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The Role of the Human Microbiome in Otolaryngology–Head and Neck Surgery: A Contemporary Review

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Abstract

Objectives/Hypothesis—The human microbiome represents the collective genomes and gene products of microbes living within and on humans. The objective of this review is to provide a summary of the current microbiome literature pertaining to otolaryngology–head and neck surgery.

Data Source—Ovid MEDLINE.

Methods—Scientific publications with clinical correlates.

Results—Human microbiome studies have been facilitated by culture-independent, high-throughput sequencing methods. Data from the Human Microbiome Project has shown that the composition of the human microbiome is specific to each body site and that each individual has a unique microbiome. Alterations in the human microbiome are associated with some disease states; thus, novel therapeutic strategies are being developed based on concepts and findings stemming from micro-biome research.

Conclusions—Although a growing body of research shows potential significance of the human microbiome for human health and disease, there is a paucity of microbiome studies in otolaryngology. More studies are required to increase our understanding of the indigenous microbiota and their effects on diseases of the head and neck.

Keywords

Microbiome; human; otolaryngology; head and neck surgery; microbiota

INTRODUCTION

The human microbiome represents the collective genomes and gene products of the microorganisms that live within and on humans.¹ Some consider the human microbiome as another component of the human genome, as humans are comprised of more microbial cells than human cells and microbes play an important role in human physiology, including homeostasis, metabolism, and immune response.¹ Furthermore, the human microbiome is site-specific, influenced by each body site's environmental conditions and the dynamic interactions between our body and the microbiome and within the microbial communities.

Given the intimate nature of the interactions between human body processes and the microbiome, it is perhaps not surprising that there is evidence in an increasing number of body systems that perturbations in the microbiome are associated with human disease.¹ For instance, antimicrobials can, in a potentially irreversible fashion, significantly modify the composition and reduce the diversity of the intestinal and oral microbiota.² Such reduction in bacterial diversity in the intestine has been associated with recurrent *Clostridium difficile* infections.³ Altered microbiota composition has also been associated with inflammatory bowel disease,⁴ necrotizing enterocolitis,⁵ and irritable bowel syndrome.⁶ These reports reinforce the notion that judicious use of antimicrobials should be practiced by all physicians, as disruption of the normal microbiome can lead to serious negative outcomes.

Major advancements in microbiome research have been facilitated by molecular technologies that have allowed the characterization of microbial communities that may include fastidious and uncultivable organisms, which resist traditional culture methods and would otherwise be masked by fast-growing species in a traditional culture-based assay.⁷ In addition, these technologies employ bioinformatics and computational statistical analyses that can process large ecological data sets.^{1,8} Metagenomics, the analysis of all the genomes of a microbial community, which may be recovered directly from clinical specimens, provides information about functionality as well as microbial community membership.⁷ Together, characterization of microbial community members and cataloging their full genomic content (metagenomes) are the main methodologies currently used in human microbiome research.⁸

To date, the most notable human microbiome study is the Human Microbiome Project (HMP), which sought to map the microbiome profiles of healthy individuals and to study host determinants that may affect micro-biome composition.^{1,9} Initiated in 2007 by the National Institutes of Health, the HMP enrolled 300 healthy, young adults and analyzed the microbial components in samples collected from five body sites, including the skin, nares, oral cavity, gut, and vagina.^{1,9}

An analysis of samples from a subgroup of 242 subjects detected approximately 600 genera and was estimated to have covered 81% to 99% of the phylogenetic range expected in

healthy individuals.^{7,9} Interestingly, although there was variation in an individual's microbiome over time, there was a much greater difference in the microbiome between participants, suggesting that a healthy individual's microbiome is unique.⁹ Although no single taxon was observed to be present in all participants and in all body sites, each body habitat in almost all subjects was dominated by one or a few specific taxa. For instance, Firmicutes and *Bacteroides* are dominant in the gut, whereas skin has more Proteobacteria and Actinobacteria, supporting the concept that the microbiome is intimately involved in body system-specific molecular processes.^{1,9} In a subset of 131 subjects, samples were taken at different time points to assess individual microbiome changes over time.⁹

Among human body sites, the microbiome of the gastrointestinal system has been most intensively studied.¹⁰ Recent studies have highlighted the diverse nature of gut microbial communities and the high interindividual variation in community composition.^{11,12} This work has underscored the importance of the gut microbiome in digestion, immunity, and both gastrointestinal and extragastrointestinal diseases.

In contrast, the head and neck region has not benefited from the same level of scrutiny. As one of the initial body sites for sampling in the HMP, the oral cavity is the only site within this region that has been studied systematically.^{1,9} This is troubling, as there are many recurrent and chronic infectious diseases of the head and neck, such as otitis media and chronic rhinosinusitis (CRS).^{13,14} Furthermore, similar to the gut, there are several open body habitats in the head and neck that are known to house diverse microbiota, even with culture-dependent methods.

The aim of this review is to provide a summary of the human microbiome studies that pertain to otolaryngology-head and neck surgery. A brief historical perspective, followed by recent findings and advancements relevant to the practicing surgeon and researchers of the head and neck is presented.

STUDY METHODS

A comprehensive literature search was conducted in Ovid MEDLINE through March of 2013 using the terms “human,” “oral,” “ear,” “otologic,” “larynx,” “chronic rhinosinusitis,” “throat,” or “tonsil” and “microbiome.” The titles/abstracts were reviewed to identify studies with clinical correlates relevant to the field of otolaryngology-head and neck surgery. The references of those studies obtained in full text were hand-searched for any additional relevant studies not identified by the original database search.

DISCUSSION

Advances in Molecular Technology

Next-generation DNA sequencing from clinical specimens is a common approach for human microbiome analysis.⁷ The process begins with careful collection of uncontaminated clinical or surgical specimens and the extraction of genetic material. Sequencing can be performed using one of two approaches. The first approach involves selective sequencing of a single gene conserved among all bacterial or fungal components. This method of analysis is cost-

effective, as a relatively short stretch of DNA is sequenced and can provide information about the species composition of complex microbial communities. For bacteria, the 16S ribosomal RNA (rRNA) gene is a ubiquitous, highly conserved gene that is used for targeted sequencing, whereas for fungal communities, the internal transcribed spacer region is widely used.^{15–19} The conserved nature of these genes allows for selective amplification of the target bacterial or fungal DNA using polymerase chain reaction (PCR) primers, without amplification of the human sequences contained in the clinical samples.⁷

Subsequent to amplification, a next-generation sequencing platform such as pyrosequencing, which is performed on the Roche (Indianapolis, IN) 454 platform,¹⁵ is used to sequence the target amplicons in parallel. Pyrosequencing detects the release of pyrophosphates as a light signal upon nucleotide incorporation, which produces an image file that can be converted into a single sequencing read for each microtiter well. As a result of its parallel sequencing, it is much more efficient than the traditional Sanger sequencing method, which employs detection of DNA chain termination by deoxynucleotide incorporation.²⁰ By incorporating sample-specific barcodes into the broad-coverage PCR primers (i.e., barcoded PCR primers) in pyrosequencing, scientists can barcode and sequence multiple samples simultaneously to generate approximately 100-fold more sequences for the same cost as traditional Sanger sequencing.^{21,22} The resulting sequences may then be matched against databases containing known marker gene sequences, including the Ribosomal Database Project or GreenGenes for bacteria.^{23,24}

The Illumina (San Diego, CA) sequencing platform, which is based on a sequencing-by-synthesis technology, can also be used for 16S rRNA (or 18S rRNA) gene sequencing.²⁵ Here, DNA is usually amplified by barcoded PCR primers to incorporate barcodes and sequencing primers. During sequencing, each amplicon's sequencing primers anneal to a complementary oligomer attached to a microfluidic cluster station, in which a PCR reaction amplifies the amplicon template in a discrete area or "cluster."²⁶ Each cluster then undergoes sequencing-by-synthesis with the addition of 3'-blocked nucleotides. As each nucleotide is added to a template strand, an imaging step identifies the base incorporated into the cluster, after which a chemical process removes the 3'-blocked nucleotide, allowing for addition of the next nucleotide(s).^{26,27} The final output, which is also an image file, is then processed to produce single sequencing reads.

Historically, pyrosequencing has been favored for 16S rRNA-based sequencing over the Illumina platform because of its longer read lengths and hence more reliable matches with reference sequences.^{22,26} However, paired-end amplicon sequencing on the Illumina platform can now produce read lengths approaching 500 base pairs that permit accurate, species-level assignment.²⁸ This, combined with its substantially lower per-nucleotide sequencing cost, will likely make Illumina the primary platform for bacterial 16S rRNA gene sequencing in the near future.

Although 16S rRNA sequencing can provide important species-level information about microbial communities and is relatively cost-effective given the short read lengths involved, it only provides information about the relative contributions of species to a community, rather than any functional information about community members. Metagenomic

sequencing, which involves direct sequencing of bacterial or fungal nucleic acid from clinical samples, although more expensive and time-consuming, can provide functional information about community members, as a greater proportion of the genome is sequenced.^{7,29} Due to the long sequencing reads required of this method, a technique called shotgun sequencing is employed, in which the microbial DNA is sheared into smaller, easily sequenced fragments and the sequences are reassembled using bioinformatic technologies.^{30,31} As with pyrosequencing, sample barcoding is employed so that multiple samples may be run at the same time. As the cost of sequencing has decreased, there has been an increase in the number of studies using metagenome sequencing.^{7,32}

Another culture-independent method that is used in microbiome research involves microarray analysis. In microbiome research, the PhyloChip is among the most commonly used microarray formats.^{33,34} Briefly, microarrays are based on oligonucleotide probes designed against desired gene targets. Each chip can consist of tens of thousands of distinct probes that could simultaneously bind to desired targets of hundreds of microbial species from a clinical specimen to generate a comprehensive profile.⁷ Although microarrays can be a relatively inexpensive, rapid method for microbiome analysis, development of the arrays can be difficult, given the conserved nature of 16S rRNA gene sequences and thus, the potential for nonspecific hybridization.³⁵ Given the improvements in DNA sequencing technology, utilization of microarray for microbiome characterization will likely decline.⁷

In addition to gathering massive amounts of data, including from fastidious and noncultivable organisms, molecular methods are also more sensitive than culturing for microorganisms living in biofilms.^{36,37} Because biofilms are encountered in many otolaryngologic conditions, these molecular techniques may be of particular importance for the head and neck surgeon.^{38,39}

Composition of the Head and Neck Microbiome

The oral microbiome—The human mouth is heavily colonized by viruses, fungi, protozoa, archaea, and bacteria. It is the only well-studied subsite within the head and neck, and the human oral microbiome database (www.homd.org) is a comprehensive resource pertaining to this topic.⁴⁰ Many inhabitants of the oral cavity have been associated with dental diseases, including periodontitis, root canal infections, and tonsillitis.^{41,42} A classic example is *Streptococcus mutans*, a common oral bacterium and a well-known cause of dental caries, which can increase secondary to regular ingestion of fermentable sugars.^{43,44} In addition, there is increasing evidence that perturbations in oral bacteria contribute to serious systemic diseases such as cardiovascular disease, diabetes, stroke, and pneumonia.^{45–48}

Analysis conducted as part of the HMP has shown that the oral microbiome is second only to the colon as the body system with the highest bacterial diversity.^{1,9} An analysis of HMP samples collected from seven dental surfaces and the tonsils, throat, and stool defined three distinct bacterial communities in the oral cavity and oropharynx: the buccal mucosa, gingivae, and hard palate had similar microbiota, whereas the saliva, tongue and throat (tonsil), and supra- and subgingival plaque contained unique community members.⁴⁹

The most dominant phyla in the oral cavity are Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes, and Fusobacteria, and oral communities tend to be similar at the phylum^{41,50–52} and even the genus level⁵³ between individuals. Although individuals' oral microbiomes frequently vary at the species level,⁵¹ the more conserved genus- and phylum-level organization lends support to the notion of a core microbiome in the oral cavity.^{50–52} The concept of a core oral micro-biome is also supported by studies that compared oral microbiomes from individuals around the world.^{50,54} An analysis of salivary microbiomes from 120 subjects in 11 countries found that although there was great diversity between individuals, microbiomes did not differ greatly between geographical locations.⁵⁴ In addition, similar oral microbiota have been found between Chinese and North American children.⁵⁰ Together, these studies suggest that macrogeographical variation in environment and lifestyle do not affect the oral microbiota as much as the microgeographic variation within the oral cavity.

Compared with the oral cavity and salivary micro-biome, the oropharyngeal microbiome has not been as thoroughly investigated. A study comparing the nasal and oropharyngeal microbiomes of seven subjects, which were analyzed by PhyloChip and 16S rRNA sequencing, found that the oropharynx had more bacterial diversity than the nostril.⁵⁵ In both the nose and oropharynx, Firmicutes accounted for the largest percentage of bacteria. However, at the family level, the nose had higher concentrations of Staphylococcaceae and Lachnospiraceae, whereas in the oropharynx, Streptococcaceae, Lachnospiraceae and an unclassified group of *Clostridia* were most prevalent.⁵⁵ A similar microbiome profile was observed in a case–control study of the laryngeal micro-biomes of 31 control and 29 laryngeal carcinoma patients.⁵⁶ Although a review of changes in the micro-biome due to malignancies is beyond the scope of this paper, analysis of the control subjects revealed several predominant phyla in the normal larynx, including Firmicutes, Fusobacteria, Bacteroidetes, Proteobacteria, and Actinobacteria. As with the oropharynx, the most abundant genus was *Streptococcus*, which was present in 36% of samples.^{55,56} Given the physical connection between the nose and oropharynx, this difference suggests a continuum of changes within the microbiome with changes in anatomic location.⁵⁵

The oropharynx houses Waldeyer's ring, which is composed of lymphoid tissue that can be considered similar to Peyer's patches of the gut as the initial sites for antigen recognition and infection control.^{57,58} A recent study showed that changes in proportional abundances of multiple oropharyngeal bacteria can contribute to recurrent tonsillitis.⁵⁹ Specifically, a comparison of the palatine tonsils between subjects with recurrent tonsillitis and tonsillar hypertrophy was performed using 16S rRNA gene-based pyrosequencing.⁵⁹ The investigators identified between 42 and 110 different oropharyngeal bacteria and a tentative core microbiome, which consisted of 12 genera. There was a difference in microbiota between subjects of different age groups, as *Streptococcus pseudopneumoniae* was present in all samples, but *Haemophilus influenzae*, *Neisseria* species, and *Streptococcus pneumoniae* were more common in children. Obligate anaerobes were also common, but *Prophyromonas* and *Prevotella* were greater in adults. This study revealed that adult recurrent tonsillitis was associated with increases in *Fusobacterium necrophorum*, *Streptococcus intermedius*, and *Prevotella melaninogenica/histicola*, whereas species

associated with acute tonsillitis, such as pyogenic *Streptococci* and *Staphylococcus aureus*, were rarely detected.⁵⁹

The otologic microbiome—Since Senturia and colleagues first cultured bacteria from middle ear effusions in 1958,⁶⁰ much attention has been given to the study of microbiology and targeted antimicrobial therapy in otitis media.⁶¹ However, to date, only two studies have analyzed the microbial profile of middle ear effusions with advanced molecular techniques.^{39,62} One study used quantitative real-time PCR analysis to compare the microbiological profile between nasopharyngeal and middle ear specimens collected from children with recurrent acute otitis media versus chronic otitis media with effusion.⁶² The results revealed no significant difference between the two groups; *H. influenzae* and rhinovirus were dominant in both the chronic and acute otitis groups. Yet, this study only targeted *S. pneumoniae*, *H. influenzae*, and *Moraxella catarrhalis*, and 15 respiratory viruses; thus, the true diversity of the middle ear microbiome cannot be concluded from this study.

Liu and colleagues performed a cross-sectional study of bacterial microbiota using 16S rRNA gene-based pyrosequencing on specimens from an 8-year-old boy with obstructive sleep apnea and chronic otitis media with effusion.³⁹ Samples were collected from the middle ear, tonsil, and adenoid for analysis. Pseudomonadaceae dominated the middle ear (82.7% relative abundance), whereas Streptococcaceae was dominant in the tonsillar microbiota (69.2%). Multiple bacteria, including Pseudomonadaceae, Streptococcaceae, Fusobacteriaceae, and Pasteurellaceae dominated the adenoid microbiota. Overlap between the middle ear and the tonsil microbiota was minimal, but the adenoid microbiota encompassed bacteria detected from both the middle ear and the tonsil.³⁹ The authors concluded that there were previously unknown bacterial communities in the studied sites, and that the adenoid tissue may be a source site for both the middle ear and the tonsil.

The sinonasal microbiome—CRS is a highly prevalent condition, involving persistent sinonasal mucosal inflammation.⁶³ Although the causes of CRS appear to be multifactorial and may include a combination of host and exogenous etiologic factors, perturbations in the sinonasal microbiome have long been hypothesized.^{64,65} Although cultured-based studies have revealed a wide range of aerobic and anaerobic bacteria in nares, oropharynx, oral cavity, and the paranasal sinuses in CRS patients,^{66–68} a high proportion of CRS cultures are either negative or result in uninformative outcomes.⁶⁹

Recent descriptive molecular studies have highlighted the polymicrobial nature of the paranasal sinuses in health and disease.^{68,70–73} In particular, these molecular studies have shown that common clinical pathogens, such as *S. aureus*, as well as less commonly known gram-negative and anaerobic bacteria, frequently cocolonize the sinuses.^{68,71,73} Although there have been some reports of differences in overall sinus microbiome composition between CRS participants and healthy controls,⁷² more studies have shown reduced overall micro-biome diversity in CRS.^{68,71,73} These findings are consistent with changes observed in microbiome profiles for other pathological conditions, in that some diseases are associated with reduced microbiome diversity.¹⁰ An association has also been shown between antibiotic use in surgically recalcitrant CRS patients and reduced microbiome

diversity, indicating that antibiotics may be ineffective in restoring normal sinus microbiome diversity.⁷⁴

Recently, Abreu and colleagues used a murine model to empirically test their finding of depleted sinus microbiome diversity in CRS, obtained using PhyloChip, which showed a proportional reduction of lactic acid bacteria and a concurrent proportional increase in *Coryne-bacterium tuberculo-stearicum*.⁷⁴ Single organism colonization by *C. tuberculo-stearicum* triggered pathological findings similar to sinusitis, but this was abated by cocolonization with *Lactobacillus sakei* (a lactic acid bacteria). These findings suggest that a potential antagonistic interaction between two different species of bacteria can confer protection against the development of CRS, and that the loss of protective bacteria may be a potential etiologic pathway in CRS.⁷⁴

CONCLUSION

New molecular sequencing techniques and metagenomic studies have underscored the importance of the human microbiome to human health and some disease states. It is clear that disease can often be associated with a disruption of the microbiota, frequently leading to the emergence of one or more pathogens.

In otolaryngology–head and neck surgery, pathologies frequently involve infections of open systems, such as the nose. More serious diseases, such as mucosal malignancies of the oropharynx, have been associated with microbiological causes (e.g., human papilloma virus). Thus, more studies are needed to first establish the composition of the head and neck microbiome in the absence of disease, and then to focus on the changes in microbial composition in disease states for possible early diagnosis and effective treatment.

Finally, studies regarding protective microbial species and microbiota manipulation, as possible strategies to reshape disease-prone microbiomes, are emerging. This promising field of metagenomic medicine will hopefully lead to improved interventions in the near future.

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