

Prevalence and abundance of *Staphylococcus aureus* in the middle meatus of patients with chronic rhinosinusitis, nasal polyps, and asthma

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Background: Chronic rhinosinusitis (CRS) is an idiosyncratic and multifactorial disease process. Bacteria play a role in some patients, by infection or stimulation of inflammation. *Staphylococcus aureus* (SA) appears to be implicated in a number of infectious and inflammatory mechanisms, and may be particularly relevant in CRS patients with nasal polyps and asthma.

Methods: Middle meatus swabs from control and CRS patients collected during endoscopic sinus surgery were analyzed by quantitative polymerase chain reaction (QPCR). Total bacterial count, SA prevalence, and SA abundance were examined with respect to patient demographics and disease characteristics.

Results: Total bacteria, as measured by QPCR, was not statistically different between controls, CRS without nasal polyps (CRSsNP), CRS with nasal polyps (CRSwNP), or CRS with asthma groups ($p < 0.09$). Total bacterial counts did not correlate with disease severity as measured by Lund-Mackay computed tomography (CT) scores ($p = 0.65$). The

prevalence of SA was similar between groups (15–25%); however, the abundance increased in CRS patients with allergic rhinitis, nasal polyps, and asthma.

Conclusion: The paranasal sinuses are not sterile. SA is implicated in a subset of CRS patients with nasal polyps and/or asthma. Further study is required to predict this subset of patients, and to define the mechanisms of SA pathogenesis. © 2012 ARS-AAOA, LLC.

Key Words:

sinusitis; chronic rhinosinusitis; bacteria; human microbiome; *Staphylococcus aureus*; asthma

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Chronic rhinosinusitis (CRS) is a highly prevalent disease, affecting 16% of the population and accounting for over 14 million annual clinic and emergency room visits in the United States.¹ CRS symptom severity can be as severe as quality of life alterations found in major diseases like congestive heart failure, angina, chronic obstructive pulmonary disease, and back pain.^{2,3} Although a number of medical and surgical therapies have been described with some degree of success, the etiology of the disease is still

poorly understood. CRS is known as a multifactorial and idiosyncratic disease process, in which bacterial infection or colonization may play some role.⁴

Bacterial superinfection of damaged mucosa has been described as the most important cause of acute rhinosinusitis, but its role in CRS pathogenesis is less clear.⁴ Most of our knowledge on bacteria in CRS comes from culture-based studies, which may not be a sensitive enough method to accurately identify microbial presence.⁵ Routine axenic culture may not be adequate to comprehensively detect organisms poorly represented in the sample swab or aspirate, those outcompeted in culture, anaerobes, fastidious organisms, and small colony variants. In contrast, reliable bacterial detection and thorough speciation can be performed with culture-independent, molecular methods such as polymerase chain reaction (PCR) or DNA sequencing. In one study of control and CRS patients, culture identified bacteria in 81% of patients and provided a mean of 1.4 organisms, whereas bacterial DNA sequencing identified bacteria in all patients with a mean of 10 organisms identified.⁶

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Staphylococcus aureus (SA) appears to be of particular interest in the etiology of CRS, with evidence for direct infection,⁷ biofilm-related disease,⁸ alterations in adaptive immunity,⁹ and induction of inflammation in nasal polyps (CRSwNP).^{10,11} Van Zele et al.¹² have shown SA colonization to be increased in CRS, and in particular in CRSwNP. In a portion of CRSwNP patients, antibodies to classical Staphylococcal enterotoxins (SAE), elevations in total immunoglobulin E (IgE) and local SAE-derived IgE antibodies, and intense eosinophilic inflammation can be found.¹³ In their work, it appears that the presence of SA is directly linked to local tissue elevations in both IgE and eosinophils.

Allergic inflammation of the upper and lower airways can coexist, and are potentially associated, especially in CRSwNP patients.^{14,15} This connection is strengthened by the facts that radiographic inflammation of the sinuses correlates with severity of asthma,¹⁶ and that treatment of CRSwNP can improve asthma-related symptoms and medication usage.¹⁷ SAE and associated IgE antibodies, similarly to CRSwNP, have also been linked to severity of asthma.¹⁸

Our previous research has indicated that the presence of SA decreases the bacterial diversity in the paranasal sinuses. Additionally, we noted that the abundance of SA was higher in the middle meatus of CRS patients when compared to normal controls, and was particularly increased in CRS patients with asthma.¹⁹ The aim of this study was to assess the bacterial burden at the middle meatus of healthy controls, CRS patients with nasal polyps, and CRS patients with asthma in a larger patient cohort.

Patients and methods

Study design and population

This cross-sectional study was approved by the Institutional Review Board of the University of Colorado (COMIRB protocol number 10-1437). The diagnosis of CRS was made according to the 2007 Adult Sinusitis Guidelines, and accordingly CRS patients were initially managed medically with a minimum of 3 weeks of antibiotics and topical intranasal steroids prior to the decision for surgery.²⁰ Antibiotics, either topical or systemic, were avoided for at least 1 month prior to surgery. Asthma was diagnosed either clinically based on history of wheeze, cough, shortness of breath, and chest tightness, or on spirometry response with bronchodilator and methacholine administration.²¹ The diagnosis of allergic rhinitis (AR) was made by positive skin prick testing or clinically by the classic constellation of symptoms of nasal congestion, sneezing, rhinorrhea, and pruritis.²² The control group consisted of patients without CRS, asthma, or AR, who underwent either (1) endoscopic sinus surgery for approach to a small unilateral skull base or orbital tumor, or (2) endoscopic septoplasty for nasal airway obstruction. All control patients with skull base or orbital lesions had radiographically normal preoperative computed tomography (CT) scans on the side that was sampled (opposite to tumor), and no evidence of in-

flammatory disease outside of the lesion. Control patients who underwent septoplasty for nasal airway obstruction had otherwise normal preoperative CT scans. Patients less than age 18 years, recent antibiotic use (within 1 month of surgery), or those with cystic fibrosis or autoimmune disease were excluded from the study.

Swabs were collected through middle meatus sampling of patients at the onset of endoscopic sinus surgery (ESS) at University of Colorado Hospital between January 2011 and January 2012. Swab specimens were obtained during surgery with ESwabs (COPAN Diagnostics, Inc., Murrieta, CA) for hospital laboratory culture and CultureSwabs (BD, Franklin Lakes, NJ) for DNA extraction. Swabs were endoscopically guided to the middle meatus or to a neighboring area of purulence when present, rotated at least 5 full turns, and placed on ice upon collection and frozen at -80°C until DNA extraction.

DNA extraction

A phenol:chloroform bead-beating method was used to extract total genomic DNA from the swab heads, as previously described.¹⁹ DNAs were precipitated by the addition of 0.5 volume ammonium acetate (7.5 M) and 1 volume of 100% isopropanol, incubated at -80°C for 10 minutes, then centrifuged ($<14,000g$; 25 minutes). Nucleic acid pellets were washed with 250 μL of 70% ethanol and centrifugation ($<14,000g$; 5 minutes), lyophilized to dry, then resuspended in 30 μL of sterile $1\times$ Tris-EDTA (pH 8.0), and stored at -80°C until polymerase chain reaction (PCR) processing.

Quantitative PCR

A duplex quantitative PCR (QPCR) assay using previously published oligonucleotide primers for Total Bacteria (16S rRNA gene, FAM reporter)²³ and SA (femA gene, TET reporter)²⁴ was conducted on an ABI 7300 thermocycler. PCR reactions contained 10 μL DyNAmo ColorFlash Probe qPCR Mastermix with ROX (Finnzymes Oy, Espoo, Finland), 3 μL water, and 1 μL each $20\times$ PrimeTimeTM primer/probe set (Integrated DNA Technologies, Inc., Coralville, IA), and 5 μL of DNA template. Thermocycling was conducted as follows: 7 minutes initial denaturation at 95°C , followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C . Standard curves were generated and absolute quantitation of DNA copy numbers per volume of template were used to compare the relative proportions of SA to total bacteria. Due to the number of samples analyzed, not all specimens could be tested concurrently by QPCR and the raw abundance data cannot be accurately compared between datasets. Alternatively, we present the relative abundances and cycling threshold (Ct) data, which are less subject to inter-run variability.

Statistics

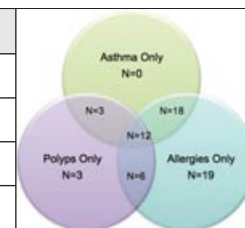
Demographic and disease characteristics were compared using analysis of variance (ANOVA) for continuous

TABLE 1. Patient demographics*

	Non-CRS	CRS	
Subjects (n)	27	74	
Female, n (%)	10 (37.0)	34 (45.9)	
Age, years (mean)	51.6	48.8	
Presence of pus, n (%)	4 (14.8)	37 (50)	

*The Venn diagram demonstrates the overlap for CRS patients with allergic rhinitis, asthma, and/or nasal polyps.

CRS = chronic rhinosinusitis.



outcomes (eg, Lund-Mackay CT scores) and Fisher's exact tests for categorical outcomes (presence/absence of medical conditions). Comparison of percent abundances of SA between groups of subjects was performed using a Wilcoxon rank sum test of the logit-transformed relative abundance values. All tests of null hypotheses were evaluated at $\alpha = 0.05$. Associations between continuous variables (eg, total bacterial load and Lund-Mackay CT scores, *S. aureus* abundance, and eosinophil counts) were tested by linear regression. All statistical analyses were performed using the R statistical package²⁵ or Microsoft Excel (Microsoft Corp., Redmond, WA).

Results

A total of 101 adult patients undergoing sinus surgery between January 2011 and January 2012 were included in the study (Table 1). The study included 74 patients with CRS and 27 controls. Of the CRS patients, 24 (32%) had nasal polyps, 33 (45%) had asthma, and 15 (20%) had both nasal polyps and asthma. Of the control patients, 14 (52%) underwent septoplasty for nasal obstruction and 13 (48%) underwent an endoscopic approach for management of skull base/orbital lesion.

The presence of bacteria was detected in all patients. The amount of total bacteria present was not statistically different between control, CRS patients without nasal polyps (CRSsNP), CRS patients with allergic rhinitis only (CRS ARonly), CRSwNP, and CRS with asthma patients (Fig. 1). CRS patients with asthma did not differ in amount of total bacteria when compared to CRS patients without asthma or to the more specific group of CRS patients without allergies, asthma, or nasal polyps ($p = 0.27$ and $p = 0.09$, respectively). In the 74 patients with a diagnosis of CRS, total bacteria did not correlate with severity of disease as measured by preoperative Lund-Mackay CT scores, as shown in Figure 2 ($R^2 = 0.003$, $p = 0.65$).

Although the prevalence of SA was similar between groups, the relative abundance of SA increased in the subgroups as more comorbidities were added (Table 2). In this comparison, CRS with and without allergies were grouped together, as were the asthma and polyp subgroups, to

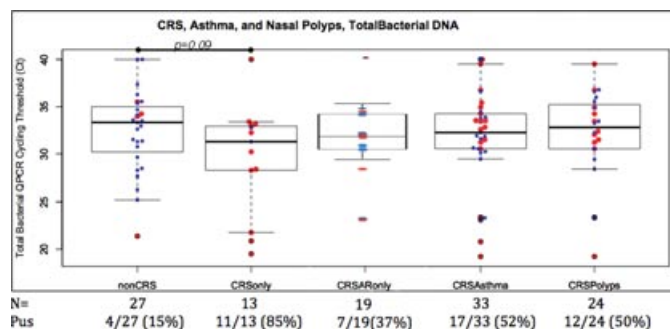


FIGURE 1. Comparison of mean bacterial loads for control patients (non-CRS), CRS without asthma or nasal polyps (CRSonly), CRS with allergic rhinitis only (CRS ARonly), CRS with asthma (CRSasthma), and CRSwNP (CRSPolyps). Red dots indicate the presence of pus when the sample was obtained. QPCR cycling time (Ct) inversely correlates to bacterial load. There were 15 CRS patients with both asthma and polyps. AR = allergic rhinitis; CRS = chronic rhinosinusitis; NP = nasal polyps; QPCR = quantitative polymerase chain reaction.

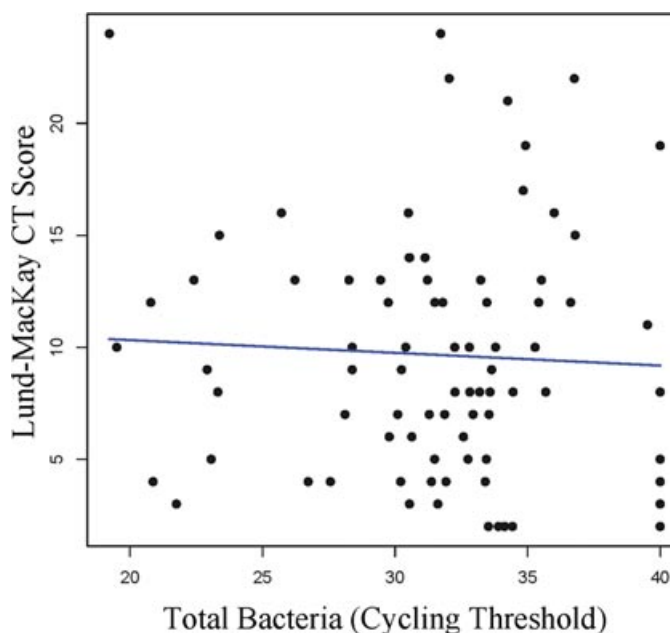


FIGURE 2. Total bacterial load does not correlate with objective disease severity as measured on preoperative computed tomography (CT) scan ($R^2 = 0.003$, $p = 0.65$). QPCR cycling time (Ct) inversely correlates to bacterial load; 3.2 cycles is roughly equivalent to a 10-fold change in bacterial concentration. QPCR = quantitative polymerase chain reaction.

TABLE 2. SA and total bacterial load for various patient groups*

	SA prevalence	SA overall abundance (mean)	SA adjusted abundance (mean)	SA adjusted abundance (median)	Total bacterial count (mean)
Non-CRS ^a	4/27 (22.2%)	0.20%	1.38%	1.26%	32.4
CRS only ^{a,b}	2/13 (15.4%)	0.19%	1.21%	1.21%	29.6
CRS AR only ^{a,b}	4/19 (21.1%)	3.17%	15.04%	9.03%	32.13
CRS asthma ^{a,b}	7/33 (21.2%)	6.22%	29.32%	5.37%	31.7
CRS polyps ^{a,b}	6/24 (25%)	6.51%	26.05%	11.20%	32.1

*The prevalence of SA was similar between groups, however, the abundance increased in the subgroups with comorbid conditions. Statistical comparisons are shown for SA adjusted abundance.

^a $p = 0.02$.

^b $p = 0.007$.

AR = allergic rhinitis; CRS = chronic rhinosinusitis; SA = *Staphylococcus aureus*.

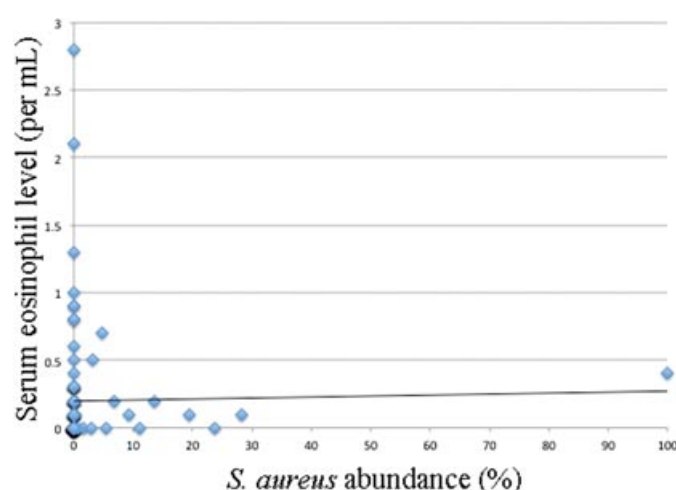


FIGURE 3. No association was discovered between *S. aureus* abundance and serum eosinophil level ($R^2 = 0.0004$, $p = 0.19$). Normal laboratory value for serum eosinophil level is less than 0.5/mL.

increase statistical power and because clinical and pathophysiologic characteristics of CRS in asthma and nasal polyp patients appears to be similar.⁴ Overall abundance was calculated for each patient as the amount of SA as a percent of the total bacteria, and adjusted abundance as the amount of SA as a percent of the total bacteria only when SA was detected. These results are shown in Table 2, demonstrating an increased abundance in CRS patients with allergies, asthma, or nasal polyps.

Serum eosinophil levels were available for 65 of the 101 patients (64%). No relationship was found between SA abundance and eosinophil level as shown in Figure 3 ($R^2 = 0.0004$, $p = 0.19$).

Discussion

As recently as 2004, a consensus panel of experts asserted that, “the paranasal sinuses are believed to be sterile under

normal circumstances.”²⁶ Since then, it has become progressively clear that healthy sinuses are not truly sterile, and this notion is echoed in our study, as similar amounts of total bacteria were found in healthy subjects and CRS patients. In addition, the total bacterial amount did not correlate with disease severity as measured by CT scan Lund-Mackay scores in the current study. Interestingly, the presence of purulence also did not appear to correlate with a higher bacterial load. It is apparent that within the CRS population, even when extensively subcategorized, not all patients exhibit similar microbiologic or inflammatory characteristics.

The presence of bacteria in the sinuses may not be the origin of disease, but it has been postulated that bacteria-host interactions may initiate or contribute to the chronic inflammatory state. Van Zele et al.¹² estimated SA colonization in control, CRS, and CRSwNP patients at 33%, 27%, and 64%, respectively. In the same study, this group further linked SA and asthma by establishing a correlation between the colonization of SA in the middle meatus of CRSwNP patients with the presence and amount of SAE-specific IgE in local tissues. They found CRSwNP patients with asthma were seven times more likely to have SAE-specific IgE found in nasal mucosal tissue. A subsequent multicenter study screened CRS patients with and without polyps for a number of staphylococcal exotoxin genes and did not find a correlation to nasal polyps.²⁷ Inflammatory mechanisms in CRSwNP may differ geographically, presumably due to genetic or environmental differences among local populations.^{28,29} Despite genetic differences in inflammatory mechanisms, the connection between tissue SAE-IgE, elevated total IgE, eosinophils, and asthma in nasal polyp patients has been asserted from these studies.

In the current study, we examined the presence of SA in CRS patients with asthma or nasal polyps as a follow-up to an earlier pilot comparison which showed that SA decreased overall microbial diversity and was more abundant in CRS patients with asthma.¹⁹ In a larger cohort, we have found SA to be more abundant in these groups, but to a lesser degree than we initially suspected. In our

Conclusion

Examination of middle meatus swabs in control and CRS patients demonstrates that the paranasal sinuses are not “sterile.” There is a complex bacterial milieu within the human upper airway that may be altered in a number of conditions. These alterations, or *dysbioses*, influence many inflammatory pathways currently implicated in CRS, such as innate immunity, immune cell recruitment, wound healing, and epithelial cell integrity. In previous studies, SA has been linked to severity of disease in CRS with polyps, and with asthma. Increased abundance of SA in subpopulations of CRS patients with asthma and nasal polyps, as shown in the current study, indicates a need for continued research in this area. As such a heterogeneous disease process, it is clear that future needs will include the appropriate classification of patients into subcategories defined by pathogenesis, which will have major implications for therapy, prognosis, and continued research. Bacterial community dynamics may be a common link to other local and systemic inflammatory pathways, and further work is required to examine these possibilities. ④

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