

Acknowledgments

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Nasopharyngeal Bacterial Interactions in Children

To the Editor: Xu and colleagues (1) examined the nasopharyngeal bacterial colonization rates in children with acute otitis media (AOM) and in healthy children. They found that *Haemophilus influenzae* colonization was competitively associated with *Streptococcus pneumoniae* and *Morexella catarrhalis* colonization in children with AOM but was not associated with *S. pneumoniae* and *M. catarrhalis* colonization in healthy children. We have a serious concern regarding their analysis.

The authors calculated odds ratios (ORs) by considering a bacterial colonization as an outcome variable and another bacterial colonization as an exposure variable. They considered an OR >1 as the presence of synergistic associations between bacteria (i.e., co-colonization is more likely to occur than it would by chance) and OR <1 as the presence of competitive associations (i.e., co-colonization is less likely to occur than it would by chance). This inference may be justified in a population-based cross-sectional study. If 2 bacterial colonizations occur independently in a stationary population, the prevalence of co-colonization will be the product (multiplication) of each prevalence, and the OR between 2 bacterial colonizations in the population (OR_{pop}) will be 1 (online Technical Appendix, wwwnc.cdc.gov/EID/article/20/2/12-1724-Techapp1.pdf).

However, the authors enrolled their case-patients according to clinical signs (i.e., AOM or healthy). Let us assume that case-patients are enrolled from a population of children during a time period of t . Let r_c be the risk for enrollment (that is, of developing the disease) among colonized children and r_n be the risk for enrollment

among noncolonized children. The OR among the enrolled case-patients ($OR_{\text{case-patient}}$) becomes r_n/r_c , which is the reciprocal of the risk ratio (RR) of developing the disease ($RR = r_c/r_n$) (online Technical Appendix). Usually, RR is >1 for diseased children and <1 for healthy children. Therefore, even in this independent colonization scenario, $OR_{\text{case-patient}}$ becomes <1 ("pseudo-competitive associations") in diseased children, and $OR_{\text{case-patient}}$ becomes >1 ("pseudo-synergistic associations") in healthy children. This is probably what the authors have observed in the study.

We cannot infer an association of multiple bacterial colonizations in a population despite an observed association in the diseased (or healthy) children, and this association is widely misunderstood (2–4). The authors' discussion regarding a potential emergence of *H. influenzae*, associated with AOM, after the introduction of pneumococcal conjugate vaccine is thus unjustifiable.

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In Response: The point made by Suzuki et al. (1) is an interesting one, but it is not directly relevant to our conclusions (2). To summarize, given bacterium 1 and bacterium 2, suppose $OR[\text{pop}]$ is the odds ratio (OR) between bacterium 1 and bacterium 2 in the population. If $r[c]$ and $r[n]$ are the risk of enrollment among colonization-positive and colonization-negative children, respectively, then $OR[\text{case}] = (r[n]/r[c]) OR[\text{pop}]$, so that $OR[\text{case}] < 1$ can be attributed to a higher risk among colonized children.

The underlying assumption of the analysis is that enrollment risk is constant for both bacteria (alone or in combination). In fact, differential risk does exist, and it cannot be separated from the issue of the relative aggressiveness of the bacterium. This can be seen by considering the ORs in the Table.

When we compared the ORs between bacteria pairs for all subjects and children with acute otitis media (AOM), we found a large decrease for pairs involving nontypeable *Haemophilus influenzae* (NTHi), in

contrast to the remaining pair, *Streptococcus pneumoniae*/ *Moraxella catarrhalis* (*Spn/Mcat*). We do not believe that these values are explainable by the effect described by Suzuki et al., especially when (as is done in that analysis) we assume a colonization-positive risk of enrollment $r[c]$ that is independent of bacterium distribution. We also point out that these estimates are instructive, but not sufficient, because each pairwise comparison may depend on interactions with the third bacterium. We therefore used a statistical model (2,3) that permits the isolation of third-order effects by modeling co-occurrence rates of 2 bacteria while controlling for a third. This allowed us to reach our conclusion, which is primarily concerned with the specific role played by NTHi, and follows from the existence of a pattern in the reported ORs, rather than the absolute value of any single OR.

It is also instructive to examine the colonization rates for AOM versus number of AOM events (nAOM) subjects: $p(\text{Spn-nAOM}) = 0.30$; $p(\text{Spn-AOM}) = 0.53$; $p(\text{NTHi-nAOM}) = 0.12$; $p(\text{NTHi-AOM}) = 0.48$; $p(\text{Mcat-nAOM}) = 0.36$; $p(\text{Mcat-AOM}) = 0.43$. As we would expect, colonization rates of each bacterium are higher for AOM patients. What is of interest is that the colonization distribution is different for children with AOM, which suggests that NTHi is more aggressive than other bacteria in some sense, and this effect is made more precise by the statistical model we used. The essential point is that the issue of competitive association cannot be isolated from differential enrollment risks, which is what our analysis reports.

Table. Comparison of the odds ratios between bacterium pairs for all subjects and children with acute otitis media*

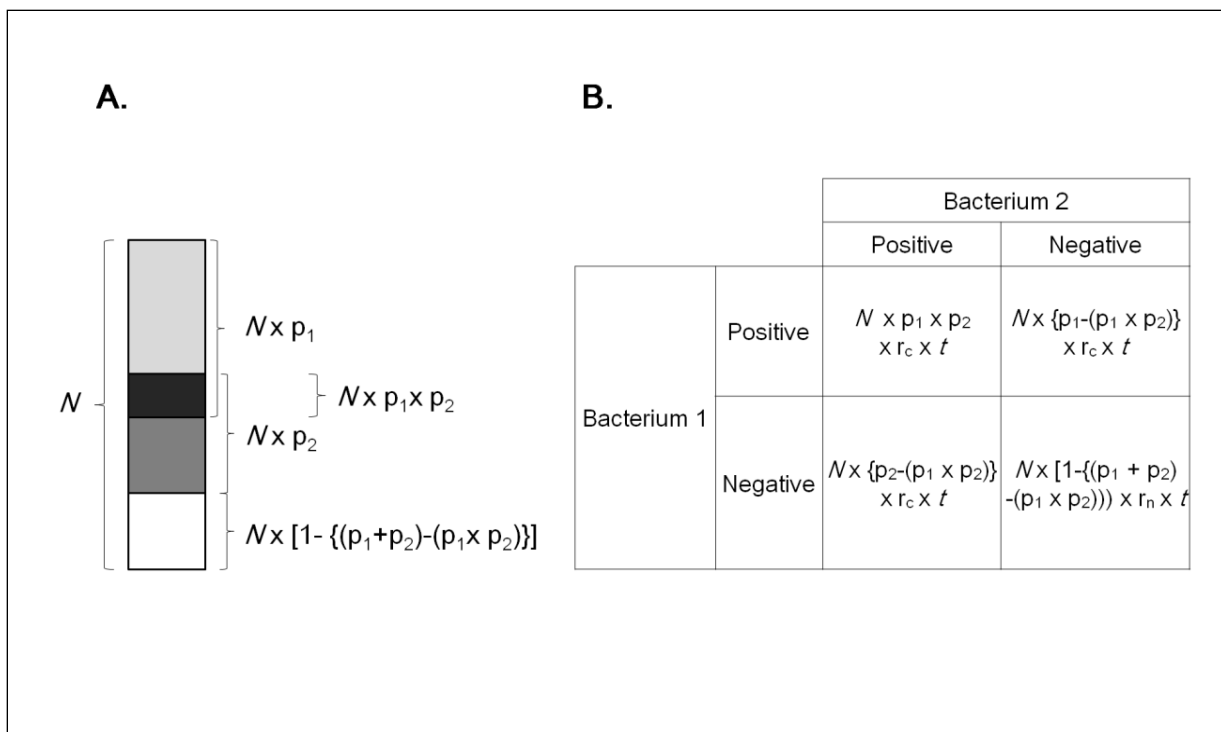
| Bacteria pair | Odds ratios, p values | |
|---------------|-----------------------|--------------------|
| | All subjects | Acute otitis media |
| NTHi/Mcat | 0.94, p = 0.6935 | 0.47, p = 0.0006 |
| NTHi/Spn | 1.58, p = 0.0004 | 0.50, p = 0.001 |
| Spn/Mcat | 1.71, p < 0.0001 | 1.53, p = 0.05 |

*NTHi, nontypeable *Haemophilus influenzae*; Mcat, *Moraxella catarrhalis*; Spn, *Streptococcus pneumoniae*.

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Technical Appendix

Figure. Number of children by bacterial colonization status in a stationary population (A) and cases enrolled during a specified time period (B).



N : the population size

p_1 : the prevalence of bacterium 1 colonization

p_2 : the prevalence of bacterium 2 colonization

r_c : the risk of enrollment among colonization-positive children

r_n : the risk of enrollment among colonization-negative children

t : a study period

Suppose the colonization of bacterium 1 and of bacterium 2 occur independently in the population.

The odds ratio between bacterium 1 and bacterium 2 in the population (OR_{pop}) will be

$$\begin{aligned} OR_{pop} &= \frac{N \times p_1 \times p_2 \times N \times [1 - \{(p_1 + p_2) - (p_1 \times p_2)\}]}{N \times \{p_1 - (p_1 \times p_2)\} \times N \times \{p_2 - (p_1 \times p_2)\}} \\ &= \frac{p_1 \times p_2 - (p_1 \times p_2) \times (p_1 + p_2) - (p_1 \times p_2)^2}{p_1 \times p_2 - (p_1 \times p_2) \times (p_1 + p_2) - (p_1 \times p_2)^2} \\ &= 1 \end{aligned}$$

The OR between bacterium 1 and bacterium 2 in the enrolled cases will be

$$\begin{aligned}
 \text{OR}_{\text{case}} &= \text{OR}_{\text{pop}} \times \frac{r_c \times t \times r_n \times t}{r_c \times t \times r_c \times t} \\
 &= \text{OR}_{\text{pop}} \times \frac{r_n}{r_c} \\
 &= \frac{r_n}{r_c}
 \end{aligned}$$

which is the reciprocal of risk ratio for enrollment (= developing the disease; r_c/r_n).