Reply to Schuhegger et al.

To the Editor—We appreciate the opportunity to respond to Schuhegger et al. [1], who highlight 2 issues in their letter. First, Schuhegger et al. [1] raise questions about the effectiveness of diphtheria toxoid vaccine in protecting against toxin-producing Corynebacterium ulcerans. It is known that there are genetic variations in the diphtheria toxin gene (tox) of Corynebacterium diphtheriae and C. ulcerans, as well as differences in the amino acid sequences of the diphtheria toxins they produce. However, given that the functional consequences of these differences have not been fully established for clinical diphtheria or diphtheria-like illness due to C. ulcerans, we believe that the use of diphtheria toxoid vaccine as a preventative measure, and especially the use of diphtheria antitoxin as part of the treatment for diphtheria-like illness due to C. ulcerans, is justified. In the case we reported, the patient who had extensive membrane in the pharynx and received equine diphtheria antitoxin recovered fully; the patient who did not receive the antitoxin had a fatal outcome.

Second, we are in agreement that a careful probe design may be useful for specifically detecting unique tox sequences, but we believe that our study has clearly documented the presence of toxin-producing C. ulcerans. We reported that the real-time PCR for detection of the C. diphtheriae tox gene [2] revealed atypical amplification of subunit A and no amplification of subunit B for both patients. Consequently, test results were reported as negative for the tox gene. However, C. ulcerans was isolated from both patients, and conventional PCR and a modified Elek test results were positive for the detection of the tox gene and the diphtheria toxin itself, respectively [3].

In summary, we support the need for a better understanding of the molecular epidemiology and characteristics of C. ulcerans and the diphtheria toxin that it produces, because the spectrum of severity of this uncommon disease is unknown, and the diagnosis of mild infections may be missed completely, especially in a non-diphtheria outbreak setting. C. ulcerans produces an exotoxin that is very similar, if not identical, to the diphtheria toxin produced by C. diphtheriae, and it can also produce typical membranous lesions in the throat and complications that are clinically indistinguishable from those of respiratory diphtheria. Most commonly, C. ulcerans is isolated as an etiologic agent while investigating for diphtheria. Because respiratory diphtheria may be severe or fatal, treatment should be immediate and should not be delayed until laboratory results are available. Specifically, diphtheria antitoxin should be administered promptly to patients with clinical respiratory diphtheria to prevent serious complications and death, even in cases in which C. ulcerans, rather than C. diphtheriae, is isolated [3].

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References


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Does Pneumococcal Conjugate Vaccine Influence Staphylococcus aureus Carriage in Children?

To the Editor—Several studies have demonstrated a negative association between carriage of Streptococcus pneumoniae (specifically, carriage of the vaccine types [VTs]) and Staphylococcus aureus in children [1–6]. This has raised concern that, similar to the occurrence of serotype replacement with non-VT S. pneumoniae [7–11], the widespread use of 7-valent pneumococcal conjugate vaccine (PCV7) and the reduction in both VT S. pneumoniae and total S. pneumoniae carriage will be followed by an increase in S. aureus carriage [12, 13].

Cohen et al. [14] have tried to address this issue in their recently published study, in which they conclude that PCV7 does not appear to influence S. aureus carriage in young children with otitis media. However, their study suffers from several flaws, and we believe that their conclusions may be unjustified.

Our main concern is methodological. Although the authors performed multivariate analysis, critical variables were not included in the analysis, thus rendering the results largely irrelevant. The choice of variables to include in a multivariate analysis is highly dependent on the question being asked. If the purpose of the study was to evaluate whether receipt of PCV7 modified the risk of staphylococcal colonization, a multivariate analysis to address this question must include this predictor. If, on the other hand, the purpose was to reassess whether S. pneumoniae carriage is
associated with *S. aureus* carriage (as several independent studies have shown), the predictor "*S. pneumoniae* carriage" should have been included in the multivariate analysis. Because neither of these analyses was performed, the authors’ 2 conclusions, that PCV7 does not affect *S. aureus* colonization and that *S. pneumoniae* carriage is not associated with *S. aureus* carriage, are not proven. Finally, age is known to be one of the most important variables associated with colonization by *S. pneumoniae* and *S. aureus* [2, 15, 16], and children who were colonized with *S. aureus* in the study by Cohen et al. [14] were younger than uncolonized children (*P* = .06). Therefore, we believe that the variable age should have also been included in the multivariate analysis. The multivariate analysis presented here is thus flawed in 2 important ways: it does not test the primary hypothesis, and it does not include probable confounding factors. Naturally, we cannot be certain what results would be obtained in a proper analysis, but the present analysis is uninformative because of these flaws.

Another concern is the method of swab transfer. The authors state that swabs were transferred to the lab within 48 h after sample collection. This is a long delay, but more worrisome is the fact that the length of the delay was not consistent. Some swabs could have been transferred within hours after sample collection, with full recovery of the bacteria, whereas others could have been transferred after a delay of 48 h, resulting in a low recovery rate. This could be a cause of misclassification bias. An additional concern is why the authors chose to perform a detailed analysis on colonization data (presented in table 2 [14]) from children >1 year of age only, despite the fact that 41% of their study population was <1 year of age. The authors give no explicit reason for this partial analysis.

Finally, the article contains numerous obvious errors or inconsistencies that make it difficult to follow and, at times, impossible to interpret. In table 2 [14], the total number of children in the subgroups exceeds the number of children in the total group (for example, 9.4% of the children carried both *S. aureus* and VT *S. pneumoniae*, whereas only 7.8% of the children carried *S. aureus*). In addition, the weighted averages are miscalculated for some of the rows (VT *S. pneumoniae* and *S. aureus* plus *S. pneumoniae*). Overall, it is difficult to determine the number of PCV7-vaccinated children in the study. At one point, the authors write that 17% were vaccinated; later, they refer to much higher numbers, whereas in table 1, they state that 82.5% of the children (1470 of 1781 children) were vaccinated [14].

It is clear that PCV7 has dramatically reduced invasive pneumococcal disease, both among vaccinated children and among nonvaccinated individuals, via indirect effects on transmission [17]. However, the growing concerns about the emergence of non-VT disease [7, 8, 10, 11] in vaccinated populations and the possible effects of this vaccine on the epidemiology of other upper respiratory pathogens, including *S. aureus*, warrant careful investigation in well-designed and adequately reported studies.

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Reply to Regev-Yochay et al.

We thank Regev-Yochay et al. [1] for their careful reading of our article. We initiated this prospective study in 2001 to follow the changes in pneumococcal carriage induced by the implementation of a 7-valent pneumococcal conjugate vaccine (PCV7) in France. To select patients with high rates of pneumococcal carriage, we chose to enroll young children with acute otitis media who had high fever and otalgia. In 2003, following the article by Veenhoven et al. [2], we added a Staphylococcus aureus culture for all nasopharyngeal samples. We would like to reassure Regev-Yochay et al. [1] about the methodological issue that they raise: all critical variables (immunization status, Streptococcus pneumoniae carriage, and age) were introduced in the multivariate analysis, but they did not appear to be statistically significant predictive factors for S. aureus carriage. The choice of the statistical method was relevant and appropriate: stepwise logistic regression. The results obtained using this method show only the statistically significant risk factors. In our population, we could conclude that vaccination with PCV7 did not affect S. aureus colonization, and we did not find an association between nasopharyngeal carriage of S. pneumoniae and carriage of S. aureus. We think that the delay between obtaining and processing nasopharyngeal specimens did not affect our results, for several reasons. First, we strictly followed the guidelines of the manufacturer of the swab culture transport system (Copan Ventury Transystem). Second, 84.2% of our samples were processed within 3 days after being obtained. Third, we observed no differences in the recovery rate of the bacteria with respect to the delay between obtaining and processing samples. Finally, the pneumococcal carriage rate found in our study is higher than those rates observed by Regev-Yochay et al. [3] and Bogaert et al. [4] in the same age groups. Furthermore, the S. aureus carriage rate is comparable to that in the study by Regev-Yochay et al. [3].

We and others have shown that the PCV7 booster dose greatly impacts pneumococcal carriage [5, 6]. Because it may potentially affect S. aureus carriage, we chose to perform a separate analysis for children >12 months of age to compare 3 vaccination status groups (unvaccinated children, children who received the primary series without booster, and children who received the primary series with booster). We did not find any differences between the 3 groups, confirming that, in our population, PCV7 vaccination does not interfere with S. aureus carriage. In a brief report, we could only show the more relevant results, and therefore, we did not present a table of data for children <1 year of age. In this age group, S. aureus carriage was 8.6% (95% CI, 3.5%–13.6%) among nonvaccinated children, 10.2% (95% CI, 5%–15.5%) among partially vaccinated children, and 10.9% (95% CI, 8.3%–13.6%) among completely vaccinated children.

The results shown in table 2 of our original article [7] are coherent, although the rates might seem to be discordant. For each population described, we calculated our percentages with different denominators of patients; for a better understanding of our original table, we propose to add the denominator for each percentage, as shown in table 1. Thus, we confirm the absence of an effect of PCV7 vaccination on S. aureus carriage. The only true error revealed by the reading of Regev-Yochay et al. [1] is a misprint: the sentence mentioned in our results section should have been “seventeen percent of the children had been immunized,” not “seventeen

Table 1. Booster dose effect on pneumococcal and staphylococcal carriage rates in children >1 year of age.

<table>
<thead>
<tr>
<th>Species</th>
<th>All children (n = 1018)</th>
<th>Unvaccinated children (n = 194)</th>
<th>Vaccinated children</th>
<th>Primary series without booster (n = 487)</th>
<th>Primary series with booster (n = 339)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proportion of children with carriage</td>
<td>Percentage of children (95% CI)</td>
<td>Proportion of children with carriage</td>
<td>Percentage of children (95% CI)</td>
<td>Proportion of children with carriage</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>79/1016</td>
<td>7.8 (4.1–10.4)</td>
<td>17/194</td>
<td>8.8 (4.9–12.7)</td>
<td>40/487</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>622/1016</td>
<td>61.2 (58.2–64.2)</td>
<td>133/194</td>
<td>68.6 (62.0–75.1)</td>
<td>308/487</td>
</tr>
<tr>
<td>Vaccine-type S. pneumoniae</td>
<td>224/622</td>
<td>36.0 (32.2–39.8)</td>
<td>83/133</td>
<td>62.4 (54.2–70.6)</td>
<td>107/308</td>
</tr>
<tr>
<td>S. aureus plus S. pneumoniae</td>
<td>47/622</td>
<td>7.6 (5.5–9.6)</td>
<td>11/133</td>
<td>8.3 (5.6–11.3)</td>
<td>28/308</td>
</tr>
<tr>
<td>S. aureus plus vaccine-type S. pneumoniae</td>
<td>21/224</td>
<td>9.4 (6.6–13.2)</td>
<td>7/83</td>
<td>8.4 (2.5–14.4)</td>
<td>11/107</td>
</tr>
</tbody>
</table>