by recombination, rather than by spontaneous mutations. According to PFGE, the SWE1-1 clone was highly related to the SWE1-2 clone, showing only minor differences. The SWE1-1 clone has been observed to emerge in Sweden, and it has been found to be a major reason for the dramatic increase in type 1 isolates that caused invasive disease in Sweden from 1992 through 1997 [18]. SWE1-2 has been shown to cause meningitis in Spain and bacteremia in Denmark. Of the minor clones of type 1 that do not belong to the SWE1-1 clonal complex, isolates of ST217 have been found to cause invasive disease both in Sweden and in Denmark, and isolates of ST250 have been found to cause bacteremia in Denmark. No isolates of type 1 were found among the 246 carrier isolates. However, in a set of isolates more recently obtained from 5 day-care centers in Stockholm during 1998–1999, 3 isolates of type 1 were found. Of interest, these 3 isolates, which were recovered from 2 healthy children (a sister and brother), belonged to the SWE1-1 clone, as determined by PFGE; this finding suggests that either the SWE1-1 clone is adapting to colonization in the nasopharynx or, more likely, that we have been successful in capturing the short period during which this clone colonizes the nasopharynx.

**Serotype 4.** Type 4 was one of the major serotypes found among invasive isolates (12% of such isolates), but this serotype was not found among carrier isolates. Of 33 invasive isolates tested, all of which were susceptible to penicillin, 23 belonged to the same clone, as determined by PFGE (figure 2B). The serotype 4 strain, for which the entire genome has been determined, also belongs to this clone [19]. A total of 6 PFGE patterns were found, and MLST performed for isolates that belonged to the major clone SWE4-1 had ST205. This clone has been found to cause meningitis and pneumonia in Denmark, the United Kingdom, and Australia, according to the MLST database.

**Serotype 7F.** Serotype 7F, like serotype 1, is not included in the 7-valent conjugated vaccine. These 2 serotypes made up 15% of all invasive isolates in the present study. Invasive isolates that belonged to type 7F showed a remarkably high degree of genetic relationship, and they were all susceptible to penicillin. All 23 invasive isolates tested (3 of which were from children) and, also, the 4 carrier isolates exhibited identical or almost identical PFGE patterns (figure 2C), a finding that suggests a close relationship. Also, the 3 isolates of type 7F that were analyzed with MLST had the same sequence type: ST191. ST191 has been associated with invasive disease in several areas, including Scandinavia, the United Kingdom, The Netherlands, and Uruguay.

**Serotype 9V.** Serotype 9V was also one of the most common types that caused invasive disease, accounting for 19 (7%) of all invasive isolates. However, only 6 (2%) of the carrier isolates were of type 9V. This serotype is also genetically homogenous, with only 3 separable clones, as determined by PFGE, and with 1 clone (SWE9V-1) dominating among invasive and carrier isolates (figure 2D). The isolates that belonged to this clone were found to have 4 different sequence types (ST156, ST162, ST1184, and ST1185), with differences found, at least, in the ddl allele (figure 2D). Some isolates with ST156 had a reduced susceptibility to penicillin and trimethoprim-sulfamethoxazole, whereas others were fully susceptible to the antibiotics tested. Isolates with ST156 showed almost the same PFGE pattern as did the internationally recognized clone Spain9V-3, also with ST156 [16].

Clonal Analysis of Serotypes 6B and 14, Serotypes Common among Both Invasive and Carrier Isolates.

Serotypes 6B and 14 are common serotypes among both invasive and carrier isolates.

**Serotype 6B.** Of the 48 isolates of serotype 6B, 33 (13%) were found among carriers, and 15 (5%) were obtained from patients with invasive disease (OR, < 1) (figure 2E). None of the invasive isolates, but 6 of the carrier isolates, had a reduced susceptibility to penicillin. A total of 10 different PFGE patterns were observed; 3 of these 10 patterns dominated (SWE6B-8, SWE6B-7, and SWE6B-6), and they were most abundant among carriers (figure 2E). SWE6B-6 and SWE6B-7 showed the same sequence type: ST176. The ST138 clone was highly related to the ST176 clone, differing at only 2 loci, as determined by MLST, and, therefore, it probably belongs to the same clonal complex. This clonal complex was represented by 39 isolates (of the 50 tested). The major invasive clone with ST138 has been found to cause invasive disease in the United Kingdom (meningitis) and Denmark (bacteremia), and the
Figure 2. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing patterns of 174 and 169 carrier pneumococcal isolates, respectively. The calculated odds ratio (OR) for different clones is included. Clones with no carrier isolates are marked (-). Shown are patterns for isolates of type 1 (A), type 4 (B), type 7F (C), type 9V (D), type 6B (E), type 14 (F), type 6A (G), type 19F (H), type 9N (I), type 19A (J), and type 35F (K). *One of the isolates with this PFGE pattern had ST129, differing at 1 locus, compared with ST124. ‡The PFGE pattern of 1 of the 5 isolates with serotype 19F is lacking. §One of the invasive isolates with this PFGE pattern had ST680, differing at 1 locus, compared with the carriage isolate with ST199. †The invasive isolate with this PFGE pattern had ST446, differing at 1 locus, compared with the carriage isolates with ST684. I/C, no. of invasive strains/no. of carrier strains.
Figure 2. (Continued.)
Figure 2. (Continued.)
related clone with ST176 has been reported to cause meningitis in the United Kingdom. Carrier isolates of type 6B that were not genetically related to the major invasive clone showed a high genetic diversity.

**Serotype 14.** Serotype 14 is one of the major serotypes associated with invasive disease worldwide (OR, >1) (figure 2F). A specific clone, which, on the basis of PFGE, was denoted “SWE14-6” (formerly, S14:1) with ST124, has been observed to be a major reason for the observed increase in invasive disease in Sweden from 1987 through 1992 [18]. Of the 50 isolates of type 14, 33 were recovered from patients with invasive disease, and 17 were recovered from carriers. A total of 9 different PFGE patterns were found; 14 of the 33 invasive isolates tested showed the PFGE pattern of clone SWE14-6, and 23 invasive isolates had the same MLST pattern, ST124 [18] (figure 2E). Also, 3 of the carrier isolates had the same PFGE pattern as did SWE14-6. The majority of the type 14 isolates (35 isolates) that were obtained from patients with invasive disease and from carriers were shown, by PFGE and MLST, to belong to the same clonal cluster as ST124. ST124 has been observed in Scandinavia, the United Kingdom, The Netherlands, and Australia, and it therefore represents one of the most successful penicillin-susceptible clones for both invasive disease and nasopharyngeal colonization. Isolates with a reduced susceptibility to penicillin were found only among carriers, clustered in separate groups from susceptible isolates (data not shown). Also, 1 clone with a decreased susceptibility to erythromycin was observed (4 invasive isolates, SWE14-4).

**Clonal Analysis of Serotypes 6A and 19F, Serotypes Commonly Found among Carrier Isolates**

Serotypes 6A and 19F were the types most commonly found among carrier isolates (34%), but they accounted for only 3% of the isolates that caused invasive disease (OR, <1) (figure 2G and 2H).

**Serotype 6A.** For the 46 isolates of type 6A that were tested (43 carrier isolates and 3 invasive isolates), 10 PFGE patterns were found. The isolates showed a high degree of genetic diversity, with 3 penicillin-susceptible clones dominating among carrier isolates (figure 2G). One of the 3 invasive isolates belonged to 1 of these carrier clones, but the other 2 invasive isolates exhibited separate patterns, a finding that suggests that isolates of type 6A are efficiently spread among carriers and are rarely associated with invasive disease. The major clone, as determined by PFGE SWE6A-4, harbored ST518, and this sequence type previously has been found among carriers in Finland. None of the invasive isolates, but 4 of the carrier isolates, had a decreased susceptibility to penicillin. These isolates belonged to separate groups, according to PFGE.

**Serotype 19F.** Of the 47 isolates of type 19F, 42 were found in carriers and only 5 caused invasive disease. A total of 15 PFGE patterns were found among the 46 isolates tested, with 2 clones (SWE19F-8 and SWE19F-11) dominating among carrier isolates (figure 2H). Two of the 4 invasive isolates clustered into the same clone as a carrier isolate. MLST revealed that this clone had the same allelic profile (ST162) as did invasive isolates of type 9V, which suggests a capsular switch. Five carrier isolates had ST236, which is the same ST sequence found in the internationally recognized multiresistant clone Taiwan19F-14. ST309 was only found among carrier isolates in the present study, but it has been recognized among invasive isolates in the study by Brueggeman et al. [9] from Oxford. Isolates with ST425 were found only among carriers in both studies.

Clonal Analysis of Serotypes 9N and 19A, Serotypes Moderately Common in Invasive Disease

Serotypes 9N and 19A are examples of types that are moderately common among individuals with invasive disease (percentage of invasive isolates, 3% and 5%, respectively) (figure 1). They are rare among carriers.

**Serotype 9N.** Of the 10 isolates of serotype 9N that were obtained in the study, 8 were from patients with bacteremia, and 2 were from carriers. Three PFGE patterns were found, for which 1 clone dominated with the sequence type ST66, including invasive and carrier isolates (figure 2I). ST66 previously has been found to cause invasive disease in Sweden (in 1995) and the United Kingdom (in 1993). In 1999, it was also found in Australia in an isolate of type 19F, which suggests a serotype switch.

**Serotype 19A.** Of the 16 isolates of type 19A, 13 were invasive isolates and 3 were carrier isolates. They showed 5 different PFGE patterns, with 2 clones dominating. One clone (ST199) was present in both invasive and carrier isolates (figure 2J). ST199 has been found in the United Kingdom and in The Netherlands, but, in the latter case, it was associated with serotype 15B. The other dominating clone has an allelic profile not previously described (ST482).

Clonal Analysis of Serotype 35F, a Serotype Rare in Both Carriage and Invasive Disease

The 5 carriage isolates of type 35F all belonged to the same clone (SWE35F-1), as did 1 of the 3 invasive isolates, according to PFGE patterns (figure 2K). Hence, even highly infrequent clones that belong to minor serotypes in the carrier population among children may be found to cause invasive disease in adults. Two sequence types, ST446 and ST694, which differed only at 1 allele, were found among isolates belonging to SWE35F-1.
A Penicillin-Non-susceptible Serotype 35B Clone Found Only among Carriers

Among carriers at 2 day-care centers, 11 penicillin-non-susceptible isolates of the unusual serotype 35B were found [20, 21], constituting 4% of the carrier isolates. All these isolates had the same PFGE pattern. No 35B isolates were found among the invasive isolates.

Antibiotic Susceptibility among All Isolates

Of the invasive isolates, <1% (n = 2; type 9V [which was also resistant to trimethoprim-sulfamethoxazole] and type 15C, respectively) were penicillin-non-susceptible S. pneumoniae, compared with 20% of the children (49 of 246 isolates) carrying pneumococci at the day-care centers. This high figure depends, to a large extent, on the fact that only day-care centers with individuals with penicillin-non-susceptible index cases were included in the present study. However, even when the index strains have been excluded, the level of penicillin-non-susceptible isolates is much higher in the carrier group (14%). The serotypes most commonly found among penicillin-non-susceptible S. pneumoniae in carriers were, in descending order, 19F (11 isolates), 35B (11 isolates), 14 (9 isolates), and 6B (6 isolates) [21]. No invasive isolates were highly resistant to penicillin, whereas 4 isolates (all from index case patients) from the carriers had an MIC ≥ 2 mg/L.

Among the invasive isolates, a decreased susceptibility to erythromycin was found in only 8 isolates (3%), 4 of which were type 14 isolates and belonged to the same clone (SWE14-4) (figure 2F). Of the invasive isolates, 7 (2%) were resistant to tetracycline and 13 (5%) were resistant to trimethoprim-sulfamethoxazole. Of the carrier isolates, only 3 of the penicillin-susceptible isolates had any antibiotic-resistance marker; 2 of these isolates were resistant to tetracycline (type 19F), and 1 was found to be resistant to trimethoprim-sulfamethoxazole (type 9N) (4 isolates of type 23F were intermediately susceptible to trimethoprim-sulfamethoxazole). The invasive isolates were also tested for resistance to ciprofloxacin, and 1 isolate was resistant. No multiresistance was found among penicillin-susceptible isolates from the carriers, but 2 invasive type 23F isolates carried resistance to erythromycin/clindamycin, tetracycline, and trimethoprim-sulfamethoxazole.

DISCUSSION

S. pneumoniae is a respiratory tract pathogen that causes severe infections, yet it may be carried in the nasopharynx of a significant number of healthy children who attend day-care centers. Disease versus carriage is poorly understood in any microbial-host system. In the present study, we made a detailed molecular comparison of pneumococcal isolates from the same geographic area (Stockholm, Sweden) during the same period (1997); the isolates were obtained from the nasopharynx of healthy children at 16 day-care centers and from the blood or cerebrospinal fluid of individuals (16 children and 257 adults) with invasive pneumococcal disease. A recent study by Brueggeman et al. [9], which compared invasive disease and carriage, was performed during a longer period (6 years), to be able to include a sufficient number of children with invasive disease. We chose to perform the study for only 1 year, because we have shown there to be great fluctuations in serotype distribution among isolates that cause invasive disease within short periods, such as 5 years [18, 22]. In a recent study, we obtained all invasive isolates from 71 children in the Stockholm area during 1998–2001, and, only during this short period, we observed fluctuations in serotype distribution that reflected that clones emerge and disappear (B.H.N., unpublished data). Also, adults have been included in the present study because we argue that children constitute a main reservoir for pneumococci and that they represent the source of spread to the adults and to elderly individuals. Herd immunity in nonvaccinated adults has been suggested to occur after widespread vaccination of children [23].

The results of the present study suggest that pneumococcal serotypes involved in invasive disease could be grouped into 2 broad, partially overlapping classes. In the first class, we included isolates belonging to major invasive serotypes (e.g., types 1, 4, 7F, and 9V) that were absent or rare among carriers. Isolates belonging to these serotypes were clonally highly related, which is in agreement with the findings of other studies [9, 11]. Because they were absent or rare among carriers, these clones were clearly efficient pathogens that, on entering the human host, had a high capacity to cause disease. Such clones are likely to possess specific attributes that allow them to be more invasive and/or more able to cope with and manipulate the innate immune system of the host. Isolates that belonged to other, minor serotypes (e.g., type 9N), which are rare among carriers, were also genetically homogeneous, a finding that suggests that they should also be regarded as primary pathogens but, potentially, with a lower capacity for spreading. The importance of highly pathogenic clones that belong to minor serotypes not included in conjugated vaccines could possibly increase in a vaccinated population.

The other major class of pneumococcal serotypes associated with pneumococcal disease was also common among carriers. Major serotypes (such as types 6A, 6B, 14, and 19F) found among healthy carriers were also found among individuals with invasive disease. Isolates that belonged to these serotypes were genetically more heterogeneous, especially those that were identified from the nasopharynx. However, in all these types, it was possible to identify, in the nasopharynx of healthy children, the presence of clones that were able to cause invasive disease. Type 14 isolates were more often associated with disease than were
type 6A and 19F isolates. Isolates that belonged to the latter 2 serotypes were also genetically more diverse than were isolates that belonged to serotype 14. Among type 19F isolates, we found 2 major clones that were only associated with carriage. We believe that clones that belong to the 4 serotypes discussed above (i.e., 6A, 6B, 14, and 19F) have a certain degree of potential for disease, although it is lower than that associated with the primary pathogens. Increased colonization of these moderately pathogenic clones within a community also is likely to increase their abundance in the disease group.

Although serotypes with a high attack rate (e.g., types 1, 4, 7F, and 9V) constituted 34% of all invasive isolates in the Stockholm area in 1997, they represented only 12% of the invasive isolates in the Oxford area during 1995–2001 [9]. Maybe pneumococcal serotypes that are less frequently associated with carriage and transmission are geographically confined, compared with serotypes that are more prone to cause carriage. Of interest, only 9% of serotypes that were found in carriers were not found among patients with invasive disease. These were all minor serotypes (each <5% of all carrier isolates). We hypothesize that clones that belong also to these presumptive carrier strains have disease potential, and we postulate that they will appear in the disease population if they increase in abundance after either immune or antibiotic selection or depending on host susceptibility. Serotype 35B, which we have described elsewhere [21], represents evidence for this. We found a penicillin-nonsusceptible pneumococcal clone of type 35B emerging in healthy children at 2 day-care centers, but none of the invasive isolates in the present study belonged to this clone, which has been observed to cause invasive disease in the United States [20]. Maybe this clone has been expanded by penicillin selection in children and may also cause invasive disease in Sweden after a significant time delay. Because type 35B is not covered by the 7-valent vaccine, an increase of this clone, which has been observed to cause invasive disease in the Stockholm area in 1997, they represented only 12% of the invasive isolates in the Oxford area during 1995–2001 [9].

The findings of the present study suggest that properties associated with a particular clonal type, in addition to capsular serotype, may be important for the potential for disease. For example, we found that isolates of serotype 14 included clones that were only found among carriers (e.g., ST230), as well as clones causing merely invasive disease (e.g., ST307); this finding suggests that these clones have different potential for disease. Also, serotype variation within 1 ST has been shown to be the result of a recombination event, and, in 2 instances, we found evidence for a serotype switch between type 19F and type 9V isolates with ST162 and ST156. ST162 was mainly found to cause invasive disease, but 1 isolate was found to colonize the nasopharynx. ST156 was only found in both types among carriers.

In conclusion, clonal properties, in addition to the capsular polysaccharide itself, may be an important factor in the ability of pneumococci to cause invasive disease. We will attempt to identify and characterize such properties genetically as well as...
functionally, focusing on the capacity of different clones to evoke inflammatory host responses.

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