



Review Article

# The microbiome and chronic rhinosinusitis

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Received 9 August 2018; accepted 13 August 2018

Available online 31 October 2018

## KEYWORDS

Sinusitis;  
Microbiome;  
Bacteriology;  
Polyposis;  
Pathogenesis;  
Inflammation

**Abstract** Chronic rhinosinusitis (CRS) is a multifactorial condition in which the microbiota plays a pathogenic role. The nature of the interaction between the microbiota and the local immune system is very complex and has not been fully elucidated. Recent improvements in the microbiological techniques have greatly advanced our understanding of the complex nature of this interaction. This paper summarizes the current state of the rapidly evolving research on this subject. Defining the nature of the role of the microbiota in CRS is important because of the associated therapeutic implications.

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## Introduction

Complex microbial communities have co-evolved with our species. At any one time, an individual can host 10 to 100 trillion microorganisms, outnumbering human cells by at least a factor of 10.<sup>1,2</sup>

Bacterial communities have been demonstrated on the mucosa of the entire respiratory tract, with the highest

concentrations being found in the upper respiratory tract (URT).<sup>3</sup> In addition to bacteria, polymerase chain reaction (PCR) based studies have revealed the presence of viral pathogens in the URT and there is evidence for the presence of fungi on healthy sinus mucosa.<sup>4–6</sup>

The interactions between the microorganisms themselves, the microorganism and the mucosa, and environmental changes influence the composition of the bacterial ecosystem. Greater mucosal biodiversity may play a vital role in limiting inflammation and protecting against infections.<sup>3,7</sup> There is some recent evidence that mucosal inflammation in the paranasal sinuses is associated with a decreased diversity of the local bacterial communities.<sup>8–12</sup> Particular microbial species known as “keystone species” may have an exceptionally large impact on the ecosystem’s function and health.<sup>13</sup> *Dolosigranulum* spp and *Corynebacterium* spp are two such examples in the URT microbiota.<sup>14,15</sup>

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Peer review under responsibility of Chinese Medical Association.



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The significant advances in sequencing technologies over the past decade have changed how we perceive the structure and function of microbial communities, and we are just starting to understand the role that these microbiomes play in the setting of sinonasal inflammation. This article summarizes our current understanding of the role of microbiomes in chronic rhinosinusitis.

## Microbiome

The term 'microbiota' describes the assemblage of microorganisms present in a defined environment. 'Microbiome' refers to the collection of genes that are encoded by the members of a microbiota. In the upper respiratory tract, an ecosystem created by bacterial, viral and fungal species interacts with the mucosal immune system.

Traditional culture-dependent techniques involve sampling the surface of the sinonasal mucosa and then growing the microbes on or in growth media. These techniques often do not capture the entire microbial diversity in a sample, as the culture media may not provide the conditions required for the growth of many organisms present. These traditional techniques are still sensitive, less expensive and allow for *in vitro* determination of the antibiotic sensitivity of pathogens.<sup>16</sup>

Newer culture-independent molecular methods include immunological, nucleic-acid based and gene-targeted or meta-omic techniques. These techniques allow for identification of microorganisms from a sample without requiring growth *in vitro* and even if they are non-viable. Immunological techniques include ELISA, serological assays and microarray.<sup>17</sup> These tests have a moderate level of sensitivity with a moderate level of specificity and have the advantages of being quick and relatively inexpensive. The disadvantages include limited detection of the microorganisms in low abundance and technical difficulties in generating highly selective antibodies.<sup>18</sup> The nucleic acid-based tests such as hybridization, PCR, sequencing and DNA/RNA – microarray have excellent specificity and have advantages of providing the most detailed, unbiased information and the potential to reveal novel organisms.<sup>19</sup>

Gene-targeted and meta-omics are two types of molecular techniques that potentially allow for a more detailed analysis of the microbiomes in the paranasal sinuses. The 16S rRNA gene of bacteria and the 18S rRNA gene of fungi are the commonly targeted genes. Meta-omics amplifies the specific targeted gene in a sample before sequencing.<sup>20</sup> This will detect the total DNA, RNA and protein content in a sample and can reveal information and functioning about the microbiome in that sample.

## Chronic rhinosinusitis and microbiome

Chronic rhinosinusitis (CRS) is an inflammatory disorder of the upper airways affecting approximately 5% of western populations.<sup>21</sup> The pathophysiology of this condition is still poorly understood with multiple environmental, host and microbial factors being implicated. Putative pathological factors include changes in the microbiota, imbalance of the local or systemic immune system, allergens, toxins and genetic predisposition.<sup>22–25</sup> The role of the microbiome in

the pathogenesis of CRS has had a renewal of interest due to the improvement in diagnostic techniques. The presence of intramucosal bacteria, biofilms, dysbiosis of microbiomes and super antigens have all been suggested to play a role in the pathogenesis of CRS.<sup>26–28</sup>

## Healthy sinus

The presence of bacteria in healthy sinuses has been demonstrated, correcting earlier assertions that the sinus mucosa is sterile.<sup>29,30</sup> Newer molecular techniques have shown rich and complex bacterial communities, including anaerobic organisms, in healthy paranasal sinuses. A germ free murine model has been used to show that the acquired sinus microbiome alters the maturation of the mucosa.<sup>31</sup> Colonization of the mucosal surfaces occurs in early infancy and the composition of the microbiome typically stabilizes by three years of age.<sup>32</sup>

Surprisingly, the total amount of bacteria present in healthy and diseased sinuses appears to be similar in adults, as determined by PCR studies.<sup>8,12,33</sup> Commonly identified bacterial genera include *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*.<sup>34–36</sup> Studies have also shown that the microbiomes in health and in CRS show high inter-individual variation. Pathogenic organisms are typically found at low abundance in healthy sinuses – they may be either a transient or permanent component of a healthy sinus microbiome.<sup>10</sup> Similar strains of *S. aureus* are seen in health and diseased mucosa. A recent study has explained a possible mechanism by which the concentration of the organism may predispose to inflammation. In low concentrations, *S. aureus* can induce the anti-inflammatory cytokine IL-10, however in high concentrations it favors a reduction in IL-10 leading to a more pro inflammatory response.<sup>37</sup> Commonly identified *Propionibacterium acnes* in healthy mucosa are shown to produce bacteriocin, which acts as an antimicrobial and antifungal compound that can modulate immune response to pathogenic bacteria.<sup>38</sup> It is likely that some micro-organisms are protective, making it more difficult for pathogenic species to become established by passively competing for space and nutrients and along with actively secreting antimicrobial compounds.<sup>11</sup>

## CRS

A number of studies have attempted to identify the links between CRS and specific bacteria. *S. aureus*, *Streptococcus* sp, *Corynebacterium* sp, *S. epidermidis* and *P. acnes* are frequently cultured in CRS patients. *H. influenzae*, *E. coli*, *Peptostreptococcus* sp, *Klebsiella* sp, and *Fusobacterium* are also seen in lower abundance.<sup>39–43</sup> However, with the exception of *S. aureus*, the association between any single species and CRS is tenuous.<sup>11,44</sup> It has been suggested that CRS results from not from the presence of a single species, but rather from changes in the composition or function of the microbial community.<sup>28</sup>

A recently performed meta-analysis of studies comparing the composition of the bacterial microbiome in CRS patients compared to healthy controls found no significant difference in species composition.<sup>11</sup> However, the microbiome of CRS patients has been found to have reduced diversity, increased

bacterial load, and to have less stable bacterial networks.<sup>8,11,38</sup> The imbalance or dysbiosis of the microbiome may be a potential cause for sinonasal inflammation. Studies have shown that *Propionibacterium acnes* act as a key bacterial species in the networks created by the diverse bacterial communities in healthy sinus mucosa.<sup>11,45</sup> Thus, the removal of this species may result in fragmentation of the community and allow for potentially pathogenic organisms such as *Staphylococcus* or *Streptococcus* to flourish. This community disruption is proposed as one of the many causes contributing to recalcitrant CRS.<sup>11</sup> Symbiosis in microbial ecosystems allows for more efficient nutrient transports and resources use resulting in decreased pathogen colonization.<sup>46</sup> Accordingly, a change in the diversity of the colonies may favor pathogenic organisms.

A biofilm is a specialized community of adherent microorganisms surrounded by a three dimensionally structured extracellular matrix.<sup>47</sup> Multiple bacterial and fungal species can live in a single biofilm.<sup>48</sup> A biofilm also allows the organisms to have metabolic cooperation that enhances efficiency, quorum sensing systems that control growth and an enlarged gene pool with more effective DNA sharing capabilities. Bacterial biofilms are detected on the sinus mucosa in up to 80% of CRS patients.<sup>49–51</sup> The common species identified in biofilms include *S. aureus*, *S. pneumoniae*, *P. aeruginosa*, *H. influenzae*, *Acinetobacter* spp, *Proteus mirabilis*, *Enterobacter* spp and coagulase negative *Staphylococci*. Biofilms are also seen on healthy sinus mucosa and their presence does not imply that they are causing mucosal inflammation. However, in the context of CRS there are several possible mechanisms by which biofilms may be pro-inflammatory including the release of planktonic organisms and the production of superantigens, which can cause ciliary dysfunction and inhibition of mucociliary clearance.<sup>52–54</sup>

Intra-epithelial microorganisms have also been identified in CRS patients, including *S. aureus*.<sup>55</sup> Microcolonies have also been identified in the interstitium just below the epithelial layer. As these colonies do not initiate a host immune response, they may act as a source for ongoing inflammation following medical and surgical treatment.<sup>56</sup> It is likely that these factors in combination with host immune system responses and disrupted epithelial lining play a role in the pathogenesis of CRS.

## Interventions

The medical treatment for CRS includes antibiotics, systemic or topical corticosteroids and saline lavage. There are a small number of studies that have examined the changes to the microbiome after antibiotics and other medical treatment. One study showed that the response following antibiotics treatment was far from consistent, although overall there was a small loss of diversity.<sup>57</sup> Colonization with taxa that were less susceptible to the prescribed antibiotics has also been observed.<sup>57</sup> A study that sought to determine the effect of topical saline or budesonide in chronic rhinosinusitis with nasal polyposis (CRSwNP) and healthy patients observed a notable change in the microbiome of the CRSwNP cohort.<sup>58</sup> A cross-sectional study that looked at factors that influence microbiome in CRS patients found that antibiotics use, reduced diversity and increased abundance of *S. aureus*.<sup>44</sup>

It is likely that antibiotics may have both positive and negative effects on the outcome of CRS. A single course of oral antibiotics has been shown to alter the gastrointestinal microbiome for up to two years.<sup>59</sup> It is conceivable that altering the sinonasal microbiome with antibiotics could unfavorably alter the clinical course of CRS. There is some indirect evidence to support this supposition.<sup>60</sup>

The impact of surgery on the sinonasal microbiome has been investigated in a small number of studies. Composition of the microbiome of patients with CRS undergoing surgery and controls undergoing transsphenoidal pituitary surgery have been compared.<sup>61</sup> At baseline, *Acinetobacter* spp had a higher abundance in patients without CRS and *S. aureus* had a much lower abundance. Interestingly, in patients who responded well to the sinus surgery, the *Acinetobacter* spp levels increased to a comparable amount to the control group.<sup>61</sup> In particular, *Acinetobacter Johnsonii* has been associated with significantly improved symptom control following surgery.<sup>61</sup> This organism is known to elaborate IL-10, an important anti-inflammatory cytokine as previously mentioned. The importance of *Acinetobacter* has also been seen in other studies and in atopic dermatitis. Another study has shown similar changes in microbiome in CRS patients after FESS.<sup>62</sup> Of note, this study reported an increase *Staphylococcus* spp despite the use of anti-staphylococcal antibiotics immediately postoperatively. The increase in *S. aureus* has also been shown to be associated with reduced bacterial diversity, higher revision surgery rates and more recalcitrant disease.<sup>42,63</sup> There is growing evidence that surgery and medical intervention can alter the microbiome in the sinus cavities. It is likely that certain commensal species such as *Acinetobacter* may help encourage microbial community stability and resistance to the establishment of pathogenic species.

## Treatments

It has been hypothesized that manipulation of the sinus microbiome may be favorably alter the course of CRS. Probiotics have been proposed to improve inhibition of pathogenic organisms and augmentation of epithelial barrier. A study has shown that the topical nasal administration of probiotics reduces the sinus inflammation caused by pathogenic bacteria in a mouse model.<sup>45</sup> Another murine study also showed that pre-treatment with *S. epidermidis* might reduce the inflammatory effects of *S. aureus* on the nasal mucosa.<sup>64</sup>

Oral probiotic use in CRS has been assessed in several studies. The use of oral *Enterococcus faecalis* showed benefit in treating CRS and recurrent acute rhinosinusitis in two studies.<sup>65,66</sup> The benefit was seen to last for up to 8 months in one randomized study in which probiotic was administered for 6 months.<sup>65</sup> Although the use of probiotics is well described in gastrointestinal research, the use in CRS remains limited.

## Virome, mycobiome and future research

A large number of DNA viruses, single-stranded RNA viruses and bacteriophages have been detected in healthy adults.<sup>67</sup>

Several studies have shown a variety of abundance of viruses in CRS including coronavirus.<sup>68–71</sup> Virus replication can damage the mucosal lining and increase the bacterial adhesion to the mucosa. These factors may play a role in the pathogenesis of CRS. As the techniques of identifying these organisms improve along with better understanding and identification of the taxa, a better understanding of sinonasal virome will develop.

Several molecular based studies have determined mycobiome of the sinus mucosa.<sup>72–74</sup> *Cryptococcus neoformans*, *Aspergillus* species and *Malassezia* species have all been identified in CRS mucosa with the *Malassezia* also being seen in healthy sinus mucosa.<sup>75</sup> The newer targets with sequencing approaches are detecting fungi more often in healthy and inflamed sinus mucosa.<sup>74</sup> Prior research has also shown that fungi can interact with *S. aureus* and *P. aeruginosa* in the sinonasal mucosa.<sup>76,77</sup> Fungi may act synergistically with pathogenic bacterial colonies to play a role in the pathogenesis of CRS.<sup>34</sup>

Our understanding of microorganisms in the paranasal sinus is still incomplete. Although there is some association between the viral, fungal and bacterial organisms and CRS, the exact nature and importance of the relationship is still unclear.

## Conclusion

The role played by microbiomes in CRS is difficult to be clearly defined at the current time due to the difficulties in the laboratory techniques and small studies with limited sample size. As larger, longitudinal and multilevel studies are being designed, the role of microbiomes in CRS and the role for specific treatments such as probiotics and “mucus” transplantation would become more clear. Rapid progress is also being made in laboratory techniques that enable determination of the composition of the sinonasal microbiome in CRS. Hopefully, the result of current investigations will soon inform antibiotic, probiotic and surgical treatments.

## Conflicts of interest

Rahuram Sivasubramaniam and Richard Douglas declare no actual or potential conflicts of interest.

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