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The Respiratory Microbiome in COPD

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Abstract

In classical teaching, the lungs were thought of as a sterile environment with the isolation of bacteria on sputum or bronchoalveolar lavage sampling felt to represent pathogenic colonisation in disease states. This teaching has been over-turned with the discovery of a rich microbiome in the respiratory tract. The respiratory microbiome is a huge target for novel research in many fields, most notable in that of airway diseases such as asthma and chronic obstructive pulmonary disease (COPD). Next-generation sequencing is a culture-independent method for microbial sampling which has transformed the accuracy and speed at which whole microbial communities can be described in studies. This has led to an explosion of knowledge regarding the human respiratory microbiome. COPD is a common, chronic disease of the respiratory system involving an irreversible airway obstruction which places huge burden on patients and healthcare systems alike. The respiratory microbiome is different in those who suffer from COPD than in those without the disease, but little is known as to the role of the microbiome in disease pathogenesis or manifestation. This chapter aims to outline the advances in sequencing methods in relation to the microbiome and establish a description of the respiratory microbiome in health and in COPD. We will describe the existing literature on the topic and discuss potential key areas for future research.

Keywords: microbiome, immune system, infection

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a common, chronic respiratory disorder which affects millions of people worldwide [1]. It causes significant morbidity and mortality and poses a huge burden on healthcare systems through recurrent hospital admissions. Indeed, it was the fifth leading cause of death worldwide in 2002 and is estimated by the WHO

to become the third leading cause of death internationally by 2030 [2]. In essence, COPD is characterised by an obstructive ventilatory deficit that is rarely reversible. Pathogenesis of the disease is most strongly associated with smoking though, certainly, there are other factors which can contribute to its development. Despite the ever-increasing prevalence of COPD worldwide, the exact roles of various causative factors and underlying disease mechanisms are not fully understood.

One such potential causative factor is the human microbiome, the collection of all the genomes of microorganisms living in association with the human body both in health and disease. The microbiota is composed of bacteria, fungi, viruses, protozoa and archaea (single-cell organisms). Microbial cells in humans are estimated to outweigh human cells by a ratio of 10 to 1, and microbial genes outweigh human genes by a factor of between 100 and 1000 [3]. Therefore, unsurprisingly, the microbiome has been implicated in many diseases across varied body systems including arthritis, colorectal cancer and inflammatory bowel disease [4, 5]. However, more recently attention has been paid to the role of the human microbiome in chronic respiratory diseases such as asthma, cystic fibrosis and COPD.

This chapter aims to explore the meaning of the microbiome and how it came to be such a current and relevant topic for high-level investigative research in the area of respiratory disease. We aim to review the existing literature on the topic and particularly explore the relationship between the microbiome and COPD exacerbation in terms of both infective and non-infective events. We will discuss the perceived importance of the gut microbiome with reference to respiratory health and disease and pay particular attention to the so-called gut-lung axis. Finally, we will discuss some potential topics for novel research in the field of microbiome analysis and discuss future directions for research.

2. The human microbiome: technological advances

Recent advances in next-generation sequencing technologies have caused a quantum leap in the analysis of microbial species in various body systems. The cornerstone of these technologies involves extraction, replication, and identification of specific highly conserved genes which are reproducible across a whole host of microbes. Total DNA is extracted from the given sample type, e.g., bronchoalveolar lavage, tissue biopsy samples, etc., and specific genes are polymerase chain reaction (PCR)-amplified using universal PCR primers to create amplicons. For bacteria, DNA coding for the 16S rRNA gene can be amplified and contains both variable sequence regions V1–V9 and the aforementioned highly conserved region present in all prokaryotes. 18S rRNA/internal transcribed spacer (ITS) genes are amplified and assessed for fungi and protozoa. The 18S rRNA gene is the conserved gene across these species, and analysis can be coupled with variable internal transcribed spacer (ITS) regions in order to achieve interspecies separation. Amplicons are then analysed using next-generation sequencing which allows rapid, simultaneous analysis, with

sequences thus clustered into operational taxonomic units (OTUs). OTUs are then identified to the level of species, family or genus, by analysis against a reference database. In this way, microbial species can be rapidly and reliably identified in a culture-independent manner. Further to this, relative abundance of species in a given sample can be calculated to establish the richness and evenness of the microbiome in a system. This advance in technology has been responsible for the identification of the components of the gut microbiome in health, and much research on the microbiome of other systems has begun from this point [6].

There are a few key limitations involved in 16S rRNA and 18S rRNA/ITS gene sequencing methods for identification of bacterial and fungal species. Both techniques rely on the availability of highly specific, non-biased PCR primers as well as rich and varied reference libraries. The quality of PCR primers and references is generally held to be very high in the case of bacterial 16S rRNA sequencing [7] but less so in the case of fungal sequencing. Furthermore, little information is gained from both these methods about the biological function of these microbes. Despite this, they are considered to be cost-effective methods for assessing microbial diversity in a given sample.

Whole-genome shotgun sequencing is an advance on the above techniques whereby long DNA strands can be sequenced and identified rapidly. DNA strands are sheared into random fragments and cloned into a vector, historically *E. coli*. Clones are then sequenced to produce reads, and reads in turn are assembled into the original sequence using software programmes. This technique not only identifies microbes but can also infer information regarding biological function encoded in the whole genome. However, due to the massive amounts of complex DNA data processed, and the long reads that tend to be generated from this method, the system is open to errors. This is also a far more expensive method for DNA sequencing.

Next-generation sequencing employs similar methods to shotgun sequencing but generates hundreds of thousands of shorter reads in a smaller timeframe. This term is synonymous with high-throughput sequencing and encompasses multiple fast, effective, accurate sequencing methods. Some potential limitations include the presence of host DNA in the original sample, high costs of these methods and some difficulty drawing meaningful conclusions from vast quantities of extremely complex data.

Both 16S rRNA sequencing and next-generation sequencing are in use in laboratories worldwide, and both contribute to the advancement of our understanding of the microbiome through quick and accurate analysis of samples. As these technologies continue to advance and become more refined, they will become more affordable and sophisticated in coming years, allowing accessible DNA sequencing to come to the fore. Further analysis of RNA and proteins can be achieved through the techniques of metatranscriptomics and metaproteomics, and this can give us information about genes and pathways in the context of microbiome sampling. See **Table 1** for a summary of terms relating to the various DNA sequencing techniques we have discussed above.

Term	Description
Microbiota	The collection of all microorganisms living in association with the human body or a specific system/environment
Microbiome	The genome of the human microbiota
Taxonomy	Classification system of organisms
Operational taxonomic unit	Categorisation of organisms into species by DNA sequence data
Metagenome	The collection of all genes obtained from microorganisms in a habitat
Next-generation sequencing	A collective term to describe high-throughput technologies. These allow rapid parallel DNA sequencing, i.e. Illumina (Solexa) sequencing, SOLiD sequencing
16S ribosomal gene	Gene encoding a 16S subunit of bacterial ribosomes that is highly conserved between different bacterial species
16SrRNA gene sequencing	Determining the DNA sequence of a 16S ribosomal gene
Metatranscriptomics	Analysis of mRNA transcripts associated with an organism
Metaproteomics	Analysis of microbial proteins
Metabolomics	Analysis of the set of metabolites present within an organism
Richness	The number of unique taxonomic units in a sample
Evenness	The relative number of taxonomic units in a sample
Alpha diversity	A measure of the richness and evenness in a sample
Beta diversity	A measure of the similarity of the bacterial composition between samples

Table 1. Summary of terms relating to DNA sequencing techniques.

3. The respiratory microbiome

For a long time, the respiratory system was thought of as a fully sterile environment. Culture isolates from sputum and bronchoalveolar lavage samples were felt to exclusively represent pathogenic colonisation in disease states and exacerbation. However, this classic teaching was challenged, only in 2009, with the publication of a study undertaken by Hilty et al. [8] which employed 16S rRNA sequencing techniques to analyse the microbiota of the lower respiratory tract. Subjects carried either a diagnosis of asthma or COPD or were healthy controls, and all underwent bronchoscopy to obtain endobronchial brushings from the left upper lobe. The microbiome of the nasal cavity and oropharynx was also sampled using surface swabs. This study found a similar abundance of microbes in all groups (with a range of 1–10 million cells per sample) but with significant differences in microbial composition between the groups studied. This was the first study of its kind and was understandably met with scepticism as it challenged the formal teaching on the subject [9]. Critics were quick to point out limitations of the study design including the fact that lower airway sampling could easily be confounded by passage of the bronchoscope through the upper airways, the oropharynx having a significantly higher bacterial load than the lower respiratory tract in the order of 1–10 billion cells

per sample. Additionally, it was felt that the relatively new (at the time) process of 16S rRNA sequencing would identify dead bacteria in most of the samples and that in order to address this potential confounder, a stronger control group was needed. Nevertheless this was an interesting and well-designed starting point for the explosion of interest in the area that was to follow.

A follow-up study which was similar in design and conducted in 'healthy smokers', and patients with COPD confirmed the presence of microbial colonies in the lower respiratory tract but again was open to the aforementioned potential confounders [10]. These confounders were first addressed by Charlson et al. [9] in 2011 who designed a study using double bronchoscopy in six healthy individuals. This involved a process of initial bronchoscopy to anaesthetise the airway and then the use of an uncontaminated bronchoscope to collect samples by brush and lavage. To further reduce the confounding potential of the environment on the scope, saline washes were used before the scope was inserted, and this saline was analysed for bacterial load. Microbiota was found to be present in all lower respiratory samples from all patients, but unlike the previously mentioned studies, the order of magnitude was far lower, 100–1000 cells per sample. The investigators performed serial lavage, results of which showed sequentially diminishing bacterial load. They inferred from this that there is marked carry-over from the upper to the lower respiratory tracts. An interesting finding was that low levels of DNA were found in the environmental control samples such as the saline washes even though these were sterile by culture. Bacterial lines were found to be more abundant in the lower airway than the upper, though overall microbiome was more rich in the upper airway.

The Hilty trial found that the bronchial tree consists of a rich and varied microbial community in all groups tested and confirmed that the individual components of this community were different in health and disease [8]. *Proteobacteria* were found in higher abundance in diseased individuals (those with asthma and COPD) than in controls, in particular the well-known respiratory pathogens *Haemophilus*, *Moraxella* and *Neisseria*. In contrast, *Bacteroidetes* such as *Prevotella* were found to be far more abundant in healthy controls. Studies since this have aimed to further refine our understanding of the now-accepted paradigm of the core respiratory microbiome. Various studies have identified varying counts of microbes, and it is generally acknowledged that the respiratory microbiome is composed of *Bacteroides*, *Proteobacteriae*, *Firmicutes* and *Actinobacteriae*. The bacterial communities of the lung are almost mirrored in the oral cavity, but not in the nose, and some species that show high prevalence in the mouth become less abundant lower in the lungs. This has led some to believe that oral microbes which migrate to the lower respiratory tract can be selectively eliminated from healthy lungs and that those which are not eliminated may contribute to low-level inflammatory processes [11].

These studies and numerous similar trials [12, 13] have established the existence of the respiratory microbiome. As technology improves, our ability to sample and analyse these complex microbial communities will continue to expand until we have a full understanding of not only the composition but also the function of the human respiratory microbiome.

4. Establishment of the microbiome and the relationship between microbiota and the immune system

We have discussed the basis of our understanding thus far of the existence of the microbiome in the lung and will go on to explore the role of the microbiome in health and disease. However we must first try to decipher the way in which the microbiome is established in humans in order to assess whether differences in development may confer lasting effects on the microbiome in later life. It has long been believed that exposure to various bacteria in youth may alter the way disease manifests in later life. The so-called hygiene hypothesis states that reduced exposure to microorganisms in infancy increases a person's likelihood to develop allergic-type diseases by suppressing the natural development of the immune system, particularly the innate immune response. It would therefore follow that interruptions in the natural development of the microbiome could confer long-lasting differences in the respiratory microbiome and that this may have knock-on effects to an individual's immunity and their propensity to develop certain diseases. Much of what is known about the development of the microbiome comes from studies of gut microbiota, and we will explore some of this literature in this section in order to assess potential links between the microbiome and immune system dysfunction.

Gut microbiota has an established role in the development and maturation of the immune response via the so-called education of mucosal surfaces and systemic immune response systems. Studies suggest that there is a critical development time between months 12 and 24 in the relationship between the gut microbiome and the building of the immune system [14]. This is demonstrated by the increased risk of the development of inflammatory autoimmune disease where dysbiosis is caused by the use of antibiotics at a critical time in infancy. For example, increased rates of immunologic disorders such as asthma and paediatric irritable bowel disease are associated with antibiotic use in infancy [15].

Traditionally, the human gut has been assumed to be sterile at birth. However, recent advances in PCR techniques as outlined above have found evidence of bacteria consistent with the maternal gut microbiota in meconium. This is believed to have been transferred via the maternal blood stream [16]. Further exposure is from diet and the surrounding environment, including the birth canal. Indeed, even the mode of delivery at birth can confer lasting changes on the developing microbiome, with those born via caesarean section found to have significantly lower abundance and diversity of gut *Bacteroides* and *Actinobacteria* species in the first 3 months of life [17]. At age 12 and 24 months, the infant gut resembles that of the adult [18]. The gut microbiome is relatively stable until late in life and has the ability to restore itself [19].

Evidence supports reduced childhood morbidity and mortality not only during the period of lactation but also beyond this period and into adulthood [20]. Facultative anaerobes create an anaerobic environment where obligate anaerobic bacteria such as *Bifidobacterium* species flourish. *Bifidobacteria* dominate the gut microbiota, and milk oligosaccharides are known to stimulate their proliferation [16]. Along with *Lactobacilli* they appear to maintain resistance to pathogenic colonisation, in short acting as so-termed good bugs. *Bifidobacteria* are rarely pathogenic and ferment bacteria to produce short-chain fatty acids (SCFA) [16].

SCFAs are thought to have many functions including important interactions with the immune system. Two mechanisms of action for this relationship have been established to

date, activation of G-protein-coupled receptors (GPR41 and GPR43) and inhibition of histone deacetylase (HDAC) via leukocytes and endothelial cell lines. SCFAs also have a regulatory role over leukocyte functions such as production of cytokines (TNF- α , IL-2, IL-6 and IL-10), eicosanoids and chemokines. In addition, SCFAs also seem to impact the migratory ability of leukocytes to travel to points of inflammation and to destroy microbial pathogens [21].

Intestinal epithelial cells are a key component of the symbiotic relationship between gut microbiota and the host. They provide a physical and chemical barrier system to spatially separate gut microbiota from the host immune system, preventing unnecessary immune responses. Immunological mediators such as cytokines and chemokines are secreted from intestinal epithelial cells which are in turn stimulated by gut microbiota-modulated host immune responses, maintaining a well-balanced relationship between gut microbes and the host immune system [22]. Gut microbiota also have a role in the maintenance of innate immunity. For example, murein lipoprotein from selective gut-symbiotic Gram-negative bacteria results in IgG targeting bacterial antigens for removal by phagocytes [23].

In addition, gut microbiota plays an important role in the development of both local and remote adaptive immunities as demonstrated by the work with germ-free (GF) mice which had significantly lower numbers of CD4+ cells. In other animal model research, the spleens and mesenteric lymph nodes of GF animals were shown to have abnormalities such as absent lymphocyte zones. Th1/Th2 imbalances have also been demonstrated in GF animal models [24].

New techniques have allowed even specific bacterial species to be associated with the development of particular T-cell subtypes. For example, *Bacteroides fragilis* is involved in the induction of the development of a systemic Th1 response via its polysaccharide A (PSA) molecules [21].

There seems to be emerging evidence to suggest a significant relationship between the gut microbiome and the respiratory microbiome proposing the existence of a 'lung-gut axis'. When depletion of gut microbiota is achieved in mouse models, the mice are prone to developing significant pneumonia, and when the gut microbiome is restored, the severity of pneumonia decreases [25]. Additionally, this relationship is strengthened by the observation that acute changes in the gut microbiota can be achieved by stimulation of the lung with lipopolysaccharide [26]. These results indicate the potential for 'cross-talk' between the gut and the lung and introduce the concept of a 'whole body microbiome', dysregulation of which can lead to disease in other organ systems.

As we can see, mostly from gut microbiome research trials, there is significant interplay between the microbial communities in the gut and the immune system, both adaptive and innate immune responses. The microbiome is established in a well-described manner but can certainly be greatly influenced by diet, microbial exposures, and even mode of delivery at birth. It is reasonable to expect the same interplay to be a feature of the respiratory microbiome, but further work is needed to establish this relationship.

5. The microbiome in stable COPD

We have explored the current knowledge of the respiratory microbiome in health and the role it plays in inflammatory processes; however, we will now discuss what is known about

the role of the respiratory microbiome in relation to COPD. *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* are known to be the most abundant colonisers in the lungs of COPD patients [27]. Consequently, they are the most frequently isolated pathogens in sputum samples and bronchoalveolar lavage in this patient group, both in health and disease. These microbes remain prominent across multiple studies of the COPD lung and are implicated in driving multiple inflammatory pathways in the diseased lung [28, 29]. This certainly has many profound clinical effects on patient groups with COPD. For example, a recent study found that patients who were chronically colonised with *H. influenzae* were more likely to have increased airway inflammation and decreased lung volumes when compared with those not colonised [30].

In healthy lungs, spatial and temporal microbial diversity in the same lung is less than the diversity seen between individuals and seems to be mostly a consequence of microbial elimination and immigration. The reverse is true in diseased lungs meaning there is wide variation in microbial communities in a given patient's lungs [31]. This is particularly true of COPD lungs where very significant temporal and biogeographical variations in diversity and abundance have been seen [32]. Microbial communities are felt to be drastically affected by local growth conditions in disease lung. The factors which can affect growth conditions are oxygen tension, pH, relative number of immune cells present in a given area and even blood perfusion. The microbiome has been linked to the clinical phenotype of COPD by one group who concluded that greater emphysema and increased immune cell infiltration were found in those COPD patients who showed decline in the diversity and richness of their respiratory microbiome [12]. Indeed more recently, correlation was shown between altered bacterial communities as seen in COPD with CT-detectable structural changes in lungs of those COPD patients [33].

Reactive oxygen species are postulated to drive the inflammatory processes which lead to COPD through activated alveolar macrophages. Bacterial activation of certain NOD-like receptors can upregulate the production of these reactive oxygen species in alveolar macrophages. Bacterial activation such as this is known to occur in the GI tract, and this potentially implicates the GI microbiota in the development of COPD [34]. If gut microbiome can potentially be implicated in driving respiratory inflammation, it is reasonable to wonder whether the use of certain probiotic bacterial strains could help to reduced lung-driven inflammatory processes. One group has shown favourable outcomes with regard to this hypothesis and has found that when alveolar macrophages phagocytose the well-known probiotic strains *Lactobacillus rhamnosus* and *Bifidobacterium breve*, certain inflammatory pathways are suppressed. In particular their use inhibits cigarette smoke-induced nuclear factor- κ B activation and the inflammation associated with this pathway [35].

6. The impact of the microbiome on infection and exacerbation

As we have discussed, there is an increasing wealth of evidence indicating the vital role that the lung microbiome plays in chronic obstructive pulmonary disease (COPD); however, our understanding of the exact role the lung microbiome plays in COPD exacerbation remains in

its infancy. While our understanding of the microbiome in humans has vastly expanded in recent years, there is still a significant dearth of certainty regarding the dynamics of the lung microbiome and its role in disease aetiology. Ambiguity arises from the heterogeneous nature of COPD coupled with the diversity and structure of the microbiome, the mediation of host inflammatory response and additional external variables such as involvement of antibiotic and steroid treatments and environmental factors.

Recent literature contributions have highlighted the central and crucial role of bacteria in COPD exacerbations with evidence to suggest that during exacerbations there is a shift in microbial diversity within the ecosystem. [36]. An increase in the relative abundance of *Proteobacteria* and a decrease in *Firmicutes* compared to non-exacerbation samples have been observed. Interestingly, there is an increase in *Moraxella* and *Haemophilus* populations during exacerbations with a concurrent decrease in *Streptococcus* identified. These shifts in taxa composition are in keeping with the findings of Wang et al. [37] who found an overall reduction in alpha diversity, the microbial diversity within a sample, during exacerbations when compared with 'stable' samples.

Network analysis has revealed potential interactions between bacterial operational taxonomic units (OTUs). Microbial network examination has revealed that a few 'hub' OTUs predominate which are highly connected with multiple other nodes of microbiota. Moreover, many of the OTUs demonstrate both 'coexistence' and 'co-exclusion' patterns between bacterial species. Most OTUs have numerous negative connections with other members of the microbiota. In particular, *Haemophilus* has a disproportionately large number of negative connections with other OTUs [38]. Consequently, any significant increase in abundance of OTUs is associated with a decrease in microbial alpha diversity. Conversely many OTUs demonstrate a strong mutual cooperative relationship in tightly connected groups. Indeed, a 'Like Will to Like' relationship of enrichment promotion among organisms of different species in an ecosystem has been well described for intestinal microbiota [39], whereby closely related phylotypes display significantly increased abundances or co-occurrences when compared with those less closely related species.

In the context of the lung microbiome, the keystone species is a topic that warrants further exploration. This inchoate paradigm, which expounds the concept describing how minimal fluctuations in the abundances of relatively few bacterial species may profoundly alter the microbial community dynamic and potentially impact the human disease state [37]. For example, *Actinobacteria*, a species whose abundance decreases during COPD exacerbations, comprises a multitude of metabolically diverse organisms which produce secondary metabolites, including those with antimicrobial activity [40]. Moreover, many *Clostridia* species are known activators of anti-inflammatory T-regulatory cells [41].

Following on, it is possible that the overgrowth of certain OTUs may drive dysbiosis of the respiratory tract through remodelling of the normal lung microbial ecosystem which could elicit a host pro-inflammatory response [37]. Through dysbiosis of the lung microbiota, pathogenic members of the genera *Haemophilus* and *Moraxella* can indirectly trigger excessive production of chemokine ligand 8/interleukin 8 (CXCL8/IL-8). This is due to pathogenic *Haemophilus* and *Moraxella* members directly causing inflammation by exposing the host to

lipopolysaccharides and other pathogen-associated molecular patterns [41]. This is coupled with depletion of species that might in fact contribute to the maintenance of community and host immune homeostasis and fight the negative effects of the above pathogenic species.

As a corollary, one potential biomarker for overall microbial lung population is sputum CXCL8/IL-8, which is directly associated with microbiome community structure and diversity. It has a prominent role in COPD [42] whereby it induces airway inflammation by recruiting neutrophils and upregulating mucin genes, causing mucus production. As such, sputum CXCL8/IL-8 levels correlate well with COPD severity [43].

Given the heterogeneous nature of COPD, identification of various phenotypes has helped in identifying the most suitable and specific treatment for exacerbations. The microbiome exacerbation phenotypes have been defined as bacterial, viral, eosinophilic, bacterial/viral combination, bacterial/eosinophilic combination or pauci-inflammatory [36]. There is a significant difference in microbiome composition at both the phylum and genus levels between different phenotypes but particularly between bacterial and eosinophilic exacerbations. Furthermore, there is a significant decrease in the *Proteobacteria/Firmicutes* ratio in the eosinophilic subgroup, compared to the other phenotypes, during exacerbations. This correlates with the hypothesis that exacerbations involving bacteria and eosinophils reflect fundamental differences in their immunopathogenesis, while virus-related exacerbations are often associated with both bacterial infections and an increase of eosinophils [36]. Consequently, the presence or absence of eosinophilic inflammation could therefore potentially be used as a biomarker for stratification of the underlying associated microbiome and subsequent treatment strategies.

With regard to COPD exacerbation treatment strategies, it has become apparent that 'standard-of-care' therapy involving antibiotics and steroids has a significant effect on the phylogenetic composition of the community. Alterations in the abundance of individual taxa are directly linked to the use of steroids and/or antibiotics. Indeed, the use of oral corticosteroids has been found to decrease microbial alpha diversity, with an enrichment of some taxa, mainly *Proteobacteria*, and an increase in the *Proteobacteria/Firmicutes* ratio has been identified. In contrast, a reversal of this trend, i.e. an increase in microbial alpha diversity and microbial composition changes, has been observed when treatment of COPD exacerbation involves antibiotics (with or without steroids) [37, 43]. Furthermore, this effect of antibiotic and steroid treatment on the microbiome structure appears to be prolonged, emphasising the importance of patient phenotype stratification.

7. A functional analysis of COPD airway microbiome

A recently developed bioinformatics tool, PICRUSt [41, 44], has enabled researchers to explore the predicted functional capacity of the microbiota involved in the changes observed during exacerbation and response to treatment. Hitherto, high-resolution metagenomic profiling has been almost prohibitively expensive and intensive computationally. PICRUSt can predict the presence and relative abundance of gene families within the microbiome. This prediction is based on 16S rRNA data and can be used at any time point or, in this case, during an

exacerbation. The intention is to identify and characterise variations in microbiome differential associated with features of exacerbations. Predicted functions that are enriched during exacerbation include pathways involved in apoptosis, p53 signalling, along with those involved in bacterial and viral infection, e.g. *Influenza A* virus and *Staphylococcus aureus*. On the other hand, depleted predicted functions include those pathways involved in the response to viral infection, e.g. RIG-I-like receptor signalling. Other known pathways depleted during COPD exacerbation include those involved in the production of betalain and indole alkaloid—known for their antimicrobial properties—and those involved in flavonoid and steroid biosynthesis, known anti-inflammatory mediators [41]. In keeping with this hypothesis, a reversal of the above compositional shifts in the microbiome can be observed during treatment and indeed may play a role in the recovery from exacerbation.

Observations suggest that while variation between test subjects in microbial community dynamics at exacerbation is seen, the compositional shifts overall for a particular microbiome are not very large. These findings suggest that exacerbations of COPD could result from the cumulative effects of many small-scale changes in the community composition. Hence, these functional predictions may describe not only an increase in pathogen-elicited inflammation but also the loss of crucial protective functions in the microbiome.

8. Conclusions and areas for future research

Throughout this chapter, we have outlined the relatively new field of microbiome analysis in respiratory disease. The lungs were once thought to be sterile, and since this presumption was first overturned in 2009, there has been a veritable explosion in the wealth of knowledge surrounding the topic. We are now beginning to understand the vital role which the microbiome plays in the development and propagation of many major respiratory diseases. This increased knowledge has been made possible by significant advances in culture-independent sequencing methods which we have outlined. As these intricate systems continue to develop and improve, our ability to analyse microbial samples will continue to strengthen with these techniques becoming more and more widespread and accessible.

Much is known about the gut microbiome, with respiratory microbiome research also on the increase. We have outlined some emerging evidence to suggest interplay between these two large micro-systems, and the hypothesis that they can mutually influence each other in health and disease is a fascinating concept. Certainly, more work needs to be done in this area through observational human research, as most of the literature involves animal models.

We now are in the position to not only name the components of a given microbiome but also to analyse their functions through next-generation metagenomics sequencing. It is not enough to know that the microbiome of the lung is altered in COPD; we must follow on to assess the function of these different bacteria and viruses in causing or preventing disease. If we can manage to do this in a meaningful and reproducible way for certain diseases such as asthma and COPD, then it is reasonable to assume that we can develop novel therapeutic targets for these chronic disease states which may alleviate the treatment burden for our patients.

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