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Between-Strain Competition in Acquisition and Clearance of Pneumococcal Carriage—Epidemiologic Evidence From a Longitudinal Study of Day-Care Children

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The state of pneumococcal carriage—that is, pneumococcal colonization in the nasopharynx of healthy persons—represents a reservoir for the spread of pneumococci among individuals. In light of the introduction of new pneumococcal conjugate vaccines, further knowledge on the dynamics of pneumococcal carriage is important. Different serotypes (strains) of pneumococcus are known to compete with each other in colonizing human hosts. Understanding the strength and mode of between-serotype competition is important because of its implications for vaccine-induced changes in the ecology of pneumococcal carriage. Competition may work through reduced acquisition of new serotypes, due to concurrent carriage in the individual, or through enhanced clearance of serotypes in carriers who harbor more than 1 serotype simultaneously. The authors employed longitudinal data (1999–2001) on pneumococcal carriage in Danish day-care children to analyze between-serotype competition. The data included observations of carriage in children who had not been vaccinated against pneumococcus, and the level of pneumococcal antibiotic resistance and antibiotic usage in the community was very low. Clearance of any single serotype was not affected by simultaneous carriage of other serotypes. In contrast, acquisition of other serotypes in already-colonized hosts was weak (relative rate of acquisition = 0.09, 95% credible interval: 0.05, 0.15).

child; day care; disease reservoirs; longitudinal studies; models, statistical; *Streptococcus pneumoniae*

Abbreviations: CI, credible interval; DCC, day-care center.

Streptococcus pneumoniae (pneumococcus) has 91 known serotypes (1). Many serotypes are commonly found in nasopharyngeal carriage, particularly among young children, who form the main reservoir of pneumococci and their transmission to new hosts (2–4). Although pneumococcal carriage is mostly transient and asymptomatic, it is also a prerequisite to pneumococcal disease (e.g., middle-ear infection, pneumonia, meningitis) (5). The pneumococcal conjugate vaccine is efficacious in preventing serious forms of pneumococcal disease caused by serotypes included in the vaccine (6–9). At the same time, its use reduces acquisition of vaccine-type pneumococcal carriage, thus inducing indirect protection against carriage and disease in the unvaccinated as well as potential for replacement of vaccine types by nonvaccine types as causes of carriage and disease (10–13).

Epidemiologic data suggest that different serotypes (strains) of *S. pneumoniae* compete with each other in colonizing human hosts. In the United States, vaccine-type carriage has been replaced by non-vaccine-type carriage during large-scale use of the pneumococcal conjugate vaccine since 2000 (11, 14–16). Such replacement implies the existence of between-strain competition that has opened a niche for nonvaccine types under the reduced carriage of vaccine-type pneumococci (17). Analyses of follow-up data about pneumococcal carriage have revealed interactions between acquisition of specific serotypes and the current status of carriage in the individual (18). Understanding the mode and strength of between-strain competition is important for interpreting past trends and predicting potential changes in the ecology of pneumococcus under vaccination pressure.

Different mechanisms of between-serotype competition have been investigated by means of theoretical modeling (17, 19). Competition may work through reduced acquisition of new serotypes, due to current (resident) carriage of other serotypes in the individual. Alternatively, clearance of pneumococcus may be enhanced in carriers who harbor more than 1 serotype simultaneously. Another type of interaction between pneumococcal serotypes may work through acquired immunity to subsequent acquisition, induced by encounters with specific serotypes (20) or pneumococci in general. Although it is possible to investigate some of these mechanisms experimentally in mouse models, it is important to reveal patterns of competition using epidemiologic data on humans.

Epidemiologic data may suffer from limitations of the method used to identify pneumococcal carriage. First, pneumococcal carriage has an episodic nature and can usually only be detected by active sampling. The sampling frequency may bias analysis of pneumococcal episodes from longitudinal data. Second, inferences about pneumococcal carriage may be affected by the nonoptimal sensitivity of pneumococcus detection (21). The standard procedure of randomly picking only 1 or 2 colonies suspected to be pneumococci for identification and typing from a plate of a nasopharyngeal specimen often yields only 1 serotype per sampling. However, double or multiple carriage occurs, with rates that may differ across different populations and methods of detection (22–26). For analyses of between-serotype competition, it is particularly important that pneumococcal carriage and multiple carriage be identified systematically with high sensitivity.

We employed longitudinal data on pneumococcal carriage in children to analyze competition between pneumococcal serotypes in colonizing human hosts. Detection of pneumococcal serotypes was based on a new method that allows more sensitive observation of simultaneous carriage of pneumococci than the standard method (27). We used the data to assess the effect of current carriage on acquisition of other serotypes and to assess the effect of co-colonizing serotypes on clearance in multiple carriers.

MATERIALS AND METHODS

Study participants

Children attending 3 day-care centers (DCCs) in Roskilde, Denmark, and employees of the DCCs were considered for enrollment in an open cohort study of pneumococcal carriage. The DCCs were nurseries for children under 3 years of age. Altogether, 123 DCC attendees (49 in DCC 1, 40 in DCC 2, and 34 in DCC 3) and 37 DCC employees (14, 12, and 11, respectively) were enrolled as study participants. The median age of children at enrollment was 1.9 years (range, 0.9–3.2). None of the participants had been immunized with a pneumococcal vaccine.

Written informed consent was obtained from the parents of each child and from the adult participants. The local ethical committee and the Department on Children and Culture in Roskilde Municipality approved the study (27).

Samples

Children in DCCs 1 and 2 were followed from April 1999 to February 2001, and children in DCC 3 were followed from December 1999 to February 2001 (see Web Figure 1, which is posted on the *Journal*'s Web site (http://aje.oxfordjournals.org/)). Deep nasopharyngeal samples were collected with a calcium alginate swab from the study participants at monthly visits to the DCCs. The first 2 sampling rounds in DCCs 1 and 2 were omitted from the present analysis because of infrequent sampling (>45 days between consecutive samples). Thus, there were 15 sampling rounds included from both DCC 1 and DCC 2 and 12 from DCC 3, involving 104 children altogether. The median numbers of children under observation were 15, 13, and 17, respectively—approximately 50% of children in each DCC. Except for 1 instance, all samples taken during a particular sampling round (visit) in a DCC were collected within 8 days. Children left the study cohort because of age (63 children; median age at exit, 2.8 years) or the end of the study (41 children; median age at exit, 2.2 years).

Altogether, 571 samples were taken from the 104 children. The average stay per child in the DCC was 0.5 years, and the median number of samples per child was 5 (range, 1–15). The 161 samples taken from the 37 employees (median age, 44 years) were used only to define serotype-specific exposure in children (see below).

Serum broth, made from beef infusion and enriched with 5% horse serum and 0.33% defibrinated horse blood, was used as a transport and enrichment culture medium. The inoculated broth was incubated overnight in air with extra 5% carbon dioxide at 35°C, and the presence of 1 or more of the 91 pneumococcus serotypes was then determined directly in the broth cultures by means of the capsular reaction test, using a panel of pneumococcal group- and type-specific antisera (27). In the analysis, serotypes 15B and 15C were considered 1 type (15B/C) because of reversible serotype switching between them (28).

Definitions and statistical methods

The individual follow-up period was defined as the time from the first sample taken in the child to the last sample. If there were more than 45 days between the collection of any 2 consecutive samples, follow-up was discontinued for the time interval between the 2 samples. For each child, serotype-specific episodes of carriage were defined for the 8 most common serotypes in this study (6B, 6A, 23F, 19F, 14, 19A, 15B/C, 11A) as series of isolations of the same serotype at consecutive visits. For the crude analysis, each episode was assumed to start midway between the first isolation of the type (serotype or ''noncarriage'') in the episode and the previous visit and correspondingly to end midway between the last isolation in the episode and the following visit. The remaining 109 isolates (23% of isolates, representing 21 serotypes and 16 nontypable isolates) were treated as a single group, and episodes for this group of *deidentified types* were determined subsequently. For convenience, in the 5 instances of 3 simultaneous serotypes in a sample, the rarest of the 3 isolates was omitted in the subsequent analyses.

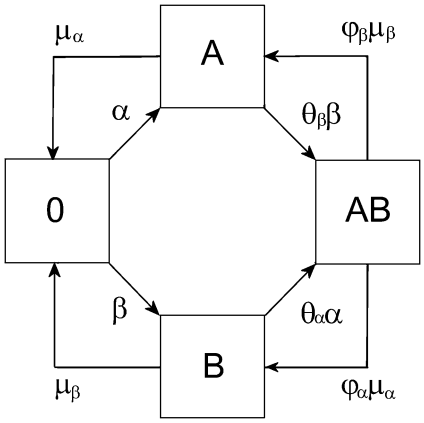

Figure 1. Epidemiologic "states" (compartments) and "transitions" for acquisition and clearance of 2 *Streptococcus pneumoniae* serotypes, A and B. State "0" denotes noncarriage, and state "AB" denotes simultaneous carriage of types A and B. The actual analysis accommodated the corresponding states: "noncarriage," "single carriage" for each of the 8 serotypes and the deidentified group (9 states altogether), and "double carriage" for all pairwise combinations of states of single carriage ($9 \times 8/2 = 36$ states). For more details, see the Web Appendix (http://aje.oxfordjournals.org/).

Samples that had been collected more than 45 days from any other samples in the individual were discarded in the episode-based analyses. These 67 samples were not different from the included samples with respect to age at sampling, prevalence of carriage, or number of siblings (data not shown).

Episodes of carriage were split as being either "single carriage" (only 1 serotype) or "double carriage" (2 simultaneous serotypes) (see Figure 1). To study whether current carriage affected pneumococcus acquisition, we estimated acquisition rates separately for noncarriers (e.g., rate α; see Figure 1) and carriers (rate $\theta_\alpha\alpha$). Likewise, to study whether pneumococcus clearance was affected by the presence of another serotype, we estimated clearance rates separately for single (e.g., rate μ_α) and double (rate $\varphi_\alpha\mu_\alpha$) carriers. The 8 target serotypes were taken to share a common rate of acquisition and a common rate of clearance, but each episode was identified individually. The deidentified group had its own rate parameters. The interval-censored times of onset and clearance of carriage were taken into account in the statistical analysis (see the Web Appendix, which is posted on the *Journal*'s Web site (http://aje.oxfordjournals.org/)).

The observed data included transitions that bypass an intermediate epidemiologic state in Figure 1. There were 72 instances in which a participant was observed to first carry a specific serotype at one visit and then another serotype at a visit made 1 month later. In addition, in 3 instances, a participant was observed to be a noncarrier at one visit and then a double carrier at the next visit; and in 5 instances, a participant was observed to be a double carrier at one visit and then a noncarrier at the next visit. In the crude analysis, the unknown intermediate states were imputed for convenience. In the actual analysis using statistical modeling, such intermediate states were treated as unknown variables (see Web Appendix). The 6 instances in which more than 2 transitions would have been required were omitted from these analyses.

Serotype-specific exposure to pneumococci was defined as carriage (yes/no) of the target type by at least 1 other person, including employees, in the DCC during the child's episode (see Web Appendix). The effect of this binary covariate was assumed to be the same for all target serotypes. Results from the statistical model are given as Bayesian posterior mean estimates of the parameters and their 95% credible intervals.

RESULTS

Carriage of pneumococci and the serotype distribution

In the children, 74.1% (423/571) of samples were positive for pneumococci. The proportion of carriers was 80% in samples taken from children under 2 years of age, as compared with 70% in the older children. The prevalence of pneumococcal carriage was relatively high in the employees (21.7%; 35/161). Carriage with multiple pneumococcal serotypes was common among children: 10.9% of positive samples (46/423) contained more than 1 serotype (25/186 in children aged <2 years and 21/237 in older children), with 41 and 5 instances of 2 and 3 simultaneous serotypes, respectively. In the employees, the proportion of carriers of multiple serotypes was likewise high, as 20% of positive samples contained more than 1 serotype.

Table 1 shows the serotype distribution among the 474 isolates in the children. The usual pediatric serotypes dominated, with the 8 most common serotypes accounting for 77% of the isolates. The serotypes observed in the employees were similar to those in the children, except for serotype 20, which was found disproportionately in the employees (9 of the 19 isolates of serotype 20 in the data).

The serotype distribution among the 298 episodes of carriage in children was similar to that in the isolates (Table 1). The serotypes in double carriers reflected those in single carriers (data not shown). Splitting the follow-up time according to carriage's being "single" or "double" resulted in 252 episodes of single carriage and 28 episodes of double carriage. The total person-time for double carriage was 25 person-months, approximately 10% of the total person-time spent carrying pneumococci. There were 167 episodes of noncarriage.

Crude analysis

Table 2 presents the crude rates of pneumococcus acquisition (any serotype) in the children, calculated from numbers of acquisitions per person-time, stratified according to current carriage status ("noncarriage" vs. "single carriage"). The crude rate of pneumococcus acquisition was 1.022 per month in a noncarrying child but only 0.085 per month in a carrying child. This difference suggests strongly reduced acquisition of other serotypes in already-colonized

Table 1. Distribution of *Streptococcus pneumoniae* Serotypes Among Children and Employees at 3 Day-Care Centers in Denmark, 1999–2001

Serotype	Isolates						Episodes[a] (Among Children)	
	Children		Employees		Total			
	No.	%	No.	%	No.	%	No.	%
23F	92	19.4	5	11.4	97	18.7	51	17.1
19F	86	18.1	2	4.5	88	17.0	54	18.1
6B	53	11.2	3	6.8	56	10.8	26	8.7
6A	45	9.5	3	6.8	48	9.3	34	11.4
19A	27	5.7	3	6.8	30	5.8	13	4.4
14	22	4.6	1	2.3	23	4.4	17	5.7
15B/C	21	4.4	5	11.4	26	5.0	14	4.7
11A	19	4.0	0	0.0	19	3.7	14	4.7
22F	18	3.8	0	0.0	18	3.5	12	4.0
33F	12	2.5	1	2.3	13	2.5	9	3.0
9V	10	2.1	1	2.3	11	2.1	5	1.7
20	10	2.1	9	20.5	19	3.7	10	3.4
Nontypable isolates	14	3.0	7	15.9	21	4.1	32	10.7
Other serotypes[b]	45	9.5	4	9.1	49	9.5	7	2.3
Total	474	100	44	100	518	100	298	100

[a] An episode of pneumococcal carriage was defined as a series of isolations of a homologous (same) serotype at consecutive visits.

[b] Serotypes in the children (total numbers of isolates): 10A (8), 18C (8), 35F (7), 3 (5), 4 (4), 16F (2), 17F (2), 18B (2), 21 (2), 35B (2), 37 (2), 7B (2), 23A (1), 31 (1), 38 (1), 7F (1), 9N (1). Serotypes in the employees (total numbers of isolates): 35F (2), 21 (1), 37 (1).

hosts. For individual serotypes, the crude acquisition rates were at least 1 magnitude smaller than the overall rate of acquisition (Table 2). The numbers of acquisitions were small, excluding the possibility of addressing possible differences across serotypes.

Table 3 presents the crude rates of clearance in the children, calculated from numbers of clearances per person-time, stratified according to carriage's being ''single'' or ''double.'' If the rate of clearance of individual serotypes is faster in double carriers than in single carriers, the rate of clearance of double carriage (to single carriage of either of the 2 types) should be more than 2-fold that in single carriers. However, the crude estimate of this ratio was only 1.5 (0.94 vs. 0.65), suggesting that such enhancement of clearance does not occur in double carriers.

Adjusted analysis

Table 4 summarizes results from the statistical model. The posterior mean rate of acquisition for a target serotype in a noncarrying child was 0.06 per month (95% credible interval (CI): 0.04, 0.09). This rate appears smaller than could be inferred from the crude analysis (Table 1). However, unlike the crude rate, the adjusted rate represents that in the absence of exposure to the type in question within the DCC. For the deidentified group, the rate of acquisition was higher (0.24 per month) than for an individual target serotype, describing the total rate of the 21 minor serotypes in the data. This rate is very similar to that from the crude analysis (neither analysis adjusted for exposure to this nonspecific ''type''). Based on the results in Table 4, the overall rate of pneumococcus acquisition in a noncarrying child was approximately 0.7 per month (i.e., $8 \times 0.06 + 0.24$).

The current status of carriage affected pneumococcus acquisition. The relative rate of acquisition (carrying vs. noncarrying children) was 0.09 (95% CI: 0.05, 0.15). For the deidentified group, the relative rate was similar (relative rate = 0.11, 95% CI: 0.02, 0.31). These results, of an approximately 10-fold higher rate of acquisition in noncarriers, are parallel to those from the crude analysis and indicate strong competition among serotypes to colonize hosts.

In addition to current status of carriage in the child, the rate of acquisition was clearly affected by exposure to pneumococci within the DCC. The relative rate of acquisition for a target type was 2.7 (95% CI: 1.7, 4.4) when a child was exposed to that type within the DCC, as compared with the case where there was no exposure to the target type.

For a target serotype, the rate of clearance was 0.63 per month (95% CI: 0.51, 0.79). The relative rate of clearance (double carrier vs. single carrier) was 0.81 (95% CI: 0.48, 1.26). For the deidentified group, the rate of clearance was 0.63 (95% CI: 0.41, 0.90), and the relative rate of clearance (double carriers vs. single carriers) was 0.80 (95% CI: 0.22, 2.17). The findings of similar clearance rates in single and double carriers again agree with those of the crude analysis, suggesting that the presence of another serotype does not affect clearance of other colonizing serotypes.

Finally, the model was used to assess the likely course of transitions in the instances of unknown intermediate states. For observed transitions from carriage of one type (e.g., A; see Figure 1) to carriage of another type (B), approximately 90% were estimated to have taken place through the state of ''noncarriage.'' This result reflects the estimated strong inhibition of current carriage against acquisition of other serotypes. Transitions from noncarriage to double carriage (target types A and B) were estimated to take place half of the time through carriage of type A, as a consequence of the assumed similarity of all target serotypes.

DISCUSSION

We employed longitudinal data on pneumococcal carriage in children to study interactions between co-colonizing serotypes. Detection of pneumococcal carriage was based on a new method that allows more sensitive measurement of carriage of multiple pneumococcal populations in the same nasopharyngeal sample than the standard method. We found that children simultaneously carrying 2 different serotypes cleared carriage of either of the serotypes at the same rate as children carrying only 1 serotype. In contrast, acquisition of another serotype was 10 times slower in children who already carried 1 serotype. These epidemiologic findings imply that between-strain competition among pneumococci works primarily through acquisition, current

Table 2. Acquisition of *Streptococcus pneumoniae* Carriage (Crude Analysis) Among Children at 3 Day-Care Centers in Denmark, 1999–2001

Target Serotype	Episodes of Noncarriage				Episodes of the Nontarget Type (Single Carriers)			
	No. of Episodes	Person-Time at Risk, months[a]	No. of Acquisitions[b]	Rate (Acquisitions per Month)	No. of Episodes	Person-Time at Risk, months[c]	No. of Acquisitions[d]	Rate (Acquisitions per Month)
6B	167	126.28	20	0.158	220	170.12	2	0.012
6A	167	126.28	8	0.063	228	179.60	2	0.011
23F	167	126.28	26	0.206	206	159.65	1	0.006
19F	167	126.28	22	0.174	207	167.05	6	0.036
14	167	126.28	10	0.079	239	187.00	1	0.005
19A	167	126.28	5	0.040	236	188.35	1	0.005
15B/C	167	126.28	7	0.055	241	195.27	1	0.005
11A	167	126.28	5	0.040	242	194.23	0	0
Deidentified group	167	126.28	26	0.206	197	158.47	3	0.019
Total	167	126.28	129	1.022	252[e]	199.97	17	0.085

[a] The total time spent noncarrying in the study cohort.

[b] Acquisition was defined as the onset of an episode of the serotype in question (the target serotype). For an observed transition from carriage of one serotype (type A) to carriage of another type (type B) (72 instances altogether), the event of clearing type A was imputed midway between the 2 observation times.

[c] The total time spent carrying some type other than the target type. For all serotypes (''Total'' line), the total time of single carriage of any of the serotypes.

[d] Acquisition was defined as the onset of carriage of the serotype in question in an already-carrying child. For an observed transition from carriage of one serotype (type A) to carriage of another type (type B) (72 instances altogether), the event of clearing type A was imputed midway between the 2 observation times.

[e] This is the total number of episodes of single carriage. For example, there were 220 non-6B episodes of single carriage and 32 6B episodes of single carriage, making the total 252; likewise for any of the other serotypes.

carriage effectively impairing acquisition of other pneumococcal strains.

The data were derived from children attending 3 DCCs in Denmark, a country with low levels of antibiotic resistance in invasive pneumococcal disease, associated with an internationally low level of antibiotic usage in all age groups (29). The 7-valent pneumococcal conjugate vaccine was introduced in the Danish childhood vaccination program in October 2007. The current study thus represented a unique opportunity to study the natural dynamics of pneumococcal carriage among children in day-care settings, without the influence of pneumococcal vaccination and with low usage of antimicrobial agents.

The data included a number of consecutive pairs of observations within an individual that bypassed an intermediate epidemiologic state in the model shown in Figure 1. Most notably, there were 72 pairs of consecutive samples taken fewer than 45 days apart, where a participant was first observed to carry one serotype and subsequently observed to carry another. In the crude analysis of acquisition (Table 2), all such transitions from carriage of one type to carriage of another were assumed to have occurred through an intermediate state of ''noncarriage.'' In this analysis, the ratio of the crude rates of acquisition (double vs. single carriage) was approximately 0.1. However, had all these transitions taken place through double carriage, the ratio would have been approximately 0.7.

To address the problem of missing data, we employed a statistical model to probabilistically impute unknown intermediate states. For example, in each of the 72 instances, the intermediate state was assigned statistically as either ''noncarriage'' or ''double carriage.'' The results of this analysis clearly suggest that a great majority (90%) of transitions from carriage of one serotype to carriage of another indeed occur as a sequence of events, where single carriage is first cleared and the other type is then acquired. In terms of model parameters, this is reflected in the strong inhibition provided by current carriage against acquisition of another type, with a relative rate of only 9% of that in noncarriers (Table 4).

It is important for analyses of between-strain competition that pneumococcal carriage be identified with sufficiently high sensitivity. Two aspects must be considered here. First, the analysis in this paper was based on episodes of carriage that were determined from repeated (monthly) within-individual measurements. It is then possible that short periods of carriage remained completely unobserved or were artificially lengthened when episodes of carriage were created, causing problems for the analysis of episodes of double carriage in particular. Second, the laboratory sensitivity to observe populations of different pneumococcal serotypes may hinder the analysis of multiple carriage.

Even if the true episodes of double carriage were shorter than those of single carriage, episodes based on sampling that is too infrequent could appear similar in length. This would result in a false conjecture on reduced acquisition (because of completely missing episodes) and clearance (because our episode definition would create artificially long

Table 3. Clearance of *Streptococcus pneumoniae* Carriage (Crude Analysis) Among Children at 3 Day-Care Centers in Denmark, 1999–2001[a]

Serotype	Carriage of a Single Serotype					Carriage of 2 Serotypes				
	No. of Episodes	Person-Time, months	Mean (Median) Duration, days	No. of Clearances	Rate (Clearances per Month)[b]	No. of Episodes	Person-Time, months	Mean (Median) Duration, days	No. of Clearances	Rate (Clearances per Month)[c]
6B	32	29.85	28 (20)	17	0.57	6	3.70	19 (18)	2	0.54
6A	24	20.37	25 (14)	12	0.59	9	5.48	18 (17)	2	0.36
23F	46	40.32	26 (18)	22	0.55	7	5.58	24 (14)	3	0.54
19F	45	32.92	22 (17)	23	0.70	11	9.08	25 (14)	3	0.33
14	13	12.97	30 (18)	10	0.77	1	1.03	31 (31)	0	0
19A	16	11.62	22 (16)	9	0.77	4	2.85	21 (19)	1	0.35
15B/C	11	4.70	13 (13)	4	0.85	4	1.65	12 (12)	0	0
11A	10	5.73	17 (14)	6	1.05	3	1.40	14 (14)	3	2.14
Deidentified group	55	41.50	23 (16)	27	0.65	11	5.25	14 (14)	3	0.57
Total	252	199.98	24 (17)	130	0.65	28	18.01	19 (14)	17	0.94

[a] For imputation to handle missing data, see the footnotes of Table 2.

[b] For a given serotype, calculated as the number of clearances of that serotype, divided by the person-time of single carriage of that serotype. For all serotypes ("Total" line), the person-time is that spent carrying any serotype.

[c] For a given serotype, calculated as the number of clearances of that serotype in double carriers, divided by the person-time of double carriage involving that serotype. For all serotypes ("Total" line), calculated as the number of clearances of either of the 2 carried serotypes divided by the person-time of double carriage. Note that for any individual serotype, the person-time is counted once for both of the carried serotypes.

episodes) for double carriage. We tested this possible explanation using simulated data with tripled rates of acquisition to and from double carriage. This leaves the prevalence of double carriage intact at the observed level of approximately 11%, while explaining competition with both reduced acquisition ($\theta \approx 0.3$) and enhanced clearance ($\varphi \approx 3$). However, the pattern of transitions in the simulated data was clearly different than what was observed. For example, double carriage continued 1 month afterwards in only 1% of the simulated data, in comparison with the 21% in the actual observations. Consequently, even though it is possible that enhanced acquisition occurs in double carriage, reduced acquisition by current carriage still seems the main mechanism of competition.

Another caveat in interpreting the data is that 100% sensitivity in detecting double carriage was assumed. However, a reasonably good sensitivity is indicated by the fact that clearance of individual serotypes in double carriers was estimated to occur at the same rate as clearance in single carriers. If true double carriers were haphazardly classified as single carriers of either of the carried types, clearance of double carriage would actually be slower than detected. Clearly, this is against the hypothesis of enhanced clearance. Good sensitivity is also indicated by the fact that there were relatively few children who carried the same serotype in another episode. Only 15% of the episodes were reacquisitions in the sense that there was more than 1 episode of the serotype in question in the child.

Only a few epidemiologic studies have previously considered between-strain competition among pneumococci. Based on follow-up of pneumococcal carriage in families, Melegaro et al. (18) suggested that current carriage of a particular serotype reduces the rate of acquisition of other serotypes. However, their model accommodated only single carriage. Even if the rates of acquisition were equal in noncarriers and carriers, it is then possible that between-serotype competition would be inferred, because infrequent sampling produces what appears as direct transitions from carriage of one type to another. In the present paper, the model of pneumococcal carriage accommodated both single carriage (only 1 colonizing serotype in an individual) and double carriage (2 simultaneously colonizing serotypes). The results still indicate that different serotypes compete, but the strength of competition may have a more mechanistic interpretation in the analysis of the present paper.

We did not consider differences across serotypes, because the numbers of episodes of double carriage were small for any individual serotype. However, we surmise that the general pattern of interactions as revealed by the present analysis could at least apply to all relatively common pneumococcal serotypes or strains. This does not exclude the possibility of differential strain-strain interactions in competition. The present analysis focused on simultaneous interactions of different serotypes, as it did not address the question of acquired immunity to subsequent acquisition or clearance. The results thus pertain to the average pattern of pneumococcal carriage in toddlers.

The biologic mechanism that inhibits simultaneous colonization by more than 1 pneumococcal strain is incompletely understood. However, a likely candidate is direct

Table 4. Rates of Acquisition and Clearance of *Streptococcus pneumoniae* Carriage (Adjusted Analysis) Among Children at 3 Day-Care Centers in Denmark, 1999–2001

Parameter	Target Serotype		Deidentified Group	
	Estimate[a]	95% CI	Estimate[a]	95% CI
Rate of acquisition in noncarrying children per month	0.06[b]	0.04, 0.09	0.24[c]	0.16, 0.33
Relative rate of acquisition in carrying children vs. noncarrying children	0.09	0.05, 0.15	0.11	0.02, 0.31
Rate of clearance in single carriers per month	0.63	0.51, 0.79	0.63	0.41, 0.90
Relative rate of clearance in double carriers vs. single carriers	0.81	0.48, 1.26	0.80	0.22, 2.17

Abbreviation: CI, credible interval.

[a] The posterior mean estimates and 95% credible intervals are given for the 2 sets of acquisition and clearance rates (target type, deidentified group) in children, obtained by fitting a longitudinal model to the data from 3 Danish day-care centers (April 1999–February 2001).

[b] The analysis of the target types was adjusted for serotype-specific exposure within the day-care center, with the mean effect of exposure (presence of the target type) on acquisition estimated at 2.7 (95% CI: 1.7, 4.4).

[c] The analysis of the deidentified group was not adjusted for exposure, explaining the higher rate of acquisition than in a target serotype (0.24 acquistions per month vs. 0.06 per month).

killing of one strain (competitor) by another (the resident strain), mediated by bacteriocins, small peptides produced by many bacterial species. In *S. pneumoniae*, the *blp* cluster of genes determines the synthesis and secretion of several putative bacteriocins and their corresponding immunity proteins, which prevent the strain from being killed by the bacteriocin it produced. A remarkable feature of the *blp* cluster is its high genetic degree of diversity, with signs of frequent gene rearrangements, fitting the proposed role as the material basis for interstrain competition (30, 31).

The *blp* cluster is under the control of a complex set of regulatory sequences closely resembling the quorum-sensing (*com*) system, which triggers genetic competence in high-density populations of pneumococci. Strain specificity is a characteristic of both regulons, based on recognition of a small peptide pheromone. The *com* system has other resemblances to the *blp* bacteriocin system: It determines several cell-wall-degrading enzymes that participate in killing of noncompetent pneumococci during transformation in a process called fratricide. Together the *com* and *blp* systems have properties that could account for the competition between pneumococcal strains. The competence system is clearly part of the regulation of genetic transformation, with the likely function of providing for gene exchange and eventual evolution. Whether this, rather than competition between strains, would be the primary function of the system remains an open question (32, 33).

The extent of serotype replacement under large-scale vaccination depends on the strength of competition between vaccine and nonvaccine strains. Using the model of Figure 1, Lipsitch (17) showed that when competition works through reduced acquisition, vaccination with a vaccine targeted against a specific serotype induces replacement of carriage by the nonvaccine type. Using a similar model, Zhang et al. (19) argued that vaccination is likely to induce notable replacement only under competition in colonizing hosts, whereas acquired cross-immunity may not be an important determinant of population-level effects. Zhang et al. (19) further argued that it may not be important to identify whether such *direct* competition works through reduced acquisition or enhanced clearance in order to predict postvaccination levels of serotype-specific carriage. However, the mechanism of replacement can be hypothesized to have a greater impact on pneumococcal disease. Replacement disease should be more prominent when competition and replacement in carriage work through acquisition, because acquisition is generally believed to pose the highest risk of developing pneumococcal disease (34, 35).

Here we have reported new evidence for between-strain competition among pneumococci, suggesting that the essential mechanism of competition works in acquisition rather than in clearance of carriage. This finding was based on the relative rarity of double (or multiple) carriage in comparison with the point prevalence of individual serotypes in the study population. These inferences should be reexamined with data from other epidemiologic settings and populations. The dependence of the strength of inferred competition on the sensitivity of pneumococcus detection should be acknowledged. Future inferences on strain competition will greatly benefit from advances in the methodology of detecting multiple carriage.

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