The Pneumococcal Pilus Predicts the Absence of *Staphylococcus aureus* Co-Colonization in Pneumococcal Carriers

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The determinants of the negative association between *Streptococcus pneumoniae* and *Staphylococcus aureus* colonization are unknown. In this matched case-control study, the odds of co-colonization with *S. aureus* were significantly lower for individuals carrying a piliated versus a nonpiliated *S. pneumoniae* strain, suggesting the pilus may be a determinant of the negative association.

*Staphylococcus aureus* and *Streptococcus pneumoniae* are important pathogens that cause significant morbidity and mortality worldwide [1, 2]. *S. aureus* is carried by 30% of healthy adults and ~10% of young children, usually in the anterior nares [2]. In contrast, *S. pneumoniae* is mainly carried by children [1], most frequently in the nasopharynx [3].

Carriage by healthy individuals is the source of transmission for both pathogens, and carriage is the initial step of invasion [4, 5]. Several epidemiologic studies have reported a negative association between *S. pneumoniae* nasopharyngeal colonization and *S. aureus* nasal colonization [6–9], which was most significant for *S. pneumoniae* serotypes included in the 7-valent pneumococcal conjugate vaccine [6, 7]. This finding raised public health concern that an increase in colonization and disease due to *S. aureus* may follow the implementation of the 7-valent pneumococcal conjugate vaccine [10].

We have been studying possible mechanisms of the negative association between these 2 pathogens. We have shown that hydrogen peroxide (H₂O₂) produced by *S. pneumoniae* is directly bactericidal to *S. aureus* in vitro [11]. Although strains that produce a high level of H₂O₂ were less likely to be found in a host co-colonized with *S. aureus*, the effect was modest and not statistically significant, thus leaving the serotype specificity of this negative association largely unexplained. Therefore, we investigated the effect of other factors described elsewhere [11, 12].

Two groups have identified the presence of a pilus-like structure in *S. pneumoniae* [13, 14]; this structure was characterized as an adhesin and an inflammatory virulence factor [13, 15]. This pilus is present mainly in 7-valent pneumococcal conjugate vaccine serotypes [16–18]. Therefore, we tested the hypothesis that piliated pneumococcal strains are less likely to co-colonize with *S. aureus* than are non-piliated strains; we used a collection of strains from a previous study on *S. pneumoniae* and *S. aureus* carriage that was performed in Israel [6]. Using a matched analysis that compared strains of the same serotype, we were able to avoid the confounding effect of serotype and to ask whether, within each serotype, the presence of the pilus was independently predictive of a lower probability of *S. aureus* co-colonization.

**Methods.** The original study [6] took place during 2002–2005 in primary care clinics of Maccabi Healthcare Services, an Israeli Health Maintenance Organization. Approximately 4500 children aged <40 months were screened for carriage of nasal *S. aureus* and nasopharyngeal *S. pneumoniae*. None of the subjects had received any pneumococcal vaccine.

The pilus operon encodes 3 structural proteins, RrgA, RrgB, and RrgC. The *rrgC* gene has been shown to be 100% conserved across strains, and its presence (detected by PCR) is an excellent predictor of the presence of the pilus by Western Blot analysis [14, 16]. To screen strains, boilates of bacterial colonies were used as DNA templates in a PCR assay for the amplification of *rrgC*. Primers were 5′-GCTCTGTGTTTTTCTCTGTGA-TGG-3′ and 5′-ATCGATCGTGGTGTGATTATTTTTA-3′.

The reaction conditions were 30 cycles at 94°C for 15 s, 55°C for 30 s, and 72°C for 1 min per kilobase, followed by 5 min at 72°C.

Two hundred seventy strains that were selected initially to assess H₂O₂ production in vaccine-type (VT) versus non-VT strains of *S. pneumoniae* were reassessed to determine the prev-
Figure 1. Prevalence of the pilus by serotype, as determined by PCR of the *rgpC* gene, among the 270 selected strains (A) and among the vaccine-type strains in the case-control (B) study. Case strains (gray bars) were isolates from patients who were co-colonized with both *Staphylococcus aureus* and *Streptococcus pneumoniae*. Control strains (black bars) were isolates from patients who were colonized with only *S. pneumoniae*. The prevalence of the pilus was highest among the VT strains (67 [52%] of 130), with the exception of serotype 18C, in which none of the 7 strains tested had a pilus. The pilus was significantly less prevalent among VT-related strains (4 [13%] of 32; 2 each of serotypes 23A and 6A; *P* < .001, compared with pilus prevalence among VT strains) or non-VT strains (2 [2%] of 108; 1 each of serotypes 17F and 11A; *P* < .001, compared with pilus prevalence among VT strains) (figure 1A).

To determine whether pneumococcal strains that carry the pilus are particularly protective against *S. aureus* carriage, we conducted a case-control study focusing on VT strains. Sixty-one case strains and 236 matched control strains were included in the analysis (table 1). The frequency of the pilus among *S. pneumoniae* strains found in children co-colonized with *S. aureus* was less than or equal to that among strains isolated from non–co-colonized children for every serotype considered (figure 1B). To further adjust for all matched factors (patient age and day care center attendance), a conditional logistic analysis was performed and determined that the odds of co-colonization with *S. aureus* were 2.13-fold lower for individuals carrying a pilated versus a non-piliated *S. pneumoniae* strain (OR, 0.47; 95% CI, 0.24–0.95).

Discussion. The mechanisms of the negative association between *S. pneumoniae* and *S. aureus* colonization remain to be defined. On the basis of prior studies [6–8, 11, 12, 19], it is likely that the mechanism is multifactorial and probably involves both host immune responses to colonization and bacterial characteristics that may promote inhibition of the other...

To determine whether pneumococcal strains from individuals who are co-colonized with *S. aureus* and *S. pneumoniae* are less frequently piliated than are strains from individuals colonized with *S. pneumoniae* only, we conducted a case-control study. Because of prevalence results in this study population, we focused only on strains of serotypes that sometimes carry the pilus (i.e., VT strains), excluding serotype 18C, which is not piliated here or in other collections [17, 18]. Case strains and control strains were selected from a collection of ∼2000 strains isolated from the nasopharynx ([6] and G.R.-Y., unpublished data). Case strains were defined as pneumococcal strains isolated from individuals who were co-colonized with *S. aureus*, and control strains were defined as strains isolated from individuals colonized only with *S. pneumoniae*. Each case strain was matched with 3–5 control strains by patient age (within 6 months), patient day care center attendance, and serotype. After limiting the full collection to only VT strains, 78 case strains were available. For several of the case strains, we could not identify 3 matching control strains; these case strains were excluded from analysis. A total of 61 case strains and 236 control strains were included. A matched analysis was performed using conditional logistic regression.

Results. The 270 selected strains consisted of 130 VT strains, 32 VT-related strains (serotypes 6A, 9A/B/N, 19A, and 23A/B), and 108 non-VT strains. The non-VT strains represented 16 different capsular types, of which 40 (37%) were serogroup 15, 10 (9%) were serotype 3, 9 (8%) were serotype 16F, and 7 (6%) were serogroup 35; the remaining 42 strains (39%) belonged to 12 different serotypes.

The pilus was present in 79 (29%) of 270 strains. The prevalence of the pilus was highest among the VT strains (67 [52%] of 130), with the exception of serotype 18C, in which none of the 7 strains tested had a pilus. The pilus was significantly less prevalent among VT-related strains (4 [13%] of 32; 2 each of serotypes 23A and 6A; *P* < .001, compared with pilus prevalence among VT strains) or non-VT strains (2 [2%] of 108; 1 each of serotypes 17F and 11A; *P* < .001, compared with pilus prevalence among VT strains) (figure 1A).

After standardization, the prevalence of a pilus in the Israeli study population is estimated to be 30%, with a pilus detected for 62.6% of VT strains, 17.8% of VT-related strains, and <1% of non-VT strains. These figures are all within 4% of the crude (unstandardized) estimates, indicating that the nonrandom strain selection did not importantly affect our estimates of pilus frequency.

To determine whether pneumococcal strains that carry the pilus are particularly protective against *S. aureus* carriage, we conducted a case-control study focusing on VT strains. Sixty-one case strains and 236 matched control strains were included in the analysis (table 1).

The frequency of the pilus among *S. pneumoniae* strains found in children co-colonized with *S. aureus* was less than or equal to that among strains isolated from non–co-colonized children for every serotype considered (figure 1B). To further adjust for all matched factors (patient age and day care center attendance), a conditional logistic analysis was performed and determined that the odds of co-colonization with *S. aureus* were 2.13-fold lower for individuals carrying a pilated versus a non-pilated *S. pneumoniae* strain (OR, 0.47; 95% CI, 0.24–0.95).

Discussion. The mechanisms of the negative association between *S. pneumoniae* and *S. aureus* colonization remain to be defined. On the basis of prior studies [6–8, 11, 12, 19], it is likely that the mechanism is multifactorial and probably involves both host immune responses to colonization and bacterial characteristics that may promote inhibition of the other...
in the study design, is that the prevalence of co-colonization is negatively associated with S. aureus. Unknown confounders can never be fully explained by at least 2 mechanisms. Because the pilus is an inflammatory structure [13], piliated strains may directly induce a host immune response that is deleterious to S. aureus colonization. A non–mutually exclusive alternative is that piliated pneumococci are more adherent [15] and are, therefore, more able to antagonize staphylococcal colonization by other (non–pilus-mediated) inhibitory effects, such as H$_2$O$_2$ secretion or some other mechanism.

Our findings that the presence of the pilus in pneumococci is negatively associated with S. aureus co-colonization could be explained by at least 2 mechanisms. Because the pilus is an inflammatory structure [13], piliated strains may directly induce a host immune response that is deleterious to S. aureus colonization. A non–mutually exclusive alternative is that piliated pneumococci are more adherent [15] and are, therefore, more able to antagonize staphylococcal colonization by other (non–pilus-mediated) inhibitory effects, such as H$_2$O$_2$ secretion or some other mechanism.

Our study is inherently limited in its ability to establish a causal relation between carriage of S. pneumoniae with a pilus and the inhibition of S. aureus colonization; a case-control study can only reveal an association that can be suggestive of a causal relation. Unknown confounders can never be fully ruled out in such studies. An additional limitation, also inherent in the study design, is that the prevalence of co-colonization was determined in a point-prevalence study, which does not provide a direct estimate of the impact of S. pneumoniae on preventing the incidence of or shortening the duration of S. aureus carriage.

Despite these limitations, this study suggests that the pilus is a determinant of the negative association between S. pneumoniae carriage and S. aureus carriage. The fact that the association was reproduced for 5 of 6 serotypes examined reduces the likelihood that some other bacterial factor that is more common in piliated strains was responsible for the negative association, because one would expect that the same factor would not be consistently associated with the pilus in unrelated serotypes. Additional mechanistic studies will be required to assess how the pilus might play such a role. In addition, ongoing epidemiologic studies of the prevalence of the pilus in different populations will be required in order to test a key prediction of this hypothesis, that the impact of pneumococcal vaccination on S. aureus carriage will depend on whether it continues to reduce the frequency of piliated strains, compared with the frequency in unvaccinated populations.

Table 1. Characteristics of the population of the case-control study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) of isolates</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Case strains (n = 61)</td>
</tr>
<tr>
<td>Isolated from adults</td>
<td>13 (21)</td>
</tr>
<tr>
<td>Isolated from children</td>
<td>48 (79)</td>
</tr>
<tr>
<td>Patient age, months</td>
<td></td>
</tr>
<tr>
<td>0–12</td>
<td>13 (21)</td>
</tr>
<tr>
<td>13–30</td>
<td>16 (26)</td>
</tr>
<tr>
<td>31–40</td>
<td>19 (31)</td>
</tr>
<tr>
<td>Patient attended day care center</td>
<td>34/49 (69)</td>
</tr>
<tr>
<td>S. pneumoniae serotype</td>
<td></td>
</tr>
<tr>
<td>23F</td>
<td>22 (36)</td>
</tr>
<tr>
<td>19F</td>
<td>15 (25)</td>
</tr>
<tr>
<td>6B</td>
<td>15 (25)</td>
</tr>
<tr>
<td>14</td>
<td>6 (10)</td>
</tr>
<tr>
<td>9V</td>
<td>2 (3)</td>
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<tr>
<td>4</td>
<td>1 (2)</td>
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NOTE. Case strains were isolates from patients who were co-colonized with both Staphylococcus aureus and Streptococcus pneumoniae. Control strains were isolates from patients who were colonized with only S. pneumoniae.

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References


