Association of Nasopharyngeal Bacterial Colonization during Upper Respiratory Tract Infection and the Development of Acute Otitis Media

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Acute otitis media occurs mostly after upper respiratory tract infection; the causative bacteria are those colonized in the nasopharynx. We studied 709 episodes of upper respiratory tract infection and found that children with no bacteria in the nasopharynx were at low risk for acute otitis media, whereas children with 3 pathogenic bacteria were at the greatest risk.

Acute otitis media (AOM) is one of the most common pediatric infectious diseases. Although the disease is primarily considered to be a bacterial infection, it is well known that viral upper respiratory tract infections (URIs) predispose children to AOM, and viruses alone can cause AOM [1]. In other words, AOM often occurs as a complication of URI. Respiratory virus infection disrupts the mucociliary system, impairing the host's primary mechanical defense against bacterial invasion. Viral infection also causes eustachian tube dysfunction, leading to reduced middle ear pressure and forcing mucus, nasopharyngeal secretions, and bacteria colonized in the nasopharynx into the middle ear [2].

The 3 most common AOM pathogenic bacteria are *Streptococcus pneumoniae,* nontypeable (NT) *Haemophilus influenzae,* and *Moraxella catarrhalis.* By 6 months of age, healthy children are colonized with ⩾1 of these pathogens [3]. Children colonized with AOM pathogens at a young age (<3 months) are at 2 times greater risk of having AOM by 6 months of age [3]. Children with AOM are more likely than children without AOM have AOM pathogenic bacteria cultured from their nasopharynxes at the time of AOM diagnosis [4]. Nasopharyngeal bacterial colonization in healthy individuals is different from nasopharyngeal colonization in individuals with URI [5] or AOM [6]. Previous studies have compared nasopharyngeal colonization in healthy individuals with colonization during URI or AOM but have not observed children from the onset of URI to the development of AOM. We studied the relationship between nasopharyngeal colonization during URI and subsequent occurrence of AOM to determine if nasopharyngeal colonization data at the onset of URI can be used to predict the occurrence of AOM complicating URI.

Methods. This study is a secondary analysis of data collected from January 2003 through March 2007 at the University of Texas Medical Branch, Galveston, during a prospective, longitudinal study of virus-induced AOM (T.C., unpublished data). The primary study was designed to capture all URI episodes occurring during a 1-year period in healthy children aged 6–35 months to study the rate and characteristics of AOM following URI. The study was approved by the University of Texas Medical Branch Institutional Review Board. At enrollment, information about demographic characteristics and AOM risk factors was collected. Parents were asked to notify the study office as soon as the child began to have a cold or URI symptoms (nasal congestion, rhinorrhea, cough, sore throat, or fever). Children were seen by a study physician as soon as possible after the onset of URI symptoms, with a follow up visit a few days later (days 3–7 of the URI), and monitored closely for 3 weeks for AOM development.

AOM complicating URI was defined as an episode of AOM occurring within 21 days after onset of the URI. AOM was defined by acute onset of symptoms (fever, irritability, or earache), signs of inflammation of the tympanic membrane, and presence of fluid in the middle ear documented by pneumatic otoscopy and/or tympanometry. Children given a diagnosis of AOM were observed or given antibiotic therapy consistent with standard of care [7].

Included in this study were URI episodes for which the child was seen by the study physician and had nasopharyngeal swab samples collected within 7 days after URI onset. Excluded from the analysis were URI episodes with nasopharyngeal swab samples for culture obtained within 7 days after receipt of antibiotic therapy and AOM episodes without a preceding URI.

Nasopharyngeal swab samples for bacterial culture were collected at enrollment, during the first visit of each URI episode,

Received 3 August 2007; accepted 4 October 2007; electronically published 9 January 2008.

Presented in part: 9th International Symposium of Recent Advances in Otitis Media, St. Pete Beach, Florida, 3–7 June 2007.

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Clinical Infectious Diseases 2008; 46:000–000

1058-4838/2008/4604-00XX$15.00
DOI: 10.1086/525856

and at the time of AOM diagnosis, using Mini-Tip Culturette kits (Becton Dickinson). The specimens were submitted for routine bacterial cultures on blood and chocolate agar plates. Isolates of *S. pneumoniae* were identified using the optochin disk susceptibility test (Taxo P; Becton Dickinson); *S. pneumoniae* isolates were not serotyped. *M. catarrhalis* isolates were identified by the API QuadFerm assay (bioMérieux), and NT *H. influenzae* isolates were identified by the Haemophilus ID Quad Plate with Growth Factors (Becton Dickinson).

χ^2 analysis was performed using Stata statistical software, version 9.0 (Stata). Logistic regression modeling was performed using SAS, version 9.1 (SAS Institute).

Results. During the study period, a total of 1295 URI episodes were documented in 294 patients who were enrolled and followed up in our study. Of the total URI episodes, 867 were seen by the study group; 709 URI episodes involving 198 patients met the inclusion criteria for this analysis. Of 198 patients, 49% were male, 58% were white, 29% were black, 10% were biracial, and 3% were Asian. Forty-three percent were of Hispanic or Latino ethnicity. The median age at enrollment was 12 months.

Nasopharyngeal cultures were positive for pathogenic bacteria in 607 cases (85.6%). Seventy-five percent of the culture samples were obtained on or before day 4 of illness. The proportion of AOM pathogenic bacteria isolated from the nasopharynx at the time of URI is shown in figure 1. *S. pneumoniae* was isolated alone and in combination during 209 episodes (34%), NT *H. influenzae* was isolated in 205 (34%), and *M. catarrhalis* was isolated in 417 (69%).

Two hundred forty-seven URI episodes (35%) were complicated by AOM (93 of them were bilateral). AOM diagnosis peaked on day 3, and 85% of AOM episodes were diagnosed within 7 days after URI onset. The ORs showed increased risk of AOM when the nasopharynx was colonized with pathogenic bacteria (*S. pneumoniae,* NT *H. influenzae,* or *M. catarrhalis*), compared with no pathogen (table 1). AOM rates were adjusted for breast-feeding, day care attendance, and cigarette smoke exposure. The 3 pathogenic bacteria, either alone or in combination, were more likely to be isolated from samples from children with AOM than from samples from children without AOM. The OR for *S. pneumoniae* was 1.8 (95% CI, 1.3–2.6) ($P < .001$), the OR for NT *H. influenzae* was 2.2 (95% CI, 1.6–3.1) ($P < .001$), and the OR for *M. catarrhalis* was 1.9 (95% CI, 1.4–2.8) ($P < .001$). Of the cases of AOM, 168 (68%) were diagnosed on the day of nasopharyngeal swab collection. To determine the predictive value of bacterial culture of nasopharyngeal specimens for AOM occurrence prior to AOM diagnosis, we excluded the URI episodes in which AOM was diagnosed at the time of nasopharyngeal swab collection. In this subset of data (table 2), ORs and adjusted ORs were similar; however, reduced numbers changed the statistical significance of differences in a few categories. Overall, compared with a child with no bacteria in the nasopharynx, a child with $\geqslant 2$ types of bacteria in the nasopharynx was 2.6 times as likely (95% CI, 1.8–3.8 times as likely) ($P < .001$) to have AOM after controlling for breast-feeding, day care attendance, cigarette smoke exposure, and number of doses of 7-valent pneumococcal vaccine (data not shown).

Discussion. Of 709 URI episodes included in this study, approximately one-third resulted in AOM. We obtained nasopharyngeal cultures early in the course of URI and clearly showed what had long been speculated: the presence of pathogenic bacteria in the nasopharynx during URI increases the risk for the complication of AOM. Children colonized with *S. pneumoniae,* NT *H. influenzae,* and *M. catarrhalis* concurrently were at the highest risk for AOM, compared with children with no pathogenic bacteria in their nasopharynxes. Our data suggest that nasopharyngeal colonization results obtained at early URI

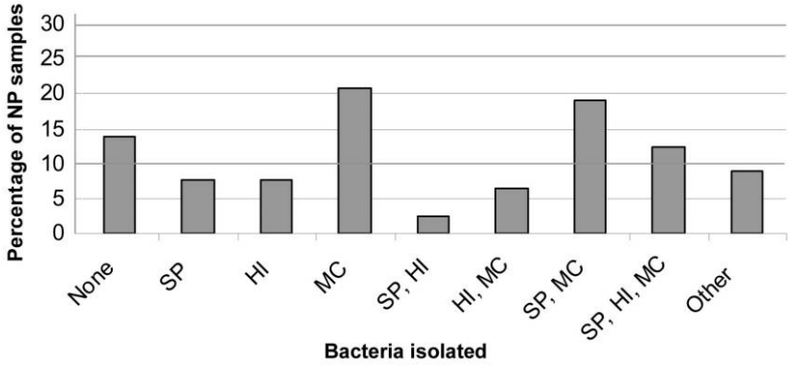

Figure 1. Acute otitis media pathogenic bacteria isolated from the nasopharynx (NP) at the time of upper respiratory tract infection. SP, *Streptococcus pneumoniae;* HI, *Haemophilus influenzae;* MC, *Moraxella catarrhalis;* other, *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae,* diptheriods, and *Bacillus* species.

Table 1. Risk of acute otitis media (AOM) complicating upper respiratory tract infection (URI) by pathogenic bacteria colonized in the nasopharynx at the time of URI.

Nasopharyngeal bacterial colonization	No. of episodes	AOM incidence, %	OR (95% CI)	*P*	Adjusted OR[a] (95% CI)	*P*
No bacteria	102	10	Ref	Ref	Ref	Ref
Sp only	55	29	3.7 (1.6–9.0)	<.01	3.8 (1.6–9.1)	<.01
Hi only	54	43	6.8 (2.9–15.9)	<.01	6.7 (2.8–15.9)	<.01
Mc only	148	32	4.4 (2.1–9.2)	<.01	4.2 (2.0–8.8)	<.01
Sp and Hi	18	50	9.2 (3.0–28.5)	<.01	8.0 (2.1–30.0)	<.01
Sp and Mc	136	41	6.4 (3.1–13.4)	<.01	5.7 (2.7–12.2)	<.01
Hi and Mc	45	51	9.6 (4.0–23.0)	<.01	8.4 (3.2–21.5)	<.01
Sp, Hi, and Mc	88	51	9.6 (4.4–20.9)	<.01	15.3 (6.0–39.6)	<.01

NOTE. Data are for a total of 646 episodes, excluding 63 cultures that yielded other bacteria, including *Staphylococcus aureus,* group A β-hemolytic streptococci, group B β-hemolytic streptococci, diptheriods, and *Bacillus* species. Multiple bacteria listed are from the same sample. In 168 (68%) of the 247 diagnoses of AOM, AOM was diagnosed at the time of nasopharyngeal swab collection. Hi, *Haemophilus influenzae;* Mc, *Moraxella catarrhalis;* Ref, referent; Sp, *Streptococcus pneumoniae.*

[a] Adjusted for breast-feeding, smoking, and day care exposure.

onset may be helpful in predicting the risk of AOM complicating URI.

Nasopharyngeal colonization is dynamic, and the rate of turnover of nasopharyngeal flora may differ among children and among bacterial strains [8]. Up to 90% of children in day care carry AOM pathogens in their nasopharynxes at some point [9]. Children who are not prone to otitis media have the same rate of nasopharyngeal colonization as children who are prone to otitis media do when healthy [10]. Nasopharyngeal colonization changes as children progress from being healthy to having URI or AOM. Harrison et al. [5] found that, during URI, children have more bacterial types and higher bacterial colony counts in the nasopharynx, compared with during a healthy period. Syrjanen et al. [6] found that 87% of healthy *S. pneumoniae* carriers had *S. pneumoniae* at the time of AOM and that only 26% of noncarriers had *S. pneumoniae* at the time of AOM. Interestingly, the majority of *S. pneumoniae* associated with AOM were newly acquired *S. pneumoniae* strains, not those found during the healthy period. This group found that AOM due to *S. pneumoniae* occurred 3.8 times more often among children with newly acquired carriage than among children with established carriage. These data demonstrate that, to better understand the causal relationship between nasopharyngeal colonization and AOM, nasopharyngeal samples for culture should be obtained closer to the onset of AOM, ideally during URI.

Children in our study were seen and nasopharyngeal samples for culture obtained as early as possible after the onset of URI symptoms, usually within 2–5 days. Because the peak incidence of AOM complicating URI is also within 2–5 days after onset

Table 2. Risk of acute otitis media (AOM) complicating upper respiratory tract infection (URI) by pathogenic bacteria colonized in the nasopharynx at the time of URI, excluding nasopharyngeal swab samples obtained at AOM diagnosis.

Nasopharyngeal bacterial colonization	No. of episodes	AOM incidence (%)	OR (95% CI)	*P*	Adjusted OR[b] (95% CI)	*P*
No bacteria	97	5	Ref	Ref	Ref	Ref
Sp only	46	15	3.3 (0.99–11.0)	.04	3.5 (1.0–12.3)	.05
Hi only	39	21	4.7 (1.4–15.6)	<.01	5.2 (1.5–17.9)	<.01
Mc only	119	16	3.5 (1.2–9.7)	<.01	3.7 (1.3–10.3)	.01
Sp and Hi	11	18	4.1 (0.7–24.2)	.09	10.5 (1.1–101.8)	.04
Sp and Mc	96	17	3.7 (1.3–10.5)	.01	3.7 (1.2–10.9)	.02
Hi and Mc	26	15	3.3 (0.8–13.4)	.07	3.6 (0.8–16.3)	.1
Sp, Hi, and Mc	53	19	4.3 (1.4–13.3)	<.01	7.5 (1.9–30.4)	<.01

NOTE. Data are for 541 episodes, excluding 54 cultures that yielded other bacteria, including *S. aureus,* group A β-hemolytic streptococci, diptheriods, and *Bacillus* species. Multiple bacteria listed are from the same sample. In 168 (68%) of the 247 diagnoses of AOM, AOM was diagnosed at the time of nasopharyngeal swab collection. Hi, *Haemophilus influenzae;* Mc, *Moraxella catarrhalis;* Ref, referent; Sp, *Streptococcus pneumoniae.*

[a] Adjusted for breast-feeding, smoking, and day care exposure.

of URI [11–13], AOM was already diagnosed at the initial visit in 68% of cases. Nevertheless, we were still able to compare nasopharyngeal culture results for 32% of children with URI prior to development of AOM (i.e., those with cases diagnosed later in the first week through the third week of URI onset) with those for children with URI who never developed AOM. There was significant correlation between nasopharyngeal colonization with multiple pathogenic bacteria and the occurrence of AOM, even after exclusion of cases of AOM that were diagnosed at the time of nasopharyngeal sample collection.

There has been some evidence to suggest that children who have been vaccinated with 7-valent pneumococcal vaccine are more likely to have *Staphylococcus aureus* in the nasopharynx [14]. In our study, 57 nasopharyngeal cultures (8%) yielded *S. aureus* alone or in combination with other bacteria. There was no difference based on pneumococcal vaccination status.

It has now been accepted that viruses play a major role in the pathogenesis of AOM [1, 15], and AOM occurs most frequently as a bacterial complication of viral URI. Viruses alone may also cause AOM [15]; this could be the case in AOM for which there were no bacteria colonized in the nasopharynx in our study. In general, both the URI-causative virus and the colonized bacteria play major roles in AOM pathogenesis [1]. Therefore, effective prevention of AOM will need to include both prevention of viral URI and prevention and/or elimination of nasopharyngeal colonization with pathogenic bacteria. Further studies are required to better understand how these interventions affect bacterial and viral interactions in the pathogenesis of virus-induced AOM. Such understanding will lead to better ways for effective prevention of this highly prevalent pediatric disease.

Acknowledgments

We thank M. Lizette Rangel, Kyralessa B. Ramirez, Liliana Najera, Rafael Serna, Michelle Tran, and Syed Ahmad, for their assistance with study subjects.

Financial support. National Center for Research Resources, National Institutes of Health (M01 RR 00073), and National Institutes of Health (R01 DC005841 and DC 005841–02S1 to T.C.).

Potential conflicts of interest. All authors: no conflicts.

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