Lack of Association between the Nasopharyngeal Carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in HIV-1–Infected South African Children

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1Centre for International Child Health and 2Immunobiology Unit, Institute in the HIV-1–positive (5.5% vs. 21.3%; ), but the rate of carriers was significantly lower than that in the noncarriers. S. aureus of 3Paediatrics and Child Health, 4Medical Microbiology, and 5Doris Duke of Child Health, University College, London, United Kingdom; Departments of 6Pediatrics and Child Health, 7Medical Microbiology, and 8Doris Duke Medical Research Institute, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa

We investigated the nasopharyngeal carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in 355 children hospitalized with severe pneumonia. Of the children, 239 (67.3%) were human immunodeficiency virus (HIV)–1 positive; 169 (47.6%) carried *S. pneumoniae*, 91 (25.6%) carried *S. aureus*, and 33 (9.3%) carried both. *S. pneumoniae* carriage was not related to HIV-1 status. The HIV-1–positive children had a significantly higher rate of *S. aureus* carriage than did the HIV-1–negative children (31.4% vs. 13.8%; ). The rate of *S. aureus* carriage in the HIV-1–negative *S. pneumoniae* carriers was significantly lower than that in the noncarriers (5.5% vs. 21.3%; = .013), but the rate of *S. aureus* carriage in the HIV-1–positive *S. pneumoniae* carriers was not significantly lower than that in the noncarriers (26.3% vs. 36.0%; = .11). We did not find a negative association between *S. pneumoniae* and *S. aureus* carriage in HIV-1–positive hospitalized children with severe pneumonia.

*Streptococcus pneumoniae* and *Staphylococcus aureus* both commonly inhabit the nasopharynx of children. Recent studies from The Netherlands and Israel have suggested that carriage of *S. pneumoniae* and *S. aureus* are inversely related [1, 2]. The mechanism of the relationship is unknown, but it has been suggested that production of hydrogen peroxide by *S. pneumoniae* inhibits *S. aureus* colonization [3].

We have analyzed data from a prospective study of children hospitalized with World Health Organization (WHO)–defined severe pneumonia in Durban, South Africa. The subjects differed from those of the previous 2 studies in that they were black African; most of them lived in poor, overcrowded housing; and 67.3% were infected with HIV-1. The children in the Dutch study were healthy and were participating in a national meningococcal vaccine campaign [1]. The children in the Israeli study were recruited through primary-care clinics in central Israel; 80% were attending a clinic because of a respiratory infection [2].

The primary aim of the overall study was to determine the response rates of children hospitalized with severe pneumonia to WHO-recommended antimicrobial therapy. These results are to be published separately. One of the secondary aims of the study was to describe the carriage of organisms in the nasopharynx in relation to HIV-1 status. The objectives of the present analyses were (1) to identify risk factors for nasopharyngeal carriage of *S. pneumoniae* and *S. aureus*; (2) to investigate any association between carriage of the 2 organisms; and (3) to determine whether HIV-1 status alters the rate of carriage in South African children admitted to the hospital with severe pneumonia.

**Subjects, materials, and methods.** Between January 2001 and December 2002, 355 children were admitted to King Edward VIII Hospital (Durban, South Africa) with WHO-defined severe pneumonia (cough or difficulty breathing, tachypnea, and chest indrawing). Immediately after study inclusion, while the child was still in the admission unit and before the first dose of antimicrobials had been administered, a flexible swab (Transwab; Medical Wire and Equipment) was inserted into the anterior nares, gently rubbed on the posterior nasopharyngeal wall, and removed. The swab was transferred to the laboratory in Amies transport medium at room temperature. Nasopharyngeal swabs were streaked onto blood agar, colistin nalidixic acid, and mannitol salt agar plates and incubated aerobically at 37°C in 5% CO2–enriched air. Colonies mor-
phologically suggestive of *S. pneumoniae* and *S. aureus* were isolated and identified using optochin susceptibility and tube coagulase testing, respectively. Pneumococci were transferred to the Pneumococcal Diseases Research Unit (Medical Research Council/National Health Laboratory Service, University of Witwatersrand; Johannesburg, South Africa) on Dorset egg medium. Serotyping was performed by the Quellung method, using antisera from the Statens Seruminstitut, Copenhagen. Vaccine types were defined as those serotypes included in the 7-valent conjugate vaccine (Prevenar; Wyeth Vaccines) as well as the cross-reactive types (i.e., 4, 6, 9, 14, 18, 19, and 23). Methicillin-resistant *S. aureus* (MRSA) was identified using 1-µg oxacillin discs on Mueller-Hinton agar supplemented with 4% NaCl.

HIV-1 infection was diagnosed by anonymous linked ELISA antibody testing (Vironostika IMPV; bioMérieux) followed by determination of HIV-1 RNA load (NucliSens HIV-1 assay; bioMérieux). For those children who were positive for HIV-1 by ELISA and had an HIV-1 RNA load of <10,000 copies, a confirmatory HIV-1 DNA polymerase chain reaction assay (Molecular Diagnostic Services) was performed. This assay detects all HIV-1 strains. There were no discordant HIV-1 DNA and RNA test results.

As part of the study-consent process, the mothers of the children received pretest HIV counseling. They were informed that neither the study team nor their provincial doctors would know the result and were encouraged to consent to a provincial HIV test. Posttest counseling was provided with this result. The present study was approved by the ethics committees of the Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, and the Institute of Child Health, University College London. Written, informed consent was obtained from the mothers of all of the study children.

Data were analyzed using SPSS for Windows (version 12.0.1; SPSS). Odds ratios (ORs) were calculated to assess risk factors for carriage of each organism, including age ≥3 months, sex, household size, number of children living in the house, number of persons sleeping in the same room as the child, previous hospital admission, history of antibiotic treatment during the current illness (maternal history), urinary antimicrobial activity, passive smoking, family history of respiratory disease during the previous 2 weeks, and 239 (67.3%) were HIV-1 positive. Eighty (39.0%) of 205 samples obtained for assessment of urinary antimicrobial activity were positive.

The HIV-1–positive children were, on average, older than the HIV-1–negative children (mean, 11.4 vs. 7.8 months; *P* = .005), had more previous hospital admissions (74/239 [31.0%] vs. 17/116 [14.7%; *P* < .001], but had fewer contacts via a family history of respiratory disease (39/239 [16.3%] vs. 32/116 [27.6%; *P* = .013). There were no other statistically significant differences between the 2 groups.

A causative organism was identified for 65.1% of the children: 52 (14.6%) had culture-proven TB, 33 (9.3%) had *Pneumocystis pneumonia*, and 116 (32.6%) had a viral etiology. Seventy-six children (21.4%) had an organism identified from their admission blood culture; 26 (7.3%) had *S. pneumoniae* bacteremia, and 13 (3.7%) had *S. aureus* bacteremia. 

*S. pneumoniae* was isolated from 169 children (47.6%) (table 1). Age ≥3 months and female sex were associated with increased *S. pneumoniae* carriage. When stratified by age, only females ≤3 months old were at increased risk (OR, 2.14 [95% confidence interval [CI], 1.02–4.16]; *P* = .024). *S. pneumoniae* carriage was less frequent in children who had received antimicrobials than in those who had not (59/145 [40.7%] vs. 110/209 [52.6%; *P* = .027). Urinary antimicrobial activity was not associated with *S. pneumoniae* carriage, possibly as a result of small sample size (37/80 [46.3%] vs. 61/124 [49.2%; *P* = .68). HIV-1 status did not affect carriage rates; 47.7% of both the HIV-1–positive and the HIV-1–negative children were *S. pneumoniae* carriers.

Serotyping results were available for 157 of the 169 children carrying *S. pneumoniae*; 9 had 2 different serotypes isolated on admission to the hospital (5 were HIV-1 positive). Figure 1 details the serotype distribution. The most common serotypes were 6A (12.8%), 6B (13.4%), 19A (12.2%), 19F (14.7%), and 23F (14.1%). Of the serotypes identified, 129 (82%) were vaccine types. Thirty-nine (75%) of the *S. pneumoniae* isolates from the HIV-1–negative children and 87 (85%) of the *S. pneumoniae* isolates from the HIV-1–positive children were vaccine types.

The frequency of *S. pneumoniae* bacteremia was higher in *S. pneumoniae* carriers than in noncarriers (19/169 [11.2%] vs. 7/186 [3.8%; *P* = .007). Neither HIV-1 status nor urinary antimicrobial activity altered this relationship.
### Table 1. Risk factors for *Streptococcus pneumoniae* and *Staphylococcus aureus* carriage in South African children, by HIV-1 status (univariate analysis).

<table>
<thead>
<tr>
<th>Variable</th>
<th>All children (n = 355)</th>
<th>HIV-1 positive (n = 239)</th>
<th>HIV-1 negative (n = 116)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. pneumoniae</em> OR (95% CI)</td>
<td><em>S. aureus</em> OR (95% CI)</td>
<td><em>S. pneumoniae</em> OR (95% CI)</td>
</tr>
<tr>
<td>Age &gt;3 months</td>
<td>2.55 (1.56–4.16) &lt;.001</td>
<td>0.82 (0.48–1.39) .43</td>
<td>3.33 (1.74–6.42) &lt;.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.61 (0.39–0.95) .02</td>
<td>0.94 (0.57–1.56) .81</td>
<td>0.46 (0.26–0.79) .003</td>
</tr>
<tr>
<td>HIV-1 positive</td>
<td>1.01 (0.63–1.62) .95</td>
<td>2.86 (1.52–5.42) &lt;.001</td>
<td>NA</td>
</tr>
<tr>
<td>History of antibiotic treatment during the current illness</td>
<td>0.62 (0.39–0.97) .027</td>
<td>0.92 (0.55–1.55) .75</td>
<td>0.67 (0.39–1.16) .13</td>
</tr>
<tr>
<td>Urinary antimicrobial activity</td>
<td>0.89 (0.51–1.56) .68</td>
<td>0.94 (0.5–1.7) .85</td>
<td>0.94 (0.47–1.86) .85</td>
</tr>
<tr>
<td>Family history of respiratory disease during the previous 2 weeks</td>
<td>1.17 (0.69–1.96) .56</td>
<td>1.03 (0.57–1.87) .90</td>
<td>1.05 (0.53–2.09) .89</td>
</tr>
<tr>
<td>Family history of TB during the previous 2 years</td>
<td>0.93 (0.6–1.45) .76</td>
<td>2.18 (1.3–3.7) .002</td>
<td>1.31 (0.77–2.22) .32</td>
</tr>
<tr>
<td>Passive smoking</td>
<td>1.21 (0.78–1.89) .37</td>
<td>0.68 (0.41–1.15) .13</td>
<td>1.44 (0.84–2.5) .16</td>
</tr>
<tr>
<td>Up-to-date immunizations</td>
<td>0.69 (0.4–1.19) .43</td>
<td>1.28 (0.67–2.45) .43</td>
<td>1.43 (0.74–2.79) .25</td>
</tr>
<tr>
<td>Household size &gt;5 people</td>
<td>0.87 (0.55–1.38) .53</td>
<td>0.94 (0.55–1.6) .81</td>
<td>1.17 (0.67–2.05) .56</td>
</tr>
<tr>
<td>Previous hospital admission</td>
<td>1.03 (0.62–1.71) .89</td>
<td>1.75 (1.0–3.04) .03</td>
<td>0.97 (0.54–1.74) .90</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> carriage</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><em>S. aureus</em> carriage</td>
<td>0.54 (0.32–0.90) .01</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; NA, not applicable; OR, odds ratio; TB, tuberculosis.

* The χ² test was used except for these values, for which Fisher’s exact test was used.
S. aureus was isolated from 91 children (25.6%) (table 1); 33 (36.3%) of the isolates were MRSA. Previous hospital admission, family history of TB during the previous 2 years, and HIV-1 infection were associated with increased carriage of S. aureus. The HIV-1–positive children had a higher rate of MRSA carriage than did the HIV-1–negative children (OR for MRSA, 4.06; OR for methicillin-susceptible S. aureus [MSSA], 0.90; OR for none, 1.0; \( P = .002 \)). Antimicrobial-resistance data were available for 28 of the 29 HIV-1–positive children carrying MRSA (for clindamycin resistance, 10 children [35.7%]; for trimethoprim-sulphamethoxazole resistance, 26 children [92.9%]; for erythromycin resistance, 23 children [82.1%]; and for vancomycin resistance, 0 children).

Thirty-three (19.5%) of the 169 S. pneumoniae carriers and 58 (31.2%) of the 186 noncarriers were colonized with S. aureus (\( P = .012 \)). The effect was mostly on MSSA carriage (OR, 0.41 [95% CI, 0.17–0.94]; \( P = .02 \)) and not on MRSA carriage (OR, 0.64 [95% CI, 0.34–1.18]; \( P = .126 \)). Previous hospital admission was associated only with an increased rate of S. aureus carriage in the HIV-1–negative children, and a family history of TB during the previous 2 years was associated only with an increased rate of S. aureus carriage in the HIV-1–positive children.

S. aureus was isolated from 6 (21.4%) of 28 children carrying nonvaccine serotypes and from 24 (18.6%) of 129 children carrying vaccine serotypes. There was no difference between the carriage rates of S. aureus, vaccine pneumococcal serotypes, and nonvaccine pneumococcal serotypes, although the relationship was significant by the \( \chi^2 \) test for trend (\( P = .012 \)).

The S. aureus carriers did not have a higher rate of S. aureus bacteremia than the noncarriers (5/91 [5.5%] vs. 8/262 [3.1%]; \( P = .29 \)). Neither HIV-1 status nor urinary antimicrobial activity altered the relationship, but sample sizes were small.

Of the 355 study children, 239 (67.3%) were HIV-1 positive. Among the HIV-1–negative children, S. aureus was isolated from 3 (5.5%) of the 55 S. pneumoniae carriers and 13 (21.3%) of the 61 noncarriers (\( P = .01 \)). However, the HIV-1–positive S. pneumoniae carriers did not have a rate of S. aureus carriage significantly lower than that of the HIV-1–positive noncarriers (30/114 [26.3%] vs. 45/125 [36.0%]; \( P = .11 \)). Among the HIV-1–positive children, there was no association between either trimethoprim-sulfamethoxazole prophylaxis or lymphocytic interstitial pneumonitis and S. pneumoniae, S. aureus, or dual carriage, and there was no association between HIV-1 RNA load and carriage of either organism. There was also no association between HIV-1 RNA load and cocolonization.

The HIV-1–negative children carrying S. pneumoniae were at decreased risk of carrying MRSA (0/55 [0%] vs. 4/61 [6.6%]; \( P = .04 \)); there was also some reduction in the rate of MSSA carriage (3/55 [5.5%] vs. 9/61 [14.8%]; \( P = .08 \)). There was no reduction in the rate of carriage of MRSA (10/114 [8.8%] vs. 19/124 [15.3%]; \( P = .1 \)) or MSSA (20/114 [17.5%] vs. 25/124 [20.2%]; \( P = .42 \)) in the HIV-1–positive children carrying S. pneumoniae.
In the multivariate analysis of all of the study children, factors positively associated with \( S. pneumoniae \) carriage were age \( \geq 3 \) months (OR, 2.62 [95% CI, 1.62–4.24]; \( P < .001 \)) and female sex (OR, 1.88 [95% CI, 1.20–2.94]; \( P = .006 \)). \( S. aureus \) carriage (OR, 0.53 [95% CI, 0.32–0.88]; \( P = .02 \)) and history of antibiotic treatment during the current illness (OR, 0.60 [95% CI, 0.38–0.94]; \( P = .025 \)) were negatively associated with \( S. pneumoniae \) carriage.

HIV-1 infection was positively associated with \( S. aureus \) carriage (OR, 2.87 [95% CI, 1.54–5.32]; \( P = .001 \)). Family history of TB during the previous 2 years (OR, 2.31 [95% CI, 1.37–3.88]; \( P = .002 \)), the number of children living in the house (OR, 1.17 [95% CI, 1.03–1.32]; \( P = .11 \)), and passive smoking (OR, 1.74 [95% CI, 1.02–2.98]; \( P = .043 \)) were also associated with \( S. aureus \) carriage. Nasopharyngeal carriage of \( S. pneumoniae \) was negatively associated with carriage of \( S. aureus \) (OR, 0.59 [95% CI, 0.35–1.00]; \( P = .05 \)).

In the multivariate analysis of the HIV-1–negative children, age \( \geq 3 \) months (OR, 2.14 [95% CI, 0.94–4.86]; \( P = .071 \)) was the only predictor of \( S. pneumoniae \) carriage. History of antibiotic treatment during the current illness (OR, 0.38 [95% CI, 0.16–0.87]; \( P = .022 \)) and carriage of \( S. aureus \) (OR, 0.17 [95% CI, 0.04–0.69]; \( P = .013 \)) were negatively associated with \( S. pneumoniae \) carriage. \( S. pneumoniae \) carriage was also negatively associated with \( S. aureus \) carriage (OR, 0.22 [95% CI, 0.06–0.81]; \( P = .024 \)) in the HIV-1–negative children.

When the analysis was restricted to the HIV-1–positive children, age \( \geq 3 \) months (OR, 3.52 [95% CI, 1.88–6.62]; \( P < .0001 \)) and female sex (OR, 2.45 [95% CI, 1.41–4.25]; \( P = .001 \)) were the only predictors of \( S. pneumoniae \) carriage; \( S. aureus \) carriage was no longer inversely related to it. \( S. pneumoniae \) carriage was also not negatively associated with \( S. aureus \) carriage.

**Discussion.** Our data confirm the inverse relationship between the nasopharyngeal carriage of \( S. pneumoniae \) and \( S. aureus \) in HIV-negative children [1, 2]. The South African HIV-negative children in the present study had rates of \( S. pneumoniae \), \( S. aureus \), and dual carriage that were similar to those of Israeli children [2]; however, the association between the nasopharyngeal carriage of \( S. pneumoniae \) and \( S. aureus \) was absent in HIV-positive children, resulting in a higher dual carriage rate of 26.3%. HIV infection is known to be a risk factor for \( S. aureus \) colonization [4, 5] and, given that \( S. aureus \) colonization is a risk factor for \( S. aureus \) septicemia, the lack of a negative association may partly explain the increased rates of \( S. aureus \) disease seen in HIV-infected patients [4, 6].

In microbiological interference, pathogens generate a niche in the host that suppresses the colonization of other microorganisms [3]. Little is known about the exact mechanism of the interaction. However, adhesion to mucosal receptors, host immunity, and exposure have been implicated in colonization [7]. HIV-infected children have reduced mucosal immunity and are known to have reduced quantitative antibody responses to pneumococcal conjugate vaccination [8]. They are also at increased risk of hospital admission and have more contacts with family members who have been hospitalized. It is possible that HIV infection results in less immunological pressure, leading to less competition between the 2 organisms combined with increased exposure to respiratory pathogens, including \( S. aureus \).

There are several limitations to the present study. First, the study children had severe pneumonia. It is possible that the negative relationship between the rates of carriage of \( S. pneumoniae \) and \( S. aureus \) remains in HIV-positive children without pneumonia. However, although \( S. pneumoniae \) carriage was associated with an increased rate of \( S. pneumoniae \) bacteraemia, \( S. aureus \) carriage was not associated with an increased rate of \( S. aureus \) bacteraemia. HIV-1 status had no effect on the relationship for either organism. Because (1) all of the study children had severe pneumonia irrespective of their HIV-1 status and (2) the association between carriage and bacteraemia was not associated with HIV-1 status, it is unlikely that the lack of association seen in our HIV-1–infected children would be different in a population without severe pneumonia. In addition, 80% of the children in the Israeli study had a respiratory infection [2].

Second, the present study was cross-sectional. A longitudinal study would be required to determine a causal relationship between carriage of \( S. pneumoniae \) and \( S. aureus \) as well as the impact of HIV status. Finally, the interaction between carriage of \( S. pneumoniae \) and \( S. aureus \) is likely to be complex, and some possible confounding factors were not measured here, including hospitalization of other family members, length of time since last hospital admission, and day-care attendance (which is unusual in this population).

Our data suggest that the negative association with \( S. pneumoniae \) carriage is strongest for MRSA. No HIV-1–negative children carrying \( S. pneumoniae \) were coinfection with MRSA. There have been increasing reports of community-acquired MRSA disease recently [9, 10], and there was an increase in the rate of nasal carriage of MRSA in the United States from 2002 to 2004 [11]. There have also been reports that children given the pneumococcal conjugate vaccine have higher rates of \( S. aureus \) otitis media than do children who have not received the vaccine [12]. Any possible increase in the frequency of MRSA disease due to pneumococcal conjugate vaccination needs to be further investigated.

In the present study, girls had higher rates of \( S. pneumoniae \) carriage than boys; however, this relationship was only statistically significant in infants \( \leq 3 \) months old. The nasal mucosa is estrogen sensitive [13, 14], and infant girls \( \leq 3 \) months old could still be responding to their intrauterine environment.

Our study confirms the inverse relationship between the nasopharyngeal carriage of \( S. pneumoniae \) and \( S. aureus \) in HIV-
negative children. However, we found this relationship to be absent in HIV-positive children. Further research is required, both on the role played by the host in bacterial interaction and on the possible implications of widespread pneumococcal vaccination.

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References