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Impact of Respiratory Viral Infections on Alpha Hemolytic Streptococci and Otopathogens in the Nasopharynx of Young Children

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Abstract

Background—We studied nasopharyngeal (NP) colonization in a cohort of children to determine the impact of viral upper respiratory infections (URI) on non-pneumococcal alpha hemolytic streptococci (AHS) and otopathogen colonization in association with acute otitis media (AOM).

Methods—NP samples were collected routinely when children were aged 6, 9, 12, 15, 18, 24, and 30 months and during episodes of AOM. NP samples were prospectively obtained from 248 children during a 5-year time span; 1,018 during routine visits, 161 at the time of AOM and 59 at follow-up visits 3 weeks after AOM.

Results—The overall NP colonization rate of AHS was 50.8% during a non-AOM visit but declined to 38.3% during a viral URI with concurrent AOM (p=0.0006). Of 56 AOM visits with paired follow-ups, 6 (10.7%) had AHS in the NP at the time of viral URI and concurrent AOM whereas 29 (51.8%) had AHS at the follow-up (p<0.001). Lower NP colonization rates with AHS were associated with significant increases in *Streptococcus pneumoniae* carriage during non-AOM visits (p<0.001) and during viral URI and concurrent AOM visits (p=0.003). AHS NP colonization rates were not different when children had a viral URI without AOM versus when they were URI negative, but NP colonization with non-typeable *Haemophilus influenzae* rates increased (p<0.001) and *Moraxella catarrhalis* decreased (p<0.001) during viral URI.

Conclusion—Respiratory viral infections alter NP carriage rates of commensal AHS and otopathogens, including prior to AOM.

Keywords

Alpha Hemolytic Streptococci; *Streptococcus pneumoniae*; *Haemophilus influenzae*; *M. catarrhalis* acute otitis media

INTRODUCTION

The upper respiratory tract microbiome may include potential bacterial pathogens that cause acute otitis media (AOM). Viral upper respiratory infections facilitate an increase in otopathogen density and alter host defenses thereby increasing the risk of AOM.(1;2) *Streptococcus pneumoniae (Spn)*, non-typeable *Haemophilus influenzae (NTHi)*, and

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Moraxella catarrhalis (M cat) are the predominant otopathogens.(3–5) The frequency that *Spn* and *NTHi* are isolated from the same nasopharyngeal (NP) specimen is significantly less than would be predicted based on their relative prevalence.(6) This suggests a competition in the NP and indeed several studies have provided explanations of how *Spn* could inhibit *NTHi* colonization.(7–9) Jacoby, et al. observed positive associations between pairwise combinations of *Spn* and *NTHi* and between *Spn* and *M cat* in healthy Aboriginal children.(10;11) This is in contrast to the observations of Pettigrew, et al, of a negative interaction between *Spn* and *NTHi*, which can shift with the presence of *M cat* allowing for all three potential otopathogens to colonize together in children with a viral URI.(11) Our group recently showed that when co-colonizing the NP, *NTHi* predominates over *Spn* to cause AOM except serotype 19A strains.(12)

Non-pneumococcal α-hemolytic *Streptococci* (AHS) also are part of the microbiome of the NP beginning at an early age.(13) AHS are normally avirulent commensals that have been shown to inhibit growth of *Spn* in the NP.(14) Previous studies have shown an increase in the rate of NP colonization with *Spn, NTHi* and *M cat* among children experiencing AOM and a decrease in the commensal flora at the time of infection.(15) Also reduced quantities of AHS have been found in otitis prone children.(16) Furthermore, loss of AHS due to antibiotic treatment raises the risk of colonization by otopathogens as well as AOM.(17–19)

Children younger than 2 years old suffer an average of six to eight respiratory infections per year, which coincides with the highest AOM occurrence.(20) Ninety-Four percent of children who develop AOM have a concurrent viral URI at diagnosis.(20;21) Respiratory syncytial virus (RSV) load in the presence of *Spn*, has been associated with increased risk of AOM in children.(22) No prior study has examined the influence of respiratory viral infections on AHS NP carriage or the associated effects on colonization with otopathogens in a prospective cohort of children at onset of AOM compared to without development of AOM. Therefore, we sought to determine alterations of the bacterial NP in the presence or absence of a respiratory viral infection with a specific focus on AHS and potential otopathogens in association with AOM.

Methods

Study design and population

The samples evaluated in this report were collected as part of a prospective study that commenced in July 2006 and is ongoing. For this analysis, we included samples collected between January 2007 and October 2011. The children were enrolled at 6 months of age and followed prospectively. NP and OP samples were obtained at 6, 9, 12, 15, 18, 24, and 30 months of age for microbial culture. Inclusion criteria were: healthy, full term birth, no craniofacial anomalies, no known immune deficits, and no AOM events prior to enrollment at 6 months of age. AOM was diagnosed by validated otoscopists relying on criteria recommended by the American Academy of Pediatrics but with a requirement for a bulging tympanic membrane.(23;24) Middle ear fluid (MEF; obtained by tympanocentesis) confirmed the diagnosis and established the etiology of AOM when a child developed their first and any subsequent AOM episodes. NP and OP samples were obtained at the time of AOM and 3 weeks later (follow-up samples). Patients enrolled prior to June 2010 received the PCV-7 vaccine, and patients enrolled after June 1, 2010 received PCV-13 vaccine. The study was approved by the University of Rochester and subsequently by the Rochester General Hospital IRB and written informed consent was obtained from parents.

Sampling NP and MEF samples were collected and processed as previously described.(25)

Demographics, Risk Factors, and Antibiotic Use

Upon enrollment, demographics and risk factors were elicited from the parents via questionnaire. The information collected included sex, breast-feeding status, tobacco smoke exposure in the household, family history of AOM, number of siblings, and participation in daycare. At every visit parents were asked if their child had antibiotics within the prior 30 days, and if so, what type, at what dose, and the duration of use.

Symptoms of Viral Upper Respiratory Infection

In the 2009–2010 and 2010–2011 winter respiratory seasons clinical viral upper respiratory infection (URI) symptoms were assessed by a physician investigator prospectively and samples for viral detection were collected. As described by Kalu *et al*, we defined a viral URI clinically by the occurrence of acute onset of rhinorrhea, cough, decreased appetite, and malaise with or without fever.(26) The number of days the symptoms were present prior to the sampling was obtained from the parents.

Bacteriology

NP swabs, nasal washes (NW), and MEF were plated on Chocolate II agar (BD) and TSA with 5% sheep blood (BD) and then isolates were selected based on colony morphology for further identification.

For the detection of *Spn*, colony morphology, α-hemolysis on trypticase soy agar (TSA) with 5% sheep blood, and inhibition by optochin were used to confirm the species identity. If inhibition by optochin was inconclusive, dissolution of colony by desoxycholate was used to test if it was *Spn*. AHS was determined by colony morphology, α-hemoylsis on TSA with 5% sheep blood, and growth in the presence of optochin were used to identify AHS. Identification of *NTHi* was based on colony morphology, gram stain, growth in quadrants III and IV on QuadID plates, no hemolysis in quadrant IV on QuadID plate, and inability to synthesize porphyrin. *M cat* identification was based on colony morphology, gram stain, oxidase reaction, and reaction to the catarrhalis disk (Remel). For each isolate, sub-culturing was used to ensure the presence of a pure isolate.

Statistical analysis

The main outcomes of interest were the relationships between AHS, in the presence or absence of URI symptoms, and other otopathogens that reside in the NP of children. All statistical analyses were conducted by using STATA version 12.0 (College Station, TX). We examined colonization of the NP by non-pneumococcal alpha hemolytic streptococci, *Spn, NTHi,* and *M cat* by using repeated measures logistic regression with an unstructured (UN) correlation structure using the command xtmelogit (a command similar to xtmixed, but for use with binary outcomes). A repeated measure design was utilized due to the longitudinal nature of this study, with children observed every 3 months from 6 months of age to 3 years, with AOM visits interspersed. This design will account for variability both within the subject and between the subjects.(27) The model for AHS colonization included the following covariates: colonization by other otopathogens (*Spn, NTHi,* and *M cat*), type of visit (Non-AOM, AOM, Follow-Up), presence of URI symptoms at visit, and antibiotic exposure 30 days prior to visit. Host factors included sex, breast-feeding (formula, <6 months, ≥6 months, combination of formula and breastfeeding), daycare attendance (home setting, center, both), exposure to tobacco smoke, and family history of ear infections. Results for the model were expressed as odds ratios with a 95% confidence interval.

Results

NP samples were obtained from 248 children; 1,018 were collected during a routine scheduled visit and 161 samples were collected at the time of an AOM event. Of the 248 children sampled, 135 (54.4%) were male, 147 (59.3%) were Non-Hispanic White, and 93 had a family history of otitis media (Table 1). The average age at enrollment was 6.9 months. Of the 161 AOM events, 106 (65.8%) patients had bacteria isolates in the MEF and an additional 29.1% detected by multiplex PCR (data not shown). In a subset analysis of available samples collected during a clinical viral URI (N=89), 47% of the samples were PCR positive for respiratory syncytial virus A or B, parainfluenza 3, or Influenzae A or B; PCR methods were not applied. The positive results suggest that the clinical diagnosis of a viral upper respiratory infection was adequately supported by PCR results in the samples available for testing.

The colonization rate of AHS and 3 potential otopathogens with and without viral URI symptoms during non-AOM visits

URI symptom data was available for 475 NP samplings during 2009–2011 when children were evaluated at pre-scheduled visits, of which 331 (69.7%) did not have viral URI symptoms while 144 (30.3%) did. Having a viral URI was not associated with any change in AHS colonization frequency (168 versus 74 out of 144 samples). Viral URI also did not significantly effect *Spn* colonization rates (83 (25.1%) vs. 48 (33.3%). Viral URI was associated with a significant increase in *NTHi* carriage, increasing from 14 (4.2%) to 30 (20.8%), $p< 0.001$. Viral URI was also associated with a significant increase in *M cat* carriage from 88 (26.6%) to 68 (47.2%), $p< 0.001$.

Colonization rate of AHS and 3 potential otopathogens in non-AOM versus viral URI infections with concurrent AOM visits

In contrast to the above findings, when viral URI progressed to AOM AHS carriage was significantly reduced (38.3% of 60 children) compared to, the 51.4% of children who had a viral URI not associated with AOM, p=0.006. The opposite occurred with otopathogens. At the time of a viral URI with concurrent AOM, 30 (50.0%), 19 (31.7%), and 29 (48.3%) carried *Spn*, *NTHi*, and *M cat*, respectively. Whereas at non-AOM, viral URI negative visits, 83 (25.1%), 14 (4.3%), and 88 (26.6%) carried *Spn*, *NTHi*, and *M cat*, respectively. The increases in *Spn, NTHi,* and *M cat* carriage during a viral URI and concurrent AOM event were significant ($p<0.001$).

Colonization rate of AHS at the time of respiratory viral infection and concurrent AOM versus recovery

Of the 56 AOM visits with paired NP samples, 6 (10.7%) of the samples had AHS in the NP at the time of viral URI and concurrent AOM whereas 29 (51.8%) had AHS in the follow-up samplings 3 weeks after recovery from infection, a significant increase ($p<0.001$).

Effect of AHS colonization on *Spn* colonization during non-AOM visits

Of the 1018 non-AOM samplings, *Spn* colonization without AHS was 44.6% (N=484), while *Spn* colonization with AHS was 13.9% (N=534 $p<0.001$). Colonization rates of *NTHi* and *M cat* with AHS were 8.1% and 37.1% respectively. Colonization rates of *NTHi* and *M cat* without AHS were 9.1% and 38.4% respectively. The difference in colonization rates by *NTHi* or *M cat* with and without AHS was not significantly different.

Multilevel modeling of NP colonization with AHS and covariates

Repeated measures logistic regression models predicting colonization by AHS are shown in Table 2. A positive association between AHS in the NP is indicated by an odds ratio (OR) ≥ 1; a negative association is indicated by an OR<1. In our model, AHS was negatively associated with *Spn* and AOM visit type. When the dataset was split into non-AOM visits and AOM visits and our model was analyzed, the same negative association between AHS and *Spn* was observed in both non-AOM and AOM visits (data not shown). Of the seven host characteristics in our model, only breast-feeding for 6 months or less was associated with colonization of NP by AHS (p=0.053). Antibiotic exposure was not associated with differences in AHS colonization.

Discussion

In this study among children in the peak age range of incidence of AOM we determined the impact of viral infection on AHS NP colonization and on NP colonization rates of *Spn*, *NTHi*, and *M cat*. The study occurred in a population of children that had been PCV7 vaccinated and herd immunity likely established since our community commenced PCV7 vaccination immediately after licensure in 2000. We found: 1) AHS and *Spn* carriage was the same, while *NTHi,* and *M cat* carriage were higher when children experienced a viral URI compared to without viral URI. 2) In contrast, AHS carriage was reduced and *Spn*, *NTHi* and *M cat* carriage was increased when children had a viral URI that was associated with AOM. 3) The frequency of isolation of AHS at onset of viral URI and concurrent AOM was decreased two-fold compared to after recovery from infection. 4) The frequency of isolation of *Spn* when AHS was present was two-fold lower than when AHS was absent.

A viral URI is known to initiate an inflammatory response in the NP, associated with damage to epithelial cells, dysfunction of cilia, increased viscosity of nasal mucus, up-regulation of epithelial cell receptors for respiratory bacteria, up-regulation of inflammatory cytokines, recruitment of polymorphonuclear neutrophils, and down-regulation of other innate and adaptive immune responses.(28–31) We have shown that viral URI perturbs the polymicrobial balance in the NP.

Viral URIs predispose children to AOM.(30) The very high rate of antecedent/concurrent viral URI we observed is consistent with many prior reports.(2;30–33) Our results suggest the NP microenvironment with viral URI and concurrent AOM must differ from that of viral URI not associated with AOM. AHS colonization rates were the same comparing children with and without viral URI but if viral URI was associated with AOM then AHS colonization rates were lower, consistent with a loss of the protective role of AHS during URIs that are followed by AOM. We interpret this finding to suggest that viral URI itself does not diminish AHS NP colonization but rather the pro-inflammatory conditions of viral URI that is associated with AOM provides a favorable environment for *Spn* to increase in density/inoculum. A second observation supporting a distinctive effect of viral URI on *Spn* was that viral URI was associated with an increase in *NTHi* and *M cat* carriage but when viral URI was followed by AOM an increase in *Spn* was also detected. A viral URI creates a more favorable micro-environment for *NTHi* and *M cat* resulting in a synergistic interaction.(34) Our results suggest that the presence of *NTHi* and *M cat* creates a more favorable micro-environment for *Spn* colonization, which then can lead to an AOM event.

As an extension of the possible protective role of AHS in the polymicrobial environment of the NP, we found that AHS colonization in the NP was significantly lower at the onset of viral URI and concurrent AOM compared to follow-up samplings in the same children after recovery from illness. The result suggests that after an episode of AOM part of the recovery

process includes AHS re-colonization. However, in some cases AHS strains that re-colonize the NP after antibiotic therapy for AOM may be more resistant to antibiotics.(17)

The influence of normal upper respiratory commensals, specifically non-pneumococcal AHS, as interfering organisms for otopathogen colonization has received prior study.(7;8;13;16;17;35) Our result regarding competition between AHS and *Spn* in the NP is consistent with earlier reports and with a protective role of AHS colonization when given to children as a probiotic therapy to prevent AOM caused by *Spn*.(14;16;35;36) Unlike a previous report, we could not correlate any inhibition of *NTHi* colonization with the isolation of non-pneumococcal AHS.(35) AHS's production of hydrogen peroxide has been suggested to be the main mechanism by which it inhibits other pathogens.(8) Similarly some *Spn* strains are also capable of producing hydrogen peroxide and have a similar inhibitory effect on other species.(9) It was unexpected that AHS was not associated with a reduction in *NTHi* or *M cat* carriage since Pericone *et al* had demonstrated that hydrogen peroxide produced by *Spn* was capable of inhibitory and bactericidal activity against *NTHi* and *M cat*.(9) However, there are many factors that have not been well studied with regard to interspecies interactions in the NP and further research is needed.(7;9;37)

Our results from the multilevel model confirm previous reports regarding the negative association between AHS and *Spn*.(17;38) This negative association between *Spn* and AHS colonization was observed in both AOM visits and non-AOM visits. The relationship between AHS and either *NTHi* or *M cat* was unclear. In our repeated measures model, a Non-AOM visit (OR=2.135; p=0.001) and follow-up visit (OR=2.122; p=0.061) were positively associated with AHS colonization. When the reference group was changed in the model (to Non-AOM), AHS colonization was negatively associated with an AOM visit (OR=0.488, p=0.002). During an AOM event, colonization by either (singly or in combination) *Spn, NTHi* and *M cat* increases, outcompeting AHS by potentially depleting the NP of necessary nutrients or creating an inhospitable microenvironment.(38) The effect of other covariates in the model, i.e. age, daycare attendance, etc., were not significantly associated with AHS colonization ($p<0.05$). This was most likely due to a lack of variability for these covariates as these samples were drawn from a middle-to-upper middle class population. Our results from the multilevel model for breastfeeding suggest that breastfeeding for less than 6 months increases AHS colonization (OR=1.787; p=0.053), which agrees with previous reports regarding the protective effect of breastfeeding.(39–41) The significance around this statistic are borderline ($p>0.05$); more samples would confirm this result (only 6.8% of visits reported breastfeeding for < 6 months). Our study has limitations regarding viral identification. As observed with many prior studies, specific identification of the viral pathogen causing viral URI illnesses was not achieved for all samples.

The dynamics of polymicrobial NP colonization with commensals, such as AHS, and potential otopathogens is not well understood. Complicating the study of polymicrobial NP colonization and the presence of different URI viruses is age of the child reflected in a maturing innate and adaptive immune response and other environmental and epidemiologic risk factors as important covariates. Despite the complexity, in this study we have been able to make distinct observations regarding the influence of clinical viral URIs on NP colonization patterns involving AHS, *Spn*, *NTHi* and *M cat*. Study of the underlying mechanisms that may account for viral infection influencing polymicrobial interactions in humans is now an area of active study by our group. The introduction of the new 13-valent pneumococcal conjugate vaccine creates a new condition in the NP. The role of AHS as a competitor in the NP niche for *Spn* epithelial cell adherence and as a donor of antimicrobial resistance genes will deserve careful study.(17;42;43)

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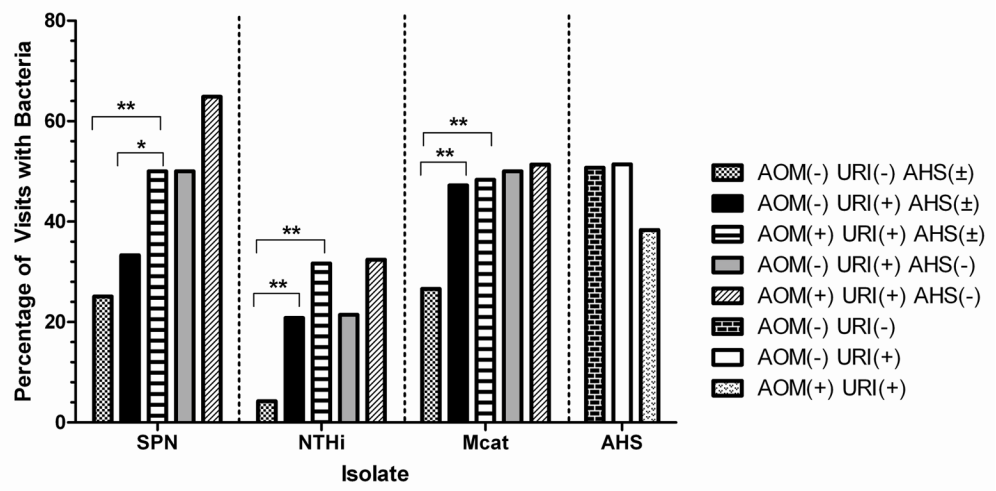

Figure 1.

Frequency of isolation of otopathogens under various criteria. AOM(±) indicates the presence or absence of an AOM event. URI(±) indicates the presence of absence of URI symptoms at the visit. AHS(±) indicates the presence or absence of AHS colonization in the NP during the visit. * indicates a significance of P = 0.05. ** indicates a significance of P < 0.001.

Table 1

Demographics of study patients at enrollment (N=248)

	No.	%
Sex		
Male	135	54.4
Female	113	45.6
Age[a] (months)		
Mean	6.9	
Range	5.8–15.2	
Race		
Non-Hispanic White	147	59.3
African American	48	19.4
Asian	2	0.8
Hispanic	12	4.8
Other	39	15.7
Family History of OM		
Maternal	34	13.8
Paternal	15	6.1
Sibling	24	9.7
>1 Family Member	20	8.1
None/Missing	155	62.3
Daycare[a]		
Home	49	60.5
Center	29	35.8
Combination (Home and Center)	3	3.7
Breast-feeding[a]		
<6 months	28	11.3
≥6 months	33	13.3
Formula	86	34.7
Combination (Formula and breast milk)	81	31.6
Antibiotic Exposure (Number of visits)		
Use in 0–30 days prior to visit	106	8.6
No antibiotic Use or 30+ days prior to visit	1133	91.4

[a] Variables collected at enrollment; daycare had 167 missing values, breast-feeding had 20 missing values

Table 2

Predicted outcome of colonization with AHS in young children (N=1146 visits)

Parameters	OR (95% CI)	P-value
Colonization by *S. pneumoniae*		
No (reference)	1.0	
Yes	**0.172 (0.125–0.238)**	**<0.001**
Colonization by *H. influenzae*		
No (reference)	1.0	
Yes	1.254 (0.803–1.957)	0.320
Colonization by *M. catarrhalis*		
No (reference)	1.0	
Yes	1.271 (0.963–1.676)	0.090
Visit type		
β AOM	1.0	
Non-AOM	**2.135(1.355–3.365)**	**0.001**
Follow Up	2.122(0.966–4.665)	0.061
Presence of URI symptoms		
No (reference)	1.0	
Yes	1.139 (0.782–1.660)	0.498
Sex		
Male	1.0	
Female	1.161 (0.868–1.554)	0.313
Exposure to tobacco smoke		
No (reference)	1.0	
Yes	0.820 (0.533–1.263)	0.369
Age (months)	1.010 (0.990–1.032)	0.316
Breastfed		
Formula(reference)	1.0	
Less than 6 months	1.787 (0.992–3.219)	0.053
More than 6 months	1.164 (0.761–1.780)	0.483
Combination (Formula + Breastfeeding)	0.883 (0.631–1.237)	0.471
Daycare		
Home	1.0	
Center	0.817 (0.477–1.401)	0.463
Both	0.362 (0.088–1.486)	0.158
Antibiotic Exposure in the last 30 days		
No (reference)	1.0	

Parameters	OR (95% CI)	P-value
Yes	0.969 (0.533–1.761)	0.920
Family History		
No (reference)	1.0	
Yes	1.150 (0.862–1.535)	0.342

Associations with a significant p-value (p 0.05) are indicated in bold lettering

βWhen the model included Non-AOM as the reference for visit type, the OR and p-value were; 0.488, p=0.002