Microbiome of the paranasal sinuses: Update and literature review

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ABSTRACT

Background: Our understanding of the resident microbiome of the paranasal sinuses has changed considerably in recent years. Once presumed to be sterile, healthy sinus cavities are now known to harbor a diverse assemblage of microorganisms, and, it is hypothesized that alterations in the kinds and quantities of these microbes may play a role in the pathogenesis of chronic rhinosinusitis (CRS).

Objectives: To review the current literature regarding the sinus microbiome and collate research findings from relevant studies published to date.

Methods: A systematic literature review was performed on all molecular studies that investigated the microbial communities of the paranasal sinuses. Methods of detection, microbiome composition, and comparative profiling between patients with and without CRS were explored.

Results: A complex consortium of microorganisms has been demonstrated in the sinuses of both patients with and without CRS. However, the latter generally have been characterized by reduced biodiversity compared with controls, with selective enrichment of particular microbes (e.g., Staphylococcus aureus). Such disruptions in the resident microbiome may contribute to disease pathogenesis by enhancing the virulence of potential pathogens and adversely modulating immune responses.

Conclusion: The advent of culture-independent molecular approaches has led to a greater appreciation of the intricate microbial ecology of the paranasal sinuses. Microbiota composition, distribution, and abundance impact mucosal health and influence pathogen growth and function. A deeper understanding of the host-microbiome relationship and its constituents may encourage development of new treatment paradigms for CRS, which target restoration of microbiome homeostasis and cultivation of optimal microbial communities.

Chronic rhinosinusitis (CRS) represents one of the most common health care problems in the United States, with one in seven adults affected and 31 million individuals newly diagnosed each year.[1,2] Since its characterization, much knowledge has been gained regarding the multifaceted inflammatory nature of CRS, with both host (*e.g.,* allergy, immunodeficiency, ciliary immotility, anatomic obstruction) and environmental factors (bacterial and/or viral infections, biofilms, pollutants) understood to be involved in its pathogenesis.[3] However, despite extensive research efforts, no clear necessary and sufficient etiology for CRS has been identified to date. Although chronic bacterial colonization has been implicated, the precise relationship between such microbes and CRS continues to be disputed, and a definitive microbial profile responsible for disease manifestation remains controversial.[4]

Microbiome refers to the genetic potential of the entire cohort of resident microorganisms (commensal, symbiotic, pathogenic) that inhabit a given niche (*e.g.,* the sinus cavities) and function as an organized community.[5] In recent years, our understanding of the significance and composition of native microbiota of the sinuses has changed considerably. Continued advances in molecular technology have expanded our ability to distinguish the numerous spectra of microbial species that inhabit host niches, far beyond the capacity of traditional culture methods. Such investigation has afforded new insights into the depth, breadth, and complexity of microbial communities that reside within the sinus cavities. Once presumed to be sterile in the healthy state, the sinuses are now known to harbor a diverse consortium of microorganisms.[6,7] Disruption of indigenous microbiota (dysbiosis) may lead to pathogen overgrowth and enhanced susceptibility to infection, similar to what has been observed in the gastrointestinal tract and lower airways.[8–10] Resident microbes may also influence the behavior of pathogenic species in a "community as pathogen" model, which further promotes development of CRS.[10]

The purpose of this article was to review the current literature regarding the sinus microbiome and collate research findings from relevant studies published to date. Methods of detection, determination of microbiome composition, and comparative profiling between patients with and without CRS were specifically explored. The potential role of commensal organisms in determining sinus mucosal health versus disease was discussed, and the clinical implications of future antibiotic versus probiotic therapy in CRS were also examined.

METHODS

A systematic literature search was performed by using the Medline, EMBASE (Elsevier, New York, NY), and Cochrane Review (Cochrane, London, United Kingdom) databases up to December 1, 2014, on studies investigating the sinus microbiome in patients with and without CRS. Keywords used in the initial screening included "microbiome," "microbe," "microbial," "community," "sinus," "sinonasal," and "sinusitis." A secondary search was also completed for specific classes of microbes by using the following keywords: "bacteria," "bacterial," "virus," "viral," "fungus," and "fungal." The literature searches were conducted by two of the authors (J.L, V.R.). References of original articles and relevant reviews were individually retrieved and perused for pertinent findings to identify those studies that specifically assessed the sinus microbiome in patients with and without CRS. CRS was defined according to the diagnostic criteria outlined by the 2007 American Academy of Otolaryngology—Head and Neck Surgery Foundation clinical practice guidelines.[11] Specifically, ≥12 weeks of two of the following signs and symptoms, *i.e.,* mucopurulent drainage, nasal obstruction, reduced smell, facial pressure, in conjunction with evidence of paranasal sinus inflammation on imaging or physical examination were required for the diagnosis of CRS.[11] Studies that used molecular diagnostics to analyze the constituents of the sinus microbiome were the primary focus of the review.

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Molecular Methods of Microbial Detection and Analysis

Although culture-dependent techniques have been the mainstay of the microbial diagnostics in CRS, it has been estimated that >70% of bacterial species inhabiting body surfaces cannot be successfully cultivated under standard culture conditions because specific microenvironments necessary for bacterial growth may not be reproducible in the laboratory.[8,12] Organisms that rely on symbiotic relationships, host immune systems, or other complex dynamics may be unable to thrive once removed from native mucosal niches. In addition, microbes that form biofilms may also go undetected because the reduced metabolic activity and phenotypic transformations can impede their capacity to grow on laboratory media.[9] Therefore, it is informative that 10–45% of CRS specimens have been reported to yield negative cultures.[13,14]

However, recent advances in molecular technology have enabled more comprehensive analysis of the sinonasal microbiome than that achieved by traditional culture.[15] Molecular methods of microbial detection are summarized in Table 1. With these techniques, DNA or RNA is extracted from the specimen, and target nucleic acids are either amplified with polymerase chain reaction (PCR) or directly interrogated. PCR assays can be designed to target either individual microbial species (or strains) or broader phylogenetic ranges of organisms. During amplification, DNA polymerase enzymically replicates selected nucleotide sequences of DNA by using oligonucleotide primers. Each cycle results in an exponential increase in the number of copies synthesized, with products of interest identified under gel electrophoresis or fluorescence. If an RNA template is used, reverse transcriptase is implemented before PCR to generate DNA needed for amplification. In this way, metabolically active bacterial species can be detected and quantified.

Gene Clone Libraries and Sequencing

The *16S* ribosomal RNA (rRNA) gene is a relatively small (1.5 k–base pairs [bp]) but highly conserved locus of the bacterial genome that contains nine hypervariable regions, which can provide sequence-dependent differentiation of individual taxa.[16–18] As such, it is one of the most frequently used genes for bacterial identification. The steps involved in *16S* rRNA gene sequencing are illustrated in Fig. 1. With this method, universal PCR primers are chosen that are complementary to the conserved regions interspersed across the gene. Amplification of conserved sequences that flank hypervariable regions allows creation of bacteria-specific primers and amplicons. Amplicons can be directly sequenced or cloned and then sequenced. Because PCR requires only nucleic acids rather than viable microbial isolates, *16S* rRNA gene amplicon libraries can be constructed from heterogeneous samples without the need for prior cultivation. The resulting sequences can then be classified by using existing taxonomic databases for phylogenetic determination.[19–27] Depending on the length of the *16S* sequences, which generally is limited by the type of sequencing platform used, such comparative analysis typically enables differentiation of microorganisms to the genus level for all major phyla of bacteria as well as classification of numerous strains according to operational taxonomic units (OTUs). OTUs are clusters of similar *16S* rRNA sequences used to define a species when only sequence data are available. They are the most commonly used diversity units to characterize microbial communities.[28] Like the *16S* rRNA gene, the *18S* and *28S* rRNA genes are similarly conserved loci in the fungal genomes that have allowed mycotic identification at the genus level and above.[22]

Although earlier studies typically sequenced the majority of the *16S* rRNA gene, introduction of parallel sequencing technologies has led to sequencing of shorter subregions at greater depth.[29] For instance,

Table 1. Molecular methods of microbial detection

	Description
Sequencing	Genes of conserved loci with hypervariable regions (*i.e.*, *16S* rRNA) are amplified, sequenced, and compared with databanks of known gene sequences for taxa identification[20]
Gene clone libraries	Bacterial vectors are used to clone gene amplicons, which are subsequently sequenced and matched to existing taxonomic databases
T-RFLP profiling	*16S* rRNA amplicons are digested with restriction endonucleases; relative abundance of resulting fragments are then depicted on electropherograms[16]
PCR-DGGE	DNA fragments from a sample are amplified with PCR; products are then subject to gel electrophoresis and increasing concentrations of denaturing reagents; unique DNA sequences will then migrate and melt at different positions along the gel[40]
Quantitative PCR	Primers and fluorescent labels are used to quantify the abundance of select DNA templates
Cepheid Xpert SA Nasal Complete	
Roche LightCycler MRSA Advanced Test	
Gen-Probe Amplified test	
MassTag PCR	PCR primers are covalently linked to a compound of unique mass; mass spectrometry is then used to measure different types of PCR products[34]
Ibis T5000 biosensor	
Microarrays	Fluorescently labeled nucleic acids are hybridized to organism-specific oligonucleotides placed onto silicon plates and visualized by using fluorometry[35]
PhyloChip	
ViroChip	
Functional gene arrays	Functional gene arrays detect genes encoding for key enzymes involved in distinct metabolic pathways[37]
GeoChip	

rRNA = ribosomal RNA; T-RFLP = terminal restriction fragment length polymorphisms; PCR-DGGE = polymerase chain reaction denaturing gradient gel electrophoresis; SA = Staphylococcus aureus; MRSA = methicillin resistant SA.
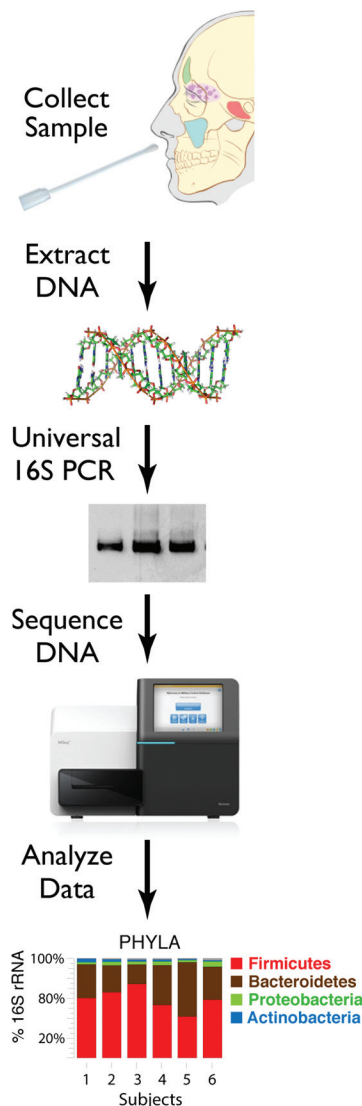
Figure 1. *Steps involved in 16S rRNA sequencing. rRNA = ribosomal RNA; PCR = polymerase chain reaction.*

the Illumina MiSeq platform (Illumina Corporation, San Diego, CA) currently generates paired sequence reads of 2 × 300 bps with higher and cheaper outputs than traditional Sanger sequencing. NextSeq and HiSeq platforms (Illumina) currently enable 2 × 150 bp reads. However, taxonomic classification based on such short gene sequences can be challenging. The efficacy of short sequencing read technologies in distinguishing taxa is dependent upon the sequencing strategy used (*i.e.,* read length, single/paired end), the region of the *16S* rRNA gene selected for sequencing, and the taxonomic classifier used. Differences in target selection can lead to inconsistencies in data sets reported and likely account, at least in part, for the marked heterogeneity observed in sinonasal microbiome studies. Instances in which distinct species have similar or identical sequences can also be a limiting factor if only short stretches of the gene are sequenced.[16]

Once considered too costly and inefficient, platforms have now been created that offer concurrent sequencing of PCR amplicons at much quicker turnaround times than previously available. MicroSeq (Boston Analytical, Salem, NH) provides partial *16S* rRNA sequencing in just 12 hours. Multiple specimens can also be analyzed simultaneously, with tags used to designate *16S* rRNA amplicon pools from different samples.[30,31] In addition, such sequencing does not require previous assumptions regarding the type of microorganisms present in a given specimen. Next-generation platforms, including those produced by Illumina, Oxford Nanopore Technology (Oxford, U.K.) and Pacific Biosciences (Menlo Park, CA) promise to offer even higher outputs and greater read depth and read length in the future.[8]

In this way, indigenous but rare microbes buried within intricate communities can be identified by using unique genetic signatures, whether bacterial, viral, or fungal.[17] Entire microbial fingerprints can be established, verifying the presence of certain microbes and quantifying their abundance within the sinuses. Such nucleic acid–based methods have revolutionized microbial identification, broadening the spectrum of detectable microorganisms and increasing the sensitivity of speciation.[12] Compared with standard culture, detection techniques that target microbial nucleic acids have raised rates of species differentiation by >10-fold.[12,25,32]

Quantitative PCR. Real-time quantitative PCR (qPCR) is the most commonly used molecular technique to directly measure microbial abundance.[8] By using target-specific primers and fluorescent labeling, identification can be performed at the strain or species level with data expressed as the number of gene copies per unit sample. The choice of oligonucleotide primers used depends on whether the abundance of total bacteria (*i.e., 16S* rRNA, FAM reporter) or specific species (*e.g., S. aureus* femA gene, TET reporter.) are being investigated.[33,34] Automated systems have been developed that enable DNA extraction plus qPCR, allowing for species identification within a matter of hours (*e.g.,* Cepheid Xpert SA Nasal Complete [Cepheid Inc., Sunnyvale, CA], Roche LightCycler MRSA Advanced Test [Roche Diagnostics, Indianapolis, IN], and the Gen-Probe Amplified tests [Gen-Probe Inc., San Diego, CA]).[35,36] However, it should be noted that the microbiome sample is not typically normalized to the amount of material obtained and would likely vary with collection method.

MassTag PCR. The Ibis T5000 (Ibis Biosciences, Carlsbad, CA) involves MassTag PCR, which is a relatively new technology for quantification of multiple target organisms in a single reaction. In this approach, each organism-specific PCR primer set is covalently linked to a compound of unique mass.[37] After amplification and purification of PCR products, mass spectrometry is then used to enumerate each type of PCR product in the mixture, from which the abundances of the target organisms can be inferred.[37] In its original description, 22 microbes could be identified with a single MassTag PCR reaction at the species level by amplifying a 50–300-bp product.[37]

Microarrays. Microbial nucleic acids can also be detected by using microarrays. With this technique, representative oligonucleotides from specific organisms are placed onto silicon plates.[15] Fluorescently labeled nucleic acids from a specimen are then hybridized to the oligonucleotides and visualized by using fluorometry.[15] Preparation of microarrays can be customized to encompass a wide variety of species. Such versatility makes this method particularly amenable to use in CRS in which numerous pathogens may be involved. However, it should be noted that species differentiation may not be achieved if cross-hybridization occurs between closely related sequences.

The *16S* rRNA PhyloChip (Affymetrix Corporation, Santa Clara, CA) is a high-density phylogenetic microarray used for comparative analysis of bacterial community composition.[5] Universal primers are used to augment the *16S* rRNA gene, which is fragmented and hybridized to fluorescently labeled probes. The PhyloChip harbors 1.2 million DNA probes that can distinguish ~60,000 taxa.[8] Similarly, the ViroChip can detect ~1800 viruses in a single run.[35] Functional gene arrays detect genes encoding for key enzymes involved in distinct metabolic pathways (*e.g.,* carbon cycle).[36] For example, the GeoChip contains >120,000 probes that target >500 functional gene families.[37] Such technology has been most effective in the analysis of biodiversity and composition of select bacterial communities, whereas newer functional gene arrays may help investigate the function of the microbiome as a whole.[5]

Clinical Application

Determining which molecular methodology is most appropriate in the clinical setting is a subject of ongoing debate. PCR (*16S* rRNA gene) with DNA sequencing likely offers the most inclusive analysis because slow-growing, fastidious organisms would be captured without requiring prior assumptions regarding identities and growth properties of microbes in the specimen. However, one drawback of molecular diagnostics is that genomes of nonviable microbes are also detected, and results, therefore, must be interpreted judiciously to distinguish between viable, potentially pathogenic and nonviable microbes or lysed cells. In addition, identification of an organism does not necessarily denote activity or pathogenicity because commensals will be amplified along with virulent bacteria. The key issue is the interpretation of such detailed information. Even today, we debate the clinical relevance of generally harmless bacteria detected on culture (*e.g.,* coagulase negative *Staphylococcus*), and a DNA sequencing report of dozens of low-abundance bacteria will cause even more confusion. Sequence-based molecular diagnostics may require more time, expense, and labor than conventional culture as well as access to specialized equipment, and, at this time, certain molecular diagnostics, including broad-range *16S* sequencing, and microarrays cannot be ordered from a laboratory to guide medical therapy. A lack of standardization, expertise, availability, turnaround time, cost, and data interpretation have been obstacles to widespread implementation in everyday clinical practice. Cost-benefit comparative analysis must also be conducted to determine utility on a routine basis. However, as technology, access, and turnaround times for processing continue to improve, such techniques may eventually become standard practice for microbial analysis of sinus samples in the future.

Composition of the Sinus Microbiome

When discussing microbial ecology, it is important to be familiar with certain terminology. Prevalence refers to the presence or absence of a particular organism within the study cohort. Abundance refers to the absolute quantity, or density, of a specific microbe within a sample, whereas relative abundance reports the percentage of the total microbes in a community represented by a given taxon. Diversity indices report the number of species and the proportion of individual species in a particular niche or anatomic site; α-diversity measures include richness, evenness, and complexity.[38] Richness describes the number of unique taxa (*e.g.,* species or genera) present in a specimen; the more distinct the taxa, the greater the richness. In contrast, evenness quantifies how similar the relative abundances of taxa are and can indicate if a sample is dominated by one or a few taxa rather than evenly spread across those present. Complexity or diversity combines both richness and evenness into a single index and is commonly measured by the Shannon diversity index and the Simpson diversity index. β-diversity measures are used to compare overall similarities (or dissimilarities) of community structure among samples. However, it should be noted that the accuracy of such measurements is not absolute given the potential for contamination during specimen collection, variability in sampling methods, differences in technique sensitivities, and patient variances. A current standard is the Good's coverage estimator, which serves as a measure of the percentage of overall species identified within a given sample and should be reported in microbiome studies.

Bacterial Microbiome. Multiple studies have begun to characterize the microorganisms that constitute the sinus microbiome.[7,12,24,39–46] Molecular diagnostics demonstrated that sinonasal microbial constituents differ significantly in health versus disease (Table 2). To assess the bacteriology of sinus cavities under normal conditions, Ramakrishnan *et al.*[7] examined middle meatal swabs from 28 healthy patients. A qPCR was used to measure bacterial loads, and PCR with *16S* rRNA sequencing was used in parallel to determine relative quantities of bacterial taxa.[7] Amplicons of the V1V3 variable regions (~500 nt) of the bacterial *16S* rRNA gene were generated by using

primers 27FYM + 3 and 534R, and sequenced on a 454/Roche Life Sciences GS-FLX instrument (Roche Life Sciences, Indianapolis, IN). Bacteria were detected in all the samples, with *Firmicutes, Proteobacteria,* and *Actinobacteria* the most prevalent (100%) and abundant phyla. (48, 25, and 23% relative abundance, respectively) [7] *Bacteroidetes* also were identified in 83% of specimens but at a much lower abundance (2.5%).[7] On the species level, *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Staphylococcus aureus* were the most prevalent (86, 92, and 68%, respectively) and abundant (11, 15, and 8%, respectively).[7] Corynebacteria had a collective prevalence of 93%, with *Corynebacterium tuberculostearicum* as the most dominant.[7] Interestingly, opportunistic pathogens were also detected (*Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis*) as well as those often associated with CRS (*Stenotrophomonas maltophilia, Enterobacter* species, anaerobes), albeit at lower abundances. These findings implied that individuals can harbor pathogens in the healthy state as long as these organisms are not given the opportunity to overpower other resident flora. Therefore, relative abundance, rather than prevalence, may have more of an impact on disease pathogenesis than the presence or absence of a particular species.[7]

Although culture-based studies have historically reported only a limited number of microbes in CRS, the advent of molecular technology has revealed a wide diversity of microbiota in this ecological niche. An early study, by Paju *et al.*[39] in 2003, used nucleic-acid–based methods for microbial identification in CRS. The *16S* rDNA (precise region unspecified) was amplified from maxillary sinus mucosa and lavage fluid of 11 patients with CRS. *S. aureus*, Gram-positive, Gram-negative organisms, and anaerobes were all detected.[39] In 2005, Power *et al.*[40] acquired middle meatal aspirates from six patients with CRS who were evaluated by using standard culture and PCR-denaturing gradient gel electrophoresis of *16S* rRNA amplicons. Greater bacterial diversity (more than three types per sample) was appreciated with PCR-denaturing gradient gel electrophoresis than culture (one colony per specimen). *S. pneumoniae* and *S. aureus* were the most commonly grown cultivars (4/6), whereas streptococci from the mitis-sanguinis group were the most frequently detected with PCR-denaturing gradient gel electrophoresis.[40]

More recently, Stephenson *et al.*[41] used both conventional culture and molecular techniques to conduct a microbial analysis of ethmoid mucosa from 18 patients with CRS and 9 controls. A 600 bp region of the *16S* rRNA gene was amplified by using bacterial primers 530F and 1100R. The *16S* rDNA sequencing detected bacteria in 100% of the CRS samples, with a mean of 10 organisms per specimen. Anerobes were the predominant microbes found in CRS, although *S. aureus* was also observed in 50% of the CRS samples. Two new genera, *Diaphorobacter* and *Peptoniphilus*, both of which were anaerobes and had never been previously associated with CRS, were also reported. In control tissue, *S. aureus*, *Corynebacterium*, and *Propionibacterium* were the most frequent organisms found. In contrast, culture detected bacteria in only 82% samples at a mean of 1.4 isolates per specimen, with *S. aureus* (18%) and coagulase-negative *Staphylococcus* species (53%) being the most common cultivars. The absence of anaerobic predominance was ascribed to limitations of culturing methods in growing anaerobic colonies. Therefore, sequencing proved to be a much more sensitive means of microbial detection than culture-based techniques, particularly for anaerobic and polymicrobial communities.[41]

In 2011, Stressman *et al.*[42] investigated the bacteriology of 73 clinical specimens (28 polyps, 15 mucus, 30 turbinate tissue) from 43 patients with CRS by using *16S* rRNA gene sequencing and terminal restriction fragment length polymorphism analysis. No control group was included. Forty-eight distinct bacterial species from 34 genera were identified, with members from *Pseudomonas, Citrobacter, Haemophilus, Propionobacterium, Staphylococcus,* and *Streptococcus* most dominant.

Table 2. Literature summary of molecular studies comparing sinus bacteriology of patients with and without CRS

Study	Study Population	Outcome Measures	Methods of Microbial Analysis	Results	Conclusion
Hauser et al.,[24] 2015	54 CRS; ethmoid swabs	Bacterial prevalence and diversity	Cx, *16S* rRNA sequencing	Cx: 3 isolates/sample; CN staph, *P. acnes*, anaerobes most common; sequencing: 21.5 sp/sample; CN staph, *Corynebacterium, P. acnes* most prevalent	Cx does not accurately represent the complete spectrum of sinus microbiota
Ramakrishnan et al.,[45] 2013	74 CRS, 27 non-CRS; middle meatal swabs	Bacterial load, SA abundance, and prevalence	qPCR	No ss dif in bacterial counts and SA prevalence between the 2 groups; ss ↑ SA abundance in CRSwNP, CRSwAR, CRS with asthma	SA implicated in subset of patients with CRSwNP with or without asthma and/or AR
Boase et al.,[12] 2013	38 CRS, 6 non-CRS; ethmoid mucosa harvested during ESS (CRS) or skull base surgery (control)	Bacterial load and diversity	Cx, Ibis T5000 biosensor, FISH	SS ↑ bacterial load and diversity in CRS (33 sp) vs non-CRS (5 sp); SA (CRS) and *P. acnes* (controls) most common bacteria detected	Prevalence and abundance of microorganisms related to disease manifestation
Aurora et al.,[46] 2013	30 CRS, 12 non-CRS: middle meatal lavage during ESS or endonasal surgery (septoplasty, pituitary, orbit)	Bacterial load, speciation, ck and immune cells in lavage, response of WBC to lavage microbiota	*16S* rRNA sequencing, ELISA (ck), flow cytometry (immune cells)	SS ↑ in bacteria load in CRS vs non-CRS, qualitatively similar microbiome composition (35% overlap), ↑IL-4/IL-5/IL-8/IL-13, eosinophils, and basophils in CRS lavage; CRS peripheral WBC show ↑ in IL-5 when exposed to lavage microbiota	CRS stems from immune hyperresponsiveness to commensal microbes
Ramakrishnan et al.,[7] (2013)	28 healthy pts; middle meatal swabs	Bacterial load, abundance, and speciation	qPCR, *16S* rRNA sequencing	Bacteria present in all pts; *S. epidermidis, P. acnes*, and SA most prevalent and abundant; opportunistic pathogens SP, *M. catarrhalis*, Hflu was also detected but in low abundance	Relative abundance may be more impactful in disease pathogenesis than presence of a particular sp

Table 2. **Continued**

Study	Study Population	Outcome Measures	Methods of Microbial Analysis	Results	Conclusion
Abreu et al.,[43] 2012	10 CRS vs 10 non-CRS; mucosal brushings of maxillary sinuses during ESS (CRS), OSA, or malocclusion surgery (controls)	Bacterial load, taxonomic distribution	qPCR, *16S* rRNA PhyloChip	No ss dif in bacterial load between the 2 groups, but ss ↓ richness, diversity, evenness in CRS; depletion of lactic acid bacteria (especially *L. sakei*) and ↑ *C. tuberculostearicum* in pts with CRS	Depleted mucosal microbiome mediates CRS; commensals may become pathogenic, depending on abundance of the local microbiota
Feazel et al.,[44] 2012	26 CRS vs 8 non-CRS; middle meatal swabs	Bacterial abundance and diversity	Cx, *16S* rRNA sequencing (15 CRS vs 5 non-CRS); qPCR SA DNA (26 CRS vs 8 non-CRS)	Cx: No ss dif in no. of isolates per sample in CRS vs non-CRS; CRS non ss ↑ SA prevalence and abundance vs controls; sequencing: CRS non ss ↓ bacteria types and less even distribution than controls, anaerobes predominant; qPCR: CRS ss ↑ prevalence and abundance of SA than non-CRS	Pts with CRS and with altered microbial composition, and ss ↑ abundance and prevalence of SA vs non-CRS
Stressman et al.,[42] 2011	43 CRS; 73 samples: 28 NP, 15 mucus, 30 turbinate	Bacterial diversity and prevalence	*16S* rRNA sequencing, TRFLP	48 distinct bacterial sp detected, PA most prevalent, SA in 50%, anaerobes also identified	Complex array of common and novel bacteria present in CRS
Stephenson et al.,[41] 2010	18 CRS vs 9 non-CRS; ethmoid mucosa	Bacterial diversity and prevalence	Cx, *16S* rDNA sequencing	Cx: 1.4 sp/sample, SA and CN staph most common in CRS; sequencing: 10 sp/sample, for CRS, anaerobes predominant, SA in 50%;, and for controls: SA, *Corynebacterium*, *Propionobacterium* most common	Molecular techniques were more sensitive than Cx, anaerobes were more common in CRS than initially presumed
Power et al.,[40] 2005	6 CRS; middle meatal aspirates	Bacterial diversity and prevalence	Cx, *16S* rRNA PCR-DGGE	Cx: 1 cultivar grown in all samples, SP and SA most common; PCR-DGGE: >3 types of bacteria were found in all samples, strep mitis-sanguinis group was most common	PCR-DGGE was more sensitive than Cx in bacterial detection

Table 2. Continued

Study	Study Population	Outcome Measures	Methods of Microbial Analysis	Results	Conclusion
Paju et al.,[39] 2003	11 CRS, maxillary mucosa and lavage	Bacterial diversity and prevalence	Cx, *16S* rDNA sequencing	Bacteria detected in 45% samples, 13 sp identified	Both aerobic and anaerobic bacteria were detected in CRS

↑ = increase; ↓ = decrease; CRS = chronic rhinosinusitis; Cx = culture; rRNA = ribosomal rRNA; CN = coagulase negative; staph = staphylococci; P. acnes = Propionobacterium acnes; sp = species; SA = Staphylococcus aureus; q-PCR = quantitative polymerase chain reaction; ss = statistically significant; dif = difference; CRSwNP = chronic rhinosinusitis with nasal polyposis; CRSwAR = chronic rhinosinusitis with allergic rhinitis; AR = allergic rhinitis; ESS = endoscopic sinus surgery; FISH = fluorescence in situ hybridization; ck = cytokine; WBC = white blood cell; ELISA = enzyme-linked immunosorbent assay; IL = interleukin; pts = patients; S. epidermidis = Staphylococcus epidermidis; SP = Streptococcus pneumonia; M. catarrhalis = Moraxella catarrhalis; Hflu = Hemophilus influenzae; OSA = obstructive sleep apnea; L. sakei = Lactobacillus sakei; C. tuberculostearicum = Corynebacterium tuberculostearicum; NP = nasal polyposis; TRLFP = terminal restriction length fragment polymorphism; rDNA = ribosomal deoxyribonucleic acid; PA = Pseudomonas aeruginosa; PCR-DGGE = polymerase chain reaction denatured gel gradient electrophoresis; strep = streptococci.

Pseudomonas aeruginosa was the most prevalent and found in 93% polyp, 92% mucus, and 90% turbinate tissue samples. In addition, multiple species not previously reported in conjunction with CRS were also observed (*i.e.*, *Aggregatibacter*, *Bradyrhizobium*). Similar to the findings by Stephenson et al.,[41] anaerobes were also detected and identified, and *S. aureus* was present in ~50% of the samples.[42]

In 2012, Abreu et al.[43] conducted a nonculture-based comparative analysis of 20 maxillary sinus specimens collected from patients with (10) and without (10) CRS. Bacterial burden and taxonomic distribution were determined by using qPCR of *16S* rRNA and a standardized phylogenetic microarray (*16S* rRNA, PhyloChip). Specifically, the total number of copies of *16S* rRNA gene per microgram of total DNA was compared in patients with CRS versus patients without CRS. No differences in overall bacterial load were detected between the two groups ($2.10 \times 10^6 \pm 1.01 \times 10^6$ in the CRS group versus $2.92 \times 10^6 \pm 2.17 \times 10^6$ in the control group; $p = 0.37$), indicating that the quantity of bacteria did not have a bearing on disease status. Pathogenic members of *Pseudomonadaceae*, *Mycobacteriaceae*, and *Lachnospiraceae* were also identified in both patients with and without CRS, suggesting that other community members may affect the activity of such pathogens.

However, patients with CRS exhibited substantially reduced bacterial richness, evenness, and diversity in comparison with controls, a finding that was independent of antibiotic therapy. Microbial communities of patients with CRS were compositionally distinct from their healthy counterparts, with 1482 OTUs significantly lower in abundance in diseased sinuses. Depletion of multiple, phylogenetically distinct lactic acid bacteria was demonstrated in patients with CRS, with a specific decrease in *Lactobacillus sakei* and concomitant increase in *C. tuberculostearicum*. It was not specified how many OTUs exhibited no difference in abundance between the two groups. In addition, certain species (*C. tuberculostearicum*) were associated with changes in the 20-question Sino-Nasal Outcome Test (a validated metric used to score sinus symptomatology), suggesting that such members may participate in physiologic processes contributing to CRS symptoms. These observations indicated a potentially protective effect of such bacteria against pathogenic species.[43]

Also in 2012, Feazel et al.[44] compared *16S* rRNA gene sequencing with standard culture in pathogen identification of 21 swabs taken from patients with CRS (15) and patients without CRS (5).[44] Results were broadly congruent between the two methodologies, but much greater biodiversity was ascertained with DNA sequencing. Fewer than 15% of organisms found with sequencing were grown in culture. No significant differences in the average number of isolates (2.8 per subject) on culture were evident between patients with CRS and controls. Coagulase-negative staphylococci (75%), *S. aureus* (50%), and *P. acnes* (30%) were the most commonly isolated organisms. In contrast, different types and quantities of bacteria were reported on phylogenetic analysis of *16S* sequences, with significantly varied distributions of sequences observed between the two groups. Patients with CRS were characterized by reduced and less even genus-level biodiversity compared with controls, although these disparities did not reach statistical significance. Coagulase-negative staphylococci (100%) were the most prevalent, followed by *Corynebacterium* species (86%), and *P. acnes* (76%). Patients with CRS also demonstrated significantly higher enrichment of *S. aureus* through both culture and molecular techniques. When present, quantities of *S. aureus* were also greater in patients with CRS versus patients without CRS, although this difference did not reach statistical significance, likely due to the small number of control subjects. When an additional 22 patients and controls were added to the initial data set, qPCR of *S. aureus* DNA was positive in 73% of patients with CRS, with a 14.8% relative abundance versus 25% and 0.4%, respectively, ($p = 0.03$) in healthy subjects.[44]

In 2013, a larger follow-up study by the same group examined middle meatal swabs from 74 patients with CRS and 27 control patients for total bacterial counts, *S. aureus* abundance, and *S. aureus* prevalence by using qPCR.[45] No significant differences in the quantity of bacteria and *S. aureus* prevalence were observed among CRS without nasal polyps, CRS with nasal polyps (CRSwNP), CRS with asthma, and control groups. There also was no correlation between total bacterial load and disease severity. However, increased *S. aureus* abundance was observed in patients with CRSwNP, CRS with allergic rhinitis, and CRS with asthma.[45]

When adding in a host component to this line of study, Aurora et al.[46] performed *16S* rRNA sequencing on nasal lavage samples from patients with CRS (30) and patients without CRS (12). Sequences were analyzed at the OTU species level, with rRNA sequences having a 95% sequence identity at the nucleotide level considered to be a single species. An overall increase in richness was reported in patients with CRS (3780 OTUs among all the patients) relative to controls (2333 OTUs among all the subjects). However, it should be noted that such differences may be due to the smaller number of patients without CRS versus patients with CRS included in the study. A 35% overlap of identified bacterial OTUs were observed between the two groups, suggesting an overall qualitative difference between the two groups. The most abundant phylum in both patients with and without CRS was, surprisingly, *Cyanobacteria* (104 species). Similar to Abreu et al.,[43] *Corynebacterium* species represented the most significant increase in CRS versus controls, although Aurora et al.[46] found elevated *Corynebacterium accolens* rather than *Corynebacterium tuberculostearicum*. Conversely, *Alicycliphilus* and *Cloacibacterium* were decreased in patients with CRS compared with controls. No clear microbial candidate

emerged as a potential pathogen responsible for inciting the inflammatory reaction seen in CRS. Therefore, the researchers indicated that CRS may stem more from aberrant host immune hyperresponsiveness to normal microbial components because this was demonstrated in their study by using a leukocyte stimulation assay.[46]

In 2013, Boase et al.[12] also compared microbial biodiversity and abundance in sinus mucosa of patients with CRS ($n = 35$) versus patients without CRS ($n = 6$). Multiple methods of analysis were used, including culture, molecular diagnostics (Ibis biosensor: PCR of 16S rDNA coupled with electrospray ionization mass spectrometry of PCR products), and fluorescence in situ hybridization. All healthy sinuses harbored microbial communities. However, unlike the aforementioned studies, more bacterial species were detected in diseased tissue ($n = 33$; mean, 3.0/patient) than in controls ($n = 5$; mean, 2.0/patient). Seventy-nine percent of patients with CRS exhibited more than one bacterial species versus 50% of healthy subjects by using the Ibis biosensor. In addition, patients with CRS also had a significantly greater total bacterial burden than controls. S. aureus (61% prevalence) and S. epidermidis (55%) were the most commonly identified bacteria in CRS, whereas P. acnes (85%) was the most frequently isolated microorganism in normal subjects. Increased microbial abundance was associated with disease as measured by the Ibis biosensor, with the patients with CRS exhibiting 10 times higher S. aureus sequences per sample versus the controls.[12]

Overall, cross-study comparative analysis of the sinus microbiome is difficult due to a lack of standardization of nucleic acid–based methods (i.e., specimen collection, DNA and RNA extraction protocols, PCR primer sets, 16S variable regions sequenced, data analysis pipelines).[47] Significant variability in microbial burden and organism profile have been reported in CRS, with some studies finding specific bacteria (i.e., P. aeruginosa, Haemophilus influenzae) to be the predominant pathogen, whereas other studies detecting those same microorganisms only in small amounts.[12,41,42] Such disparities are challenging to reconcile but may be attributed to differences in detection methodology, patterns of antibiotic usage, CRS subtype, disease severity, geography, and other comorbidities.

Viral Microbiome. Viral components of microbial communities have remained largely understudied, particularly in normal subjects, but are likely to contribute to the microbiome and its functions. The first study to use DNA sequencing to investigate the presence of viruses in healthy patients was published by Wylie et al.[48] in 2014. A total of 706 samples from 102 normal subjects were obtained at multiple time points (30–359 days) from five body sites: nose, oral cavity, skin, vagina, and stool. The Illumina platform (Illumina, San Diego, CA) was used to generate whole genome shotgun sequence data sets from the sample sites. These were then sequenced and matched to reference genomes for viral identification. Eukaryotic double-stranded DNA viruses were the focus of the analysis. An average of 5.5 viral genera was detected per patient, with at least one virus observed in 92% of individuals. Seven viral families were identified, including *Herpesviridae*, *Papillomaviridae*, and *Adenoviridae*. Unique viral profiles were evident in each subject, which demonstrated the high interpersonal diversity that characterizes the human virome. Some of the viruses appeared to represent stable components of a particular individual's flora, whereas other viruses were only seen at single time points.[48] Additional investigation is needed to better elucidate the composition and dynamics of the human virome and its potential impact on immune homeostasis. Thus far, no published studies have specifically examined the sinus virome in patients with and without CRS.

Fungal Microbiome. The presence of fungi in the sinonasal cavities of patients with and those without CRS has been well established by using both culture-based and molecular detection techniques.[49,50] Fungi are reported to be equally prevalent in patients with and without CRS.[51] In addition, qPCR studies demonstrated that the quantity and types of fungal taxa are similar in subjects with and without CRS.[49,52–54] However, other studies have reported conflicting results.[12,46] Consequently, determining the role of fungi in the pathogenesis of CRS remains elusive. Studies investigating the mycology of the sinus microbiome utilizing molecular techniques are summarized in Table 3.[12,46,52–56]

In a study by Murr et al.,[53] the mycology of the middle meatus was compared in 74 patients with CRS (37) and those without CRS (37). Endoscopically guided brush samplings were collected and analyzed by using qPCR for 82 fungal species. No significant difference in the presence or absence of fungus was found, with fungal recovery prevalences of 45.9% for both groups. Molds typically recovered in a water-damaged environment were classified as group 1, with other fungi ubiquitous in the household setting referred to as group 2.[53] Fifteen species were detected, with *Cladosporium cladosporioides* and *Aureobasidium pullulans* the most common. No statistically significant difference in the distribution of either fungal species or groups 1 and 2 molds were evident between diseased and control populations. Cross-referencing also failed to demonstrate any correlation between the presence of a particular fungus and CRS.[53]

In a follow-up study by the same group, sinus samples from 73 patients with CRS and 16 patients without CRS were evaluated with qPCR to determine the presence and abundance of 36 fungi.[54] As in their previous study,[53] rates of detection were very similar for brush specimens from patients with CRS and patients without CRS. *Alternaria alternata* and *C. cladosporioides* types 1 and 2 were found in higher concentrations than other species, but the differences were not statistically significant between the two groups. However, in a small subset of patients with CRS (16 of the 73 patients), one or more of seven fungal species were found to be >1000 times the concentration of the average in patients without CRS, which indicated that a subgroup of patients with CRS was particularly susceptible to fungal growth and colonization.[54]

Cleland et al.[56] also attempted to characterize the fungal sinus microbiome. Swabs were collected from 23 patients with CRS and 11 patients without CRS at the time of surgery and 6 and 12 weeks after surgery. A total of 207 fungal genera were detected through 18S rDNA sequencing, with fungi detected in all the patients. No significant difference in fungal richness or overall prevalence was observed between the control and CRS groups from intraoperative specimens. *Malassezia*, which had not been previously reported in the sinuses, was the most abundant genus and was observed in every subject. Notably, the abundances of *Alternaria* and *Aspergillus* genera were low. Interestingly, the only genus that demonstrated a significant difference in prevalence in patients with and without CRS was *Scutellospora* (5% versus 36%, respectively).[56]

In the study by Aurora et al.,[46] the mycology of the sinus cavities was also compared between patients with and without CRS using 18S rRNA sequencing. Greater fungal β-diversity was observed in CRS samples (132 species) versus controls (106 species), with an overlap of 17.5% between the two groups. *Cryptococcus neoformans* was the dominant species in both CRS (90%) and normal (61%) samples, followed distantly by *Rhodosporidium diabovatum*, which was only detected in the CRS group (2.5%).[46] Because responses to *C. neoformans* infection can range anywhere from asymptomatic colonization of the airways to meningitis or disseminated disease in patients who are immunocompromised, it is unclear what the clinical significance of this finding will be in the future. Similar to the study by Murr et al.,[xx] a comparison of interpatient microbiomes yielded a high degree of similarity between patients with and without CRS.[46] Likewise, Scheuller et al.,[52] reported that the amount of fungal DNA in the middle meatus did not differ significantly between patients with CRS ($n = 19$) and without CRS ($n = 19$) and that fungal elements were present in an equivalent number of patients with CRS and controls.

In contrast, in the study by Boase et al.,[12] fungi were found in only a small proportion of patients with CRS ($n = 35$) and was completely absent in patients without CRS ($n = 6$). It was rarely detected in patients with CRSwNP and not found in patients with CRS without nasal polyps. Culture, PCR, and fluorescence in situ hybridization

Table 3. Literature summary of molecular studies comparing sinus mycology of CRS vs non-CRS patients

Study	Study Population	Outcome Measures	Methods of Microbial Analyses	Results	Conclusion
Cleland et al.,[56] (2014)	23 CRS, 11 non-CRS; middle meatus or anterior ethmoid swabs taken during ESS, 6 and 12 wk postop	Fungal prevalence, richness, abundance	*18S* rDNA sequencing	No ss difference in richness or overall prevalence between the 2 groups; 207 genera identified; *Malassezia* most abundant; *Scutellospora* was the only genus with an ss difference in prevalence (36% control vs 5% CRS); ss ↓ in richness after surgery	No significant differences in fungal microbiome of pts with CRS vs pts without CRS
Aurora et al., [46] 2013	30 CRS, 12 non-CRS: middle meatal lavage at the beginning of ESS or endonasal surgery (septoplasty, pituitary, orbit)	Fungal load, speciation	*18S* rRNA sequencing	Quantitative ↑ and greater diversity in CRS (132 sp) vs healthy pts (106 sp); 17.5% overlap; qualitatively similar fungal microbiome composition with *Cryptococcus neoformans* most dominant sp in both CRS and controls	CRS stems from immune hyperresponsiveness to commensal microbes
Boase et al.,[12] 2013	38 CRS, 6 non-CRS; ethmoid mucosa harvested in ESS (CRS) or skull base surgery (control)	Fungal prevalence, speciation	Culture, FISH, Ibis biosensor - PCR with mass spectrometry	Fungus rarely detected in pts with CRSwNP, not detected in CRSsNP or controls; *Aspergillus fumigatus* (3) and *Bipolaris papendorfii* (1) were the only 2 fungal sp identified by Ibis biosensor	Fungi may play a role in the subset of pts with CRSwNP
Murr et al., [54] 2012	73 CRS, 16 non-CRS; middle meatal swabs	Fungal prevalence, abundance	q-PCR	No ss difference in presence or abundance of fungi between the 2 groups; but 7 fungi were found at high concentrations in 16 pts with CRS	Disproportionately high fungal concentration in subset of pts with CRS indicate fungi may thrive in susceptible pts

Table 3. **Continued**

Study	Study Population	Outcome Measures	Methods of Microbial Analyses	Results	Conclusion
Murr *et al.,* [53] 2006	32 CRS, 32 controls; middle meatal swabs	Fungal prevalence, distribution, speciation	q-PCR	Fungal recovery rate 46% for both groups; no ss difference in types or distribution of fungal sp; *Cladosporium cladosporioides* and *Aureobasidium pullulans* were most common	CRS not ascribed to the presence or type of particular fungus
Scheuller *et al.,* [52] (2004)	19 CRS, 19 controls; middle meatal brushings	Fungal load, prevalence	q-PCR	No difference in the presence or quantity of fungal DNA between the 2 groups	Presence or quantity of fungus did not explain pathogenicity in pts with CRS

$\downarrow$ = decrease; $\uparrow$ = increase; CRS = chronic rhinosinusitis; ESS = endoscopic sinus surgery; postop = postoperative; rDNA = ribosomal DNA; ss = statistically significant; pts = patients; rRNA: ribosomal RNA; sp: species; FISH = fluorescence in situ hybridization; PCR = polymerase chain reaction; CRSwNP = chronic rhinosinusitis with nasal polyposis; CRSsNP = chronic rhinosinusitis without nasal polyposis; q-PCR = quantitative polymerase chain reaction.

techniques were used, and all three methods exhibited similar sensitivities. Only two fungal species were identified: *Aspergillus fumigatus* (3) and *Bipolaris papendorfii* (1) by the Ibis biosensor. Culture was able to detect two of the *Aspergillus* cases, and an additional two patients with *Penicillium chrysogenum* and *Trichosporon*, respectively. Fluorescence in situ hybridization results were positive in three patients with *Aspergillus,* the patient with *Penicillium chrysogenum*, and two other patients.[12]

Mechanism of Disease

Although molecular diagnostics have clearly demonstrated a rich and diverse sinus microbiome, the roles that such native microorganisms play in promoting healthy versus disease status remain uncertain. Although recent studies report somewhat conflicting results, it seems apparent that CRS is characterized by significant disruption of the resident microbial community, with reduced richness, alterations in composition, and skewed abundances of indigenous microbes.[43–45] Because of such findings, views on the pathogenesis of CRS have evolved beyond that of a disease state that arises from infection by specific pathogens into a complex condition associated with disturbances of the baseline microbiome.

Microbes interact in mutualistic and antagonistic ways with each other and their host. These interactions include nutrient consumption, secretion of antimicrobial factors, and attachment site competition.[12] Any factor (*e.g.,* viral infection) that perturbs this delicate ecosystem may disrupt immune homeostasis and create an environment conducive to CRS development.[12] Although it remains unclear what the clinical implications of reduced biodiversity in sinus microbiota are and whether such loss enhances susceptibility to infection, a similar occurrence has been seen in the gastrointestinal tract, as in *Clostridium difficile* infections, secondary to oral antibiotic use.[57] Gut commensals also directly impact the adaptive immune system, regulating the balance of T-helper (Th) and T-regulatory cells.[58] Loss of native gut microbiota is believed to incite aberrant host responses, potentially

directly resulting in development of inflammatory bowel disease.[59–61] Likewise, in the lower airways, perturbations in the lung microbiome have been found to increase disease severity in pulmonary disorders (*e.g.,* cystic fibrosis).[62,63] Diminished biodiversity, greater bacterial burden, and a predominance of particular airway microbiota (*e.g.,* Proteobacteria) have been observed with chronic asthma.[64] Bacterial colonization and microbiome changes have also correlated with accelerated loss of lung function and more frequent exacerbations in patients with chronic obstructive pulmonary disease.[65] Following the unified airway hypothesis (in which the entire respiratory system is considered as a single functional unit), it is plausible that a similar phenomenon may also occur in the sinus cavities, with detrimental shifts in microbial balance contributing to the onset, progression, or refractory nature of CRS.[66] However, it should be noted that the reduced biodiversity evident in CRS may also represent an adverse effect of medical therapy.

Multiple mechanisms of disease that stem from disturbances of the resident microbiome have been postulated. The mere presence of a known pathogen in a given niche does not necessarily denote pathogenicity. In the largest survey of sinuses of control subjects, opportunistic pathogens typically associated with CRS were detected in the middle meati of non-CRS patients, albeit at low abundance.[7] This finding supports the concept that diversity in the healthy state may be protective and that disruption of the baseline microbiome (*i.e.,* dysbiosis) may be a critical requirement for the onset of disease. This was illustrated in the study by Abreu *et al.*[43] who examined the effects of *C. tuberculostearicum* in a murine model. Although historically perceived as innocuous skin microbiota, the addition of *C. tuberculostearicum* in the presence of a replete sinus microbiome resulted in a modest increase of goblet cells and mucus hypersecretion. However, the introduction of *C. tuberculostearicum* in the absence of indigenous microbiota led to profound goblet cell hyperplasia, which exhibited amplified pathogenic effects in a depleted microbial environment.[43] Although mucus hypersecretion and goblet cell hyperplasia are non-

specific responses, the experiment, nonetheless, indicated that an intact microbiome appears to be pivotal in dictating behavior of potential pathogens ("pathobionts") and modulating their effects on sinus epithelium.[43]

Conversely, certain commensals have been shown to exert protective effects on sinus mucosa, which contribute to host defense. In the study by Abreu et al.[43] co-instillation of *L. sakei* with *C. tuberculostearicum* negated the goblet cell hyperplasia and mucin hypersecretion induced by the latter, even in the context of a depleted microbiome. The mechanism by which such bacteria provide mucosal protection is still under investigation. A species-rich microbiome may resist surface colonization of potential pathogens and restrict fluctuations of core microbiota. It has also been theorized that products (*i.e.,* bacteriocin, lactic acid) elaborated by these probiotics may impede pathogen overgrowth through competitive inhibition. *P. acnes*, which has been identified in 80% of control patients, secretes bacteriocin, which not only has antibacterial and antifungal properties but also modulates innate immune responses to infection.[67] This lends credence to the hypothesis that patients who possess a richer, denser baseline sinonasal microbiome may be less prone to infection.

As in the gut, perturbations of the sinus microbiome may also impact host inflammatory responses in CRS. In the sinus cavities, a multitude of intrinsic and adaptive immune responses participate in host defense.[68] The mucociliary blanket continuously ushers potential pathogens out of the sinonasal tract and contains a broad spectrum of antimicrobial substances, including surfactants, defensins, enzymes protease inhibitors, *etc*. Synthesis of such innate immune effector molecules can be stimulated by activation of toll-like receptors expressed on dendritic and epithelial cells. Dysregulation of innate immune mechanisms has been postulated to contribute to the persistent inflammation seen in CRS. The type and severity of CRS has also been shown to be governed by Th1 and Th2 immunogenic pathways, with predominance of the latter found to be associated with nasal polyposis.

Diminished bacterial diversity may promote initiation and sustenance of sinus inflammation through increased pathogen susceptibility or through direct effect on mucosal immune homeostasis. Local inflammation, in turn, can compromise epithelial barriers and further facilitate colonization (Fig. 2).[69] Innate immunity, acquired immunity, mucosal integrity, wound healing, and other host-microbial interac-

tions may all be disrupted from microbial imbalances. Thus, the resident microbiome may not only be critical for pathogen exclusion but also serves as a disease modifier through its regulatory effects on the host immune system.[70] Consequently, preserving the homeostasis of the resident microbial community may be integral to maintaining general health status and preventing infection of the sinuses.

Factors that Affect the Sinus Microbiome

Myriad factors have been shown to impact constituents of the sinus microbiome, which makes it extremely challenging to formulate universal protocols for CRS research and therapy. Beyond a significant intersubject variation, age and smoking affect the composition and distribution of microbial species, respectively.[45] Frequent courses of antimicrobial agents may also disrupt the precarious equanimity of the microbiome, leading to selection of organisms outside the coverage spectrum and possible superinfections.[71] In a study by Liu et al.,[71] sinus microbiota of six patients with CRS were compared before and after administration of maximal medical treatment. All the patients had active mucosal inflammation and previous antrostomies, allowing maxillary swabs to be collected before and after therapy, which were subsequently analyzed by using *16S* rRNA sequencing. Before treatment, a wide spectrum of sinus microbiota was identified; after treatment, no uniform microbial profile emerged. Responses to therapy were highly divergent, with shifts in microbiota composition varying from subject to subject, despite similar clinical outcomes. However, the patients were found to be colonized more frequently with taxa less sensitive to prescribed antibiotics. Significant diminution in bacterial diversity and evenness were also observed consistently after medical therapy. It is unclear, though, if effects were secondary to antibiotics specifically or from resolution of disease.[71]

In a cross-sectional study by Feazel et al.,[44] antibiotic use, asthma, and previous surgery were all demonstrated to impact sinus microbial ecology. Antibiotics and asthma correlated with significant reductions in bacterial diversity and increased *S. aureus* abundance, whereas previous surgery was associated with decreased richness.[44] Such observations support the hypothesis that prolonged, repetitive antibiotic administration can diminish the complexity of a microbial community and lead to emergence of a few dominant bacteria. Consequently, it is possible that the reduced biodiversity evident in
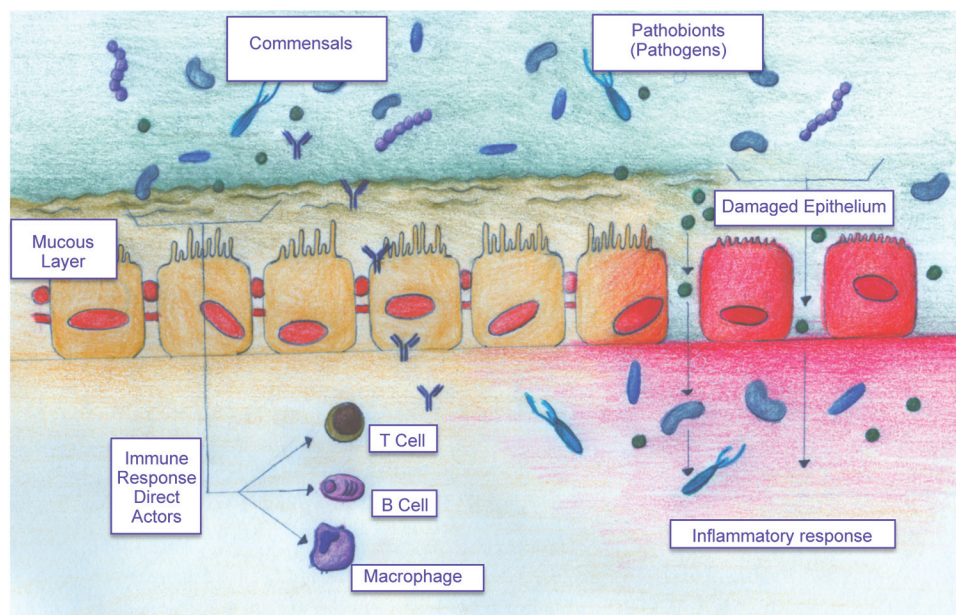

Figure 2. Overview of microbiome-host interactions hypothesized to degrade mucosal barrier function and incite inflammation in chronic rhinosinusitis.

patients with CRS may be due, in part, to repeated antibiotic therapy. Further investigation is needed to determine if medications, surgery, and/or the natural course of time are responsible for such changes in microbial composition and diversity.

Multiple reports revealed similar effects on fungal populations after sinus surgery.[54,56] In a study by Murr et al.,[54] postoperative brush samplings from four patients with CRS revealed much lower concentrations of fungi by qPCR than had been present before surgery. However, new fungi that were not detected before surgery also appeared, which made it difficult to draw any definitive conclusions.[54] Likewise, in a study by Cleland et al.,[56] endoscopic sinus surgery was shown to significantly reduce fungal richness and diversity. The prevalence of *Fusarium* and *Neocosmospora* also diminished, with decreased abundance of the latter as well.[56] Improved ciliary function and saline solution irrigation distribution after surgery may partially account for this observation because inhaled fungi are more efficiently cleared.[56] In addition, it is likely that surgery alters physicochemical properties within the sinus microenvironment (*e.g.,* humidity, partial pressure of atmospheric gases, pH). Thus, such factors taken in aggregate may represent the primary drivers of microbiome shifts seen in CRS as opposed to the actual disease *per se.*

Implications of Antibiotic versus Probiotic Therapy

Given increasing antibiotic resistance and challenges with new drug development, new treatment modalities are urgently needed.[72] The collective findings from microbiome studies have broad-reaching implications for future pharmaceutical therapy of CRS. Antibiotics have traditionally been the primary medical treatment for CRS, although evidence to support their efficacy is still lacking. More than 90% of otolaryngologists continue to use prolonged systemic antibiotics as "maximal medical therapy" before surgical intervention.[73] However, given our better understanding of the potential role of the sinus microbiome in promoting sinus health, such nonselective medications may disrupt the dynamics of the entire bacterial community. Rather than prescribing antibiotics to eradicate pathogenic bacteria, timely probiotic or prebiotic (nonviable food components that modulate microbiota to benefit the host) supplementation may emerge as a new mode of therapy to competitively inhibit pathogens or to facilitate sinus recolonization with desirable commensals, in a similar vein to gastrointestinal disease.[74] Such probiotics may be administered either orally to mediate systemic immunity through the gut or topically to modulate local immune responses.

Probiotics involve administration of live microbes in sufficient amounts to directly confer beneficial physiologic effects on the host.[75] The mechanisms by which such microorganisms provide protection against invading pathogens are multifold and generally involve modifications of host immunity through the gut ecosystem. Commensals have been shown to reinforce the integrity of the mucosal barrier, induce secretion of antimicrobial peptides, and competitively inhibit bacterial adherence and colonization.[75] Systemically, ingestion of probiotics has been reported to enhance production of γ-interferon, and interleukin-2 lymphocyte responses, and to shift the balance of Th cells toward an increased Th1:Th2 ratio.[76–78] Because allergic diseases, asthma, and CRSwNP have been associated with Th2 skewed responses, such effects could help protect against CRS as well. A decrease in T-regulatory cells and the presence of immunoglobulin E for the *S. aureus* superantigen have also been reported in CRSwNP.[79] Oral intake of probiotic microorganisms may preferentially augment Th1 and T-regulatory reactions, which, in turn, could help counterbalance the excess Th2 activity characteristic of these conditions.[80]

The use of probiotics for the prevention and management of diseases of the gastrointestinal tract has been studied extensively, with some encouraging results.[81] More recently, administration of probiotics for use in ailments outside of the gut has also been investigated. Probiotics have been effective in the treatment of atopic dermatitis and pollen allergy.[82,83] Patients with allergic rhinitis who were given *Lactobacillus paracasei*-33 reported improved quality of life versus con-

trols.[84] *Streptococcus salivarius*, a nonpathogenic oral microbe that produces bacteriocin, diminishes colonization of bacteria involved in upper respiratory tract infections and has been used as a probiotic. Oral formulations of *S. salivarius* K12 are already commercially available.[85] Such therapies may be extrapolated to the sinuses as well, with commensal organisms used as probiotics to inhibit pathogen colonization and overgrowth. Cleland et al.[86] investigated the effects of intranasal *S. epidermidis* on CRS by using a mouse model. Mice co-inoculated with both *S. epidermidis* and *S. aureus* had significantly lower goblet cell counts versus those who received *S. aureus* alone. Likewise, Abreu et al.[43] showed that topical *L. sakei* protected against *C. tuberculostearicum* in a murine model of CRS. Uehara et al.[87] also demonstrated that nasal administration of *Corynebacterium* species was able to eradicate *S. aureus* in 71% of patients. It should be noted that, in the aforementioned studies, none of the bacteria were probiotics, *per se,* in that they did not improve health when administered alone. Instead, these studies demonstrated that, when these organisms were coadministered with pathogens, a reduction of induced disease was observed. However, in a recent double-blind, randomized controlled trial of 77 patients with CRS, no significant differences in the 20-question Sino-Nasal Outcome Test scores or symptom frequency was observed between those who received the oral probiotic *Lactobacillus rhamnosus* R0111 ($n = 39$) versus placebo ($n = 38$) for 4 weeks.[88] It should be noted that both patients with CRS without nasal polyps and patients with CRSwNP were included and analyzed together, so it is unclear if probiotics had varying effects on different CRS subtypes.

The wide spectrum of microbial profiles in CRS, lack of uniform posttherapy microbiota, and highly individualized responses to medical treatment make development of generalized therapeutic protocols for CRS difficult.[71] Different CRS subtypes may also harbor divergent sinus microbiomes that contribute to variable responses to treatment, which highlights the potential futility of seeking a universally applicable antimicrobial regimen. An initial challenge in the institution of probiotic therapy for CRS is selection of the appropriate microbe(s) for a particular disease subtype because immunoregulatory effects will likely be strain and concentration dependent. Extensive study is then needed to identify virulence determinants and to investigate any metabolic, enzymic, or hemolytic activity potentially harmful to the host. In addition, a topical probiotic must also adhere to sinonasal tissue and not have any deleterious effects on other resident microbiota. Despite these challenges, such innovative research opens the door to a plethora of novel, ecologically based therapies designed to cultivate a natural microbial community. If proven effective, then the coming years may witness a paradigm shift in management of CRS away from attempts to eradicate bacteria toward restoration of native sinus ecology.

Future Directions

Although we recognize that complex microbial communities are present in the sinuses, further investigation is necessary to better understand how fluctuations in the baseline microbiome contribute to disease pathogenesis. Understanding the dynamics of sinus colonization likely will help clarify how microorganisms may precipitate CRS. Additional study is needed to characterize the virulence profile of known and newly identified microbes, their role in the pathophysiology of CRS, and relationship to disease severity. In addition, extensive research is required to elucidate the ecologic and environmental pressures that influence the sinus microbiome and how specific microbial species and/or strains may influence health or disease status. Better delineation of the complex dynamics between resident microbiota and the host immune system is also critical to guiding future medical therapy.

As our understanding of the intricate dynamics of microbial communities expands, treatment of CRS may be customized according to pathophenotype. Specific probiotic and prebiotic therapies may be tailored to treat imbalances unique to a patient's individual dysfunc-

tional microbiome. Because there may be homeostatic fluctuations that are unique from one patient to another, large cohorts with longitudinal sampling will be needed to accommodate these interpersonal variations. Strategic, rational, personalized manipulations of bacterial communities may emerge as a more sophisticated approach to management of recalcitrant CRS. Therefore, increased knowledge of the resident microbiome can help shape future treatment approaches to CRS, particularly those with refractory disease.

CONCLUSION

The advent of culture-independent molecular diagnostics has led to a greater appreciation of the intricate microbial ecology of the sinus microbiome. Complex polymicrobial communities reside within the sinus cavities of both patients without CRS and patients with CRS, with the latter often characterized by reduced diversity compared with controls. Dysbiosis of endogenous microbiota may influence mucosal health and disease severity, with various species exerting pathogenic or protective effects, depending on permissive conditions. Probiotic formulations provide an exciting frontier of topical and systemic therapies geared toward strategic manipulation of host bioburden to promote immune homeostasis. A deeper understanding of the host-microbiome relationship may lead to evolution of novel diagnostic, prognostic, and treatment paradigms, targeting restoration of native sinus ecology and cultivation of optimal microbial consortia.

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