MAJOR ARTICLE

Association of Serotype with Risk of Death Due to Pneumococcal Pneumonia: A Meta-Analysis

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Background. The 92 capsular serotypes of *Streptococcus pneumoniae* differ greatly in nasopharyngeal carriage prevalence, invasiveness, and disease incidence. There has been some debate, though, regarding whether serotype independently affects the outcome of invasive pneumococcal disease (IPD). Published studies have shown variable results with regard to case-fatality ratios for specific serotypes and the role of host factors in affecting these relationships. We evaluated whether risk of death due to IPD is a stable serotype-associated property across studies and then compared the pooled effect estimates with epidemiologic and biological correlates.

Methods. We performed a systematic review and meta-analysis of serotype-specific disease outcomes for patients with pneumonia and meningitis. Study-specific estimates of risk of death (risk ratio [RR]) were pooled from 9 studies that provided serotype-specific data on pneumonia and meningitis using a random-effects method with serotype 14 as the reference. Pooled RRs were compared with RRs from adults with low comorbidity scores to evaluate potential confounding by host factors.

Results. Significant differences were found in the RR estimates among serotypes in patients with bacteremic pneumonia. Overall, serotypes 1, 7F, and 8 were associated with decreased RRs, and serotypes 3, 6A, 6B, 9N, and 19F were associated with increased RRs. Outcomes among meningitis patients did not differ significantly among serotypes. Serotypes with increased RRs had a high carriage prevalence, had low invasiveness, and were more heavily encapsulated in vitro.

Conclusions. These results suggest that IPD outcome, like other epidemiologic measures, is a stable serotype-associated property.

Streptococcus pneumoniae, or pneumococcus, is an important cause of pneumonia, meningitis, otitis media, and septicemia and is associated with significant morbidity and mortality worldwide. There are 92 known pneumococcal serotypes, and each produces a unique polysaccharide capsule that protects the bacterium against host immune effectors [1]. Serotype affects many aspects of pneumococcal epidemiology. The rank orders of serotypes found in nasopharyngeal carriage [2] and invasive disease [3, 4] are similar worldwide, with a few exceptions. Likewise, the invasiveness of a serotype, or the frequency with which it causes invasive disease per carriage episode, is a stable property [5]. There is an inverse relationship between the carriage prevalence of a serotype and its invasiveness [5] and between disease severity and invasiveness [6].

The outcome of a case of invasive pneumococcal disease (IPD) can be affected by both bacterial factors, such as serotype, and host characteristics, such as old age, very young age, low socioeconomic status, quality

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of care, alcoholism, immunodeficiency, and other underlying conditions [7–10]. Some studies have found that even after controlling for relevant host factors, certain serotypes are independently associated with more severe outcomes [11–13]. Likewise, experimental studies in mice have shown that serotypes differ in their ability to cause severe disease [14], and strains with larger capsules are more virulent in animals than strains of the same serotype with smaller capsules [15, 16]. It was long ago noted that differences in polysaccharide production among serotypes 1–3 correlated with the case-fatality ratios (CFRs) for these serotypes in humans [7, 17].

Although a number of studies have investigated the relationship between serotype and disease outcome, they differ in the kinds of clinical syndromes included, the age of the populations studied, and the covariates included when deriving effect estimates. As a result, published studies differ in the magnitude and direction of effect estimates for certain serotypes, and these studies have not previously been compared to determine whether stable patterns of virulence exist. We performed a systematic review and meta-analysis of IPD outcome by serotype to evaluate the stability of these estimates among studies. We found that clinical outcome in bacteremic pneumonia, such as carriage prevalence and invasiveness, is a stable serotype-associated property, and we hypothesize the biological reasons for these patterns.

METHODS

Literature review, inclusion criteria, and sources of data. We performed a search of the PubMed database with combinations of the terms "pneumococcus," "pneumococcal," "invasive pneumococcal disease," "serotype," "type," "fatality," "mortality," and "severity" and reviewed abstracts for content. Additional studies were identified from reference lists and from published texts [7]. English-language studies from 1928 to the present were considered that provided serotype-specific data on the number of invasive disease cases and number of deaths, although many of the older studies were excluded because they did not use contemporary diagnostic procedures and did not require the isolation of bacteria from sterile sites. A number of studies, including some with high-quality data, were excluded. Reasons for exclusion included the following: results were not stratified by syndrome, case definitions did not require the isolation of bacteria from a normally sterile site, or the study did not contain fatalities in the reference group, did not contain extractable data in the publication, or overlapped with other studies included in the analysis. In addition, given our primary focus on bacteremic pneumonia, we did not seek or include studies that focused exclusively on meningitis. For studies that did not provide sufficient detail in the original text, we attempted to contact the corresponding authors of the studies for additional data.

Patients from the identified studies were included if they had pneumococcal isolates obtained from blood or cerebrospinal fluid with a clinical diagnosis of pneumonia or meningitis. Nonbacteremic pneumonias were not included in this analysis. We used the diagnoses reported in the original studies, among which the clinical case definitions of these syndromes varied. Patients diagnosed as having both pneumonia and meningitis were classified as having meningitis.

Additional data on pneumonia outcome in adult patients with no known comorbidities (Charlson comorbidity score of 0) were derived from the dataset recently described by Harboe et al. [11]. Serotype-specific estimates of invasiveness and carriage frequency (number of times a serotype was detected in the population) were extracted from published data as described in the text.

As previously described, we measured the degree of encapsulation in vitro using capsule-switch variants that were created in the laboratory on the TIGR4 genetic background [18]. The strains were grown on TSAII plates, resuspended in phosphate-buffered saline, and mixed with fluorescein isothiocyanate (FITC)–dextan, a large macromolecule that is excluded from the dense capsular region. We then measured the area of FITC-dextran exclusion for 100–250 bacteria with a Nikon Eclipse 80i. Degree of encapsulation measurements is presented as the mean area of the zone of FITC-dextran exclusion in pixels.

Selection of serotypes for comparison. To be included in the meta-analysis, we only considered serotypes that were found in at least 3 different studies with at least 10 isolates in each study for the pneumonia meta-analysis or at least 2 different studies for the meningitis meta-analysis. All available studies, regardless of their sample size, were used to calculate the pooled effect estimates and to examine heterogeneity.

Calculation of the risk ratio (RR). We calculated the serotype-specific risk of death (RR) and 95% confidence intervals (CIs) compared with serotype 14 [5]. Serotype 14 was chosen because it is a common cause of IPD and is the only serotype that contained nonzero numbers of fatalities in all studies. In situations where there were no fatalities, a value of 0.5 was added to each component of the RR before calculation.

Statistical analysis. Pooled RRs for each serotype were calculated by using a random-effects model [19] using the "metan" package in Intercooled Stata v9.2 (StataCorp). There could be true differences in the RRs among study locations attributable to different circulating bacterial strains or to differences in the host population. Although a fixed-effect approach calculates weights based only on the variance of the studies and

Table 1. Characteristics of Studies Included in the Meta-Analyses

Characteristic	Site (reference)									
	New York[a] [20]	Chicago [21]	Multicenter[a,b] [9]	The Netherlands[b] [22]	Denmark[b] [11]	Denmark[b] [11]	Kenya[b] (unpublished)	Germany[b] [12]	Israel[b] (unpublished)	The Gambia[b,c] [23]
Study years	1952–1962	1967–1970	1998–2001	2004–2006	1997–2007	1997–2007	1994–2008	1997–2003	1999–2006	2000–2004
Age, years										
Median	…	…	52	69.4	…	…	1.75	1.5	1.16	0.98 (0.2–2.5)
Range	>12[d]	>14[e]	14–97	18–100	⩾12	<12	0–12	0–15	0–15.7	…
No. of cases	321	204	582	841	5374	137	436	264	66	28
Pneumonia, no. dead/total no. by serotype										
1	6/78	6/21	10/75	5/60	53/853	…	7/82	0/15	…	0/1
3	18/35	9/18	13/55	14/53	98/330	…	4/9	0/3	…	…
4	8/43	7/33	4/68	14/88	63/587	…	2/11	0/3	…	0/1
5	2/23	3/22	2/27	0/5	4/49	…	1/18	…	…	1/6
6A	3/10	1/5	3/18	2/22	31/134	…	3/24	0/2	…	0/1
6B	…	…	9/35	5/16	33/135	…	4/30	0/4	…	1/3
7F	…	4/20	0/14	10/115	41/482	…	0/1	1/7	…	…
8	8/54	2/33	0/19	9/73	35/347	…	0/2	…	…	…
9N	2/6	4/14	2/11	4/17	50/210	…	…	…	…	…
9V	…	…	8/30	11/85	43/396	…	1/7	0/2	…	1/1
12F	7/32	3/21	1/22	0/8	31/214	…	2/6	…	…	…
14	1/14	1/7	13/70	18/123	95/605	…	3/32	1/31	…	1/6
19A	…	…	3/20	3/28	27/111	…	2/18	0/4	…	0/4
19F	6/14	1/5	9/28	4/17	41/115	…	5/12	0/2	…	0/1
22F	1/6	0/5	2/12	2/13	21/158	…	…	…	…	…
23F	3/6	…	7/37	6/49	38/184	…	5/20	1/3	…	2/4
Meningitis, no. dead/total no. by serotype										
1	…	…	5/6	1/2	6/34	…	27/71	0/5	1/8	…
6A	…	…	1/3	2/5	8/32	0/11	7/17	1/5	1/7	…
6B	…	…	0/3	0/4	7/21	3/45	8/16	2/24	0/9	…
7F	…	…	1/3	1/8	11/59	1/32	1/2	4/15	0/2	…
8	…	…	1/1	3/8	9/38	…	…	0/1	0/2	…
9N	…	…	0/1	2/4	6/28	…	…	0/5	…	…
9V	…	…	1/4	2/5	15/39	…	1/2	1/10	0/2	…
12F	…	…	4/5	1/3	18/76	…	3/6	…	0/5	…
14	…	…	1/5	0/6	12/44	1/35	7/20	7/72	1/9	…
19A	…	…	…	1/2	6/15	…	1/3	0/10	0/3	…
19F	…	…	1/3	3/7	5/33	0/14	4/11	1/21	0/5	…
23F	…	…	2/5	6/15	11/45	…	6/16	2/20	1/14	…

NOTE. The data from New York, Chicago, Kenya, and the multicenter study come from hospital studies. The data from The Netherlands, Denmark and Germany come from surveillance systems in the respective countries. The data from The Gambia comes from the control arm of a vaccine trial.

[a] Pneumonia without extrapulmonary focus.

[b] Additional data provided by investigators.

[c] Unvaccinated controls only.

[d] A total of 9.6% of patients 12–29 years old, 38.8% were 30–49 years old, 33.8% were 50–69 years old, and 17.8% were >70 years old.

[e] A total of 11.5% of patients were 14–29 years old, 49.6% were 30–49 years old, 33.2% were 50–69 years old, and 5.7% were >70 years old.

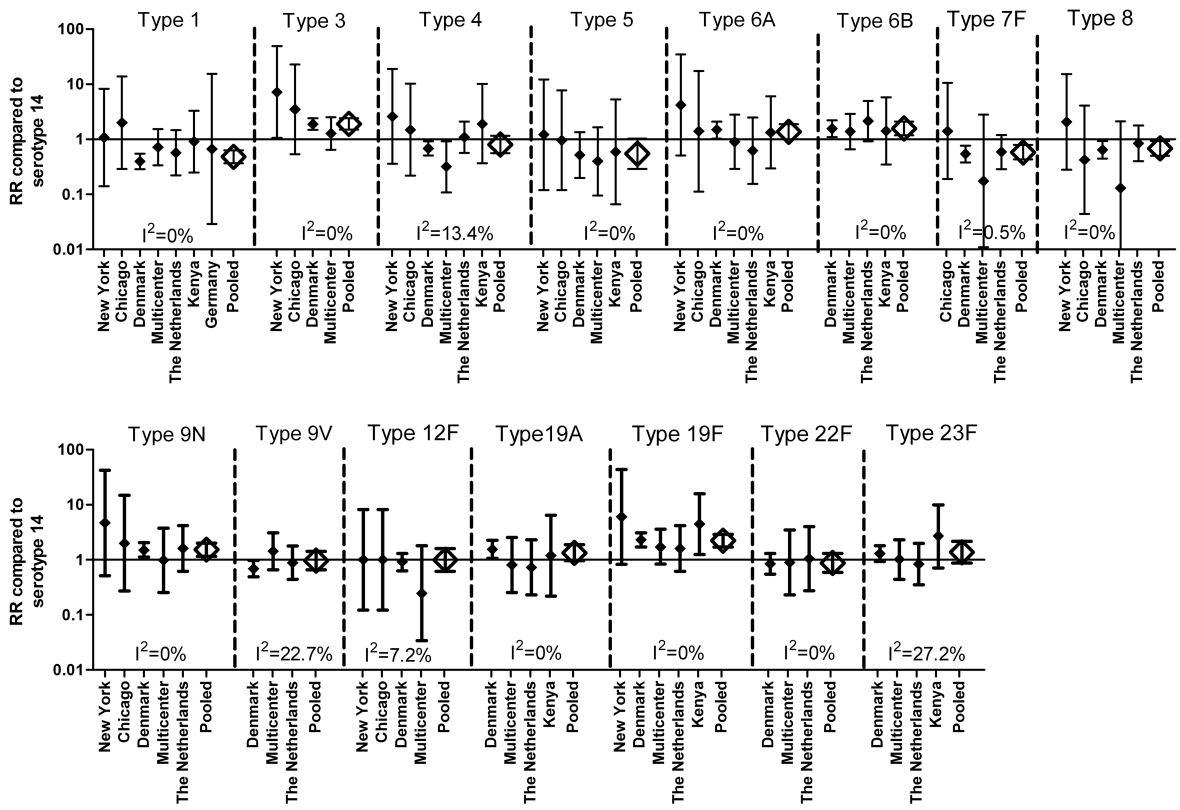

Figure 1. Study-specific and pooled risk ratios (RRs) for death due to bacteremic pneumonia compared with serotype 14. *Closed diamonds* represent study-specific RR (95% confidence interval [CI]). *Open diamonds* represent the pooled RR (95% CI). I^2 denotes the amount of variation in the RR due to heterogeneity. Only studies with ⩾10 isolates of the serotype are shown, although all studies were used to calculate the RR and evaluate heterogeneity.

assumes that all of the RRs are drawn from the same underlying population, the random-effects model accounts for potential differences among studies and distributes the weights more equally. Separate analyses were performed for patients with pneumonia and meningitis, and we have stratified the meta-analysis by age group—pediatric or adult as defined in Table 1—of the population. Although it would be preferable to use smaller age subgroups, we did not have sufficient data for such an analysis. To further evaluate whether serotype is associated with outcome independent of age, we used data from the Dutch study [22] and the multicenter study [9] in logistic regressions to calculate the odds ratio of death for each serotype compared with serotype 14 and performed the regression either with or without a predictor for patient age (cubic spline). We found that adjusting for age did not greatly affect the odds ratio in the 2 studies we examined (Appendix [available online],), suggesting that serotype independently affects disease outcome, which is consistent with published findings [11].

In an effort to evaluate the influence of host comorbidities, we compared RRs from adult Danish patients with no known comorbidities with the overall pooled RRs, which were recalculated to exclude these patients.

Spearman's rank correlations were used to compare the pooled RRs with carriage prevalence and invasiveness data and with in vitro measurements.

Heterogeneity among the studies was evaluated using the I^2 approach, which determines the percentage of variability among studies that can be attributed to true heterogeneity rather than random variation [24]. It has been suggested that an I^2 value of <25% reflects low levels of heterogeneity [24].

RESULTS

Risk of death due to pneumonia. We analyzed 9 studies (summarized in Table 1) drawn from the United States, Europe, Africa, and the Middle East covering 1952 to the present. Five of the datasets included pediatric patients.

Among bacteremic pneumonia patients, we found significant differences in the pooled RRs among serotypes (Figure 1; Appendix [available online], Table A1). Overall, patients with pneumonia caused by serotypes 3, 6A, 6B, 9N, and 19F were significantly more likely to die than those patients with serotype 14. In addition, for serotypes 19A and 23F, the RR was increased, albeit not statistically significantly. In contrast, patients

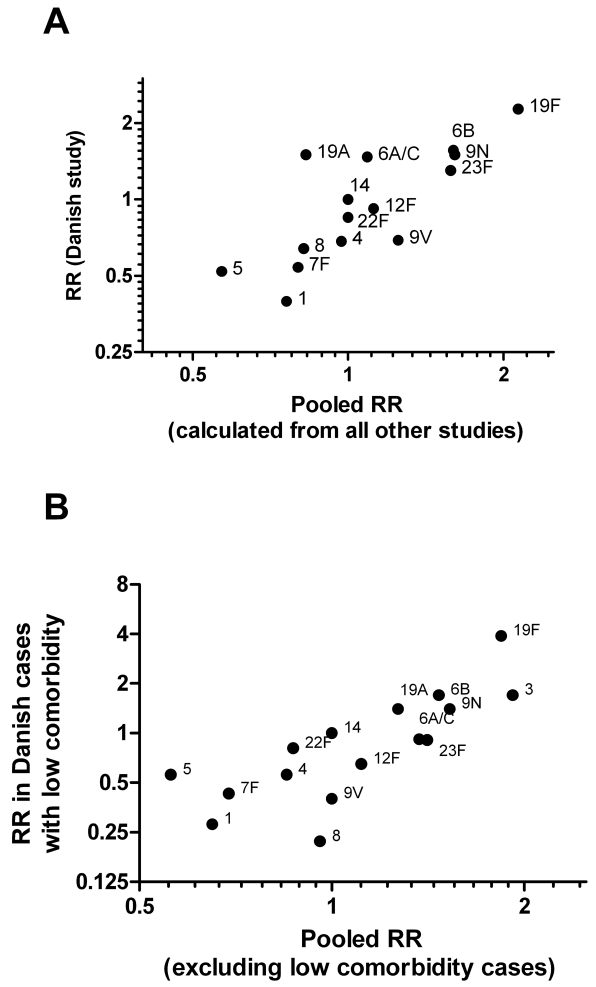

Figure 2. *A,* Comparison of the risk ratios (RRs) calculated among Danish adult bacteremic pneumonia patients compared with the pooled RRs from all other studies. *B,* Comparison of RRs calculated among adult Danish bacteremic pneumonia patients with no known comorbidities with the overall pooled RRs representing all studies except for the Danish low-comorbidity cases.

infected with serotypes 1, 7F, and 8 were significantly less likely to die than those infected with serotype 14, and the RRs for serotypes 4 and 5 were also decreased but not significantly so. In most instances, we did not observe a significant difference between the RR estimates among adults and children (Appendix [available online], Table A1), although the estimates for children were less precise because of the relatively small number of pediatric deaths included in this meta-analysis. For some serotypes the RR appeared to be substantially higher among children than among adults. However, this could be attributed, in part, to the bias introduced in the small pediatric studies by the correction factor of adding 0.5 to the components of the RR in instances when there were no fatalities. This correction leads to the appearance of an artificially large RR for serotypes with few isolates. All of the serotypes exhibited low levels of heterogeneity among studies based on the I^2 values, with the exception of serotypes 9V and 23F, which exhibited moderate levels of heterogeneity (Figure 1; Appendix [available online], Table A1).

Among patients with meningitis, we found that the differences in the RRs among serotypes were less pronounced than for pneumonia, and there was no serotype for which the RR was significantly different from 1 (Appendix [available online], Table A2).

Given that nearly 70% of the patients identified in the pneumonia meta-analysis were derived from the Danish study, we considered whether the pneumonia RRs could be dominated by the inclusion of this heavily weighted study. We recalculated the pooled effect estimates without the Danish data and found a strong correlation between the RRs calculated without the Danish data and the Danish estimates alone ($\rho = 0.82$; $P<.001$) (Figure 2*A*), indicating that the estimates are not unduly influenced by the inclusion of this large study.

Correlation of pooled pneumonia estimates with RRs from patients with no known comorbidities. Host comorbidities could affect the outcome of IPD and may make it appear that serotypes that are more frequently found in patients with preexisting conditions are more virulent [6, 9]. As a result, we evaluated whether cases without known comorbidities would have a similar pattern of disease severity among serotypes compared with the overall pooled RRs. Approximately 47% of the adults with bacteremic pneumonia in the Danish study did not have a diagnosed comorbidity. We calculated RRs from these patients and also recalculated the overall pooled RRs to exclude these patients with no known comorbidities. There was a strong correlation ($\rho = 0.83$) (Figure 2*B*) between the RRs of the patients with no known comorbidities and the recalculated overall pooled RRs, indicating that the RRs are unlikely to be strongly biased by host comorbidities and could reflect true differences between serotypes.

Association of serotype-specific RR with carriage prevalence, invasiveness, and degree of encapsulation of the infecting serotypes. Next, we compared the pooled RRs from bacteremic pneumonia patients with serotype-specific carriage prevalence. The pooled RRs also correlated with pre–pneumococcal conjugate 7 carriage prevalence data from studies in England (<2 years; $\rho = 0.78$; $P<.001$) [25], The Gambia (9–15-month placebo controls; $\rho = 0.67$; $P<.01$) [26], the Netherlands (<20 years old; $\rho = 0.66$; $P<.01$) [27], United States (<7 years old; $\rho = 0.67$; $P<.01$) [28], and Canada (most <5 years old; $\rho = 0.71$; $P<.01$) [29]. Among children from Kenya, this associa-

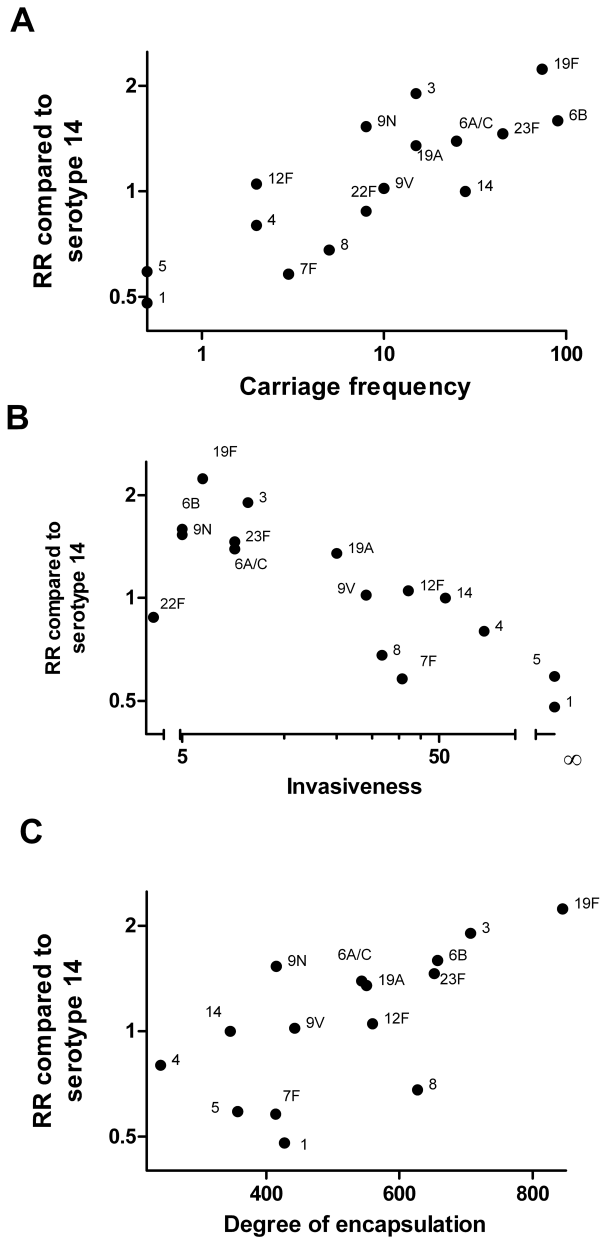

Figure 3. Relationship between serotype-specific risk ratios (RRs) and epidemiologic and microbiological measures. The serotype-specific RR among pneumonia patients is related to carriage prevalence in a pediatric study in England (number of nasopharyngeal isolates) (*A*) [22], invasiveness (*B*) [22], and degree of encapsulation (area in pixels) (*C*) [18]. There were no carriage isolates for types 1 or 5.

tion was stronger in those younger than 5 years ($\rho = 0.64$; $P < .01$) than in older children from the same population ($\rho = 0.46$) [30].

Interestingly, serotypes with high carriage prevalence and high RRs tended to be less invasive [25]. Finally, we compared the pooled RRs for bacteremic pneumonia with in vitro measurements of capsule size and found that heavily encapsulated serotypes tended to be associated with increased RRs ($\rho = 0.70$; $P < .01$) (Figure 3).

DISCUSSION

In this study, we found that among bacteremic patients with pneumonia, the risk of death varies by serotype and, at least in adults, is stable among studies across time and in diverse geographic locations. In addition, we found that the RR estimates were directly correlated with the carriage prevalence of the serotypes and inversely correlated with invasiveness. Finally, the serotypes that were more frequently associated with fatal outcomes tended to be more heavily encapsulated in vitro. Although it was long ago suggested that there is a link between the degree of encapsulation and virulence [7], this is the first time, to our knowledge, that such a comparison has been formally made across a large number of serotypes.

The findings of this study suggest a potential mechanism for the epidemiologic relationships among serotypes. More heavily encapsulated serotypes tend to be more prevalent among pediatric carriage isolates and are less likely to cause bacteremic invasive disease, but they tend to cause more severe disease when they do invade. The thick capsule could allow the bacterium to persist in the nasopharynx, lungs, and blood by protecting against host immune effectors. However, a large capsule could hinder the invasion process itself because invasion into tissues or the bloodstream could involve either direct transcytosis across epithelial cells [31] or the induction of an inflammatory response that disrupts the epithelial barrier [32]. In either case, a heavily encapsulated strain would interact with the host less efficiently and might be less likely to cross the epithelium.

We excluded several preantibiotic era studies that evaluated the relationship between serotype and disease outcome [32–34]. These studies found a pattern similar to what we report here, with type 3 having a higher CFR and serotypes 5 and 14 having lower CFRs, and this is despite major changes in diagnostic procedures since that time. Our results are also consistent with some other individual studies that have addressed this topic but did not meet our inclusion criteria [6, 35, 36] (Appendix [available online], Table A3).

Bacterial factors in addition to capsule likely contribute to disease severity. Animal studies demonstrate different strains of the same serotype differ in virulence [37]. Consequently, regional differences or changes over time of the circulating clones could influence the virulence associated with a specific serotype.

The measurements of capsule size described here were performed on strains created in the laboratory and then isolated

from the noses of mice and were confirmed using clinical carriage isolates (data not shown). It is possible, though, that among disease isolates, the capsule size measurements would follow a different pattern.

Although we have focused on the importance of microbiological characteristics, host factors also have an important role. A number of studies have found that serotype independently affects disease outcome, whereas others have suggested that host factors were more important [6, 9–12]. Although we did not have sufficient data to adjust for age and comorbidity before pooling the RRs, we found good agreement between our pooled RRs and estimates from patients with pneumonia who have no known comorbidities.

The relatively low fatality rate in children makes estimating the pooled RRs difficult. However, in many instances in which the data were sufficient, the directions of the RRs were the same in adults and children. To generalize our findings to neonates, in whom there is a high burden of disease, or to subsets of patients with specific syndromes, such as empyema, further study would be required.

Differences in socioeconomic status, access to health care, the prevalence of human immunodeficiency virus infection, or inherent host differences could potentially affect the virulence of the serotypes and could contribute to the heterogeneity that we see among study populations. However, although the absolute CFRs differ among studies, the directions of the RRs are similar in populations from North America, Europe, and Africa, suggesting that serotype-associated factors have an independent effect on disease outcome.

Aside from age and chronic comorbidities, recent infections with respiratory viruses could influence the risk of death due to pneumococcal pneumonia. If specific serotypes have an increased tendency to infect individuals with recent respiratory illnesses, then it might appear that these serotypes are more virulent when in fact they might just have different host preferences.

Antimicrobial resistance tends to be associated with serotype, leading to the possibility that differences in resistance patterns could influence outcome differences among serotypes. However, Denmark has extremely low levels of antimicrobial resistance (2%–5%) [11], and we see similar mortality patterns in the Danish study and in the other datasets. This finding suggests that resistance is unlikely to lead to the mortality patterns observed among serotypes.

We found a notable difference in the patterns of disease outcome among patients with pneumonia and those with meningitis. This finding could reflect the fact that bacteremic pneumonia encompasses a broad range of disease severities, whereas meningitis is, by its nature, a more serious condition with distinct pathophysiologic features, so serotype might have a different and less important role in this context. In addition, there tends to be a higher prevalence of comorbidities among adult patients with meningitis that could overshadow the difference among serotypes.

Overall, we did not find substantial heterogeneity among studies. However, some differences were found in the magnitude and direction of the estimates. Some estimates are based on small numbers and are subject to considerable uncertainty. In particular, death due to IPD was relatively rare in the pediatric studies included in this review, so the 95% CIs are especially wide around these estimates. In addition, our decision to use a single serotype as the reference group could account for some differences among studies. In most instances, the CFR for serotype 14 was similar to the mean from the study, but for Austrian studies [8], serotype 14 had a substantially lower CFR than the mean CFR for all patients, potentially biasing some estimates.

The results of this study support an important and stable role for serotype in determining the outcome of pneumonia. Given the correlations between serotype-specific disease outcome, carriage prevalence, and invasiveness, there is likely a common microbial explanation for these stable patterns. Understanding these patterns of disease severity and transmission will help to determine the potential benefits of using vaccines with increased coverage that might target serotypes associated with higher mortality.

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