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Review

Microbiota - The Unseen Players in Adult Asthmatic Airways

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Abstract

Modulation of human lung airway physiology by commensal microbiota has become one of the key mechanisms involved in the pathogenesis of adult asthma. Recent evidence suggests that the composition of respiratory microbiota plays a significant role in the manifestation of adult asthma; however, scientific evidence about the relationship between airway microbial diversity and phenotypes of adult asthma is limited. Further research is needed to understand the interactions between the airway microbiota and host immune response to develop microbiota-based strategies in management of adult asthma. This study reviews the advances in culture-independent methods for detection of airway microbiome, the current data about airway microbiota in healthy individuals and in adult patients with asthma with a focus on bacterial communities, and the future research directions in airway microbiome.

KEYWORDS: Airways, asthma, adult asthma, microbiota, microbiome

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INTRODUCTION

The human body hosts a complex community of commensal microorganisms comprising of bacteria, viruses, archaea, and fungi, all together termed as microbiota [1]. Advances in culture-independent techniques (metagenomics) and DNA sequencing technology (such as next-generation sequencing) have revolutionized the investigations of microbiota in human health and disease. Recent evidence using these technologies have shown that these commensal microbes maintain our immune system and perform various biochemical and metabolic functions complementing the host [1]. The main hypothesis about the interaction with the host and microorganisms is that the change in microbial compositions might have causal roles, protective roles, or both, against health and disease.

Asthma has become very common in both children and adults around the world in recent decades [2], and the increase in the prevalence of asthma has been associated with changes of gut and airway microbiota as a result of antibiotic use and dietary differences in westernized countries [3]. Emerging evidence suggests that exposure to microorganisms and/or their components during early life may provide protection against asthma in childhood, whereas exposure to pathogenic species, such as respiratory viruses and *Chlamydia pneumonia*, is associated with the risk of exacerbation in childhood asthma [4]. Although the results of studies on childhood asthma have been highly variable because of the different methods used to assess bacterial populations, advanced scientific knowledge on microbiome in the recent years have provided extensive data on childhood asthma. In contrast to childhood-onset asthma, less is known about the role of microbiome on adult asthma, and we need further investigation to define a core healthy or dysbiotic microbiota in adult asthma. This study discusses the advances in culture-independent methods for detection of airway microbiome, the current data about airway microbiota in healthy individuals and in adult patients with asthma with a focus on bacterial communities, and the future research directions in airway microbiome.

Molecular Methods for the Analysis of Airway Microbiome

Comparative studies based on culture-dependent and independent techniques have shown that the majority of microbes in the airways (more than 95%) are fastidious bacteria and, thus, are "unculturable" using the routine laboratory culture techniques [5]. The culture-independent techniques or metagenomics is based on the molecular identification of assemblage of microorganisms directly from the clinical or environmental sample (for example, bronchoalveolar lavage (BAL) fluid, stool, soil, and so on) circumventing the need to culture these microbes in the laboratory [6]. With the advent of metagenomics and huge advancements in DNA sequencing technologies, computational resources, and bioinformatics, there has been a spurt in microbiome studies revealing its importance in environmental and human health. The high-throughput next-gen-

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eration DNA sequencing (NGS) technology is broadly based on 2 features: short read sequencing (for example, offered by Illumina Inc.) and long-read sequencing (for example, offered by PacBio - Pacific Biosciences of California, Inc., Oxford Nanopore Technologies). These technologies have aided researchers to perform massive parallel ultra-deep sequencing, generating hundreds of megabases and have transformed the landscape of microbial genomics [7].

The most common sequencing approach to analyze the microbiome using molecular tools, is based on the sequence analysis of the 16S ribosomal RNA (rRNA) gene. The 16S rRNA gene is a component of the rRNA operon, which is comprised of the internal transcribed spacer (ITS) and 23S rRNA gene. This *rrn* operon (16S rRNA – ITS – 23 rRNA) is reported to be evolutionary conserved and present in bacteria [8]. Before the advent of long-read sequencing technology, analysis of the smaller variable regions of 16S rRNA (for example, V1-V3, V3-V5, V4) was a preferred modality, using short read sequencing technology (for example, Illumina, Roche 454) (Figure 1). The 16S primarily consists of 9 variable regions that distinguishes bacterial taxa. Usually, degenerate primers targeted at the preceding section of the variable regions are used to amplify the variable regions to identify the landscape of the microbes [9, 10]. However, this may not hold true as the degenerate primer or different variable regions may not generate a comparable data [11, 12]. For example, recently the primer sequences targeting the V4 region have been updated by adding more degenerate sequences to increase the detection of bacterial taxa [13, 14]. For instance, sequencing V1-V3 and V4 region may identify the same taxa but in different relative abundance ratio (Figure 1). It should be noted that the NGS technologies have been evolving rapidly, and Roche 454 has been phased out; however, comparable data have been reported using the same primer region [15]. Alternatively, the 16S rRNA PhyloChip or phylogenetic arrays, a high-density array containing probes, can be employed to identify bacteria [16, 17]. With the long-read sequencing technology being readily available, researchers are now exploring methods to sequence the full 16S rRNA or the 16S-ITS-23S amplicon (Figure 1), which may provide much higher resolution of the microbial species in the samples [18]. By sequencing the longer amplicons using PacBio or Nanopore platforms, we can alleviate the biases introduced with sequencing smaller variable regions [19, 20].

There are multiple advantages of the amplicon-based techniques; for example, sample processing and sequencing are cost-effective, requires less computational and bioinformatics resources for analysis, and standard web-servers are available for data analysis [21]. However, one of the major limitations is the accuracy of taxonomic assignment of the 16S sequences. This technique confidently and accurately assigns at the genus rank, although many sequences that are distant from the commonly used reference sequences or that are taxonomically ambiguous can only be assigned to class, order, or family rank and can be clustered together into operational taxonomic units (OTUs). The OTU is defined as a group of 16S rRNA sequences sharing >97% of sequence homology [6]. Although, amplicon sequencing ability to identify virus or to offer any insights into the predicted functional potential of the microbial genome is limited, it has significantly aided to identify the bacterial and fungal diversity in the microbiome studies its [6].

An alternative to the amplicon sequencing method is the shotgun metagenome sequencing or shotgun metagenomics [6]. In this approach, the high molecular weight metagenomic DNA is directly isolated from the clinical or environmental sample and fragmented into smaller fragments. According to the NGS platform, a compatible adaptor and barcodes are then added to the fragmented DNA, which is then sequenced using the appropriate NGS platform on the basis of short read sequencing chemistry. Alternatively, the high molecular weight metagenomic DNA can be sequenced directly using the long-read sequencing platform. The major advantage of the shotgun metagenomics is the accurate and high confidence of the taxonomic assignment of the bacteria at the species rank. In addition, viruses, fungi, archaea, and other single cellular eukaryotic organisms can be identified in the sequencing data [6, 22]. The functional potential of the microbiota can also be predicted as random fragments of the genome are sequenced. However, shotgun metagenomics is more expensive than amplicon sequencing and requires more extensive computational and bioinformatics resources for data analysis. In addition to the microbial sequences, the host genome (for example, mouse or human) are also sequenced [23, 24]. This could also add to the extent of deep sequencing to obtain a significant amount of microbial data in the host abundant DNA (low biomass samples such as cord blood, bronchoalveolar lavage fluids, blood, urine, and so on). Metatranscriptomics (microbial RNAseq) has also enabled the researchers to investigate the functional potential of the microbiota by identifying the expression of microbial genes and further delve into the crosstalk with other microbes and host. In this method, the total RNA is isolated, and ribosomal RNA is depleted and can be enriched for mRNA. The mRNA is converted to cDNA and sequenced using the shotgun sequencing approach [22,

MAIN POINTS

- The respiratory tract contains a heterogeneous microbiota that decreases in biomass from the upper to the lower tract. Spatial variation within the lungs of healthy individuals is mild.
- The most prevalent phyla in healthy airways are Bacteroidetes and Firmicutes.
- Microbiota of asthmatic airways commonly include a greater prevalence of Proteobacteria and Firmicutes, in particular, *Haemophilus* spp, *Neisseria* spp, and *Moraxella* spp.
- Specific members of the airway microbiota is associated with different phenotypes of asthma, such as obesity-associated asthma, eosinophilic asthma, corticosteroid-responsive asthma, or Th17-associated airway inflammation.
- Airway microbiota dysbiosis seems to be associated with steroid resistance, disease severity, and the type of inflammation such as neutrophilic or eosinophilic in adult asthma.

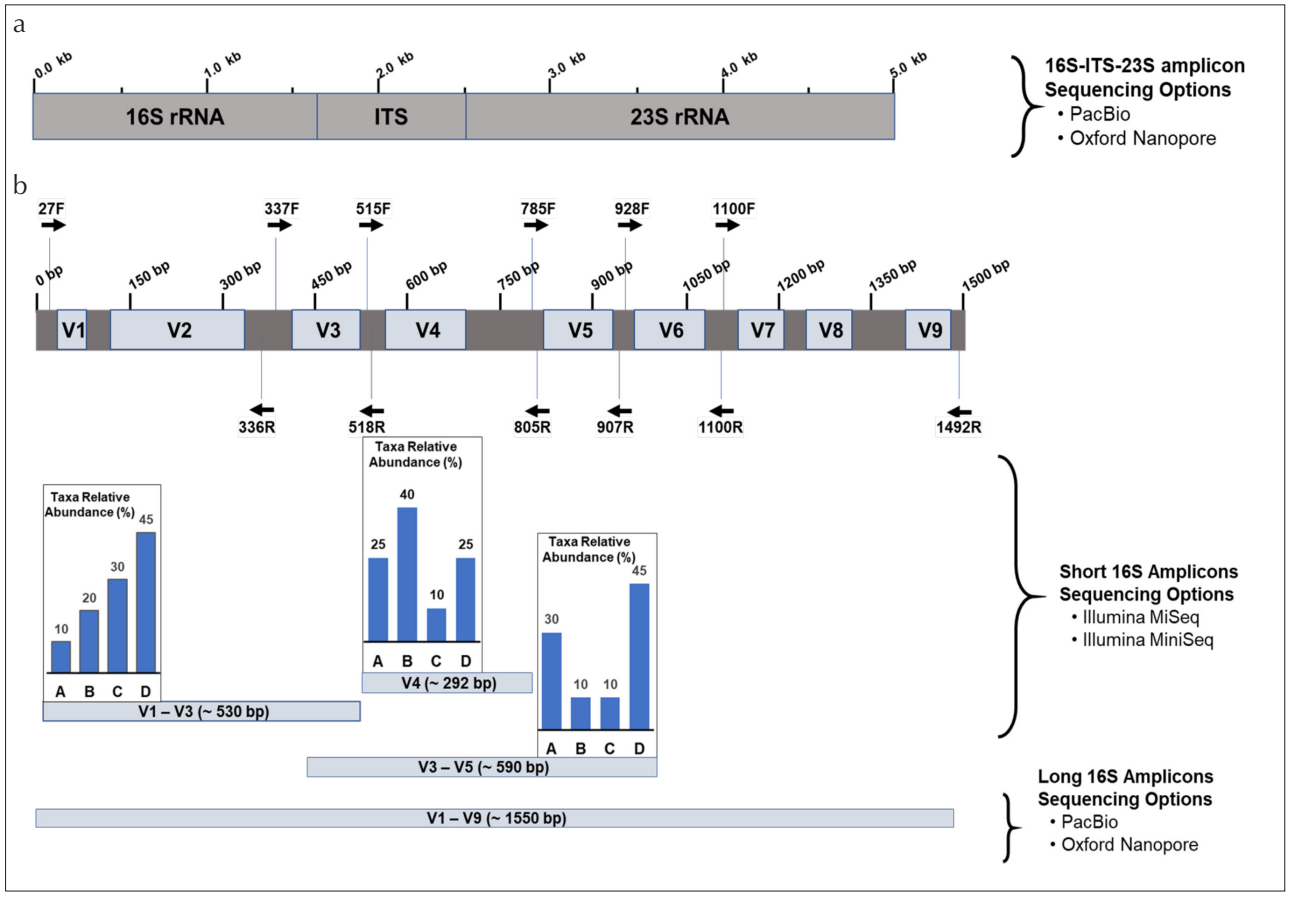

Figure 1. a, b. Molecular methods for the analysis of airway microbiome. (a) Schematic illustration of Bacterial/Archaeal 16S-ITS-23S ribosomal RNA operon. (b) Detailed view of 16S rRNA variable regions. Note, sequence analysis of different variable regions may affect the identification bacterial taxa, due to primer bias. Note, base pairs not to scale, E. coli rRNA operon used as reference

25]. A point to note is that the depletion of rRNA may not be efficient, and the total RNA can also be sequenced. However, sequencing the total RNA can be costly as >95% is rRNA and may not provide any functional information. Undoubtedly, these technologies have significantly enabled the researchers to understand the role of microbiota in human health and pulmonary diseases, particularly in asthma.

Microbiome in Healthy Lung

In utero, the lungs are filled with amniotic fluid, and bacterial DNA has been detected by new non-cultured methods in amniotic fluid and placental specimens [26, 27]. It has been hypothesized that prenatal lung development occurs in the presence of microbial communities [28]. Studies have reported a significant similarity between the microbial communities of the oral and placental microbiomes [27]. It is suggested that the placental microbiome is likely established by a hematogenous spread of oral microbiota [27]. Microbes in the mouth are more similar to the lung bacterial microbiome than the communities at other body sites. This similarity supports the notion that the upper respiratory tract microbiomes can colonize in the lungs as a result of micro-aspiration [28]. The human nasal, oral, and pharyngeal cavities consist of hundreds of microbial species, including 25-40 families of bacteria. In the pharynx, 5 major bacterial phyla have been identified by the human microbiome project (HMP): Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria. In the human pharynx, the most common genera in descending order are *Prevotella, Capnocytophaga, Campylobacter, Veillonella, Streptococcus, Neisseria,* and *Haemophilus,* according to the HMP data [29].

Unlike the oral microbiome, the microbiome of the nose closely resembles that of the skin than that of the lungs [30]. The bacterial communities of the nasal cavities are Actinobacteria, Firmicutes, and in some cases, Proteobacteria. Corynebacteriaceae and Propionibacteriaceae are the most prevalent families of Actinobacteria in the nasal cavity [30]. Nasal cavity communities such as Propionibacteriaceae and Staphylococcaceae are undetectable in most oral cavity samples [30]. There are 3 types of microbial populations defined within the nasal cavity: (1) Actinobacteria (mainly *Propionibacterium* and *Corynebacterium* spp.) is dominant in healthy adult nares; (2) *Staphylococcus aureus* is the dominant nasal species in *S. aureus* colonized individual nares with concomitant reductions in the prevalence of Actinobacteria; (3) *S. epidermidis* is the dominant species in *S. aureus* non-colonized individuals with reduced levels of Actinobacteria [31].

The respiratory tract has a surface area of 2.8 m^2 at birth, 32 m^2 at 8 years of age, and 75-100m^2 at adulthood [32]. The surface area of the lungs is approximately 30 times greater than the skin [33]. Airways are directly exposed to allergens, microbes, and other irritants, and inhaled air contains 10^4–10^6 bacterial cells per cubic meter [33]. Although the lower airways in humans have long been thought to be sterile, new

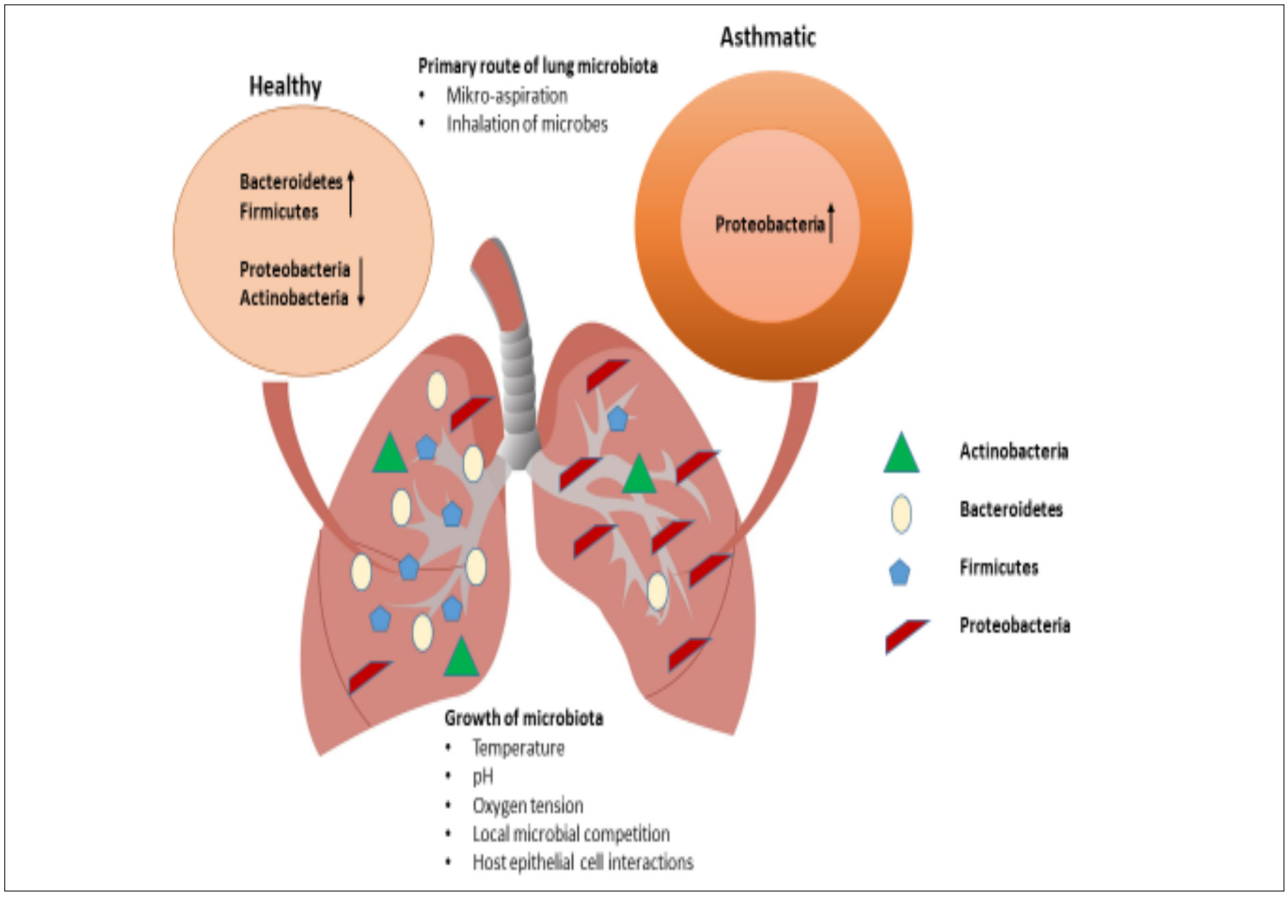

Figure 2. Factors influencing lung microbiota

studies have detected various microbial communities in the lower airways of healthy individuals using culture-independent techniques. Lung microbiota occurs by the balance of three factors, that is, microbial immigration, microbial elimination, and the relative reproduction rates of its members [34]. In a healthy lung, the microbiome is determined largely by the balance of immigration and elimination. The relative reproduction rates of its members have relatively little contribution on the lung microbiota. The primary routes of microbial immigration to the lungs are micro-aspiration, inhalation of bacteria, and direct mucosal dispersion. Temperature, pH, oxygen tension, nutrient availability, local microbial competition, host epithelial cell interactions, activation of inflammatory cells, and concentration of inflammatory cells, all influence growth rates of microbiota members. The main microbial elimination mechanisms are cough, mucociliary clearance, and innate and adaptive host response. These microbes are commensals, and the infection by these microbes only develops when normal pulmonary defense mechanisms are impaired [34]. A healthy lung has a diverse microbiota, and the most prevalent phyla in the airways are Bacteroidetes and Firmicutes. Proteobacteria and Actinobacteria are less prevalent in the airways [1]. Streptococcaceae (Firmicutes), Veillonellaceae (Firmicutes), Prevotellaceae (Bacteroidetes), Fusobacteriaceae (Fusobacteria), Neisseriaceae (Betaproteobacteria), Porphyromonadaceae (Bacteroidetes), and Lachnospiraceae (Firmicutes) are the most prevalent bacteria found in healthy lung airways [1]. Factors influencing the lung microbiome is summarized in Figure 2.

The respiratory tract contains a heterogeneous microbiota that decreases in biomass from the upper to the lower tract [35]. Spatial variation within the lungs of healthy individuals is mild [36]. Various methods, including BAL, protected brushings and biopsy taken at bronchoscopy, and sputum induction can be used for detection of microbiota in the airways [37]. Sputum sampling is the easiest method for microbiota analysis of the lung. However, sputum diversity may not always reflect the diversity of the lung microbiome in the lower airways [37]. BAL fluid or protected specimen brushings are the preferred methods for minimizing carryover from the upper airway [37]. However, the passage of a bronchoscope through the upper respiratory tract may have a theoretical risk of contamination. Some investigators use a 2-bronchoscope method, in which a first bronchoscope is used to sample the upper airway, and a second bronchoscope is used to sample the lower airways to minimize contamination by upper airway organisms [38]. The microbiome differs dramatically in different locations such as the mouth and the nose. However, the route of bronchoscope insertion (oral versus nasal) has no detectable influence on the BAL microbiome [38].

Microbiome in the Airways of Adult Asthmatics

Respiratory microbiome studies in adult patients with asthma have mainly used induced sputum, BAL, or bronchial brushing for analysis. Studies using bronchial biopsy is very limited. Adult studies of the airway microbiota suggest that microbiota of patients with asthma commonly include a greater prevalence of Proteobacteria and Firmicutes, in particular,

Haemophilus spp, *Neisseria* spp, and *Moraxella* spp [39-46]. The presence of *Moraxella catarrhalis* (phylum Proteobacteria), *Haemophilus* spp (phylum Firmicutes), and *Streptococcus* spp (phylum Firmicutes) in the airways has been associated with poor lung function, high neutrophil counts, and elevated IL-8, IL-1β, IL-8, and IL-17 concentrations in the sputum and BAL [43-45]. Increased abundance of pathogenic Proteobacteria, such as *Neisseria, Haemophilus, Pseudomonas*, and *Ricketssia spp* were also associated with obstructive status and oral corticosteroid resistance [39-43]. The relative abundance of Proteobacteria members of the Comamonadaceae, Sphingomonadaceae, Oxalobacteraceae, and other bacterial families was highly correlated with bronchial hyperresponsiveness [44]. Most of these studies have analyzed airway microbiota in patients with asthma when they were using inhaled steroids. Although inhaled steroids may have a theoretical risk on commensal airway microbiota, the impact of long-term inhaler corticosteroid use on bacterial diversity of asthmatic airways is currently unknown. Denner DR et al. [41] have investigated the oral steroid effect on the diversity and composition of the airway microbiome of patients with asthma using generalized linear models. They have demonstrated significant differences according to the corticosteroid treatment. The combination of inhaled and oral corticosteroids has increased the abundance of Proteobacteria and the genus *Pseudomonas* and decreased the abundance of Bacteroidetes, Fusobacteria, and *Prevotella* species. Durack J et al. [46] have investigated the bacterial component of microbiota in the bronchial brushing of adult asthmatic airways. They clustered patients as steroid-naive atopic asthma, atopy but no asthma, and non-atopic healthy subjects and compared the results. The study has found similar results with other studies investigating airway microbiome in patients with asthma using inhaled corticosteroids. Steroid-naive patients with asthma had a significantly higher proportion of Proteobacteria members (for example, *Haemophilus* and *Neisseria* spp) like patients with asthma using inhaled corticosteroids, which suggests that the association between Proteobacteria members and asthma is free of steroid treatment.

Asymptomatic nasopharyngeal bacterial colonization of the respiratory tract with *Streptococcus, Haemophilus,* or *Moraxella* has been associated with an increased risk of persistent wheezing in children, which suggests a potential role for nasopharyngeal microbiome in asthma pathogenesis [47]. Although the nasopharyngeal microbiome has been studied more frequently in childhood asthma, less is known about it and the factors associated with adult asthma. A study by Fazlollahi M et al. [48] showed differences in nasal microbiota composition among adult patients with exacerbated asthma, non-exacerbated asthma, and healthy controls. Taxa from Proteobacteria and Bacteroidetes were found enriched in subjects with exacerbated and non-exacerbated asthma relative to healthy controls. *Prevotella* (phylum Bacteroidetes), *Alkanindiges* (phylum Proteobacteria), and *Gardnerella* (phylum Actinobacteria) were more abundant in patients with exacerbated asthma, whereas *Dialister* (phylum Firmicutes) was significantly more abundant in patients with non-exacerbated asthma. Chronic rhinosinusitis with nasal polyps (CRSwNP) is closely associated with the development of asthma. Chalermwatanachai T et al. analyzed microbiota communities from 17 healthy individuals, 21 patients with CRSwNP and without asthma (CRSwNP-A), and 20 patients with CRSwNP and with co-morbid asthma (CRSwNP+A). *Propionibacterium acnes*, *S. aureus*, and *E. coli* were found to be significantly abundant in the healthy group, in the CRSwNP-A group, and in the CRSwNP+A group, respectively. At the species level, *Moraxella catarrhalis* was more prevalent and abundant in the CRSwNP+A group than the CRSwNP–A group. There was an association between Proteobacteria (such as *H. influenza, E.coli,* and *M. catarrhalis*) and CRSwNP disease, especially in patients with CRSwNP+A. Parallel results in upper and lower airway microbiota studies support the notion that the presence of *Moraxella catarrhalis* and *Haemophilus influenza* in the upper or lower airways seems to play a role in adult asthma. However, this is in contrast to the results of a study by Ramakrishnan VR et al. [49] in which Firmicutes, Proteobacteria, and Actinobacteria were detected in the middle nasal meatus of healthy subjects in the absence of chronic inflammation or antibiotic exposure.

Specific members of the airway microbiota may also be associated with different phenotypes of asthma, such as obesity-associated asthma, eosinophilic asthma, corticosteroid-responsive asthma, or Th_{17}-associated airway inflammation. A study by Huang YJ et al. [40] has demonstrated that Proteobacteria was associated with poor asthma control and sputum total leukocyte counts in patients with severe asthma. Body mass index (BMI) was found to be strongly correlated with Bacteroidetes and Firmicutes. TH_{17}-related genes were associated with Proteobacteria. Patients with asthma who had more severe symptoms had an increased number of Actinobacteria in their airways than healthy controls or patients with mild-to-moderate asthma [40]. A study by Goleva E et al. [39] investigated the influence of *Haemophilus parainfluenzae* on the expression of corticosteroid-regulated genes of BAL macrophages in an *in vitro* co-culture model. In this study, the presence of *H. parainfluenzae* resulted in inhibition of the steroid response. The study by Simpson JL et al. [50] demonstrated that adults with neutrophilic asthma had reduced bacterial diversity and species richness combined with a high prevalence of *H. influenza. Tropheryma whipplei* was identified as the predominant genera in patients with eosinophilic asthma. In another study of sputum microbiota in severe asthma, Bacteroidetes and Fusobacteria were found reduced in both non-severe and severe asthmatic groups compared with the healthy group, whereas Firmicutes was markedly increased in the severe asthmatic group. *Streptococcus* spp was correlated with asthma severity [51]. Millares L et al. [52] have assessed the bacterial composition of the bronchial mucosa in patients with severe chronic IgE-mediated asthma. Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria were the most abundant phyla, and *Prevotella* and *Streptococcus* spp were the most predominant genera. Genus *Legionella* was also detected in bronchial biopsies of these patients. The results of this study suggest that the increase of Proteobacteria is important in both neutrophilic and eosinophilic asthma phenotypes. However, microbiota show prominent differences at the species level. A recent study with a large study population confirmed this result demonstrating

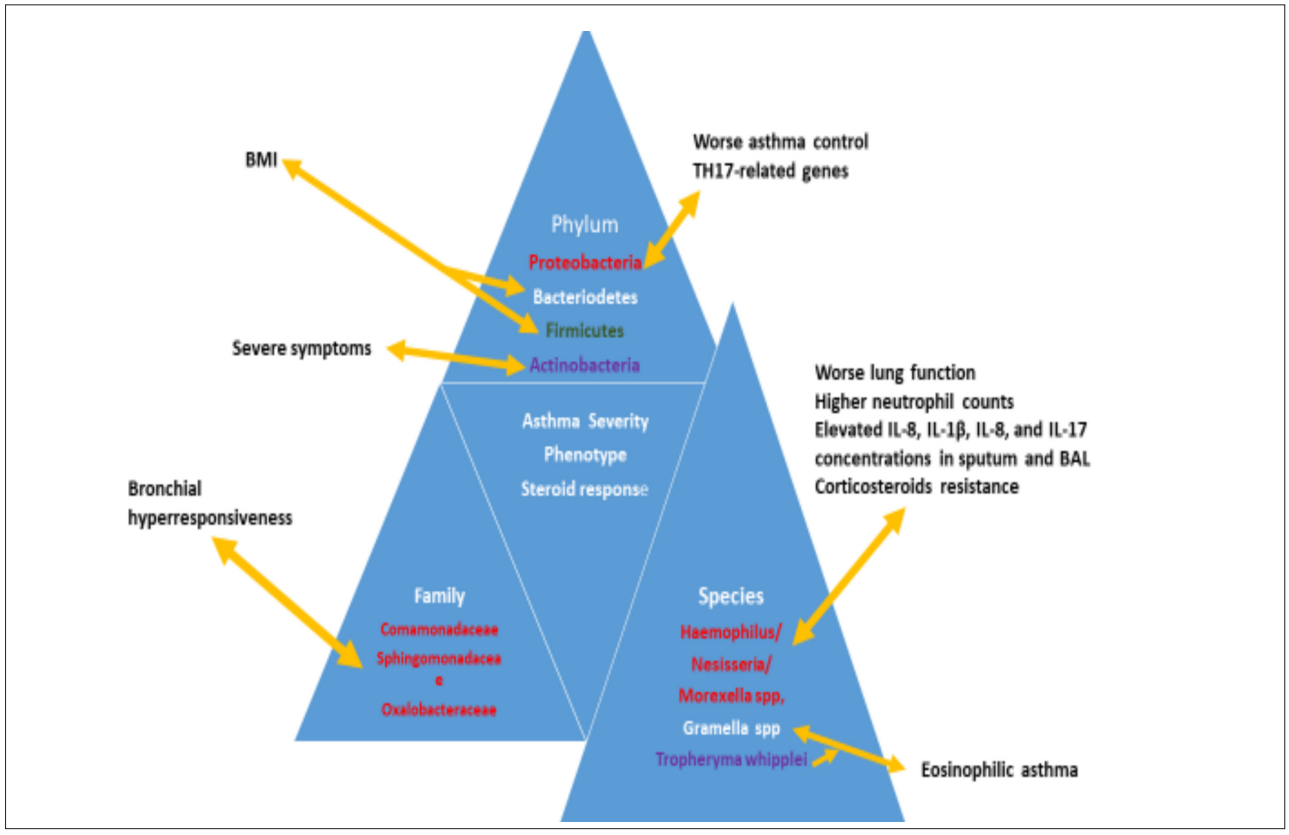

Figure 3. Network of associations between features of adult-onset asthma and airway microbiota members *The same colors represents the same bacterial taxonomy. Yellow is positive association in the network. BMI: Body mass index

that *Haemophilus* and *Moraxella* taxa were more prevalent in the neutrophilic asthma phenotype, whereas *Gemella, Streptococcus, and Neisseria* taxa were more prevalent in the eosinophilic phenotype [53].

Cigarette smoking can affect neutrophilic inflammation in asthma, and smoking-induced changes in the lung microbiome may be related to the neutrophilic asthma phenotype. The mouth microbiome differs in non-smokers and smokers, but smoking does not seem to change the bacterial composition of the lung in healthy smokers compared with non-smokers [54]. Munck C et al. [55] have randomized 44 patients with asthma, who were smokers, to receive either varenicline or a placebo for 12 weeks. Induced sputum samples were collected for microbiota analysis at 0 and 12 weeks. The cigarette smoking status of the patients was evaluated at weeks 0, 6, and 12. Induced sputum microbiome results of patients with asthma were compared with 20 healthy controls. Streptococcaceae and Spirochaetaceae were found to be significantly more abundant in the patients with asthma than in the controls. Of the 44 patients with asthma, 25 had quit cigarette smoking at week 12. However, the study did not reveal any significant difference in the changes in bacterial communities between patients with asthma who quit cigarette smoking and those who did not. Another study with the limitation of a small sample size also could not demonstrate any differences in bacterial communities of induced sputum between patients with asthma who were ex-smokers and non-smokers [42]. However, Simpson JL et al. showed that patients with asthma, who had smoked in the past, had a higher abundance of Actinobacteria and Bacteroidetes and a lower abundance of Proteobacteria in their induced sputum than never-smokers [50]. The network of associations between features of adult-onset asthma and airway microbiota members is shown in Figure 3.

The microbial flora differs between healthy individuals and patients with asthma. However, airway microbiome of adult and pediatric patients with asthma seems to be similar. Proteobacteria, including *Haemophilus*, *Moraxella,* and *Neisseria* species are the predominant bacteria in adult and pediatric patients with asthma [4]. Results of childhood asthma microbiome studies suggest that airway microbiota dysbiosis is associated with asthma development and risk of exacerbation, whereas adult asthma microbiome studies suggest that some airway bacterial communities may be associated with steroid resistance, disease severity, and the type of inflammation such as neutrophilic or eosinophilic in adult asthma [4]. Although Th2 inflammation is more specific to childhood asthma and airway microbiome, the neutrophil predominant pattern is dominant in adult asthma, which is associated with increased bacterial pathogen load such as *H. influenzae, S. aureus,* and *M. catarrhalis* [4]. Complex interactions between these bacterial species and atopy, cigarette smoking, and disease development and progress need further investigation.

The two major limitations in these studies that could be addressed by future research programs include the small-scale observational design of the recent studies and the 16S rRNA gene sequencing method used in most of the above studies, which does not allow for detailed functional assessment of the airway microbiome.

Table 1. Future research directions in airway microbiome

- Microbial ecology of various indoor and outdoor environment and their impact on airway microbiome needs to investiagted for a comprehensive understading of enviroment-microbes-pulmonary health crosstalk.
- A better understanding of other microorganisms such as virus and fungi and their impact on adult asthma is an imortant realm of investigation.
- Large-scale and population wide metagenomic studies are needed to assess the impact of specific microbial functions on human physiology and adult asthma.
- Large longitudinal observational studies in adult asthma with different severity to determine the relationship between airway microbial symbiosis and development or progression of lung or airway damage.
- A better understanding of the effect of medications used in the treatment of asthma, such as corticosteroids and antibiotics on the lung microbiota is required.
- Studies on microbiome based treatment strategies in prevention and in personalized medicine of asthma.

FUTURE RESEARCH DIRECTIONS AND CONCLUSION

Omics technologies and novel bioinformatics techniques have helped to understand the association between respiratory microbiome and asthma; however, the interaction between the microbial ligands and metabolites and the host immunity and their protective or causal role in childhood and adult asthma pathogenesis are still unanswered. We are at the beginning phase of defining the significance of airway microbiota in asthma development and course. Studies identifying the bacterial, viral, and fungal composition of healthy upper and lower airways and their relationship with asthma are warranted. Future studies must also account for standardization of collection and sequencing methods and data analysis, geographic and environmental diversity, and the inherent variability in respiratory microbiota. The effect of inhaled steroids and antibiotics on respiratory microbiota is not well established and should be studied rigorously. Evidence suggests a causal relationship between asthma and smoking, but how smoking affects the respiratory microbiome is not yet defined. Future large-scale cross-sectional studies will be crucial to characterize the functional effects of microbiota in different phenotypes of asthma, such as obesity-linked asthma, smoking-related asthma, and childhood/adult asthma. Longitudinal studies investigating the relationship between airway microbiota and asthma onset or asthma progress are also needed. The ultimate goals of these studies would be to answer the clinically relevant questions, such as which bacterial communities are important in disease progression or asthma development; can we predict the development or exacerbation of asthma by using a microbiome biomarker; when does airway microbiome modulation begin, prenatally or after birth; and can microbiome-mediated therapeutics or strategies shape asthma management. Future research directions in airway microbiome are summarized in Table 1. A better understanding of microbiome-driven pathophysiology will help develop novel preventive or curative strategies in asthma management and may lead to the discovery of microbial or immunological targets to control adult/childhood asthma in the future.

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