

The Microbiome and Chronic Rhinosinusitis



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KEYWORDS

- Microbiome • Sinusitis • Chronic rhinosinusitis • Anaerobe • Mucin fermentation
- Animal model of CRS • *Pseudomonas*

KEY POINTS

- The dysbiosis hypothesis (alteration of microbial composition associated with perturbation of the local ecological landscape) has been widely implicated in chronic rhinosinusitis (CRS).
- CRS might develop through a defined series of temporally dependent events: Impaired mucus clearance → anaerobic microenvironments → anaerobe proliferation → increased nutrient availability for sinus pathogens.
- There remains a need for continued CRS research with longitudinal sampling prior to and during disease initiation and application of robust preclinical models.

INTRODUCTION

The upper airways play a critical role in the respiratory system by conditioning and clearing contaminants from the inspired airstream before it accesses the lower respiratory system.¹ Large particulate matter is removed from inhaled air in the anterior nares or nasal vestibule, a relatively dry environment lined by skinlike squamous epithelial cells and containing sebaceous glands and vibrissae. Smaller particulate matter, including bacteria and hydrophilic aerosolized compounds, are trapped in a flowing mucus blanket covering the sinonasal mucosa deeper in the nasal cavity and sinuses. Sinonasal mucociliary function is a key host defense mechanism that clears the inhaled particulate matter. Characterized by impaired mucociliary clearance (MCC), bacterial colonization may play some role in the initiation or sustenance of the inflammatory process in chronic rhinosinusitis (CRS).²

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In recent years, growing understanding of the fundamental role of the microbiome in the initiation, adaptation, and function of the human immune system has revolutionized the field of mucosal immunology.³ Although each inflammatory disease can be differentiated by exclusive genetic and biological mechanisms, many inflammatory diseases, including CRS, are associated with significant shifts in the resident microbiota from a healthy to a diseased state.³ The dysbiosis hypothesis—alteration of microbial composition associated with perturbation of the local ecological landscape—has been widely suggested as a mechanism involved in CRS pathogenesis. This hypothesis is supported by several studies identifying a healthy local environment with particular keystone species, or microbes that normally maintain a stable and interactive community.^{4–6} Yet, sinus microbiome studies are in their infancy; many findings have not been replicated due to small study cohorts and variable experimental methods. In addition, results across studies are difficult to interpret in aggregate, and observed associations do not establish causality between the presence of certain microbial communities in the airways and the development of CRS.⁷ Many of these difficulties are intrinsically related to the broad diagnostic parameters of CRS and lack of a universally appropriate animal model.

High-impact microbiome studies from other organ systems (eg, gut) have been conceptually applied to the respiratory field.⁸ Although seemingly reasonable, nucleic acid–based surveys of airway microbial communities and their proposed role(s) in CRS remain to be addressed using appropriate model systems. Should an etiological role of specific community structures hold true, opportunities will arise for novel therapeutic interventions with potential for personalized, microbiome-based treatment strategies. This review discusses major concepts that highlight the complex role of the microbiota in sinus health and disease and explores future directions for study.

CONSIDERATIONS IN SINUS MICROBIOME INVESTIGATION

Sampling locations vary between studies, reflecting subtly different microenvironments throughout the upper airway, and making cross-study meta-analyses a challenge.⁹ It is clear that the anterior nasal cavity microbiome is distinct from the middle meatus and sphenoethmoid recess in the healthy state,¹⁰ but the most representative single sampling site in the nasal cavity is often argued.^{4,11} How can a single site encompass the complexities of the many anatomic niches and account for differences in local immune and disease properties? The middle meatus often is used as a representative sampling site for the deeper sinuses, given

1. Its high agreement in culture comparison studies with the maxillary sinus¹²
2. Its location as a common drainage pathway of the 3 major (maxillary, anterior ethmoid, and frontal) sinuses
3. Its accessibility for sampling^{9,13}

In the gastrointestinal (GI) microbiome field, stool often is studied as a convenient single sample that overrepresents the cecal contribution, acknowledging there are likely biogeographical differences between the upper GI tract, duodenum, jejunum, ileum, and large intestine. To address this concern in the context of CRS, the authors compared 12 sites from 8 subjects with CRS at the time of surgical intervention and found a fair concordance between the middle meatus and underlying sinuses. These data suggest that, if there is interest in single-site representation, then the middle meatus would be a reasonable proxy for the entire upper airway.¹⁴

In terms of bacterial detection, it is clear that molecular methods are superior to traditional culture-based approaches in CRS, because identification of even the

most fastidious of organisms can be achieved with DNA-based detection and classification by variable regions with the 16S ribosomal RNA (rRNA).^{15–17} Hauser and colleagues¹⁶ demonstrated that bacterial detection using 16S rRNA gene sequencing allows for greater sensitivity and provides more information on bacterial diversity than standard clinical swab culture in CRS. Although clinical laboratory culture has been the gold standard for decades and offers useful information, these techniques are unique to institutional laboratories and may miss bacteria that are present in disease. The true clinical utility of culture-independent molecular techniques remains, however, to be determined. The ability to detect bacteria that are present more accurately may allow for more effective treatment regimens and allow for an improved basis for clinical and laboratory research into CRS. Nevertheless, there are some shortcomings of culture-independent molecular techniques. 16S rRNA gene sequencing measures total or relative abundance of bacterial DNA and does not differentiate between actively growing, dormant, or dead biomass.¹⁸ As with all tests, it is important to be aware of such biases. To better understand *in vivo* bacterial activity, culture-independent approaches must be improved and new innovative techniques should continue to be integrated, for instance, by separating active cells from extracellular DNA and inactive microbial subpopulations.^{19,20}

Currently, there are 2 main gene sequencing approaches used for studying microbial communities:

1. Targeted sequencing of specific marker genes (ie, 16S rRNA gene for bacteria and 18S rRNA or internal transcribed spacer regions for fungi)
2. Shotgun sequencing of the metagenome

16S rRNA gene sequencing currently is the most widely used approach for characterizing bacterial community membership and comparing phylogeny between samples. This method is based on the premise that 9 hypervariable regions within the 16S rRNA gene harbor sufficient sequence diversity to differentiate bacterial taxa down to the genus or species level. Flanking these regions are highly conserved sequences across bacteria and archaea that facilitate the use of universal polymerase chain reaction primer sets.^{21,22} Although costlier, shotgun sequencing methods are useful for characterizing microbial communities more broadly, including viruses and fungi that also have been implicated in the development of upper airway disease. Shotgun metagenomics, the study of whole-community DNA extracted directly from samples, increasingly has been used in various settings, particularly as sequencing costs decrease and output increases.^{23,24} Furthermore, relative to targeted amplicon assays (eg, 16S rRNA gene sequencing), shotgun metagenomics offers potential for both higher-resolution identification of organisms and the study of microbial communities without introduction of sequencing bias due to unequal amplification of the target gene.^{24,25} Moreover, shotgun approaches capture details of the microbial metagenome (ie, antibiotic resistance and virulence factors) not provided using single-marker gene studies.^{22,26} Sequencing technologies have undergone rapid advances during the past several years to attempt to resolve biases associated with current methods and to obtain a better balance between data yield, read length, and cost.²² These efforts have resulted in third-generation sequencing technologies (eg, Oxford Nanopore, Technologies Oxford Science Park, UK and PacBio platforms, Pacific Biosciences, Menlo Park, CA), which are single-molecule and real-time technologies that reduce amplification bias as well as short read length limitations.^{22,27,28} Reduction in cost and time presented by these sequencing

methods are valuable assets, and certainly future incorporation of new technologies and bioinformatics is expected.

Based on the anatomic location and local disease environment, viruses and fungi have hypothesized interactions with the bacterial community, which have been borne out in a prior study.²⁹ The relative absence of fungal and viral study at the current time may be a simple lag behind the bacterial microbiome research explosion, because the early microbial detection techniques focused primarily on numerically dominant bacteria. Multiple studies have demonstrated the presence of viruses and fungi in CRS.^{30–35} Virus replication can result in epithelial damage and increase bacterial mucosal adhesion, whereas fungi may act synergistically with pathogenic bacteria to play a role in the pathogenesis of CRS.^{32,36} The precise roles of these organisms in the pathogenesis of CRS and etiologic importance remains poorly understood.³²

DYSBIOSIS OF SINUS MICROBIOTA IN CHRONIC RHINOSINUSITIS

Analysis of the normal state of the microbiome in sinus cavities is crucial, because there is a clear role for commensals in pathogen exclusion and in the modulation of the healthy host-microbial immune response.³⁷ The deeper nasal cavity and sinuses have unique local microenvironments (PO₂, pH, and so forth) and host immune properties.^{11,38,39} Although Yan and colleagues¹⁰ recently examined deeper anatomic subsites in healthy human nasal cavities and Ramakrishnan and colleagues¹⁴ compared upper airway subsites and sinuses in CRS, there has been no thorough comparison within normal sinus cavities to date, perhaps owing to the requirement of a more invasive approach.

In the healthy state, commonly identified bacterial genera from the upper airways include *Staphylococcus*, *Corynebacterium*, *Peptoniphilus*, and *Propionibacterium*.^{2,6,32,36,40,41} Total bacterial load present in healthy and diseased sinuses appear surprisingly alike across adults. Furthermore, high interindividual microbiome variation often is observed in healthy controls and CRS patients.^{42,43} Many opportunistic pathogens are found at low abundance in healthy sinuses and, therefore, have the potential to create disease after an acute alteration in the stable baseline microbial community (ie, dysbiosis).^{2,32}

Disruption of stable microbiota may contribute to the exacerbation of chronic inflammatory disease in the absence of acute infection.^{11,44} Dysbiosis can lead to benign microbial communities becoming proinflammatory or invasive or allowing overgrowth of pathogens. There also is growing evidence that dysbiosis of the sinus microbiota is associated with CRS pathogenesis.⁴⁵ Human studies have revealed that the CRS microbiome is characterized by loss of diversity compared with healthy controls,^{5,43,46} indicating the opportunity for prosperity of pathogens.⁴⁷ Results from these and other sequence-based studies have transformed understanding of the role of microbial community composition and dynamics in CRS pathogenesis.

Disruption of healthy commensal interactions with the local immune system seems to be a critical determinant of CRS progression. Linear discriminant analysis identified the genus *Corynebacterium* as a potential biomarker that was significantly increased in abundance in CRS patients; however, this genus also was omnipresent in healthy subjects from other studies.^{4,11} Using a murine model challenged with *C tuberculo-tearicum* after antibiotic-mediated microbial depletion, Abreu and colleagues⁴⁸ demonstrated goblet cell hyperplasia and mucin hypersecretion, 2 important histologic hallmarks of CRS. In this study, however, there were only 7 samples from CRS patients, and another study subsequently reported opposing findings that CRS patients with enriched *C tuberculo-tearicum* colonization at the time of endoscopic sinus

surgery showed improved surgical outcomes.^{6,49} Regarding host interaction with local immune system, another group found that nasal lavage samples of microbiota collected from CRS patients stimulated the induction of proinflammatory cytokines, such as interleukin 5, in peripheral leukocytes isolated from healthy controls.^{11,50} Together, these data suggest that the CRS state represents an altered ecological landscape interacting with an aberrant immune response. To this concept, a recent cross-sectional study of CRS and non-CRS patients who underwent endoscopic sinus surgery demonstrated a correlation between the loss of bacterial species richness and diversity and the severity of inflammation and tissue eosinophilia.⁵¹ Whether dysbiosis is causative or a result of the disrupted local immune system remains to be determined.

A preponderance of anaerobes has been consistently observed in studies of CRS, which may be explained

1. By selective pressure of antimicrobial agents enabling anaerobic organisms to flourish⁵²
2. From the existence of conditions appropriate for anaerobic growth (ie, sinus hypoxia)⁵³

Anaerobic taxa, such as *Peptoniphilus*, *Anaerococcus*, and *Prevotella*, have been reported as abundant taxa in multiple CRS studies.^{4,42,54–56} Ambient conditions within the sinus cavities may not be hypoxic, especially after endoscopic sinus surgery has opened the cavities. Expansion of anaerobes in CRS may be indicative, however, of underlying tissue hypoxia, or may suggest that discrete microenvironments within mucus or bacterial biofilms in CRS also can be oxygen limited, allowing anaerobes to thrive.^{4,53,57} It is likely that, similar to mucus plugs in the lower airways of individuals with cystic fibrosis (CF), oxygen levels within sinus mucus are dynamic and driven by both host and microbial processes.⁵⁸ Whether anaerobic bacteria have an etiologic role in CRS disease progression has been addressed only marginally and is an emerging area of research in chronic airway disease.

MICROBIAL INTERACTIONS IN CHRONIC RHINOSINUSITIS

Understanding the complexity and dynamics of interspecies and interkingdom relationships represents a major challenge in microbiome research but has the potential to help clarify effects in several chronic respiratory diseases including CRS.⁸ Symbiosis in healthy microbial ecosystems allows for efficient nutrient utilization and results in decreased pathogen colonization.⁴⁷ Most microorganisms face a constant battle for resources, and there are diverse mechanisms by which bacterial species can coexist with, or dominate, other organisms competing for the same pool of resources.⁵⁹ Understanding of microbial interactions will be crucial in establishing the function of microbial communities in CRS and implementing new therapeutic strategies.

Yan and colleagues¹⁰ studied the interaction between *S aureus* and *Corynebacterium* in the healthy human nasal cavity and showed that *Corynebacterium* spp are involved in both mutualistic and inhibitory interactions with *S aureus*. *C accolens* and *S aureus* appear to be adapted to each other and mutually promote each other's growth in vitro, whereas *C pseudodiphtheriticum* may interfere with colonization of *S aureus* and was observed to inhibit *S aureus* growth in vitro. Within the nasal cavity, these reciprocal interactions suggest the possibility for niche competition and possible protection against *S aureus* nasal colonization.

Pseudomonas aeruginosa also is an important respiratory pathogen and often carries intrinsic and/or acquired resistance to many classes of antibiotics. Its

appearance and recalcitrance in a portion of CRS subjects is an ongoing clinical challenge. Flynn and colleagues⁶⁰ investigated the role of airway mucins as the microbial carbon source in the CF airway and characterized their potential to stimulate the growth of *Pseudomonas*. Their group demonstrated that coculture of *P aeruginosa* with an anaerobic bacterial consortium facilitates robust growth of *P aeruginosa* using mucins as a sole nutritional carbon source. These data support an ecological role for anaerobes in shaping the landscape of the human airway for progression of chronic disease (eg, CRS) and these data a model for the role of anaerobes in disease pathogenesis.⁶⁰ In this model, potential pathogens that cannot degrade mucins (eg, *P aeruginosa* and *S aureus*) do not establish an airway infection until mucin-fermenting bacteria (anaerobes) have colonized (Fig. 1). Numerous 16S rRNA gene sequencing studies in CRS have demonstrated a previously unrecognized abundance of anaerobes in the disease state.^{6,48} Based on this hypothesis, chronic airway disease could develop through a defined series of dependent events:

1. Impaired mucus clearance
2. Generation of anaerobic microenvironments
3. Dysbiosis with mucin-fermenting anaerobes
4. Mucin degradation to carbon source nutrients (eg, short-chain fatty acids [SCFAs])
5. Proliferation of sinus pathogens

In this context, the authors preliminarily tested whether there is evidence of mucin fermentation in human CRS by analyzing the presence of SCFAs in the mucus of subjects during acute exacerbations. Using gas chromatography–mass spectrometry, 3 SCFAs (acetate, propionate, and butyrate) were quantified in human mucus

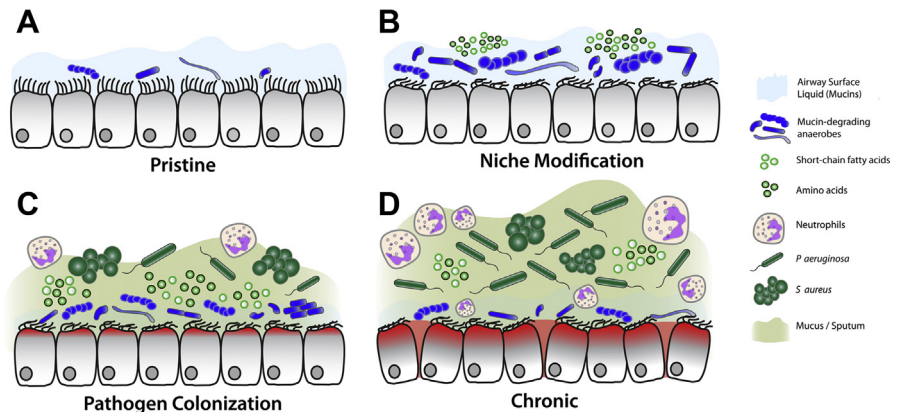


Fig. 1. Model for the role of mucin-fermenting bacteria in the progression of CF lung disease, as applied to CRS. (A) In early life, airway surface liquid harbors a low number of bacteria. Numerous factors allow for establishment of personal local microbiota. (B) Local insult resulting in impaired MCC and defective immune responses results in hypoxic environment ideal for expansion of anaerobes. In turn, their ability to degrade and ferment respiratory mucins further modifies the airway environment for secondary colonizers. (C) The abundance of fermentation byproducts facilitates pathogen colonization, heightened inflammation, neutrophil recruitment, and further hypoxia. (D) In late stages of disease, host inflammatory responses and epithelial damage increase the abundance of pathogens, whereas healthy commensals are eliminated by the host and via broad-spectrum antibiotic therapies. (Data from Flynn JM, Niccum D, Dunitz JM, et al. Evidence and Role for Bacterial Mucin Degradation in Cystic Fibrosis Airway Disease. PLoS Pathog 2016;12(8):e1005846.)

samples collected from 6 controls and 9 CRS patients during acute exacerbation episodes. SCFAs were found at millimolar concentrations in all mucus samples and at significantly higher concentrations in CRS compared with healthy subjects (Fig. 2). Given that SCFAs are derived predominately from bacterial fermentation, this evidence suggests that mucin-fermenting bacteria are able to generate carbon source nutrients for pathogenic bacteria in CRS, similar to proposed mechanisms of disease progression in the lower airways.⁶⁰ Based on these data, it is intriguing to consider that the growth of canonical airway pathogens (eg, *S aureus* and *P aeruginosa*) might be inhibited by targeting co-colonizing microbiota that potentiate their growth and virulence.

DEVELOPING PRECLINICAL MODELS

The study of dysbiosis in human CRS is especially challenging because medical therapies used in disease treatment are likely to affect resident bacterial communities.^{45,61,62} Observed alterations in CRS local microbiota in cross-sectional studies have been unable to account for the repeated and prolonged medical therapies that are common in study subjects. Unfortunately, small animals are not universally accepted in CRS because they do not develop upper airway phenotypes (eg, CF murine models), possibly from absence of submucosal glands,⁶³ and their small size precludes thorough examination of sinus pathology.⁶⁴ Thus, there remains a need for a robust preclinical model of CRS for longitudinal sampling prior to and during disease initiation. Although there are some limitations when applying animal findings to human pathophysiology, preclinical models have played a significant role in the process of understanding CRS pathophysiology.^{65–67}

Many different animals (eg, murine, rabbit, sheep, and pigs) have been used to establish acute and chronic sinus inflammation in prior studies. Small animal models used in CRS microbiome research include the murine model of sinusitis described previously in investigation of *C tuberculo*stearium as a potential pathogen on the sinus microbiota. By inoculating *C tuberculo*stearium into the nasal cavity with and without preceding antibiotic treatment, this study showed the capability of *C tuberculo*stearium to induce a CRS phenotype, particularly in conjunction with a depleted host commensal community. Co-inoculation of *C tuberculo*stearium with *Lactobacillus sakei*, a putative probiotic, resulted in a reduced abundance of *C tuberculo*stearium.⁴⁸ In addition, mice have been used to understand the dynamics of sinonasal infection and the role of the mucosal microbiome in short-term and long-term responses after topical inoculation of human pathogens (eg, *P aeruginosa*).⁶⁸ Mice are

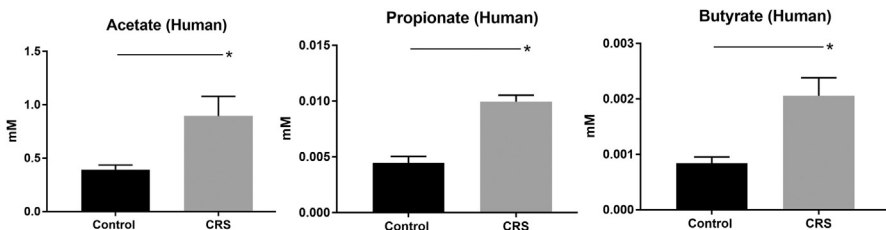


Fig. 2. Concentrations of SCFAs in human mucus samples from CRS with acute exacerbation versus healthy controls. All 3 SCFAs were significantly higher in CRS (n = 9) compared with control (n = 6): (left) acetate = 0.89 mM ± 0.19 mM versus 0.39 ± 0.04 mM (P<.05); (middle) propionate = 0.01 mM ± 0.00 mM versus 0.0045 mM ± 0.00 mM (P<.0001); and (right) butyrate = 0.002 mM ± 0.00 mM versus 0.0008 mM ± 0.00 mM (P<.01). * indicates as P<0.05.

easy to work with in the laboratory and carry many advantages of experimental application that have been extensively documented. Murine CRS models are limited, however, due to animal size, unclear similarity of commensal microbes to human counterparts, and poorly defined ecological properties of stability and resilience and because mice do not reproduce key aspects of human airway physiology. They do not have true sinuses, for instance, essential for the analysis of the pathophysiologic mechanisms of CRS.⁶⁹ Furthermore, immune responses in mice are notably different from those in humans.⁷⁰ Compared with mice, rat models are much larger, which makes acquiring larger tissue specimens easier and ameliorates the technical limitations of smaller models.⁷¹ Transgenic rat models useful for CRS are rare, however, and the CF transmembrane conductance regulator (CFTR) knockout rat model (*Rattus norvegicus*; SD-CFTRtm1sage) does not develop spontaneous sinusitis.⁶⁴

As an alternative small animal model, the in vivo rabbit sinusitis model is established and may be well suited for studies of therapeutic intervention. The rabbit sinusitis model

1. Can recapitulate histopathologic features of sinusitis
2. Is of sufficient size to study spatial and temporal microbial changes
3. Has been used to explore experimental ostial obstruction and/or microbial inoculation in the development of the disease⁷²

Cho and colleagues⁷³ developed a rabbit model of sinusitis by blocking the maxillary sinus ostium for 2 weeks in the absence of infection to create an anaerobic environment with decreased MCC, resulting in the infiltration of sinus epithelium with acute inflammatory cells (neutrophils). When followed for another 12 weeks after removal of ostial obstruction, those rabbits exhibited a chronic inflammatory phenotype at week 14 (Fig. 3). In this model, the mucin-fermenting anaerobic phyla *Firmicutes* and *Bacteroidetes* dominated at week 2 but were followed by a significant microbial shift to pathogenic Proteobacteria (eg, Burkholderiales, and Pseudomonadales) during the development of chronic inflammation by week 14. Such a model provides the opportunity to study microbial host interactions with a level of experimental control that is not achievable in mouse or humans and also permits multiple longitudinal samplings because the nasal cavity is accessible by nasal endoscopy.

FUTURE DIRECTIONS IN CHRONIC RHINOSINUSITIS MICROBIOME RESEARCH

Standardization in Sampling Procedures

Many protocols have been utilized and advocated for different reasons. The ideal sampling protocol depends on the question being addressed. Mucus swab of the middle

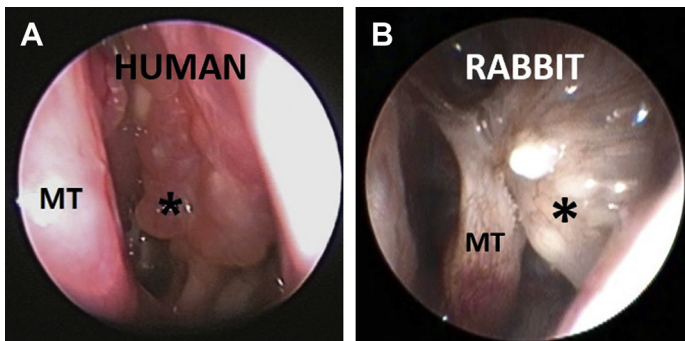


Fig. 3. Middle meatus (asterisk) of human (A) versus rabbit (B) CRS. Similar significant polypoid mucosal changes (asterisk). MT, middle turbinate.

meatus or ethmoid cavity may be the simplest approach for longitudinal study of the sinus microbiome, considering that it can be obtained from a wide range of subjects and does not require invasive procedures. Whether microbes are sampled by swab, brush, or tissue biopsy, sequencing provides a general picture of the composition of the bacterial community, while an accompanying clinically meaningful and functional physiologic approach is still required.

Healthy Microbiome Patterns

What is the healthy sinus microbiome consortium and what defines “normal”? How do healthy microbiota protect against potential pathogens, either passively through niche competition or actively through or metabolic processes or secretion of antimicrobial compound? Are these organisms susceptible to changes that occur in the sinus environment as a result of the CRS disease process or iatrogenic manipulation? Normality patterns for viruses and fungi still need to be defined in the upper respiratory system.

Further Characterization of Nonculturable and/or Nonpathogenic Bacteria

16S rRNA gene analyses have shown discrete patterns of nonculturable microorganisms obtained from patients with CRS. Conventional sequencing methods do not differentiate, however, between actively growing, dormant, or dead biomass nor do they capture in situ activity at the transcriptional and/or protein level. Detailed characterization of CRS-associated microbial communities therefore requires further innovative assessment. As an example, bioorthogonal noncanonical amino acid tagging (BONCAT) can be used to fluorescently label actively growing bacteria within samples prior to gene sequencing and has the potential to enhance traditional sequencing methods by characterizing bacterial activity at the protein level.¹⁹ Other methods, such as stable isotope probing or single-cell transcriptional analyses coupled with in situ imaging, also carry potential for generating unprecedented insights into the microbial basis of CRS disease progression.^{74,75}

Local versus Systemic Microbial Interactions

Future studies may need to address the contributions of both local and systemic microbial communities (ie, local and GI occupants). New studies, including bacteriophage, viral, and fungal contributions to functional host immune processes, are eagerly anticipated.

Interventions

Bacterial supplementation and modulation of the microbiota through prebiotics or probiotics and equivalents are opportunities for thoughtful and ethical clinical research. Whether probiotics directly target inflammatory processes within the sinonasal epithelium or aim to restore normal upper airway microbiota by mucus transfer, novel strategies to address pathogens in CRS are needed.

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REFERENCES

1. Shusterman D. The effects of air pollutants and irritants on the upper airway. *Proc Am Thorac Soc* 2011;8(1):101–5.
2. Ramakrishnan VR, Feazel LM, Gitomer SA, et al. The microbiome of the middle meatus in healthy adults. *PLoS One* 2013;8(12):e85507.
3. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell* 2014;157(1):121–41.
4. Copeland E, Leonard K, Carney R, et al. Chronic rhinosinusitis: potential role of microbial dysbiosis and recommendations for sampling sites. *Front Cell Infect Microbiol* 2018;8:57.
5. Wagner Mackenzie B, Waite DW, Hoggard M, et al. Bacterial community collapse: a meta-analysis of the sinonasal microbiota in chronic rhinosinusitis. *Environ Microbiol* 2017;19(1):381–92.
6. Ramakrishnan VR, Hauser LJ, Feazel LM, et al. Sinus microbiota varies among chronic rhinosinusitis phenotypes and predicts surgical outcome. *J Allergy Clin Immunol* 2015;136(2):334–42.e1.
7. Ramakrishnan VR, Frank DN. Microbiome in patients with upper airway disease: moving from taxonomic findings to mechanisms and causality. *J Allergy Clin Immunol* 2018;142(1):73–5.
8. Faner R, Sibila O, Agustí A, et al. The microbiome in respiratory medicine: current challenges and future perspectives. *Eur Respir J* 2017;49(4) [pii:1602086].
9. Ramakrishnan VR, Hauser LJ, Frank DN. The sinonasal bacterial microbiome in health and disease. *Curr Opin Otolaryngol Head Neck Surg* 2016;24(1):20–5.
10. Yan M, Pamp SJ, Fukuyama J, et al. Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and *S. aureus* carriage. *Cell Host Microbe* 2013;14(6):631–40.
11. Proctor DM, Relman DA. The landscape ecology and microbiota of the human nose, mouth, and throat. *Cell Host Microbe* 2017;21(4):421–32.
12. Dubin MG, Ebert CS, Coffey CS, et al. Concordance of middle meatal swab and maxillary sinus aspirate in acute and chronic sinusitis: a meta-analysis. *Am J Rhinol* 2005;19(5):462–70.
13. Lund VJ, Stammberger H, Fokkens WJ, et al. European position paper on the anatomical terminology of the internal nose and paranasal sinuses. *Rhinol Suppl* 2014;24:1–34.
14. Ramakrishnan VR, Gitomer S, Kofonow JM, et al. Investigation of sinonasal microbiome spatial organization in chronic rhinosinusitis. *Int Forum Allergy Rhinol* 2017;7(1):16–23.
15. Feazel LM, Frank DN, Ramakrishnan VR. Update on bacterial detection methods in chronic rhinosinusitis: implications for clinicians and research scientists. *Int Forum Allergy Rhinol* 2011;1(6):451–9.
16. Hauser LJ, Feazel LM, Ir D, et al. Sinus culture poorly predicts resident microbiota. *Int Forum Allergy Rhinol* 2015;5(1):3–9.
17. Rhoads DD, Cox SB, Rees EJ, et al. Clinical identification of bacteria in human chronic wound infections: culturing vs. 16S ribosomal DNA sequencing. *BMC Infect Dis* 2012;12:321.

18. Willis AL, Calton JB, Carr TF, et al. Dead or alive: deoxyribonuclease I sensitive bacteria and implications for the sinus microbiome. *Am J Rhinol Allergy* 2016; 30(2):94–8.
19. Couradeau E, Sasse J, Goudeau D, et al. Probing the active fraction of soil microbiomes using BONCAT-FACS. *Nat Commun* 2019;10(1):2770.
20. Nelson MT, Pope CE, Marsh RL, et al. Human and extracellular DNA depletion for metagenomic analysis of complex clinical infection samples yields optimized viable microbiome profiles. *Cell Rep* 2019;26(8):2227–40.e5.
21. Bent SJ, Pierson JD, Forney LJ, et al. Measuring species richness based on microbial community fingerprints: the emperor has no clothes. *Appl Environ Microbiol* 2007;73(7):2399–401 [author reply: 2399–2401].
22. Malla MA, Dubey A, Kumar A, et al. Exploring the human microbiome: the potential future role of next-generation sequencing in disease diagnosis and treatment. *Front Immunol* 2018;9:2868.
23. Manichanh C, Chapelle CE, Frangeul L, et al. A comparison of random sequence reads versus 16S rDNA sequences for estimating the biodiversity of a metagenomic library. *Nucleic Acids Res* 2008;36(16):5180–8.
24. Yang X, Noyes NR, Doster E, et al. Use of metagenomic shotgun sequencing technology to detect foodborne pathogens within the microbiome of the beef production chain. *Appl Environ Microbiol* 2016;82(8):2433–43.
25. Shah N, Tang H, Doak TG, et al. Comparing bacterial communities inferred from 16S rRNA gene sequencing and shotgun metagenomics. *Pac Symp Biocomput* 2011;165–76. https://doi.org/10.1142/9789814335058_0018.
26. Tessler M, Neumann JS, Afshinnekoo E, et al. Large-scale differences in microbial biodiversity discovery between 16S amplicon and shotgun sequencing. *Sci Rep* 2017;7(1):6589.
27. Fichot EB, Norman RS. Microbial phylogenetic profiling with the Pacific Biosciences sequencing platform. *Microbiome* 2013;1(1):10.
28. Kasianowicz JJ, Brandin E, Branton D, et al. Characterization of individual polynucleotide molecules using a membrane channel. *Proc Natl Acad Sci U S A* 1996;93(24):13770–3.
29. Robinson CM, Pfeiffer JK. Viruses and the microbiota. *Annu Rev Virol* 2014;1: 55–69.
30. Liao B, Hu CY, Liu T, et al. Respiratory viral infection in the chronic persistent phase of chronic rhinosinusitis. *Laryngoscope* 2014;124(4):832–7.
31. Ramadan HH, Farr RW, Wetmore SJ. Adenovirus and respiratory syncytial virus in chronic sinusitis using polymerase chain reaction. *Laryngoscope* 1997;107(7): 923–5.
32. Sivasubramaniam R, Douglas R. The microbiome and chronic rhinosinusitis. *World J Otorhinolaryngol Head Neck Surg* 2018;4(3):216–21.
33. Wood AJ, Antoszewska H, Fraser J, et al. Is chronic rhinosinusitis caused by persistent respiratory virus infection? *Int Forum Allergy Rhinol* 2011;1(2):95–100.
34. Zhao YC, Bassiouni A, Tanjararak K, et al. Role of fungi in chronic rhinosinusitis through ITS sequencing. *Laryngoscope* 2018;128(1):16–22.
35. Zhang I, Pletcher SD, Goldberg AN, et al. Fungal microbiota in chronic airway inflammatory disease and emerging relationships with the host immune response. *Front Microbiol* 2017;8:2477.
36. Gevers D, Knight R, Petrosino JF, et al. The Human Microbiome Project: a community resource for the healthy human microbiome. *PLoS Biol* 2012;10(8): e1001377.

37. Lee JT, Frank DN, Ramakrishnan V. Microbiome of the paranasal sinuses: Update and literature review. *Am J Rhinol Allergy* 2016;30(1):3–16.
38. Seshadri S, Rosati M, Lin DC, et al. Regional differences in the expression of innate host defense molecules in sinonasal mucosa. *J Allergy Clin Immunol* 2013;132(5):1227–30.e5.
39. White LC, Weinberger P, Coulson H, et al. Why sinonasal disease spares the inferior turbinate: An immunohistochemical analysis. *Laryngoscope* 2016;126(5):E179–83.
40. Kaspar U, Kriegeskorte A, Schubert T, et al. The culturome of the human nose habitats reveals individual bacterial fingerprint patterns. *Environ Microbiol* 2016;18(7):2130–42.
41. Mahdavinia M, Engen PA, LoSavio PS, et al. The nasal microbiome in patients with chronic rhinosinusitis: analyzing the effects of atopy and bacterial functional pathways in 111 patients. *J Allergy Clin Immunol* 2018;142(1):287–90.e4.
42. Biswas K, Hoggard M, Jain R, et al. The nasal microbiota in health and disease: variation within and between subjects. *Front Microbiol* 2015;9:134.
43. Hoggard M, Biswas K, Ziong M, et al. Evidence of microbiota dysbiosis in chronic rhinosinusitis. *Int Forum Allergy Rhinol* 2017;7(3):230–9.
44. Dickson RP, Martinez FJ, Huffnagle GB. The role of the microbiome in exacerbations of chronic lung diseases. *Lancet* 2014;384(9944):691–702.
45. Orlandi RR, Kingdom TT, Hwang PH, et al. International consensus statement on allergy and rhinology: rhinosinusitis. *Int Forum Allergy Rhinol* 2016;6(Suppl 1):S22–209.
46. Wilson MT, Hamilos DL. The nasal and sinus microbiome in health and disease. *Curr Allergy Asthma Rep* 2014;14(12):485.
47. Cardinale BJ, Duffy JE, Gonzalez A, et al. Biodiversity loss and its impact on humanity. *Nature* 2012;486(7401):59–67.
48. Abreu NA, Nagalingam NA, Song Y, et al. Sinus microbiome diversity depletion and *Corynebacterium tuberculo*steaericum enrichment mediates rhinosinusitis. *Sci Transl Med* 2012;4(151):151ra124.
49. Chalermwatanachai T, Vilchez-Vargas R, Holtappels G, et al. Chronic rhinosinusitis with nasal polyps is characterized by dysbacteriosis of the nasal microbiota. *Sci Rep* 2018;8(1):7926.
50. Aurora R, Chatterjee D, Hentzleman J, et al. Contrasting the microbiomes from healthy volunteers and patients with chronic rhinosinusitis. *JAMA Otolaryngol Head Neck Surg* 2013;139(12):1328–38.
51. Rom D, Bassiouni A, Eykman E, et al. The association between disease severity and microbiome in chronic rhinosinusitis. *Laryngoscope* 2019;129(6):1265–73.
52. Barenfanger J, Drake CA, Lawhorn J, et al. Outcomes of improved anaerobic techniques in clinical microbiology. *Clin Infect Dis* 2002;35(Suppl 1):S78–83.
53. Brook I. The role of anaerobic bacteria in sinusitis. *Anaerobe* 2006;12(1):5–12.
54. Cleland EJ, Bassiouni A, Vreugde S, et al. The bacterial microbiome in chronic rhinosinusitis: Richness, diversity, postoperative changes, and patient outcomes. *Am J Rhinol Allergy* 2016;30(1):37–43.
55. Stephenson MF, Mfuna L, Dowd SE, et al. Molecular characterization of the polymicrobial flora in chronic rhinosinusitis. *J Otolaryngol Head Neck Surg* 2010;39(2):182–7.
56. Ivanchenko OA, Karpishchenko SA, Kozlov RS, et al. The microbiome of the maxillary sinus and middle nasal meatus in chronic rhinosinusitis. *Rhinology* 2016;54(1):68–74.

57. Kim YJ, Cho HJ, Shin WC, et al. Hypoxia-mediated mechanism of MUC5AC production in human nasal epithelia and its implication in rhinosinusitis. *PLoS One* 2014;9(5):e98136.
58. Cowley ES, Kopf SH, LaRiviere A, et al. Pediatric cystic fibrosis sputum can be chemically dynamic, anoxic, and extremely reduced due to hydrogen sulfide formation. *MBio* 2015;6(4):e00767.
59. Hibbing ME, Fuqua C, Parsek MR, et al. Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol* 2010;8(1):15–25.
60. Flynn JM, Niccum D, Dunitz JM, et al. Evidence and role for bacterial mucin degradation in cystic fibrosis airway disease. *PLoS Pathog* 2016;12(8):e1005846.
61. Dethlefsen L, Huse S, Sogin ML, et al. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 2008;6(11):e280.
62. Liu CM, Soldanova K, Nordstrom L, et al. Medical therapy reduces microbiota diversity and evenness in surgically recalcitrant chronic rhinosinusitis. *Int Forum Allergy Rhinol* 2013;3(10):775–81.
63. Wine JJ, Joo NS. Submucosal glands and airway defense. *Proc Am Thorac Soc* 2004;1(1):47–53.
64. Tipirneni KE, Cho DY, Skinner DF, et al. Characterization of primary rat nasal epithelial cultures in CFTR knockout rats as a model for CF sinus disease. *Laryngoscope* 2017;127(11):E384–91.
65. Jia M, Chen Z, Du X, et al. A simple animal model of *Staphylococcus aureus* biofilm in sinusitis. *Am J Rhinol Allergy* 2014;28(2):e115–9.
66. London NR Jr, Lane AP. Innate immunity and chronic rhinosinusitis: what we have learned from animal models. *Laryngoscope Investig Otolaryngol* 2016;1(3):49–56.
67. Shin HW. Animal models in CRS and pathophysiologic insights gained: a systematic review. *Laryngoscope Investig Otolaryngol* 2016;1(5):116–23.
68. Cope EK, Goldberg AN, Pletcher SD, et al. A chronic rhinosinusitis-derived isolate of *Pseudomonas aeruginosa* induces acute and pervasive effects on the murine upper airway microbiome and host immune response. *Int Forum Allergy Rhinol* 2016;6(12):1229–37.
69. Lindsay R, Slaughter T, Britton-Webb J, et al. Development of a murine model of chronic rhinosinusitis. *Otolaryngol Head Neck Surg* 2006;134(5):724–30 [discussion: 731–2].
70. Liang KL, Jiang RS, Wang J, et al. Developing a rabbit model of rhinogenic chronic rhinosinusitis. *Laryngoscope* 2008;118(6):1076–81.
71. Mashimo T, Serikawa T. Rat resources in biomedical research. *Curr Pharm Biotechnol* 2009;10(2):214–20.
72. Al-Sayed AA, Agu RU, Massoud E. Models for the study of nasal and sinus physiology in health and disease: a review of the literature. *Laryngoscope Investig Otolaryngol* 2017;2(6):398–409.
73. Cho DY, Mackey C, Van Der Pol WJ, et al. Sinus microanatomy and microbiota in a rabbit model of rhinosinusitis. *Front Cell Infect Microbiol* 2017;7:540.
74. DePas WH, Starwalt-Lee R, Van Sambeek L, et al. Exposing the three-dimensional biogeography and metabolic states of pathogens in cystic fibrosis sputum via hydrogel embedding, clearing, and rRNA labeling. *MBio* 2016;7(5) [pii:e00796-16].
75. Kopf SH, Sessions AL, Cowley ES, et al. Trace incorporation of heavy water reveals slow and heterogeneous pathogen growth rates in cystic fibrosis sputum. *Proc Natl Acad Sci U S A* 2016;113(2):E110–6.