Association of the sinonasal bacterial microbiome with clinical outcomes in chronic rhinosinusitis: a systematic review

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Background: The association between sinonasal microbiome and clinical outcomes of patients with chronic rhinosinusitis (CRS) is unclear. We performed a systematic review of prior studies evaluating the CRS microbiome in relation to clinical outcomes.

Methods: Computerized searches of PubMed/Medline, Cochrane, and EMBASE were updated through October 2019 revealing a total of 9 studies including 244 CRS patients. A systematic review of the literature was performed, including data extraction focusing on sample region, sequencing platforms, predominant organisms, and outcomes measures.

Results: Nine criterion-meeting studies included 244 CRS patients, with varied results. Eight studies used 16s–ribosomal RNA (16s-rRNA) gene sequencing to assess the sinonasal microbiome and 1 used 16s-rRNA PhyloChip analysis. Seven studies used Sino-Nasal Outcome Test scores, 1 applied another CRS symptom metric, and 1 used need for additional procedures/antibiotics as the primary clinical outcome. Three studies suggest that baseline abundance of phylum *Actinobacteria* (specifically genus *Corynebacterium*) was predictive of better surgical outcome. One study found *C. tuberculostearicum* was positively correlated with symptom severity. Another study revealed genus *Escherichia* was overrepresented in CRS and had positive correlation with increased symptom scores. In addition, 1 study identified *Acinetobacter johnsonii* to be associated with improvement in symptom scores while supporting *Pseudomonas aeruginosa* as having a negative impact on quality of life.

Conclusion: Microbiome data are varied in their association with clinical outcomes of CRS patients. Further research is required to identify if predominance of certain microbes within the microbiome is predictive of CRS patients' outcomes. © 2020 The Authors. International Forum of Allergy & Rhinology published by Wiley Periodicals, Inc. on behalf of American Academy of Otolaryngic Allergy and American Rhinologic Society.

Chronic rhinosinusitis (CRS) is an inflammatory disorder of the paranasal sinuses that is defined based on the presence of characteristic sinonasal symptoms such as nasal and extranasal symptoms for at least 12 weeks.[1-4] CRS-associated symptoms are differentially associated with the downstream disease consequences of decreased quality of life (QOL) and decreased productivity.[5-7] A wide range of hypotheses have been described with regard to

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the pathogenesis of CRS, with both environmental and innate host defense mechanisms implicated in the etiology of the disease.[8,9] Local environmental and microbial factors within the paranasal sinuses that could drive or modulate the CRS disease process include presence of biofilms, *Staphylococcal* superantigens, fungi, and more recently, dysbiosis of the microbiome.[10]

The concept of dysbiosis is a complex entity but has been defined as microbial imbalance, change in the microbiota, or misrecognition of normal microbiota that may contribute to disease.[11,12] Dysbiosis has been implicated in various inflammatory diseases including CRS and respiratory disease as well as inflammatory bowel disease, rheumatoid arthritis, and psoriasis.[11-13] Specifically, in the CRS population, colonization by pathogenic organisms, and resultant microbial imbalance have been cited as triggers of a dysfunctional and chronic immune response.[14] On the other hand, dysbiosis may be a result of dysfunctional immune barriers and resultant inflammation that establish an environment amenable to overgrowth of pathogenic bacteria that then promote a state of dysbiosis.[14]

The concern for microbiome dysbiosis has prompted extensive study to elucidate specific components of the sinonasal microbiome. In healthy sinuses, the microflora includes aerobic and anaerobic bacteria in addition to fungi, including but not limited to: *Staphylococcus*, *Streptococcus*, *Haemophilus*, *Propionibacterium*, *Corynebacterium*, *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Peptostreptococcus*, *Candida*, *Aspergillus*, *Streptomyces*, *Penicillium*, *Nocardia*, and *Mucor*.[15-17] Studies have illustrated that the microbiomes of patients with CRS are qualitatively similar, but with alterations in population diversity. Multiple studies have had contrasting results with regard to microbial diversity in CRS compared to control subjects.[13,18-23] Overall, it has been proposed that in CRS, the microbiome is disrupted, with pathogenic bacteria overtaking commensal bacteria, which may lead to development of chronic sinonasal inflammation and associated sinonasal symptoms.[10,24]

Despite the extensive study of the composition of the sinus microbiome, there is a paucity of studies examining the clinical application of these findings. Various QOL measures including the Sino-Nasal Outcome Test (of which the 22-item version [SNOT-22] is most frequently used currently) have been validated to accurately reflect the burden of CRS symptomatology experienced by patients.[25,26] To have a clinically relevant bearing on patient care, any pathophysiologic mechanisms of disease would ideally translate to clinical outcomes. At present it is unclear how the sinonasal microbiome in CRS translates to clinical outcomes; eg, in relation to medical and surgical management of CRS.[19,20,27] In this systematic review, we seek to synthesize the current literature on the sinonasal microbiome in CRS and how it may affect clinical outcomes related to QOL. We believe that further exploration of this relationship may serve to propel the field from simply defining the
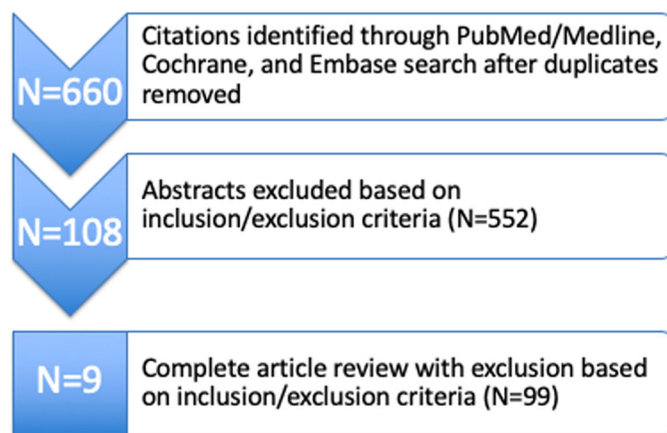

FIGURE 1. Flow diagram of citation sources.

microbiome to utilizing its composition in a way that will affect patient care.

Methods

Literature search
Computerized PubMed/Medline, Cochrane, and EMBASE searches ranging from 1965 through October 2019 were performed to identify all relevant manuscripts. Articles mapping to the exploded medical subject heading rhinosinusitis or containing sinusitis were combined into one group. Medical subject headings bacteria, microbiome, 16s–ribosomal RNA (16s-rRNA) gene sequencing, and microarray were exploded and the manuscripts were collected into a second group. Medical subject headings outcomes, QOL, 20-item Sino-Nasal Outcome Test (SNOT-20), SNOT-22, Chronic Sinusitis Survey (CSS), or Rhinosinusitis Disability Index (RSDI) score were combined into a third group. The 3 groups were then cross-referenced. Non-human studies and studies not in English were excluded. The initial combined searches yielded 660 references. Titles and abstracts were then evaluated according to the inclusion/exclusion criteria described in the next section. Two individual reviewers (J.C.W. and C.A.M.) performed searches independently, blinded to each other's results, with the search results additionally reviewed by the senior author (A.R.S.). Titles and abstracts for all identified studies were reviewed, and 9 articles were ultimately included in the analysis (Fig. 1).

Inclusion and exclusion criteria
Articles identified by the search strategy in the previous section were evaluated to meet these inclusion criteria: (1) studies that included CRS; (2) evaluation of microbiome via 16s-rRNA gene sequencing and/or microarray; and (3) QOL assessment or outcomes assessment including use of SNOT-20, SNOT-22, CSS, or RSDI scores.

Articles were excluded if they: (1) assessed biofilm development but not microbiome, (2) addressed acute

rhinosinusitis rather than CRS, (3) had no QOL outcomes assessment, (4) did not utilize 16s-rRNA gene sequencing or microarray, and (5) they were abstracts without subsequent full manuscript publication.

Data extraction

Extracted data included: (1) study design; (2) number of subjects; (3) location of microbial swabs; (4) sample type; (5) microbial phylum, genus, and/or species identified; (6) richness, diversity, prevalence, and mean relative abundance (MRA); and (7) outcomes measures.

Results

Study populations

The 9 publications represented 244 CRS patients (range, 10 to 56 per study) and 67 control patients (range, 0 to 26 per study). The patient, microbiome results, and QOL results are recorded in Table 1. The patient populations studied were from varying regions and climates including: Sydney and Adelaide, Australia; Mangalore, India; Auckland, New Zealand; and Aurora and Boulder, CO, Scottsdale, AZ, and San Francisco, CA, in the United States (Table 2).

Methodology

Table 2 summarizes some of the varying technical aspects utilized in each study to allow for better analysis and comparison between publications. The third column of Table 2 lists the analysis platform used for each study. Illumina MiSeq (MiSeq; Illumina, San Diego, CA) and Roche 454 pyrosequencing (454 Life Sciences, Roche Applied Sciences, Branford, CT) were the most commonly used platforms. One study used PhyloChip as an alternative method of assessing the microbiome using 16s-rRNA. The fourth and fifth columns of Table 2 lists the primers used by each study for polymerase chain reaction (PCR) amplification and 16s-rRNA gene regions for bacterial identification. Each study used swabs as the means of sampling with some variability in the site used, while Karunasagar et al.[28] and Joss et al.[29] also included mucosal biopsies. Most studies included the middle meatus (MM) or anterior ethmoids as the sampling site, while Abreu et al.[13] collected from the maxillary sinus. Joss et al.[29] and Copeland et al.[22] collected swabs from all sinuses as well as from the nostrils. Lal et al.[21] was the only group to include swab samples taken from the inferior meatus (IM).

Diversity analyses of microbiota in CRS

Important to a discussion on microbiome is the shared language and measures of microbial populations across these studies. Diversity of a sample is a measure of the bacterial richness and evenness, often expressed using a metric known as the Shannon index. Richness in this context refers to the number of unique genera or taxa per sample; evenness represents the difference in abundance across those genera. Standard laboratory culture-based techniques likely underestimate the diversity of species within the microbiome, with reported 1% to 10% of known microorganisms capable of being cultured; therefore, sequencing-based techniques afford a more complete assessment of the predominant microbes within a given population, independent of culturing.[30]

Our included studies showed differing results when comparing measures of richness, evenness, and diversity between CRS patients and healthy controls. Three of the included studies suggested that the healthy sinonasal microbiome is more rich and diverse compared to CRS patients. Abreu et al.[13] showed that the CRS microbiome is less rich, less even, and shows less diversity. Ramakrishnan et al.[31] showed that "optimal" surgical outcomes in CRS patients were associated with increased richness, evenness, and complexity of their preoperative baseline microbiota. Similarly, Lal et al.[21] demonstrated that diversity of MM samples is lower in CRS compared to control patients. They also showed reduced diversity in the MM compared to the inferior middle meatus in CRS patients where there is no sinus drainage pathway, and is therefore less likely to be involved in the CRS disease process.[21]

Contrary to the aforementioned studies, three other included studies did not support reduced microbiome diversity in CRS. Cleland et al.[32] did not find any significant difference in richness or diversity between CRS or control patients. They also showed decreased diversity in CRS patients after endoscopic sinus surgery—when symptoms are expected to be improved—compared to the preoperative microbiota. Copeland et al.[22] showed less diversity in controls and in CRS with polyps patients compared to CRS patients without polyps. Although multiple studies suggest a possible trend of changes in diversity being related to CRS pathogenesis, larger trials are needed to confirm a consistent pattern.

Sinonasal microbiome in CRS and controls

The most significant communities found repeatedly across multiple studies included members of the phyla Firmicutes, Actinobacteria, and Proteobacteria. These phyla include many organisms commonly associated with CRS. Firmicutes includes *Staphylococcus* and *Streptococcus* species, Actinobacteria includes *Corynebacterium* and *Propionibacterium*, and Proteobacteria includes *Moraxella* and *Haemophilus* species.

The genus *Corynebacterium* emerged as an important population across studies, although with inconsistent results. Joss et al.[29] found *Corynebacterium* to be the most significant community in 13 of 19 CRS patients. Cleland et al.[32] found two *Corynebacterium* species to be more prevalent in control patients compared to CRS patients: *Corynebacterium confusum* (73% control vs 26% CRS, $p = 0.023$) and *Corynebacterium fastidiosum* (64% control vs 22% CRS, $p = 0.026$). Jain et al.[27] demonstrated *Corynebacterium* or *Staphylococcus* as the

TABLE 1. Studies describing the CRS microbiome in the context of QOL

Study	Design	Sample type	Microbiome findings	QOL correlation
Karunasagar et al.[28] (2018)	20 CRS and no control patients. Cross-sectional study comparing molecular results in culture-negative CRS	1. Swab of discharge from the MM 2. Mucosal biopsies from the "middle meatal region"	Bacteria detected in all culture-negative cases. Staphylococcus, Enterobacter, Pseudomonas were dominant groups.	Patients with severe SNOT-22 scores (>80), 5 with PCR for Staphylococcus species, 2 with Pseudomonas, 1 with Klebsiella, 1 with Escherichia coli.
Joss et al.[29] (2016)	19 CRS and no control patients. Cross-sectional study comparing molecular and culture methods across sample locations	1. Swab from nostril, MM, maxillary sinus, ethmoid sinus, sphenoid sinus, and frontal sinus. 2. Biopsy from each sinus bilaterally	Corynebacterium most significant community in 13/19 patients. Staphylococcus high in most patients. Cultured organisms often had high sequence count. Staphylococcus would culture even when sequence counts low. Relative abundance variable between sinuses.	SNOT-22 scores with mean of 53. Patient with highest score 95 had 29% of their sequence counts show Escherichia and 53% Corynebacterium.
Ramakrishnan et al.[31] (2015)	56 CRS and 26 control patients. Longitudinal study comparing molecular methods among disease and control patients	1. Swabs "endoscopically guided to the ethmoid region, a neighboring sinus, or both when purulence was present."	CRS with asthma carried lower abundance Prevotella, Fusobacterium, and Campylobacter, but higher Staphylococcus, Acinetobacter, and Ralstonia compared to CRS without asthma. Purulence associated with expansion of obligate anaerobes Fusobacteria and Bacteroidetes. No anaerobes isolated from nonpurulent secretions	QOL based on need for revision procedures, need for additional antibiotics or steroids postoperatively. Patients with optimal outcomes showed enrichment of Actinobacteria, including Corynebacterium species, particularly Corynebacterium tuberculostearicum.
Cleland et al.[32] (2016)	23 CRS and 11 control patients. Longitudinal study	1. Swabs from MM and/or anterior ethmoid, repeated for CRS patients at 6 and 12 weeks postoperatively	Acinetobacter johnsonii and Corynebacterium confusum more prevalent in control population. No species statistically more prevalent in CRS. Staphylococcus aureus significantly increased relative abundance in CRS vs controls.	A. johnsonii significant improving effect for both VAS and SNOT-22; associated with improvement in SNOT-22 and VAS postoperatively. Pseudomonas aeruginosa significant negative effect on SNOT-22.
Jain et al.[27] (2018)	20 CRS patients receiving doxycycline or prednisone compared to 6 untreated CRS patients. Longitudinal study	1. MM swabbed at initial visit and again 7 days later	Bacterial profiles dominated by Corynebacterium and Staphylococcus in all 26 patients. Treatment with doxycycline or prednisone had variable and unpredictable changes. Average relative abundance of Propionibacterium increased after doxycycline and Corynebacterium decreased after prednisone.	No bacterial taxa significantly correlated with changes in SNOT-22 scores after treatment.
Jain et al.[20] (2017)	23 CRS and no control patients. Longitudinal study	1. MM swabs intraoperatively and ~120 days postoperatively	Richness increased after surgery for most patients. No significant postoperative changes in diversity. Samples dominated by Firmicutes (Staphylococcus, Streptococcus), Proteobacteria (Haemophilus, Moraxella), Actinobacteria (Corynebacterium). Few changes at phylum level postoperatively, but at genus level there was increases in Staphylococcus consistently after surgery while most other genera had reductions in relative abundance, including Streptococcus. 179 different taxa composed <1% of abundance.	5-symptom CRS scores used. Average improvement after surgery. Statistically significant changes in Staphylococcus and Streptococcus postoperatively did not correlate with symptom score changes. Finegoldia increased in patients with worse postoperative symptom scores, but was associated with a reduction in average abundance in most patients postoperatively.

(Continued)

TABLE 1. Continued

Study	Design	Sample type	Microbiome findings	QOL correlation
Copeland et al.[22] (2018)	21 CRS and 12 control patients Cross-sectional study	1. Swabs from all 8 sinuses in CRS patients. 1 to 5 swabs in healthy subjects. Left and right MM and nostrils also swabbed.	Diversity similar among sinuses. Largest variation was between individuals. Primary species included Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes. Proteobacteria significantly more abundant in CRS. At genus level only *Escherichia* was significantly different with higher abundance in CRS. Actinobacteria significantly more abundant in controls. Disease positively correlated with several facultative anaerobes including *Finegoldia, Anaerococcus, Peptoniphilus,* and *Lactobacillus.*	18 OTUs positively correlated with SNOT-22 scores, 9 of which were *Escherichia.* One OTU negatively correlated with SNOT-22 - *Corynebacterium*
Lal et al.[21] (2017)	46 CRS, 11 AR, and 8 controls Cross-sectional study	1. Swabs from MM and IM in office	Bacterial diversity significantly reduced in the MM compared to the IM in CRSsNP patients. MM diversity lower in CRSsNP patients compared to healthy or AR subjects. No changes in diversity in the IM across all groups. Most abundant groups included *Corynebacterium, Staphylococcus, Propionibacterium, Streptococcus,* and *Alloiococcus*	SNOT-22: mean 28.2 in healthy subjects; 30.6 in AR. 50.82 in CRSsNP, 48.43 in CRSwNP. Linear regression analysis based on SNOT-22 scores did not reveal any statistically significant differences for Shannon (richness and evenness) or Faith's phylogenetic diversity.
Abreu et al.[13] (2012)	10 CRS and 10 control patients Cross-sectional with secondary mouse model given *C. tuberculostearicum.*	1. Swabs of lateral, central, and medial maxillary sinus	CRS patients with less richness, evenness, and Shannon diversity compared to controls. 1482 taxa detected in significantly lower relative abundance in CRS patients. Most significant reductions in relative abundance among Lactobacillales. *Corynebacterium tuberculostearicum* exhibited significant increase in abundance in CRS patients.	228 groups correlated with lower SNOT-20 scores. Among these included Lactobacillaceae, Enterococcaceae, Aerococcaceae, and Streptococcaceae. Two taxa positively correlated with increased symptom severity, both *Corynebacterium, C. tuberculostearicum* most significantly.

AR = allergic rhinitis; CRS = chronic rhinosinusitis; CRSsNP = CRS without nasal polyps; CRSwNP = CRS with nasal polyps; IM = inferior meatus; MM = middle meatus; OTU = operational taxonomic unit; PCR = polymerase chain reaction; QOL = quality of life; SNOT-20 = 20-item Sino-Nasal Outcome Test; SNOT-22 = 22-item Sino-Nasal Outcome Test; VAS = visual analogue scale.

TABLE 2. Sequencing platforms and primers

Study	Location of study	Analysis platform	Primers	16S-rRNA gene region
Karunasagar et al.[28] (2018)	Mangalore, India	ABI Prism 3100 Genetic analyzer	16s: 5′-AAGAGTTTGATCCTGGCTCAG-3′ and 5′-TACGGCTACCTTGTTACGACT-3′; 1503 bp	Not reported
Joss et al.[29] (2016)	Sydney, Australia	Illumina MiSeq	16s forward AATGATACGGCGACCACCGAGATCTACAC (8bp_barcode)TATGGTAATTGTGTGCCAGCMGCCGCGGTAA Reverse CAAGCAGAAGACGGCATACGAGAT(8bp_barcode) AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	V4
Ramakrishnan et al.[31] (2015)	Aurora and Boulder, CO, USA	Pyrosequencing; Roche 454	16s: rRNAgeneV1V3 variable region (approximately 500 bp; primers 27FYM13 and 515R	V1–V3
Cleland et al.[32] (2016)	Adelaide, Australia	Pyrosequencing; Roche 454	16s: 27Fmod (AGRGTTTGATCMTGGCTCAG) and 519Rmodbio (GWATTACCGCGGCKGCTG)	V1–V3
Jain et al.[27] (2018)	Auckland, New Zealand	Illumina MiSeq	16s: 341F and 806R	V3–V4
Jain et al.[20] (2017)	Auckland, New Zealand	Illumina MiSeq	16s: 341F and 806R	V3–V4
Copeland et al.[22] (2018)	Sydney, Australia	Illumina MiSeq	16s: 338F and 806R positions of the *Escherichia coli*	V3–V4
Lal et al.[21] (2017)	Scottsdale, AZ, USA	Illumina MiSeq	16s: S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21	V3–V4
Abreu et al.[13] (2012)	San Francisco, CA, USA	Phylochip	16s: 27F and 1492R universal primers	Not applicable

dominant species in all 26 study patients before treatment. They showed a reduction in average relative abundance of *Corynebacterium* after treatment with prednisone with unknown significance. In another study by Jain et al.,[20] reductions in *Corynebacterium* were associated with worse symptom scores. The species *Corynebacterium tuberculostearicum* was identified in three studies. Abreu et al.[13] showed a significant increase in *C. tuberculostearicum* abundance in CRS patients, while Ramakrishnan et al.[31] identified enrichment of this species as associated with optimum outcomes following endoscopic sinus surgery. Further, Cleland et al.[32] did not support either of these findings, with *C. tuberculostearicum* having an overall MRA <1% with no significant changes in any subjective or objective measures. Last, Copeland et al.[22] noted Actinobacteria as having significantly elevated abundance in controls compared to CRS patients.

Karunasagar et al.[28] did not report the full microbiome data of each patient, but reported on the dominant species isolated by 16s rRNA gene sequencing analysis in CRS patients that were otherwise culture-negative. Bacteria were detected in all culture negative cases. *Staphylococcus*, *Enterobacter*, and *Pseudomonas* were the dominant groups. Ramakrishnan et al.[31] found that swabs of purulence were associated with expansion of obligate anaerobes *Fusobacteria* and *Bacteroidetes*, while no obligate anaerobes were isolated from nonpurulent secretions.

Lal et al.[21] uniquely included the IM as a swab site. They showed that bacterial diversity was significantly reduced in the MM compared to the IM in CRS without nasal polyposis (CRSsNP) patients. The diversity of the MM was also significantly reduced in CRSsNP patients compared to the MM of healthy or allergic rhinitis subjects. There were no changes in diversity among the IM across all patients.[21] Similarly, Abreu et al.[13] showed overall less richness, evenness, and diversity among CRS patients compared to controls.

Finally, Copeland et al.[22] took samples across all 8 sinuses in CRS and control patients. They found that diversity was similar among the sinuses and that the largest variation was between individuals rather than sampling sites. They identified the genus *Escherichia* as having significantly higher abundance in CRS patients.

QOL findings

The studies reported and analyzed QOL outcomes in different ways. The majority used SNOT-20 or SNOT-22 questionnaires. Jain et al.[20] used a 5 symptom score questionnaire, each scored on a 0 to 5 scale in their 2017 article. Ramakrishnan et al.[31] used the need for revision endoscopic sinus surgery or additional antibiotics or oral steroids postoperatively as their outcome measure. Ramakrishnan et al.[31] defined patients as having an optimal outcome if they did not require revision endoscopic sinus surgery, additional antibiotics or additional oral steroids after 6 months of follow-up. They found that patients with optimal outcomes showed enrichment of the phylum Actinobacteria, including *Corynebacterium* species, particularly *C. tuberculostearicum*. Optimal outcomes were associated with patients who had

increased richness, evenness, and complexity of their baseline microbiota.[31]

Cleland et al.[32] identified *Acinetobacter johnsonii* as a species of particular interest. This species was significantly more prevalent in controls compared to CRS patients (82% vs 26%; *p* = 0.0003) and was in fact the most abundant species in the control group (MRA 22% in controls vs 4% in CRS). Prevalence of this species among CRS patients was also associated with decreased SNOT-22 scores (less CRS symptom burden). Consistent with this finding, there was a noted increase in prevalence of this species postoperatively—when symptom burden would be expected to be reduced—that was mirrored by an increase in MRA of *A. johnsonii*. Significant improvement in SNOT-22 and visual analogue scale (VAS) scores persisted after controlling for the beneficial effect of performing surgery. No significant correlations were found between species richness and SNOT-22 scores. The relative abundance of 22 species were associated with changes in SNOT-22 scores, but most of these were present at an MRA <1% and thus would not be expected to have clinical significance; these species were therefore not fully reported in their manuscript. Last, the abundance of *Pseudomonas* was identified as having an association with higher SNOT-22 scores (worse CRS symptom burden).

The included 2018 study by Jain et al.[27] compared stable CRS patients treated with doxycycline, prednisone, or no treatment. Changes in patient sinus microbiota were variable and unpredictable after treatment. Pretreatment and posttreatment symptom scores were not significantly different and had no statistically significant correlation with microbiota changes.

The 2017 study by Jain et al.[20] compared microbiota in CRS patients before and 120 days after endoscopic sinus surgery. CRS QOL outcomes were assessed as the severity of 5 nasal symptoms (nasal obstruction, anterior and posterior rhinorrhea, sinus pain/pressure, and anosmia) experienced by patients. They found that *Staphylococcus* species increased in relative abundance postoperatively and *Streptococcus* species decreased in relative abundance postoperatively. The significant changes in *Staphylococcus* and *Streptococcus* postoperatively did not have any correlation with symptom score changes. They did find that reductions in *Corynebacterium* species were associated with an increase (worsening) in symptom scores.[20]

Copeland et al.[22] and Abreu et al.[13] both found a significant association between the presence of *Corynebacterium* and QOL scores. Copeland et al.[22] included 12 healthy controls and 21 CRS subjects and found only 1 genus, *Corynebacterium*, that correlated with decreasing SNOT-22 scores. Conversely, Abreu et al.[13] identified 2 taxa that were correlated with worsening SNOT-20 scores and both of these were *Corynebacterium* species. Most significantly was *C. tuberculostearicum*, which showed significantly higher abundance in CRS patients compared to controls. Copeland et al.[22] identified

Escherichia as being associated with higher SNOT-22 scores.[22] Abreu et al.[13] identified 228 taxa that were significantly correlated with lower SNOT-20 scores. Most of these were members of Lactobacillaceae, Enterococcaceae, Aerococcaceae, and Streptococcaceae.

Karunasagar et al.[28] and Joss et al.[29] reported SNOT-22 scores for their patients but did not report any further analysis related to the microbiome and the survey results. However, Karunasagar et al.[28] showed that of the 9 patients with severe SNOT-22 scores (>70), 5 had *Staphylococcus* species as their most abundant, 2 had *Pseudomonas*, 1 had *Klebsiella*, and 1 *Escherichia*. Among the CRS patients studied by Joss et al.,[29] the patient with the highest SNOT-22 score of 95 had 29% of their sequence counts show *Escherichia* and 53% *Corynebacterium*. Lal et al.[21] did not find any statistically significant differences after linear regression analysis for SNOT-22 when compared with Shannon or Faith diversity measures. Table 3 summarizes the QOL assessment tool utilized by the study and its association to the microbiome.

Asthma, polyps, and aspirin-associated respiratory disease

Important to consider are possible differences that may exist between various subgroups of CRS such as those with comorbid asthma, aspirin-associated respiratory disease, or nasal polyposis. Of the selected articles, some failed to delineate these subgroups, others controlled for these subgroups, and a few specifically analyzed differences that may exist between these populations.

Two studies that commented on differences in an asthmatic population with CRS compared to a nonasthmatic population found significant differences in bacterial diversity and species abundance.[20,31] Ramakrishnan et al.[31] found that asthmatics with CRS had a lower abundance of *Prevotella*, *Fusobacterium*, and *Campylobacter* species compared with nonasthmatic patients with CRS. On the contrary, *Staphylococcus, Acinetobacter*, and *Ralstonia* had higher abundance in asthmatic patients with CRS. On the other hand, Joss et al.[29] failed to find a difference between asthmatics and nonasthmatics.

In discussion of bacterial differences in patients with CRS with nasal polyposis (CRSwNP) compared to those without nasal polyposis (CRSsNP), the evidence is contradictory. Copeland et al.[22] showed less diversity in CRSwNP, while Lal et al.[21] found that CRSsNP patients exhibited decreased microbial diversity. Multiple authors failed to find a significant difference in bacterial composition between CRSwNP and CRSsNP.[22,29] Also of note, Karunsagar et al.[28] noted that in 2 patients with recurrent polyposis who were culture-negative, *Staphylococcus* was detected in rRNA PCR, highlighting the importance of molecular techniques in this population. On the other hand, other studies have failed to find a difference between asthmatics and nonasthmatics.[29]

TABLE 3. QOL assessment tool utilized by studies and its association to the microbiome

Study	QOL assessment tool	Microbiome results				Association of microbiome with QOL
Karunasagar et al.[28] (2018)	SNOT-22	Reported SNOT-22 scores but did not perform analysis with microbiome data				Not applicable
Joss et al.[29] (2016)	SNOT-22	Reported SNOT-22 scores but did not perform analysis with microbiome data				Not applicable
Ramakrishnan et al.[31] (2015)	QOL based on need for revision procedures, need for additional antibiotics or steroids postoperatively	"Optimal" surgical outcomes in CRS patients	Richness ↑	Evenness ↑		Enrichment of *C. tuberculostearicum* as associated with optimum outcomes following endoscopic sinus surgery
Cleland et al.[32] (2016)	VAS and SNOT-22	CRS microbiome compared to controls	Richness no change		Diversity no change	*Corynebacterium tuberculostearicum* had no significant changes in any subjective or objective measures
		CRS after ESS			Diversity ↓	Prevalence of *Acinetobacter johnsonii* among CRS patients was associated with decreased SNOT-22 scores Abundance of *Pseudomonas* was identified as having an association with higher SNOT-22 scores
Jain et al.[27] (2018)	SNOT-22	Patients treated with either doxycycline or prednisone	Richness no change		Diversity no change	Significant differences in clinical scores, bacterial community richness, diversity, and bacterial abundance were not seen after treatment.
Jain et al.[20] (2017)	5-symptom CRS symptom score survey				Diversity no significant postoperative change	Reductions in *Corynebacterium* were associated with worse symptom scores Significant changes in *Staphylococcus* and *Streptococcus* postoperatively did not have any correlation with symptom score changes
Copeland et al.[22] (2018)	SNOT-22	In controls and CRS with polyps compared to without polyps			Diversity ↓	*Escherichia* as having significantly higher abundance in CRS patients Presence of *Corynebacterium*, that correlated with decreasing SNOT-22 scores
Lal et al.[21] (2017)	SNOT-22	CRS microbiome compared to controls			Diversity ↓	No significant difference in SNOT-22 when compared with Shannon or Faith diversity measures
Abreu et al.[13] (2012)	SNOT-20	CRS microbiome compared to controls	Richness ↓	Evenness ↓	Diversity ↓	228 taxa significantly positively correlated with lower SNOT-20 scores (less severe symptoms). Among these were members of Lactobacillaceae, Enterococcaceae, Aerococcaceae, and Streptococcaceae. 2 taxa significantly positively correlated with increasing SNOT-20 scores, both from Corynebacteriaceae with *C. tuberculostearicum* most significantly

CRS = chronic rhinosinusitis; ESS = endoscopic sinus surgery; QOL = quality of life; SNOT-22 = 22-item Sino-Nasal Outcome Test; VAS = visual analogue scale.

Discussion

Clinical significance

CRS is characterized by persistent mucosal inflammation in the sinonasal cavity. CRS has historically been viewed in the context of persistent infection, and although this framework has been disavowed, there are clearly changes in the sinonasal microbial flora in CRS.[33] The clinical significance of these changes remains unknown. The primary outcome measure by which CRS patients are evaluated is QOL, which can be directly impacted by CRS through sinonasal symptomatology and exacerbations of lower airway disease.[7,34-36] Elucidation of the relationship between the CRS microbiome and the impact of CRS on decreased QOL is crucial to understanding the clinical significance of changes to the microbial flora in CRS.

Staphylococcus

Previous culture-based studies found the prevalence of *S. aureus* to be higher in CRS patients vs controls and higher in CRS patients with more severe disease, such as those who progressed to revision surgery.[37,38] Our review suggests that *Staphylococcus* species' perceived role as a pathogen in CRS may be amplified by the ease with which it is cultured. Joss et al.[29] showed that *Staphylococcus* species were often cultured from sinuses even when sequencing revealed very low *Staphylococcus* abundance. In their study, most other organisms were only found in culture when their sequence counts were high, suggesting that *Staphylococcus* species are easily cultivable in a laboratory or were contaminants. Jain et al.[20] showed that patients had increases in *Staphylococcus* abundance after endoscopic sinus surgery, despite improved symptom scores compared to baseline. Cleland et al.[32] had results that correlated with prior culture-based studies, in that *S. aureus* relative abundance was higher in CRS relative to controls; however, this abundance did not correlate with subjective or objective disease severity measurements. *S. aureus* was approximately 3-fold more abundant in patients with poorer outcomes in the study by Ramikrishnan et al.[31] although this result was not statistically significant. Culture techniques may be more sensitive in identifying *Staphylococcus*, but it must be considered that DNA extraction is not as effective in detecting gram-positive bacteria.[29]

Role of anaerobes

Anaerobes are difficult to culture secondary to specific environmental conditions necessary for their growth and are often not included in initial diagnostic testing. Copeland et al.[22] showed several taxa that are strict and facultative anaerobes, including *Finegoldia*, *Anaerococcus*, *Peptoniphilus*, and *Lactobacillus*, with 5 CRS patients having high levels of these anaerobic genera (10-80% relative abundance) across all or most sinuses. Joss et al.[29] showed a high abundance of obligate anaerobes including *Finegoldia*, *Anaerococcus*, and *Peptoniphilus*, as well as fac-

ultative anaerobes such as *Escherichia* and *Haemophilus*. Additionally, Ramakrishnan et al.[31] demonstrated expansion of anaerobes including *Fusobacteria* and *Bacteroidetes* only when the swab sample was of purulence. No anaerobes were isolated from nonpurulent samples. These studies suggest a need for more research into anaerobic contributions to CRS pathogenesis and consideration for anaerobic antibiotic coverage when purulence is present.

A. johnsonii

In the study by Cleland et al.[32] *A. johnsonii* was associated with improved QOL scores for both the VAS ($p = 0.019$) and SNOT-22 ($p = 0.006$) scoring systems. Interleukin 10 (IL-10), an important anti-inflammatory cytokine, has been previously shown to have a positive correlation with the abundance of the genus *Acinetobacter*. In healthy compared to atopic individuals, IL-10 expression was positively correlated with abundance of *Acinetobacter* on the skin.[39] These findings suggest that individual microbes could be further studied for their potential role as specific immunomodulators and possibly even for topical therapy of the sinuses.

Role of *Corynebacterium*

Corynebacterium species are often disregarded clinically as normal flora when identified on culture. This genus was identified as a significant population in almost all studies, often comprising the most abundant genus represented in both CRS and control populations. When discussed on a genus level, increases in abundance were associated with improved outcomes and reductions were associated with worse symptom scores in multiple studies.[20,22,31] Abreu et al.[13] identified *C. tuberculostearicum* as a potential pathogen with increases significantly correlating with worse symptom scores, whereas the work of Ramakrishnan et al.[31] and Cleland et al.[32] did not support this finding. Further study is needed to identify possibly pathogenic species within this seemingly beneficial genus.

Pseudomonas

Discussion of the genus *Pseudomonas* in relation to QOL was relatively limited throughout the studies. Nevertheless, Cleland et al.[32] identified an association between *Pseudomonas aeruginosa* and poorer QOL in CRS patients. Cleland et al.[32] found an MRA of 4% in CRS patients and <0.01% in controls. Among CRS patients, higher SNOT-22 scores as an indication of worse QOL, were associated with this microbe. Further studies are needed to characterize this potential association with this possibly pathogenic genus.[32]

Limitations

Sampling of the sinuses relies on swabs through the nasal cavity; therefore, contamination with nontarget microbes requires consideration (eg, nasal mucosa when attempting to swab the maxillary sinus). Variability of specimens'

sites collected among studies makes it difficult to generalize results. The most consistently swabbed region was the MM (7/8 studies). Five studies swabbed from additional places as well. However, 1 study had 22 subjects, of which only 4 had MM analyses using 16s-rRNA gene sequencing conducted.[29] Another study reported results of swabs of MM and/or with anterior ethmoids, confounding the results if attempts were made to compare studies.[32]

The rapid turnover of mucus covering respiratory epithelium is also a confounding factor to sampling of the microbiome, because DNA persists after bacteria death, which is still detected via 16s-rRNA gene sequencing.[27] The capability of molecular techniques to precisely identify living vs dead bacterial communities in a sample is unclear. It is widely accepted that levels of bacterial DNA do not correlate with viability.[40] In 16s-rRNA gene sequencing, employment of denoising, a signal processing method to remove noise and preserve useful information, can reduce the number of potentially spurious sequencing. Such processing can result in biases in identification of bacterial genus and species.[41] As shown in Table 2, the variability of differences in primer bias as well as variable gene regions of 16s-rRNA utilized across studies is another confounding factor.

There is substantial variation in the native microbiome between individuals, which is another limitation of analyzing the microbiome.[33,42] High interindividual variation has also been identified as a factor in CRS microbiome studies.[27] In addition, studies have reported that variability between sinuses within the single CRS patient is significantly less than variability across different patients.[43,44] Age and smoking status may also contribute to variability in sinus microbial diversity.[45] The use of perioperative and postoperative antibiotics, steroids, and nasal irrigations confounds changes of the microbiome. The studies overall take mucosal sampling from varying sinuses, making it challenging to compare the data extracted. The small sample sizes of the studies included in this analysis makes it difficult to make definitive conclusions.

Conclusion

Next-generation sequencing research is greatly expanding our understanding of sinonasal microbiome in normal and CRS patients. Several studies allude to the "beneficial" role of *Corynebacteria* within the microbiome in CRS because it is associated with QOL improvements, although this varies based on whether it is at the genus vs the species level. Contrarily, staphylococci, classically known as CRS pathogens, were not consistently associated with poorer QOL outcomes. At present, there is no clear and consistent relationship between the sinonasal microbiome of CRS patients and their QOL outcomes. Larger studies are needed with a focus on QOL correlations to allow for application of microbiome findings in CRS to clinical practice. ⚕

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