Association Between Carriage of Streptococcus pneumoniae and Staphylococcus aureus in Children

Gili Regev-Yochay, MD
Ron Dagan, MD
Meir Raz, MD
Yehuda Carmeli, MD, MPH
Bracha Shainberg, PhD
Estela Derazne, MSc
Galia Rahav, MD
Ethan Rubinstein, MD

Streptococcus pneumoniae and Staphylococcus aureus are common inhabitants of the upper respiratory tract in children and are responsible for common infections. Carriage of S aureus and S pneumoniae can result in bacterial spread and endogenous infections.1-3 Streptococcus pneumoniae is carried in the nasopharynx by most children at least once during early childhood1 but not frequently by adults.5 Staphylococcus aureus is carried by 10% to 35% of children5-7 and by approximately 35% of the general adult population.3 Various studies have explored bacterial interference—the suppression of one species by another.8-11 However, studies examining possible interference between S aureus and S pneumoniae are noticeably absent. An association between these 2 pathogens may suggest epidemiologic changes that could follow widespread vaccination with pneumococcal conjugate vaccines.

We investigated the possible association between the 2 pathogens by studying their prevalence and risk factors for carriage in young children in a region where pneumococcal conjugate vaccination is not practiced.

METHODS
The study was approved by the Sheba Medical Center Ethics Committee, Ramat-Gan, Israel. Informed consent was obtained from all parents.

Study Population
During February 2002, children aged 40 months or younger seen at primary care clinics in central Israel during February 2002.

Context Widespread pneumococcal conjugate vaccination may bring about epidemiologic changes in upper respiratory tract flora of children. Of particular significance may be an interaction between Streptococcus pneumoniae and Staphylococcus aureus, in view of the recent emergence of community-acquired methicillin-resistant S aureus.

Objective To examine the prevalence and risk factors of carriage of S pneumoniae and S aureus in the prevaccination era in young children.

Design, Setting, and Patients Cross-sectional surveillance study of nasopharyngeal carriage of S pneumoniae and nasal carriage of S aureus by 790 children aged 40 months or younger seen at primary care clinics in central Israel during February 2002.

Main Outcome Measures Carriage rates of S pneumoniae (by serotype) and S aureus; risk factors associated with carriage of each pathogen.

Results Among 790 children screened, 43% carried S pneumoniae and 10% carried S aureus. Staphylococcus aureus carriage among S pneumoniae carriers was 6.5% vs 12.9% in S pneumoniae noncarriers. Streptococcus pneumoniae carriage among S aureus carriers was 27.5% vs 44.8% in S aureus noncarriers. Only 2.8% carried both pathogens concomitantly vs 4.3% expected dual carriage (P = .03). Risk factors for S pneumoniae carriage (attending day care, having young siblings, and age older than 3 months) were negatively associated with S aureus carriage.

Conclusions Streptococcus pneumoniae carriage, specifically of vaccine-type strains, is negatively associated with S aureus carriage in children. The implications of these findings in the pneumococcal vaccine era require further investigation.

JAMA. 2004;292:716-720
ish population. Each child was included once; accompanying adults (usually parents) were also screened. None of the children or their contacts received pneumococcal vaccine.

Study Design
Nasopharyngeal and nasal swabs were obtained from children and their accompanying adults, who also responded to an interviewer-administered questionnaire including demographic characteristics, number of young siblings (aged <6 years), day care attendance, prior antibiotic treatment, and smoking habits of family members. The physician’s diagnosis on the screening day and medical and immunization histories were obtained from patients’ files. The diagnoses were categorized as respiratory infections, skin diseases (including infections), other infections (including urinary tract infections and enteric infections), and noninfectious diagnoses.

Laboratory Procedures
Nasopharyngeal cultures were obtained with a rayon-tipped wire swab and nasal cultures of both nares were obtained with a sterile cotton polyester swab. Swabs were placed in Amies transport medium (Copan, Brescia, Italy). All specimens were processed within 6 hours.

Nasopharyngeal swabs for *S pneumoniae* isolation were streaked onto tryptic soy agar plates with 5% sheep blood and 5 µg/mL of gentamicin (HyLabs, Rehovot, Israel) and incubated aerobi cally at 35°C in 5% CO₂-enriched air. Suspect colonies were isolated and identified according to National Committee for Clinical Laboratory Standards recommendations. Serotyping of *S pneumoniae* was performed using antisera (Statens Serum Institute, Copenhagen, Denmark). Vaccine types were defined as serotypes included in the current 7-valent conjugate vaccine as well as the cross-reactive types (ie, serogroups 4, 6, 9, 14, 18, 19, and 23).

Nasal swabs for *S aureus* isolation were streaked onto tryptic soy agar plates with 5% sheep blood. *Staphylococcus aureus* was identified by morphology, β-hemolysis, catalase, DNAase, and coagulase production.

Statistical Analysis
We expected 15% of children to carry *S aureus* and 50% to carry *S pneumoniae*. To detect a difference of at least 7% in *S aureus* carriage rates among *S pneumoniae* carriers and noncarriers with α = .05 and 80% power, a sample size of 353 children in each group was needed.

Odds ratios (ORs) and Fisher exact tests were calculated to assess risk factors for carriage of each organism including age, sex, young siblings, dwelling density, passive smoking, day care attendance, respiratory tract infection diagnosis, chronic and recurrent diseases, *S pneumoniae* or *S aureus* carriage by the accompanying adult, steroid treatment, number of clinic visits and hospitalization in the last 6 months, and antibiotic treatment in the last month. Mantel-Haenszel common ORs and the Breslow-Day test for homogeneity were used to control for possible confounding variables (age and day care attendance).

A multivariate logistic regression model with stepwise backward elimination was performed separately for each pathogen. Variables with P < .10 in the univariate analysis were included. Interactions of *S pneumoniae* with day care attendance, age, and having young siblings were also included in the model for *S aureus* carriage. The criterion for entering into the model was a score statistic of *P* = .05. A Wald statistic of *P* = .10 was used to remove a variable from the model. −2 Log likelihood, the Nagelkerke *R*², and the Hosmer-Lemeshow test were used to assess goodness of fit. Risk factors were checked for confounding and collinearity. Cross-validation was used to assess overfitting. All tests used were 2-tailed, and *P* < .05 was considered statistically significant. Computations were performed with SPSS software, version 11.0 (SPSS Inc, Chicago, III) and S-Plus, version 6.2 (Insightful Corp, Seattle, Wash).

©2004 American Medical Association. All rights reserved.

RESULTS
A total of 790 children (90% of children approached) aged 5 days to 40 months (median, 1.3 years) were screened. Fifty-five percent were male. A total of 6.1% came for healthy check-up visits and 80% were diagnosed as having a respiratory tract infection. Chronic or recurrent disease (ie, asthma, recurrent otitis media, recurrent pneumonia, or skin disorders) was present in 27.8%.

*S phagolyticus* aureus and *S pneumoniae* were isolated in 80 children (10.1%) and 340 children (43.0%), respectively. The proportion of vaccine-type strains among *S pneumoniae* carriers was 74.2%. *Staphylococcus aureus* carriage among *S pneumoniae* carriers was 6.5% vs 12.9% in *S pneumoniae* noncarriers. *Streptococcus pneumoniae* carriage among *S aureus* carriers was 27.5% vs 44.8% in *S aureus* noncarriers. If carriage of the 2 organisms were independent (ie, occurring at random), the expected dual carriage would be 4.3%. However, dual carriage was found in only 22 children (2.8%) (OR, 0.47; 95% confidence interval [CI], 0.28-0.78; *P* = .03 by Fisher exact test).

Seven hundred four adults (621 mothers [88%], 77 fathers, and 6 other family members) aged 18 to 45 years (median, 30 years) were screened for *S aureus* nasal carriage and 693 for both pathogens. *Sphagolyticus aureus* and *S pneumoniae* were isolated from 182 (25.9%) of 704 and 35 (5.1%) of 693, respectively. The proportion of vaccine-type strains among *S pneumoniae* carriers was 66.7%. *Staphylococcus aureus* carriage among adults was similar in *S pneumoniae* carriers and noncarriers (25.7% and 25.3%, respectively), and *S pneumoniae* carriage was similar in *S aureus* carriers and noncarriers (51.5% and 50.0%, respectively). Dual carriage was found in 1.3%, the same as the expected prevalence (OR, 1.02; 95% CI, 0.47-2.23; *P* = .96 by Fisher exact test).

The highest *S aureus* carriage rate (30%) was observed in children aged 3 months or younger, in whom *S pneumoniae* prevalence was lowest (9%)
ASSOCIATION OF S PNEUMONIAE AND S AUREUS IN CHILDREN

(Figure 1). The highest S pneumoniae carriage rate (approximately 50%) was in children aged 7 to 40 months, in whom S aureus prevalence was the lowest (5%-9%).

In a univariate analysis, age older than 3 months, having young siblings, day care attendance, respiratory tract infection at screening, and prior steroid treatment were risk factors for S pneumoniae carriage in children (Table). These same factors were inversely associated with S aureus carriage. Being an S pneumoniae carrier was inversely associated with S aureus carriage and vice versa (P = .003). Antibiotic treatment during the prior month reduced carriage of both pathogens. Having a parent who was an S aureus carrier was a risk factor for carrying S aureus, but having a parent who was an S pneumoniae carrier was not associated with S pneumoniae carriage. To assess for confounding effects, the inverse relation of carriage was analyzed while controlling for age and day care attendance. In children, the prevalence of S aureus was lower among S pneumoniae carriers compared with S pneumoniae noncarriers in all age groups (Mantel-Haenszel OR, 0.51; 95% CI, 0.29-0.89; P = .003) (Figure 2). There was no evidence that this association was modified by age (Breslow-Day \( \chi^2 = 1.716; P = .63 \)).

The association between S pneumoniae and S aureus carriage was stratified by day care attendance. Among day care attendees, S aureus carriage was significantly lower among S pneumoniae carriers compared with S pneumoniae noncarriers (3.0% vs 9.8%; OR, 0.28; 95% CI, 0.11-0.73; P = .003). Among non–day care attendees, S aureus carriage rates were 11.3% and 11.7% in S pneumoniae carriers and noncarriers, respectively (OR, 0.96; 95% CI, 0.48-1.94; P = .54; conditional independence Mantel-Haenszel \( \chi^2 = 2.858 \), P = .09; homogeneity Breslow-Day \( \chi^2 = 4.313; P = .04 \)).

Risk factors for S pneumoniae carriage in a multivariate logistic analysis

Figure 1. Staphylococcus aureus and Streptococcus pneumoniae Prevalence by Age Group

![Graph showing carriage percentage of S aureus and S pneumoniae by age group.]

The association between age and the proportion of carriers was examined by trend test for linear association. A significant descending trend in S aureus carriage between age 3 months or younger and age 13 to 24 months (P < .001) and an ascending trend thereafter (P = .04) was found. For S pneumoniae, a significant increase was found among children older than 3 months vs younger children (P < .001).

Table. Risk Factors for Staphylococcus aureus and Streptococcus pneumoniae Carriage by Children (Univariate Analysis)

<table>
<thead>
<tr>
<th>Variables</th>
<th>S pneumoniae Carriers</th>
<th>S aureus Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥3 mo</td>
<td>733 / 57 (45.7)</td>
<td>63 / 17 (46.8)</td>
</tr>
<tr>
<td>Reference: age ≤3 mo</td>
<td>8.75 (3.46-22.17)</td>
<td>0.22 (0.12-0.41)</td>
</tr>
<tr>
<td>Day care attendance</td>
<td>2.32 (1.73-3.11)</td>
<td>0.39 (0.24-0.65)</td>
</tr>
<tr>
<td>Reference: no day care attendance</td>
<td>2.32 (1.73-3.11)</td>
<td>0.39 (0.24-0.65)</td>
</tr>
<tr>
<td>Have siblings aged &lt;6 y</td>
<td>1.75 (1.32-2.33)</td>
<td>0.55 (0.34-0.88)</td>
</tr>
<tr>
<td>Reference: no sibling aged &lt;6 y</td>
<td>1.75 (1.32-2.33)</td>
<td>0.55 (0.34-0.88)</td>
</tr>
<tr>
<td>RTI at screening</td>
<td>1.44 (0.99-2.09)</td>
<td>0.60 (0.35-1.02)</td>
</tr>
<tr>
<td>Reference: no RTI at screening</td>
<td>1.44 (0.99-2.09)</td>
<td>0.60 (0.35-1.02)</td>
</tr>
<tr>
<td>Antibiotic treatment in last month</td>
<td>0.73 (0.53-0.99)</td>
<td>0.55 (0.31-0.97)</td>
</tr>
<tr>
<td>Reference: no antibiotic in last month</td>
<td>0.73 (0.53-0.99)</td>
<td>0.55 (0.31-0.97)</td>
</tr>
<tr>
<td>Steroid treatment in last month</td>
<td>1.63 (1.09-2.43)</td>
<td>0.42 (0.18-1.00)</td>
</tr>
<tr>
<td>Reference: no steroid treatment in last month</td>
<td>1.63 (1.09-2.43)</td>
<td>0.42 (0.18-1.00)</td>
</tr>
<tr>
<td>Male</td>
<td>1.19 (0.89-1.58)</td>
<td>1.60 (0.98-2.58)</td>
</tr>
<tr>
<td>Reference: Female</td>
<td>1.19 (0.89-1.58)</td>
<td>1.60 (0.98-2.58)</td>
</tr>
<tr>
<td>S pneumoniae carrier</td>
<td>1.19 (0.89-1.58)</td>
<td>1.60 (0.98-2.58)</td>
</tr>
<tr>
<td>Reference: noncarrier of S pneumoniae</td>
<td>1.19 (0.89-1.58)</td>
<td>1.60 (0.98-2.58)</td>
</tr>
<tr>
<td>S aureus carrier</td>
<td>1.19 (0.89-1.58)</td>
<td>1.60 (0.98-2.58)</td>
</tr>
<tr>
<td>Reference: noncarrier of S aureus</td>
<td>1.19 (0.89-1.58)</td>
<td>1.60 (0.98-2.58)</td>
</tr>
<tr>
<td>Have S pneumoniae--carrying guardian</td>
<td>1.17 (0.59-2.31)</td>
<td>0.3 (0.86-2.58)</td>
</tr>
<tr>
<td>Reference: guardian not S pneumoniae carrier</td>
<td>1.17 (0.59-2.31)</td>
<td>0.3 (0.86-2.58)</td>
</tr>
<tr>
<td>Have S aureus--carrying guardian</td>
<td>0.89 (0.63-1.25)</td>
<td>0.50 (0.36-0.68)</td>
</tr>
<tr>
<td>Reference: guardian not S aureus carrier</td>
<td>0.89 (0.63-1.25)</td>
<td>0.50 (0.36-0.68)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NA, data not applicable; OR, odds ratio; RTI, respiratory tract infection.
were day care attendance (OR, 2.18; 95% CI, 1.56-3.06; P < .001); having young siblings (<6 years) (OR, 1.86; 95% CI, 1.34-2.56; P < .001); and age older than 3 months (OR, 5.93; 95% CI, 2.28-15.42; P < .001); antibiotic treatment during the previous month was inversely related (OR, 0.60; 95% CI, 0.43-0.85; P = .004). Sex, S pneumoniae carriage by parents, respiratory tract infection at screening, and S aureus carriage did not significantly affect S pneumoniae carriage (−2 log likelihood = 868.59; Nagelkerke R² = 0.131; Hosmer-Lemeshow χ² = 1.199; P = .95).

The only significant risk factor for S aureus carriage in a multivariate logistic analysis model was having an S aureus carrier parent (OR, 2.60; 95% CI, 1.48-4.55; P = .001), while several factors were inversely related: carrying S pneumoniae while attending day care (OR, 0.27; 95% CI, 0.10-0.72; P = .009); having young siblings (OR, 0.52; 95% CI, 0.30-0.91; P = .02); and age older than 3 months (OR, 0.51; 95% CI, 0.21-1.26; P = .15) for age 4-6 months; OR, 0.31; 95% CI, 0.13-0.74; P = .009 for 7-12 months; OR, 0.14; 95% CI, 0.05-0.36; P < .001 for 13-24 months; and OR, 0.35; 95% CI, 0.15-0.35; P = .02 for 25-40 months. Sex and skin disease did not have a significant influence (−2 log likelihood = 367.63; Nagelkirk R² = 0.178; Hosmer-Lemeshow χ² = 9.448; P = .31). When the analysis was repeated but restricted to vaccine-type S pneumoniae carriage, the results were almost identical (OR, 0.22; 95% CI, 0.07-0.74; P = .02 for being a vaccine-type S pneumoniae carrier and attending day care). Analysis of non–vaccine-type S pneumoniae did not yield any associations with S aureus carriage.

**COMMENT**

We have shown an inverse relation between S aureus and S pneumoniae carriage (specifically of vaccine-type strains) in children. Factors positively associated with S pneumoniae carriage were negatively associated with S aureus carriage. The distribution of S aureus carriage by age was a mirror image of that of S pneumoniae, an observation also reported in older children. In addition, concurrent carriage of S pneumoniae and S aureus in children was significantly lower than expected.

This apparent inverse relationship could be due to bacterial interference or could be a consequence of confounding effects. Age was considered a possible confounder because the association between the 2 pathogens was not demonstrated in the adult group and the tendency to carry different pathogens at different age groups is well known (eg, pharyngeal Streptococcus pyogenes at age 6-14 years; nasopharyngeal meningococcus in adolescents). Nevertheless, when controlling for age, the negative association between S pneumoniae and S aureus carriage among children persisted.

Although close contacts and poor hygienic conditions (eg, in day care centers and among young siblings) are considered to increase S aureus transmission, in our study day care attendance was surprisingly inversely related to S aureus carriage (when combined with S pneumoniae carriage). A possible explanation is that day care attendance may indicate prolonged S pneumoniae carriage (the inhibitory factor against S aureus), while this study measured point prevalence solely. Alternatively, day care attendance could be an indicator of the presence of another bacterial or viral pathogen that interferes with S aureus.

Bacterial interference could explain the inverse relation between S aureus and S pneumoniae. This phenomenon has been reported between S aureus and coagulase-negative staphylococci, Clostridium perfringens, and viridans group streptococci. Hydrogen peroxide has been suggested to be the inhibitory factor of viridans streptococci on S aureus and of S pneumoniae on other respiratory tract bacteria.

Pneumococcal conjugate vaccines reduce nasopharyngeal carriage of vaccine-type S pneumoniae. Our finding of an inverse relationship between vaccine-type S pneumoniae and S aureus may imply an upcoming shift, not only toward nonvaccine S pneumoniae serotypes but also toward higher S aureus carriage rates in children. This would be particularly disturbing in light of the emergence of community-associated methicillin-resistant S aureus. This possibility is supported by a recent report of an increased rate of S aureus culture-positive draining ears in vaccinated children compared with controls.

Limitations of this study include being a point prevalence study of children visiting primary care clinics that may underrepresent healthy pediatric populations; other pathogens potentially involved in bacterial interference were not studied and antibiotic use was examined only for the prior month. Longitudinal studies including older children and additional pathogens could further refine this association.

Our study suggests a protective role of S pneumoniae carriage against S aureus carriage. Studies measuring the effect of vaccination on S pneumoniae epidemiology should also examine concurrent changes in S aureus.

**Author Contributions:** Dr Regev-Yochay had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. **Study concept and design:** Regev-Yochay, Dagan, Raz, Carmeli, Shainberg, Derazne, Rahav, Rubinstein.
Acquisition of data: Regev-Yochay, Raz, Derazne. Analysis and interpretation of data: Regev-Yochay, Dagan, Raz, Derazne.

Drafting of the manuscript: Regev-Yochay, Raz, Carmeli, Rahav, Rubinstein.

Critical revision of the manuscript for important intellectual content: Regev-Yochay, Dagan, Shainberg, Derazne, Rahav, Rubinstein.

Statistical analysis: Carmeli, Derazne.

Funding/Support: This study was financially supported by the Israeli National Institute for Health Policy and by Maccabi Healthcare Services.

Role of the Sponsors: The study sponsors played no role in the design and conduct of the study, in the collection, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.

Previous Presentation: This study was partially presented at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy meeting, September 14-17, 2003, Chicago, Ill (abstract G-2048).

Acknowledgment: We thank Erica Pinco, MSc, for laboratory assistance and Nurtal Porat, PhD, and Ronit Trefler, BSc, for serotyping. We gratefully acknowledge the attending physicians of Hashfela District of Maccabi Healthcare Services, whose cooperation was crucial.

REFERENCES


The true measure of a human is how he or she treats his fellow man. Integrity and compassion cannot be learned in college, nor are these qualities inherited in the genes.

—Ann Landers (1918-2002)