Clonal and Capsular Types Decide Whether Pneumococci Will Act as a Primary or Opportunistic Pathogen

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(See the editorial commentary by Crook on pages 460–2)

Background. *Streptococcus pneumoniae* is a major cause of morbidity and mortality worldwide. The role of the different capsular and clonal types in invasive disease severity remains to be defined.

Methods. Disease severity and disease type were correlated to age, underlying disease, capsular serotype, and clonal type of the causative agent for 494 adult patients with invasive pneumococcal disease.

Results. Pneumococcal isolates of serotypes 1 and 7F were genetically homogenous, had the highest potential to infect previously healthy individuals, and were not causing deaths. Also, type 1 isolates were only found among younger adults, whereas other serotypes were mainly found among elderly persons (e.g., type 23F). Some serotypes and/or clones were more prone to cause more-severe disease, as observed by high APACHE II scores calculated at admission, and were also associated with a high mortality (e.g., clones of type 3 and 11A). We found no evidence of an impact of penicillin resistance on disease severity and disease type.

Conclusions. We suggest that clones with capsular types 1 and 7F, which are known to have a high invasive disease potential, behave as primary pathogens, whereas clones with other capsular types with a lower relative risk of causing invasive disease are more opportunistic, primarily affecting patients with underlying disease. Disease caused by the latter group, however, was more severe, even in previously healthy individuals.

Streptococcus pneumoniae is a major contributor to morbidity and mortality worldwide [1–3]. It causes diseases ranging from mild respiratory tract infections to more-severe diseases, such as pneumonia, septicemia, and meningitis, and sometimes it leads to a fatal outcome. Despite being a devastating pathogen, it harmlessly colonizes the nasopharynx of up to 60% of healthy children attending day-care centers [4–5]. The major virulence factor has been suggested to be the capsular polysaccharide, permitting the bacteria to resist phagocytosis [6–7], and opsonizing antibodies to capsular polysaccharide are protective against invasive disease and also diminish colonization. Pneumococci can be divided into at least 90 serotypes, depending on the capsular structure. The serotype distribution among invasive pneumococcal isolates differs by geographic area and time period studied [8]. Recent epidemiological studies comparing the distribution of invasive isolates with carriage isolates have shown that the potential for pneumococci to cause invasive disease differs by serotype [9–10]. This conclusion was strengthened in a meta analysis conducted by Brueggeman et al. [11], which comprises 7 studies from different countries that calculated ORs for different serotypes/serogroups causing invasive disease. Certain serotypes, such as types 1 and 7F, are mainly observed to cause invasive disease and are rarely observed among carriers, whereas other serotypes are predominately observed to cause carriage (such as types 19F and 23F).

We have previously described an association between

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Table 1. Severity of disease, as measured by APACHE II scores >11, mortality, and prevalence of pneumonia and meningitis, by serotype.

Serotype	No. (%) of isolates	Isolates from patients with an APACHE II score >11, % (95% CI)	Case-fatality rate (95% CI)	Isolates obtained from patients with meningitis, %	Isolates obtained from patients with pneumonia, %
1	29 (6)	21 (8–40)	0 (0–12)	3	97
3	38 (8)	63 (46–78)	32 (18–49)	18	92
4	31 (6)	35 (19–55)	10 (2–26)	6	81
6A	12 (2)	67 (35–90)	33 (10–65)	17	75
6B	21 (4)	76 (53–92)	19 (5–42)	10	80[a]
7F	34 (7)	26 (13–44)	0 (0–10)	3	91[a]
8	14 (3)	43 (18–71)	7 (0–34)	14	77[a]
9V	49 (10)	40[b] (25–54)	10 (3–22)	6	89[a]
11A	12 (2)	92 (62–100)	25 (6–57)	0	73[a]
14	72 (15)	62[b] (49–72)	10 (4–19)	11	93[a]
18C	15 (3)	47 (21–73)	7 (0–32)	20	60
19A	19 (4)	58 (34–80)	11 (1–33)	5	84
19F	21 (4)	71 (48–89)	29 (11–52)	10	76
22F	15 (3)	60 (32–84)	13 (2–41)	20	73
23F	40 (8)	48 (32–64)	10 (3–24)	10	73
Other	72 (15)	57[b] (42–66)	18 (10–29)	12[c]	73[a]
All	494 (100)	52[b] (47–56)	14 (11–17)	10[d]	83[a]

NOTE. $P < .001$ for the percentage of isolates obtained from patients with an APACHE II score of >11; $P = .45$ for the percentage of isolates obtained from patients with meningitis; $P = .009$ for the percentage of isolates obtained from patients with pneumonia; and there was no convergence with logistic regression for the case-fatality rate.

[a] The prevalence of pneumonia was calculated on the basis of the following data: 20 of 21 cases among patients with type 6B isolates; 33 of 34 cases among patients with type 7F isolates; 13 of 14 cases among patients with type 8 isolates; 47 of 49 cases among patients with type 9V isolates; 11 of 12 cases among patients with type 11A isolates; 71 of 72 cases among patients with type 14 isolates; 26 of 72 cases among patients with isolates of other serotypes; and 436 of 494 total cases.

[b] Calculations regarding APACHE II scores were made on the basis of 47 of 49 isolates for type 9V, 71 of 72 isolates for type 14, 68 of 72 isolates for other serotypes, and 487 of 494 isolates for overall values.

[c] Data were based on 69 of 72 isolates.

[d] Data regarding total values were based on 491 of 494 isolates.

capsular serotype and mortality [12] and found that infections caused by serotypes 3, 6B, and 19F were associated with a higher mortality (25%), compared with the mortality associated with infections caused by serotypes 1 and 7F (0%). Recently, we have shown [13] in animal models (mice) that the capsular type is important for virulence and that other bacterial factors, as well as host factors, are important for colonization, invasiveness, and severity of disease. In the present study, the severity and type of disease for 494 adult patients with invasive pneumococcal disease (IPD) were correlated to age, underlying diseases, capsular serotype, and clonal type of the microbe.

MATERIAL AND METHODS

Clinical Data and Pneumococcal Isolates

Pneumococcal isolates were collected during 2 prospective clinical studies of IPD in adult patients. The first clinical study was performed during 1993–1995, during which time hospitals in 5 countries (Canada, the United Kingdom, Spain, Sweden, and the United States) participated [12, 14]. All adult patients (>17 years old) with pneumococcal bacteremia who had not been treated in a hospital during the 30 days before hospitalization were eligible. A total of 460 patients were included during the 2 study years, and 354 isolates were available for further characterization, because 106 isolates died or could not be retrieved. Treatment routines and clinical parameters were recorded [14].

The second clinical study was performed in the Stockholm area during 1999–2000. One hundred two adult patients (>17 years of age) with IPD who were admitted to the Department of Infectious Diseases (Karolinska, Sweden) and Danderyd Hospitals (Stockholm, Sweden) and who had not been treated in a hospital within 30 days before the present hospitalization, were included [15]. In addition, we retrospectively included 38

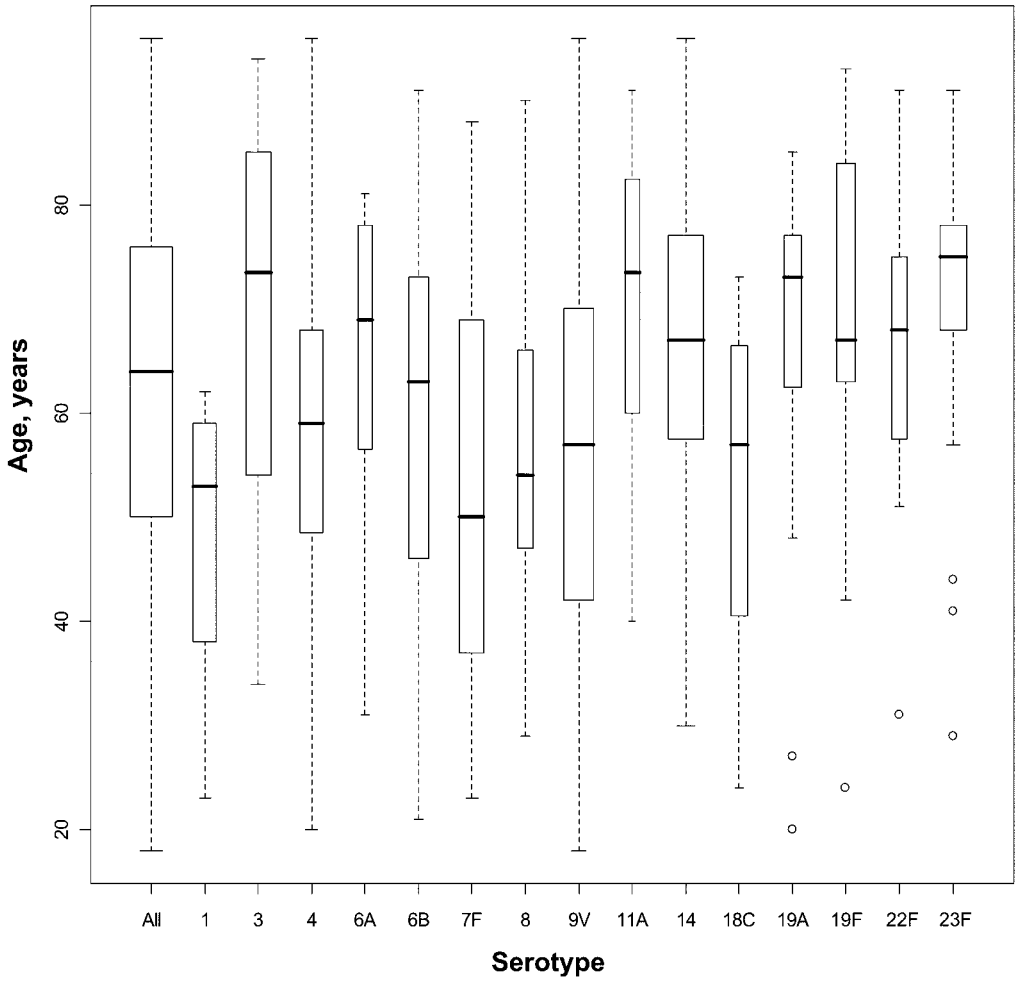

Figure 1. Box plots for age for 494 patients, according to serotype. Box length indicates interquartile ranges; box width is proportional to the square root of the number of patients. *Circles,* outlier values; *thick horizontal lines,* median values; *whiskers,* range of values within 1.5 interquartile range.

adult patients with IPD who were treated during the same time period in other departments of the Karolinska hospital. A total of 140 patients were included, and all isolates were available.

Severity of Disease

APACHE II scores were used to estimate severity of disease at the time that patients were admitted to the hospital. A higher score indicated more-severe disease. The APACHE II scores were divided into 3 strata (0–11, 12–24, and >24) for correlations to serotype and clonality [14–17].

All strains were serotyped at the Swedish Institute for Infectious Disease Control (Solna, Sweden) by gel diffusion, using 46 type- or group-specific typing serum samples [18] obtained from the World Health Organization (WHO) Collaborating Center for Reference and Research on Pneumococci at Statens Seruminstitut (Copenhagen, Denmark). Isolates that could not be typed or that belonged to a group that included several types were examined by capsular reaction testing (Quellung test) and/or gel diffusion with type-specific antisera [19, 20] obtained from the WHO Collaborating Center.

Drug Susceptibility Testing

All isolates were tested for drug susceptibility using an agar dilution method, as described elsewhere [21]. Serotyping of the isolates was performed by gel diffusion using 46 type or group serum samples obtained from the WHO Collaborating Center for Reference and Research on Pneumococci at the Statens Seruminstitut (Copenhagen, Denmark). All pneumococci belonging to a group that included >1 type were examined by the capsular reaction test with type-specific sera.

Molecular Typing Methods

Three molecular typing methods were used for characterizing the clinical isolates: Box fingerprinting, PFGE, and multilocus

Table 2. Underlying disease among patients with invasive pneumococcal disease, by serotype.

	Patients with underlying disease, %									
Serotype	Any[a]	Cardiac[b]	Lung[c]	Renal[d]	Liver[e]	Cancer[f]	HIV infection[g]	Immunodeficiency[h]	Alcohol abuse[i]	Other[j]
1	28	17	7	0	0	3	0	0	0	10
3	68	37	26	0	8	16	3	0	11	13
4	48	19	23	6	3	3	0	0	7[k]	16
6A	83	25	25	0	8	25	8	17	8	17
6B	71	19	14	0	5	33	14	14	0	19
7F	50	18	21	0	0	0	6	0	15	15
8	71	14	29	7	7	14	0	14	14	14
9V	67	33	12	0	4	22	4	10	14	18
11A	100	50	33	8	33	33	17	25	8	33
14	78	43	14	1	8	11	6	10	17	25
18C[l]	73	27[l]	36[l]	9[l]	0[l]	18[l]	9[l]	27[l]	9[l]	27[l]
19A	84	47	26	5	11	21	5	0	11	32
19F[m]	79	16[m]	26[m]	16[m]	21[m]	32[m]	16[m]	16[m]	11[m]	16[m]
22F[n]	75	50[n]	33[n]	0[n]	0[n]	25[n]	0[n]	8[n]	8[n]	0[n]
23F[o]	76	50[o]	34[o]	0[o]	11[o]	16[o]	5[o]	11[o]	11[o]	26[o]

[a] $P < .001$
[b] Defined as chronic heart condition (arteriosclerotic or hypertensive heart disease with or without heart failure as defined by the New York Heart Association classification of cardiovascular disease) ($P = .008$).
[c] Defined as asthma or chronic obstructive pulmonary disease ($P = .12$).
[d] Defined as regular medical control for renal failure (with or without dialysis) ($P = .05$).
[e] Defined as liver disease (with clinical diagnosis or biopsy verification, with or without liver failure) ($P = .03$).
[f] With or without active treatment ($P = .002$).
[g] Recorded when known ($P = .27$).
[h] Defined as autoimmune diseases (other than HIV infection) or receipt of high-dose steroid therapy ($P < .001$).
[i] Defined as active alcoholism ($P = .39$).
[j] Defined as receipt of regular treatment for diabetes mellitus, neurological disorders, and other chronic disorders.
[k] Based on 30 of 31 isolates.
[l] Data for this serotype are based on 11 of 15 isolates.
[m] Data for this serotype are based on 19 of 21 isolates.
[n] Data for this serotype are based on 12 of 15 isolates.
[o] Data for this serotype are based on 38 of 40 isolates.

sequence typing (MLST). The Box fingerprinting method and PFGE have been shown to have approximately the same discriminatory power [22]. Pneumococcal isolates belonging to the major serotypes (1, 3, 4, 6A, 6B, 7F, 9V, 11A, 14, 19F, and 23F) were subjected to molecular typing with Box fingerprinting and/or PFGE. Representatives of the clones identified were further characterized with MLST. A total of 173 isolates were analyzed using Box fingerprinting, 247 isolates were analyzed using PFGE, and 105 isolates were analyzed with MLST.

Box Fingerprinting

Box patterning was performed, as described by Hermans et al. [22]. Isolates with a banding pattern of >90% similarity were defined as being closely related and of possible clonal origin.

PFGE. The PFGE method was adapted from the procedure described by Hermans et al. [22]. Gels were analyzed using the software BioNumerics, version 2.5 (Applied Math), with the Jaccard coefficient and the unweighted pair group method with arithmetic mean (UPGMA) dendrogram type. The optimization setting was 1.75% with a position tolerance of 2.25%. The clones were defined using the criteria described by Tenover et al. [23] (i.e., isolates that differed by ⩽3 bands were considered to be closely related).

MLST. A total of 105 isolates selected on the basis of being different according to PFGE and Box fingerprinting were analyzed using MLST. This method was adapted from the procedure described by Enright et al. [24]. Chromosomal DNA obtained from pneumococcal isolates were subjected to sequencing of 7 different house-keeping genes (*aroE, gdh, gki, recP, spi, xpt,* and *ddl*) using an Applied Biosystems Prism 3700 automated sequencer (PE Applied Biosystems). The sequences obtained were submitted to the online MLST database [25], thereby assigning alleles at each locus and a sequence type.

Invasive Disease Potential among Serotypes

Brueggeman et al. [11] have performed a meta analysis of 7 studies from different countries to calculate the ORs for different serogroups/serotypes causing invasive disease. In this study, serotype 14 was chosen as the reference and set to 1. Serogroups/serotypes 1 and 7 had ORs >1, whereas serogroups/

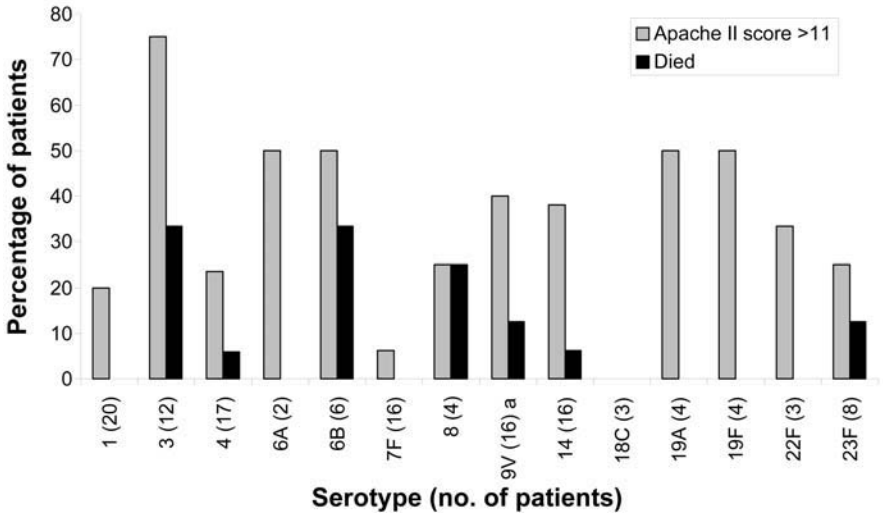

Figure 2. Case-fatality rate and APACHE II scores among previously healthy patients, by major serotype. APACHE II scores for patients infected with type 9V were calculated with data for 15 of 16 patients.

types 4, 18, and 9, in descending order, had ORs <1 but greater than those of the third group, including serogroups/serotypes 8, 19, 6, 23, and 3. In the present study, we used these results to divide the serotypes into 3 different groups with different invasive disease potentials (high, medium, and low).

Statistical Analysis

Significant differences between the serotypes were determined by performing an analysis of variance (ANOVA) from fitting a logistic regression for the dichotomous variables, whereas an ANOVA from a simple linear model was used for age. In addition, residual analysis was conducted to verify the assumptions of the linear models. The confidence intervals for the proportion of patients were calculated with the exact binomial distribution [26]. The association between 2 categorical variables was tested with Fisher's exact test, and, in the analysis of invasive disease potential, the trend test for proportions was used. The trend test was calculated by assuming a simple linear model, and the goodness-of-fit of the linearity was evaluated by comparing the χ^2 statistics obtained from a test for independence with the statistic obtained from the trend test [27]. All analyses were carried out in the statistical software R, version 2.1.1 [28], and the significance level was set at .05.

RESULTS

Disease severity and disease type differ by serotype. The 15 most common serotypes among the 494 patients with IPD represented 85% of the isolates. Serotype 14 was the most abundant (15% of isolates), followed in descending order by types 9V (10%), 23F (8%), 3 (8%), 7F (7%), 4 (6%), and 1 (6%) (table 1). Severity of disease differed by serotype ($P < .001$) (table 1). On admission to the hospital, patients infected with types 1, 4, or 7F had less-severe disease, with APACHE II scores >11, in only 21%–35% of cases, compared with patients infected with type 11A or 19F, among whom as many as 92% and 71% of the patients, respectively, had APACHE II scores >11 (table 1). Furthermore, nobody died from infection with pneumococci belonging to serotypes 1 and 7F, whereas the case-fatality rates of patients infected with serotypes 6B, 11A, 19F, 3, and 6A, in ascending order, ranged from 19%–33%.

Forty-nine (10%) of the 491 pneumococcal strains were isolated from CSF samples (sources of 3 strains were unknown), whereas the remaining 442 isolates were isolated from blood samples only. Most of the isolates obtained from patients with meningitis belonged to the 14 most common serotypes in the study. Pneumonia was found in 83% of all patients. Types 1, 14, 3, and 7F were usually associated with pneumonia (>90% of isolates) (table 1), and a significant difference in prevalence of pneumonia was found between patients infected with different serotypes ($P = .009$).

Table 3. Serotypes, case-fatality rates, and underlying disease among patients with invasive pneumococcal disease, by invasive disease potential.

Invasive disease potential	Serotypes	Proportion (%) of cases with fatality	Proportion (%) of patients with underlying disease
High	1, 7F	0/63 (0)	27/63 (43)
Medium	4, 9V, 14, 18C	16/167 (10)	111/163 (68)
Low	3, 6A, 6B, 8, 19F, 23F	31/146 (19)	106/142 (75)
Total	…	47/376 (13)	244/368 (66)

NOTE. Invasive disease potential was determined according to the findings of Brueggeman et al. [11]. All data were not available for all patients.

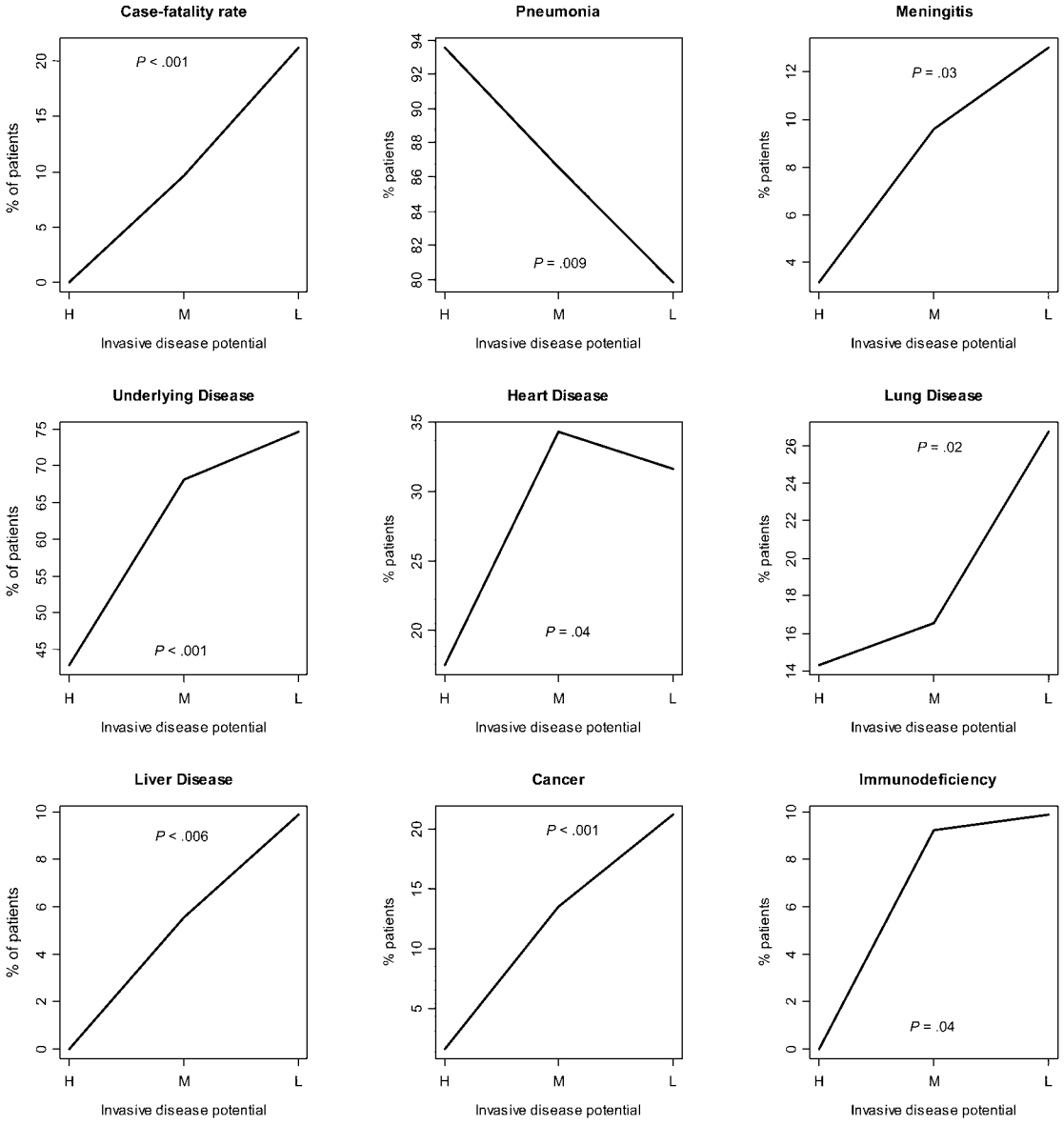

Figure 3. Trend analysis according to invasive disease potential. *P* values correspond to those obtained in the trend test for proportions, except those for heart disease, which resulted from the Fisher's exact test (nonsignificant trend). *H,* high invasive disease potential; *M,* medium invasive disease potential; *L,* low invasive disease potential (see Material and Methods).

Serotype varies by age and the presence of underlying disease. Fifty percent of patients were >64 years of age (figure 1), and there was a correlation between serotype and age ($P < .001$). Invasive disease caused by serotype 1 was found only among patients <65 years old, whereas serotype 23F was mainly found in patients >65 years old (78%) (figure 1).

The percentage of patients with underlying disease differed by serotype ($P < .001$), ranging from 31%–53% among patients infected with serotypes 1, 4, and 7F, to 67%–100% among patients infected with other serotypes (table 2). None of the patients infected with type 11A were previously healthy. Chronic heart and lung diseases and cancer were the most common underlying diseases and were found in 32%, 20%, and 16% of patients, respectively. Twenty-three (5%) of 435 patients were HIV infected. Significant differences in serotype distribution were found among isolates obtained from patients wth cardiac disease ($P = .008$), liver diseases ($P = .03$), cancer ($P = .002$), and immunodeficiencies ($P < .001$) (table 2).

Disease severity in previously healthy individuals. Among previously healthy individuals, we found that the majority of patients infected with serotypes 3, 6A, 6B, 19A, and 19F had APACHE II scores >11, whereas the majority of patients infected with types 1, 4, and 7F had low scores. Serotypes 3 and 6B were associated with the highest mortality (figure 2), whereas

Table 4. Major pneumococcal clones found by means of molecular typing and their association with clinical parameters.

Sequence type	Clone	Serotype	No. of isolates	Isolates obtained from patients with underlying disease, %	Percentage of patients, by APACHE II score			Case fatality rate
					<12	12–24	>24	
306	STO 1-A	1	17	24	82	18	0	0
227	STO 1-B	1	8	38	63	38	0	0
180	STO 3-A	3	21	67	33	67	0	43
260	STO 3-B	3	9	67	33	44	22	22
232	STO 3-C	3	3	33	67	33	0	0
205	STO 4-A	4	24	38	67	33	0	8
247	STO 4-B	4	4	75	75	25	0	25
1378	STO 6A-A	6A	4	100	25	50	25	25
138	STO 6B-A	6B	7	57	14	86	0	29
176	STO 6B-B	6B	6	83	17	83	0	0
191	STO 7F-A	7F	34	53	74	24	3	0
53	STO 8-A	8	10	70	50	50	0	10
156/162/838/1184	STO 9V-A	9V/14/19F/24A	54	70	56[a]	42[a]	2[a]	11
62	STO 11A-A	11A	12	100	8	83	8	25
124	STO 14-A	14	41	76	37	49	15	15
13	STO 14-B	14	4	75	50	50	0	0
9	STO 14-C	14	5	60	50[b]	50[b]	0	0
36	STO 23F-A	23F	8	75	50	50	0	13
977	STO 23F-B	23F	5	100	0	100	0	0
440	STO 23F-C	23F	5	100[c]	40	60	0	0
439	STO 23F-D	23F	3	50[d]	67	33	0	33
33	STO 23F-E	23F	3	100	67	33	0	0
81	STO 23F-F	23F	5	80	40	60	0	20
37	STO 23F-G	23F	3	100	100	0	0	33

NOTE. Only clones with >2 isolates are included.

[a] Data are based on 52 of 54 isolates.
[b] Data are based on 4 of 5 isolates.
[c] Calculated on the basis of 4 of 5 isolates.
[d] Calculated on the basis of 2 of 3 isolates.

only 1 of the previously healthy patients infected with serotypes 1, 4, and 7F died.

Serotypes/serogroups with elevated ORs for causing invasive disease behave as primary pathogens, whereas those with a low risk for invasive disease behave as opportunistic pathogens. We divided the 15 most common serotypes into 3 groups with different invasive disease potentials, according to criteria used by Brueggeman et al. [11]. Types 1 and 7F comprised the first group, which had a high invasive disease potential. Types 4, 9V, 14, and 18C comprised the middle group, and types 3, 6A, 6B, 8, 19F, and 23F comprised the least invasive group [11]. A low percentage of patients infected with serotypes belonging to the group with the highest invasive disease potential had an underlying disease (43%; in these patients, the strains behave like primary pathogens), compared with percentages of patients in the other 2 groups, and nobody died from infection (table 3). In contrast, patients infected with the least invasive group had a high percentage of underlying disease (75%; in these patients, the strains behave like opportunistic pathogens) and experienced a high mortality rate (19%) (table 3). Trend analysis for the 3 groups with different invasive disease potentials showed significance with respect to case-fatality rate, pneumonia, meningitis, and underlying diseases (figure 3).

Clonal characteristics of serotypes behaving as primary pathogens and of serotypes behaving as opportunistic pathogens. By using molecular methods, Box fingerprinting, PFGE, and MLST to study genetic relationships, we found that isolates of serotypes with a high invasive disease potential (i.e., isolates of types 1 and 7F), were genetically highly related, whereas isolates belonging to serotypes with less invasive potentials (i.e., types 19F and 23F) were much more diverse. We found no clones of type 19F by means of PFGE that included >2 isolates (table 4).

Both clones of serotype 1 (determined by PFGE) were ge-

netically related, because they belonged to the same clonal cluster (differing by only 1 allele), according to MLST. Most often, they caused a mild disease with low APACHE II scores and caused no fatalities, even though there was a tendency for infected patients to acquire more-severe disease with clone STO 1-B (table 4).

Although type 11A behaved as an opportunistic pathogen (because all infected patients had an underlying disease) all isolates of type 11A belonged to the same clone as determined by PFGE and MLST. In contrast, isolates of another opportunistic serotype, type 23F, were genetically highly diverse, with only a few patients being infected by each clone. Differences, albeit not significant, were observed between the clones with respect to clinical outcome, even though a majority of patients had an underlying disease.

The most common clone observed in the study by means of PFGE, STO 9V-A, comprised 54 isolates belonging to 4 different serotypes (9V, 14, 19F, and 24A) (table 4) and, therefore, represented a highly successful clone that has undergone a number of different capsular switches. The majority of isolates were of type 9V ($n = 45$) and included both penicillin-susceptible and nonsusceptible isolates. MLST confirmed that all of the isolates belonged to the same clonal cluster, differing in only 1 and the same allele. A high percentage (70%) of patients infected with this clone had an underlying disease, suggesting that this clone acts primarily as an opportunistic pathogen. However, we noticed that 3 (75%) of 4 patients infected with the STO9V-A clone of type 14, 1 (25%) of 4 patients infected with type 19F, and 23 (40%) of 45 patients infected with type 9V had no underlying disease. Thus, this suggests that there may be differences in the potential to cause inasive disease in previously healthy individuals within a single clone.

Penicillin resistance has no major impact on the outcome of IPD. In our study, 32 (6%) of 494 patients had pneumococci with a reduced susceptibility to penicillin. There were no significant differences in case-fatality rate (13% died) or in underlying disease when patients infected with clones of pneumococci with a reduced susceptibility to penicillin or clones with other resistant traits were compared with patients infected with susceptible clones belonging to the same serotypes (data not shown). None of the serotype 1 and 7F isolates had any resistance traits.

DISCUSSION

In this article, we demonstrate that pneumococcal clones of serotypes 1 and 7F, which were previously shown to have a high risk of causing invasive disease, primarily infect previously healthy individuals and, therefore, behave as "primary pathogens." Nevertheless, most of these patients did not have a severe invasive disease, and there were no deaths. This was in sharp contrast to many other clones belonging to serotypes with a lower potential for causing invasive disease. In the majority of cases, infections with such clones behaved more like "opportunistic pathogens" causing disease in patients with underlying disease, and were associated with more-severe disease and significant mortality. Also, when comparing invasive diseases in previously healthy individuals, trend analysis of the 3 groups with different invasive disease potentials demonstrated statistical significance with respect to case-fatality rate and APACHE II scores (data not shown). Therefore, after we eliminated the bias of underlying disease, clones of serotypes 1 and 7F were observed to cause milder diseases than clones belonging to the less invasive groups. We have recently infected mice with several of these clones and found that clones belonging to the latter groups caused a robust proinflammatory cytokine response in serum after intraperitoneal challenge. This was not the case for serotype 1 and 7F clones [12]. Thus, it appears that primary and opportunistic *S. pneumoniae* isolates may differ from one another in their ability to activate innate immune responses. Such responses are required for bacterial clearance but may also contribute to disease severity once invasive disease has been established.

To prevent IPD and less-severe diseases caused by pneumococci, vaccines based on the capsular polysaccharide have been used for decades. A recently licensed 7-valent conjugated vaccine includes only a limited number of different capsular polysaccharides, and the potential coverage rate differs by geographic area and time period, because fluctuations in the serotype distribution have been observed [8, 29, 30]. The present 7-valent vaccine does not include the highly clonal primary pathogens, serotype 1 and 7F. However, in view of our findings, this may have an impact mainly on morbidity and, to a lesser extent, on mortality associated with IPD among adults. Serotype 11A is another example of a type not included in the 7-valent conjugated vaccine. In this study, 11A was causing invasive disease only in patients with underlying disease. This serotype is relatively rare among carriers, but the frequency of this serotype could possibly increase in vaccinated groups. Interestingly, infections caused by this strictly opportunistic serotype were only represented by a single clone. This was in contrast to many of the other more opportunistic serotypes (i.e., 19F and 23F) that were represented by several clonal types among the patients.

Because of the limited number of patients infected with a given clonal type, we could not firmly resolve whether variations in disease severity and ability to cause invasive disease in previously healthy individuals should be attributed to the capsular type, to the clonal type, or to both. The largest clonal cluster in the study was STO 9V-A, including both penicillin-susceptible and resistant isolates. Even though the number of isolates analyzed was small, isolates belonging to STO9V-A with serotype 14 appeared to be more prone to infect previously

healthy individuals than were isolates with serotype 19F, which is in agreement with the elevated OR of the former serotype for causing invasive disease [11]. However, for any given serotype, there were also differences with respect to degree of underlying disease and disease severity, suggesting that properties other than capsular type may affect these parameters. A number of pathogenicity islands have recently been described for *S. pneumoniae* [31]. The presence of such islands within different clonal types and different serotypes is currently being determined in our laboratory, and the result should provide valuable new information regarding the genetic properties of pneumococci that are important for invasive disease severity.

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