Bacterial pathogens in the nasopharynx, nasal cavity, and osteomeatal complex during wellness and viral infection

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ABSTRACT

Background: Viral sinusitis can precede acute bacterial sinusitis, but the influence of viral infection on bacterial colonization is unclear. The objective of this study was to evaluate the presence of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in the osteomeatal complex (OMC), nasal cavity, and nasopharynx in adults during wellness and viral upper respiratory illness (URI).

Methods: Subjects were recruited for the study during wellness and at the time of acute viral rhinosinusitis. Swab cultures were obtained from the OMC, nasal cavity, and the nasopharynx. Swab eluates were inoculated on selective agars to detect *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*.

Results: The study included 237 subjects, 100 adults with URI and 137 well adults. Positive culture results were found for any site in 70% ($n = 70$) of ill subjects and 64% ($n = 88$) of well subjects ($p = 0.393$). Of the 91 OMC cultures, positive cultures were over five times more likely to be found in ill subjects than in well subjects (31% versus 8%; $p = 0.010$). The nasal cavity cultures were positively statistically significant more often in ill subjects versus well subjects (39% versus 25%; $p = 0.022$). The overall nasopharyngeal cultures did not show a statistically significant difference (65% versus 60%; odds ratio, 1.2; $p = 0.461$). *S. pneumoniae* was positively cultured in at least one site in 15% of ill subjects and 31% of well subjects ($p = 0.006$). *H. influenzae* was positively cultured in at least one site in 45% of ill subjects and 31% of well subjects ($p = 0.027$). *M. catarrhalis* was positively cultured in at least one site in 42% of ill subjects and 27% of well subjects ($p = 0.018$).

Conclusion: This study defines the carriage rates of the three most common bacterial pathogens for acute sinusitis in the nasopharynx, nasal cavity, and OMC during illness and in the healthy state.

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Bacterial sinusitis is often thought to be secondary to viral infection. The normal mucociliary clearance of the nasal and paranasal sinus mucosa is important for preventing infection by clearing bacterial pathogens. Viral upper respiratory infection (URI) is a common illness that can have a significant effect on this process by altering the normal clearance patterns, allowing bacterial colonization and subsequent infection.¹

Although the paranasal sinuses and osteomeatal complex (OMC) are usually sterile, the nasopharynx often harbors bacterial pathogens responsible for both middle ear and sinus infections. The nasopharynx is the end point for mucociliary clearance of bacterial pathogens from the middle ear and paranasal sinuses and is normally colonized by both pathogenic and nonpathogenic bacteria in the adult population.^{2,3}

This study was designed to determine the carriage rate of the three common bacterial pathogens—*Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*—in the OMC, nasal cavity, and nasopharynx in well adults and in adults with URI.

METHODS

Design

A case-control study was designed to compare the carriage rate of well adult subjects with adults with URI symptomatology. The study was approved by the University of Virginia School of Medicine Human Investigation Committee. This study includes data that were previously published for secondary analysis of sinonasal pathogens in well patients and those with URI.

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Subjects

Advertisements were used to invite people >18 years of age from the general population to participate in the study. Subjects were recruited from 1999 to 2006 and were provided compensation for their participation. The subjects were required to participate in a single office visit with a study physician; no follow-up visit was required. Ill adults were defined as individuals with self-reported URI symptomatology (nasal congestion, rhinorrhea, sore throat, or cough) for ≤ 8 days as documented by a physician. Well adults were defined as individuals with no URI symptomatology at least 3 weeks before study enrollment. Exclusion criteria included age <18 years or any history of previous sinus surgery, chronic sinusitis, or inhalant allergy. Subjects were excluded from the control group if they had used topical or oral antibiotics within 4 weeks before the study or had nasal symptoms within 14 days before the study. Exclusion criteria specific to the study group included fever, nasal polyposis, or use of antibiotics within 14 days before study enrollment.

Sampling Technique

1. For samples from the OMC, the nasal cavity was first inspected with a nasal speculum. Under visualization with a 0° rigid endoscope, a culture swab (rayon tip on aluminum wire shaft; Mini-Tip Culturette; Becton Dickinson, Cockeysville, MD) was passed into the OMC, rotated, and removed. The sample was then eluted into 1 mL of sterile saline.
2. For samples from the nasal cavity, the nasal cavity was inspected with a nasal speculum and a culture swab was passed beside the inferior turbinate along the floor of the nose until the posterior wall of the nasopharynx was reached. The swab was rotated, removed, and eluted into 1 mL of sterile saline.
3. For samples from the nasopharynx, the shaft of a culture swab was bent at a 45° angle. Under direct visualization with the tongue depressed, the culture swab was passed behind the soft palate and swept over the posterior nasopharyngeal wall. There was no contact of the culture swab with oral cavity or oropharyngeal mucosa. The swab was then eluted into 1 mL of sterile saline.

All samples were taken without the use of topical anesthetic or decongestant medication. The samples in the early group of well

subjects were collected under supervision of one of the senior authors (B.W.). The samples of the late group were collected by another senior author (J.K.H.).

Microbiology

All samples were plated on selective culture media. Each plate was inoculated with 0.1 mL of sample, which was spread over the agar. Plates were then incubated for 48 hours at 35°C. *S. pneumoniae* was isolated using gentamicin agar (tryptone blood agar base with 5% sheep blood and 5 µg/mL of gentamicin). *H. influenzae* was isolated on GCYSB agar (GC agar base with chocolate sheep blood, yeast autolysate, vancomycin, bacitracin, and clindamycin). *M. catarrhalis* was isolated on B-AVTA media (brucella agar base with 5% sheep blood, amphotericin B, vancomycin, trimethoprim, and acetazolamide). Selective media were used because these have been shown to have higher sensitivity compared with nonselective media.⁴ The plates for *S. pneumoniae* and *H. influenzae* were incubated in a candle extinction jar and plates for *M. catarrhalis* were incubated in air. Cultures with appropriate colony and Gram stain morphology were identified by routine methods.⁴ The number of colonies of bacteria on each plate was used to determine bacterial titer expressed as colony-forming units per milliliter of sample.

Statistical Analysis

Analyses were conducted using SPSS (Version 16.0; SPSS, Inc., Chicago, IL). Culture results were dichotomized into either positive (1) or negative (0). Odds ratios (OR)s with 95% confidence levels were calculated for each variable. Pearson chi-square and Fisher's exact tests were used to analyze the difference in proportions, using a two-tailed α -level of 0.05 to determine significance.

RESULTS

The study sample included a total of 237 subjects, consisting of 100 adults with self-reported early URI symptoms and 137 well adults who had no sinonasal symptoms for at least 3 weeks before the study. One well subject was excluded from the data analysis because the culture results showed overgrowth of *Proteus* species and was con-

sidered unreliable. Because the protocol was modified during recruitment to obtain OMC samples, this site was cultured in only 51 ill subjects and 40 well subjects. Ages of study subjects ranged from 20 to 62 years with a mean age of 38 years.

Table 1 provides the culture results for the ill and well subjects. At least one of the studied bacterial pathogens was found in at least one of the sites evaluated in 158 subjects. Overall, positive culture results were found for any site in 70% ($n = 70$) of ill subjects and 64% ($n = 88$) of well subjects, which does not represent a statistically significant difference ($p = 0.393$; 95% CI, 0.732–2.214).

Overall Carriage Rate by Site

Of the 91 OMC cultures, positive cultures were over five times more likely to be found in ill subjects than in well subjects (31% versus 8%; OR, 5.6; $p = 0.010$). Two hundred thirty-seven cultures of the nasal cavity and nasopharynx were performed. The nasal cavity cultures were positively statistically significantly more often in ill subjects versus well subjects (39% versus 25%; OR, 1.9; $p = 0.022$). The overall nasopharyngeal cultures did not show a statistically significant difference (65% versus 60%; OR, 1.2; $p = 0.461$).

Bacterial Pathogens by Site

Bacterial pathogens were studied at each site as well. Ninety-one swabs of the OMC were taken, consisting of 40 well subjects and 51 ill subjects. Nineteen positive cultures of the OMC were reported. Of these, only one of the cultures was positive for two different bacteria, *H. influenzae* and *M. catarrhalis*. There was only one positive culture, taken from an ill subject, for *S. pneumoniae* in the OMC; there were 9 cultures from the OMC that grew *H. influenzae*. All of these were taken from subjects with URI. There were no positive cultures for *H. influenzae* in the control group ($p = 0.004$). All of the cultures that were positive in the OMC were positive in the nasal cavity, nasopharynx, or both. There were 10 positive cultures for *M. catarrhalis* in the OMC. Three (8% of) well subjects grew *M. catarrhalis* and seven (14% of) subjects with URI were positive ($p = 0.503$). All 10 subjects who cultured positive for *M. catarrhalis* in the OMC also cultured positive for the same bacteria in the nasal cavity, nasopharynx, or both.

Table 1 Results of bacterial cultures, all subjects

Positive Bacterial Culture	Ill n (%)	Well n (%)	OR (95% CI)	p Value#
n	100	136		
Any site, any organism	70 (70)	88 (64)	1.273 (0.732–2.214)	0.393
OMC,* any organism	16 (31)	3 (8)	5.638 (1.511–21.040)	0.010§
NC, any organism	39 (39)	34 (25)	1.918 (1.097–3.353)	0.022§
NP, any organism	65 (65)	82 (60)	1.223 (0.716–2.089)	0.461
<i>Streptococcus pneumoniae</i> , any site	15 (15)	42 (31)	0.395 (0.204–0.763)	0.006§
<i>Haemophilus influenzae</i> , any site	45 (45)	42 (31)	1.831 (1.071–3.130)	0.027§
<i>Moraxella catarrhalis</i> , any site	42 (42)	37 (27)	1.938 (1.120–3.352)	0.018§
OMC,* <i>S. pneumoniae</i>	1 (2)	0 (0)		1.000
OMC,* <i>H. influenzae</i>	9 (18)	0 (0)		0.004§
OMC,* <i>M. catarrhalis</i>	7 (14)	3 (8)	1.962 (0.474–8.129)	0.503
NC, <i>S. pneumoniae</i>	5 (5)	13 (10)	0.498 (0.172–1.445)	0.200
NC, <i>H. influenzae</i>	16 (16)	10 (7)	2.400 (1.039–5.542)	0.040§
NC, <i>M. catarrhalis</i>	25 (25)	15 (11)	2.689 (1.333–5.425)	0.006§
NP, <i>S. pneumoniae</i>	12 (12)	35 (26)	0.394 (0.192–0.805)	0.011§
NP, <i>H. influenzae</i>	44 (44)	39 (29)	1.954 (1.136–3.361)	0.015§
NP, <i>M. catarrhalis</i>	38 (38)	32 (24)	1.992 (1.131–3.507)	0.017§

OMC cultures were obtained in only a portion of the subjects (ill: $n = 51$; well: $n = 40$).

#Two-tailed α -level of 0.05 used to define statistical significance. Fisher's exact test was used if any cells had expected counts of <5 ; otherwise, Pearson χ^2 was used.

§Statistical significance.

OMC = osteomeatal complex; NC = nasal cavity; NP = nasopharynx; OR = odds ratio.

There were 237 swabs taken in both the nasal cavity and the nasopharynx. In one patient, both sites had cultures overgrown with *Proteus*. This patient was not included in the data analysis. Of the 236 remaining subjects, 136 were in the control group and 100 were in the study group. In the nasal cavity, there were 18 cultures that grew *S. pneumoniae*. Thirteen (72%) of these cultures were from the well group with only 5 (28%) coming from the ill group. Ten percent of cultures from well subjects were positive and 5% from ill subjects were positive ($p = 0.200$). *H. influenzae* was cultured in the nasal cavity in 26 subjects. Ten (7%) well subjects cultured positive for *H. influenzae* and 16 (16%) ill subjects cultured positive ($p = 0.040$). There were 40 positive cultures for *M. catarrhalis* in the nasal cavity, consisting of 15 (11%) well subjects and 25 (25%) ill subjects that cultured positive ($p = 0.006$).

In the nasopharynx, 47 cultures grew *S. pneumoniae*. Thirty-five (74%) positive cultures were from the control group (well group) and the remaining 12 (26%) were from the study group (ill group). Twenty-six percent of cultures in the well group grew *S. pneumoniae* along with the 12% of ill patients that grew *S. pneumoniae* ($p = 0.011$). *H. influenzae* was cultured in 83 patients. Thirty-nine (29%) well subjects cultured positive for *H. influenzae* and 44 (44%) ill subjects cultured positive ($p = 0.015$). There were 70 cultures that grew *M. catarrhalis*. Thirty-two (24%) subjects in the control group cultured positive and 38 (38%) subjects in the study group cultured positive ($p = 0.017$).

Bacterial Pathogens Overall

S. pneumoniae was positively cultured 57 times overall from the various sites. Fifteen (26%) of these cultures were from ill subjects and 42 (74%) were from well subjects. Fifteen percent of ill subjects grew *S. pneumoniae* in at least one site and 31% of well subjects cultured positive in at least one site ($p = 0.006$). There were 87 positive cultures overall for *H. influenzae* with 45 (52%) from the ill group and 42 (48%) from the well group. Forty-five percent of ill subjects cultured positive for *H. influenzae* and only 31% of well subjects cultured positive ($p = 0.027$). Finally, there were 79 positive cultures for *M. catarrhalis* with 42 (53%) in ill subjects and 37 (47%) in well subjects. Forty-two percent of ill subjects had positive cultures for *M. catarrhalis* compared with only 27% of well subjects culturing positive ($p = 0.018$).

DISCUSSION

Overall, there were more numbers of positive cultures for all sites in well subjects when compared with ill subjects. However, there was no significant difference in the percentage of well subjects with positive cultures (64%) and the percentage of ill subjects with positive cultures (70%). There were more well subjects in the study than ill subjects, which explains the greater raw number of positive cultures in the well group. The percentage of positive cultures was higher in the nasopharynx in both the ill and the well groups than that of the nasal cavity or OMC. This is likely because the nasopharynx serves as a reservoir for both nonpathogenic and pathogenic bacteria during both wellness and during periods of illness.³ The sampling technique may also explain the high percentage of positive cultures from the nasopharynx. Previous studies have found no significant difference in the prevalence of *M. catarrhalis* between the oropharynx and naso-

pharynx.⁵ One study found the prevalence of *H. influenzae* to be higher in the nasopharynx than on the tonsils.⁶

There were significantly more positive cultures for the various bacterial pathogens in the OMC in the ill group compared with the well group. Thirty-one percent of ill subjects cultured positive when compared with 8% of well subjects. In addition, there was a statistically significant difference in percentage of positive cultures for *H. influenzae* in the OMC. The OMC is usually sterile in adults without infection. In a study by Chow, there were no positive cultures of the OMC in 10 healthy adults.⁷ In addition, Klossek *et al.* showed only 4 positive cultures in 139 healthy adults.⁸ Gwaltney *et al.* indicated that nose blowing may propel nasal and nasopharyngeal fluid into the paranasal sinuses.⁹ Impaired mucociliary clearance may also contribute to the increased incidence of positive cultures of the OMC in the ill group. Sakakura *et al.* showed that rhinovirus infection caused decreased mucociliary clearance.¹ These factors may all contribute to the increased incidence of bacterial pathogens in the OMC in the ill group.

There were 19 positive cultures of the OMC. Of these, 9 cultured positive for *H. influenzae* and 10 subjects cultured positive for *M. catarrhalis*. One of the patients who cultured positive for *H. influenzae* also cultured positive for *M. catarrhalis* as well. All of the subjects who grew *H. influenzae* in the OMC also grew the same pathogen in the nasal cavity, nasopharynx, or both. In addition, all of the subjects who cultured positive for *M. catarrhalis* in the OMC also cultured positive in the nasal cavity, nasopharynx, or both. This is further indication that the nasopharynx and nasal cavity may be reservoirs for bacterial pathogens (Table 2).

We also found a significant difference between the two groups in the nasal cavity. Thirty-nine percent of subjects in the study group had positive cultures and 25% of subjects in the control group had positive cultures ($p = 0.022$). The nasal cavity, although not sterile, does not have a significant burden of bacterial pathogens. Ylikowski *et al.* showed that only 6% of well adults cultured positive for either *S. pneumoniae* or *H. influenzae*.³ There was also a statistically significant difference at this site for *H. influenzae* and *M. catarrhalis*. Only 7% of well subjects cultured positive for *H. influenzae* in the nasal cavity when compared with 16% of ill subjects ($p = 0.040$). Twenty-five percent of cultures of the nasal cavity were positive for *M. catarrhalis* in the ill group when compared with 11% in the well group ($p = 0.006$). One may expect to find a difference in the nasal cavity flora during periods of illness as opposed to during wellness. Jousimies-Somer found that the three most common bacterial pathogens in sinusitis—*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*—were rarely isolated from the nasal cavity in well individuals. The same bacteria were cultured in significantly higher percentages in patients with acute maxillary sinusitis.¹⁰ The nasopharyngeal reservoir of bacterial pathogens along with the impaired mucociliary clearance may predispose to infection or colonization of the nasal cavity by these bacteria. The selective agar media used in this study may also play a role. Previous authors have shown the efficacy of using the particular media used in this study for *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*, respectively.^{11–14}

Because the nasopharynx already serves as a reservoir of bacteria, one would not expect a significant increase in the carriage rate during illness. Indeed, the well and ill groups in this study were similar. Sixty-five percent of ill subjects had a positive culture for one of the three bacterial pathogens and 60% of well subjects had a positive

Table 2 Results of positive cultures of the OMC

Positive Cultures	OMC	NC	NP	NC/NP
Total <i>n</i>	19	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
<i>Streptococcus pneumoniae</i>	1	1/1 (100)	1/1 (100)	1/1 (100)
<i>Haemophilus influenzae</i>	9	7/9	8/9	9/9 (100)
<i>Moraxella catarrhalis</i>	10	8/10 (80)	9/10 (90)	10/10 (100)

Distribution is shown of positive cultures of the OMC by bacterial pathogen and indicates the occurrence of the same pathogens in the nasal cavity and nasopharynx.

OMC = osteomeatal complex; NC = nasal cavity; NP = nasopharynx.

culture. However, the carriage rate of specific pathogens was different in the ill group. Although the percentages of positive cultures was consistent for each of the three pathogens in the well group, the percentage of cultures positive for *S. pneumoniae* in the ill group was 12% when compared with 44 and 38% for *H. influenzae* and *M. catarrhalis*, respectively. There was a significantly different percentage of positive cultures between the two groups for each bacterium. There were significantly more positive cultures for *S. pneumoniae* in the well group when compared with the ill group. This does not necessarily reflect a protective factor for the nasopharynx with respect to *S. pneumoniae* and does not indicate clinical significance. The opposite was true of *H. influenzae* and *M. catarrhalis*. This may be caused by the sampling technique for the nasopharynx. Although Chi *et al.* showed the current technique to be the most sensitive overall for the nasopharynx, they did find that it was not as helpful for detecting *S. pneumoniae* when compared with the other pathogens.¹⁵

We also found statistically significant differences between the ill and well groups with regard to the three bacteria throughout all sample sites. *S. pneumoniae* was cultured in 31% of well patients and in only 15% of ill patients. This would indicate a protective factor of *S. pneumoniae*. However, the majority of positive cultures for *S. pneumoniae* were cultured from the nasopharynx and although there may be a statistically significant difference, the clinical significance is unclear. Although bacterial pathogens are frequently present in the nasopharynx, they rarely cause infection at this site. There was also a statistically significant difference between the two groups with regard to *H. influenzae* and *M. catarrhalis*. Forty-five percent of ill subjects overall cultured positive for *H. influenzae* whereas only 31% of well subjects overall cultured positive ($p = 0.027$). These findings were similar for *M. catarrhalis*, with 42% of all ill subjects and 27% of all well subjects culturing positive ($p = 0.018$). This is consistent with the difference between carriage rates of these pathogens at the various sites evaluated in this study including the nasopharynx and nasal cavity.

There are several limitations of this study. First, bacteria may be undercultured. Other techniques such as polymerase chain reaction or microarray may be more sensitive for detecting the presence of bacteria compared with standard culturing techniques.¹⁶ Post *et al.* developed a multiplex polymerase chain reaction-based assay to reliably detect *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* in the clinical laboratory.¹⁷ Other investigators have shown similar sensitivities with microarray techniques in detecting these pathogens from middle ear fluid and throat swab samples compared with those of standard culture techniques.¹⁸ In addition, other techniques for culturing the various sites may have altered our results. For example, Chi *et al.*¹⁵ cultured the nasopharynx in a variety of ways including passing swabs through the mouth and using aspiration of nasopharyngeal secretions. They found that per oral swabs of the nose provided the highest sensitivity for detecting presence of bacteria overall. However, this technique was deficient in detecting the presence of *S. pneumoniae* specifically.¹⁹ Second, only the most common bacteria were evaluated. Studying other common bacterial pathogens may have a significant impact on the outcome. Finally, culturing for common viral respiratory pathogens may have added further significance to these results. In this study, subjects were included in the study group after diagnosis by a physician based on history and physical exam.

CONCLUSION

This study defines the carriage rates of the three most common bacterial pathogens in sinus and middle ear disease in the nasopharynx, nasal cavity, and OMC during illness and in the healthy state. It also defines the nasopharynx as a reservoir for bacterial pathogens in the OMC and nasal cavity during wellness and URI. The OMC and nasal cavity are more likely to harbor bacterial pathogens during viral

upper respiratory illness than during wellness. These pathogens may be transferred to the OMC or nasal cavity from the nasopharynx by nose blowing and may also be associated with impaired mucociliary clearance caused by viral infection.

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