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Review article

Integrating the microbiota of the respiratory tract with the unified airway model

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ABSTRACT

The unified airway model has developed from indications that the upper and lower respiratory tracts share key elements of pathogenesis. These shared traits likely extend to similar niche characteristics that support bacterial communities, and as such, we suspect that similar microbes exist on upper and lower respiratory tract epithelium. Over the past decade and a half there have been significant improvements in microbiological identification and analysis due to the development of new molecular technologies, including next-generation sequencing. In this review, we provide an overview of the modern collection and sequencing methods involved in respiratory microbiota research, and outline the specific microbial communities that have been found to be associated with the healthy and diseased human respiratory tract. Demonstration of a remarkable similarity between the upper and lower respiratory tract in terms of microbiological presence adds further corroboration to the existence of a unified airway.

Contents

1. Introduction

Rhinitis and asthma share key elements of pathogenesis and have long been noted to co-occur, suggesting that the upper and lower respiratory tracts are more than just physically connected [1,2]. Indeed, the unified airway model suggests that immunological responses in one section of the respiratory tract can be linked to responses in other areas [3–5]. Data from the Human Microbiome Project (HMP) presents further supportive evidence of this model. Bacterial communities in healthy lungs have been shown to be highly similar to those in the upper respiratory tract, and shifts in one anatomical location may be associated with changes in others [6,7]. This is perhaps not surprising due to the ecologically similar

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niches found throughout the respiratory tract, many of which are covered in a continuous mucosal layer, and bathed in mucus and saliva.

Research in the past few decades has shown that many of the roughly 10^{14} microbial cells that live in and on our bodies are necessary for our wellbeing [8]. This consortium of microbes, termed the microbiota, serve a number of functions including priming the immune system, digesting food, providing nutrients and vitamins, and protecting us from potential pathogens [9]. Bacteria are estimated to outnumber human cells 10 to 1, and contain more collective genetic content than that found in human cells. Indeed, every surface of the human body in contact with the outside world is coated in microbes, including the gastrointestinal tract from the mouth to the anus, the respiratory tract from the mouth to the lungs, the entirety of our skin, even our eyes [6,7,10,11]. Interestingly, out of the about 50 known bacterial phyla, humans generally only associate with members from ten of these phyla, including Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, Tenericutes, TM7, and Verrucomicrobia [12].

The methods used to study these bacterial communities has changed drastically in the past decade, due in large part to a vast increase in computing power coupled with the advent of new molecular technologies, often called “next-generation sequencing” (NGS), that generate thousands to millions of sequences per sample [13]. While standard culture-dependent work certainly laid the groundwork for this field of research, NGS has opened the door to a better understanding of the breadth and depth of the human microbiota. Understanding what grows attached to surfaces of the human body may give us not only a better view of what it means to be healthy, but also open avenues to a better understanding of chronic diseases that may be associated with an altered microbiota, and may aid in development of better treatments and perhaps even preventative measures.

The goals of this review are three-fold: 1) give a brief overview of how human microbiota research is conducted in general and specifically in the respiratory system; 2) review the microbial community found associated with the healthy and diseased human respiratory tract; and 3) discuss how this corroborates the unified airway model.

2. Human microbiota – modern sequencing methods

Study of the human microbiota starts with sample collection. Samples may be collected in many different ways, depending on the site being studied and the questions researchers are trying to answer. Swabs and brushes have both been used to physically remove bacteria from any surface the implement can reach, along with instruments that more literally scrap the epithelia. Likewise, liquids (saliva, sputum, vaginal secretions, and gastric juices) and solids (feces) can be used. Historically, and still today in many labs, these samples were used in culture based assays, where an attempt was made to grow bacteria on agar plates or in liquid media, and then identify the bacteria present in the original sample. However, today we know that many bacteria associated with humans are not easily cultured, and this older method of sampling missed the vast majority of bacteria associated with humans [14]. The inability to culture bacteria from some areas of the body, such as the stomach and lungs, led to the belief that these sites were sterile. This idea has been shown to be incorrect, though many of these bacteria remain elusive in terms of required culturing conditions [14].

The discovery of the 16S rRNA (16S) gene and the advent of polymerase chain reaction (PCR) birthed a new era in microbial ecology research. Pioneering work by Carl Woese demonstrated that distinct groups of bacteria could be identified based on the 16S gene [15]. The 16S gene, universally present in all bacteria, is about 1500 base pairs long and contains nine hypervariable regions flanked by highly conserved regions (Fig. 1). This gene structure is highly amenable for identifying bacteria; universal primers can be designed for the conserved regions, while the intervening regions can be used for sequencing and identification of bacteria. Out of this came a number of techniques to explore microbial communities using molecular techniques, such as clone libraries, denaturing gradient gel electrophoresis (DGGE), and terminal restriction fragment length polymorphism (tRFLP), amongst others [16,17]. The drawbacks of these techniques have made them less popular, and they have mostly been replaced by newer sequencing technologies.

The advent of NGS techniques, so named because they further the ideas of Sanger sequencing, made possible the description and exploration of microbial communities like never before [18,19]. Where before a successful clone library may have yielded a few hundred clones from a handful of samples, we can now recover millions of sequences, easily multiplexed across numerous samples. Sequencing platforms such as the MiSeq and HiSeq from Illumina, and SMRT sequencing from Pacific Biosciences, in addition to others, make this possible. In short, for bacterial community analysis using the 16S gene, PCR products are produced targeting relatively short regions of the 16S gene using universal bacterial primers that also contain sequences specific for the sequencing platform being used. Primers are usually also designed with barcodes for each sample allowing each sample to be labeled with a unique 4-12 bp DNA fragment that, after sequencing, can be tied back to the original sample [20]. This also allows PCR products from numerous samples to be pooled, sequenced en masse, and the sequences for each sample to be separated later based on the assigned barcode using bioinformatics programs such as mothur or QIIME [21,22].

These current sequencing techniques are not without bias. Results from experiments may vary based on the sampling technique, DNA extraction protocol, polymerase used for PCR along with the primer choice and region of the 16S gene sequenced [23–25]. Likewise, multiple protocols and programs exist for processing sequences [21,22], checking sequence quality and removing noise [26,27], and detecting chimeras [28,29]. Sequence identification can also vary, as the array of databases for identification purposes each have their own strengths and weaknesses [30–32]. Even how sequences are aligned to these databases is important [33]. Each step in the overall protocol for how samples are handled introduces bias and studies done using different protocols are not always easily comparable to each other.

3. Sampling the respiratory system as a niche for bacteria

For the purposes of this review, we use the term ‘respiratory tract’ loosely to include all epithelial surfaces associated with respiration and the path air takes to reach the lungs, including surfaces from the anterior nares into the nasal cavity and sinuses, back to the nasopharynx, the soft tissue of the oral cavity, back to the oropharynx, down through the larynx, trachea, and finally, to the lungs (Fig. 2). Two types of epithelia dominate these surfaces. Ciliated pseudostratified columnar epithelium lines the sinuses, nasal cavity, nasopharynx, larynx, and trachea, while the oral cavity, oropharynx, and vocal folds are lined with stratified squamous epithelium. Mucin producing goblet cells are found only in ciliated pseudostratified columnar epithelium, while submucosal glands found throughout the respiratory tract are also known to produce mucus.

Mucin, a heavily O-glycosylated protein, is found in both saliva and the continuous mucus layer that covers all epithelial surfaces of