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REVIEW ARTICLE

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The airway microbiome in COPD, bronchiectasis and bronchiectasis-COPD overlap

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Abstract

Objective: To review the airway microbiome in chronic obstructive pulmonary disease (COPD), bronchiectasis and bronchiectasis-COPD overlap (BCO).

Data source and study selection: Relevant studies were selected from PubMed, Google scholar, EMBASE and Web of Science. All studies involving human microbiomes, published in the English language, and using the search terms "COPD", "Chronic Obstructive Pulmonary Disease", "Bronchiectasis", "BCO" or "Bronchiectasis and COPD overlap", AND "microbiome", "mycobiome" or "metagenomics" were included.

Results: Despite variability in sampling methods and specimen types used, microbiome composition remains relatively comparable in COPD and bronchiectasis with prominence of Proteobacteria, Firmicutes and Bacteroidetes. Alterations to airway microbiomes occur in association to disease severity and/or exacerbations in COPD and bronchiectasis. Decreased alpha diversity and *Haemophilus*-predominant microbiomes are associated with poorer survival in COPD, while, in bronchiectasis, *Pseudomonas*-predominant microbiomes demonstrate high exacerbation frequency and greater symptom burden while *Aspergillus*-dominant mycobiome profiles associate with exacerbations. The role of the microbiome in BCO remains understudied.

Conclusion: Use of next-generation sequencing has revolutionised our detection and understanding of the airway microbiome in chronic respiratory diseases such as COPD and bronchiectasis. Targeted amplicon sequencing reveals important associations between the respiratory microbiome and disease outcome while metagenomics may elucidate functional pathways. How best to apply this information into patient care, monitoring and treatment, however, remains challenging and necessitates further study.

KEYWORDS

BCO, bronchiectasis, COPD, metagenomics, microbiome, overlap

1 | INTRODUCTION

Advances in sequencing technologies has enabled the identification of unique genetic signatures of microbiota in the airway.[1-4] Airway microbiota play a crucial role in the development, progression and exacerbation of lung disease.[4] The characteristic airway microbiomes detected in patients with chronic respiratory diseases including chronic obstructive pulmonary disease (COPD) and bronchiectasis are altered when compared to non-diseased (healthy) individuals.[1]

This is demonstrated in the upper and lower respiratory tract with minor differences.[2,5] Measurable bacterial and fungal colonisation are frequent in COPD and bronchiectasis, and identifiable by conventional culture, however, these lack sensitivity.[6] Use of culture independent sequencing overcomes this and reveals the resident airway microbiome communities and their interaction in health and disease. This current review discusses the existing evidence-base for microbiome profiling and its translational relevance to disease phenotyping in COPD, bronchiectasis and bronchiectasis-COPD overlap (BCO).

1.1 | Objective

To review available evidence including composition, diversity and clinical translatability of the airway microbiome in COPD, bronchiectasis and BCO considering different sample types and methodologies used including in relation to clinical outcomes.

1.2 | Data source and study selection

PubMed, Google scholar, EMBASE and Web of Science were searched using the keywords "COPD", "Chronic Obstructive Pulmonary Disease", "Bronchiectasis", "BCO" or "Bronchiectasis and COPD overlap", AND "microbiome", "mycobiome" or "metagenomics". Only human microbiome studies published in the English language were included.

2 | RESULTS

2.1 | Specimen type and microbiome composition in COPD

Oropharyngeal swabs and washes are commonly used across multiples studies to characterise oral microbiomes. In COPD, the dominant oral microbiota include Bacteroidetes, Proteobacteria, Firmicutes, Fusobacteria and Ascomycota phyla, and the genera *Veillonella, Haemophilus, Streptococcus, Neisseria, Prevotella, Lactobacillus, Pseudomonas* and *Rothia*.[7-11] In contrast to sputum, the COPD oral bacteriome exhibits higher diversity, but is minimally different when diversity and composition of the fungal microbiome (the mycobiome) is considered.[10,12] In comparison to healthy individuals, increased *Veillonella, Pseudomonas* and *Lactobacillus* are reported in COPD, indicating potential relevance in pathogenesis and clinical course.[8,11]

Sputum is widely used to assess the airway microbiome, due to accessibility, 'low-risk' procedures for its collection and non-invasiveness. Reduced microbiome diversity in sputum with resultant overgrowth of specific bacterial taxa are observed in COPD.[13] In addition, diversity is further reduced as disease progresses, and is also reported during acute exacerbations (AECOPD).[13-17] The dominant microbial phyla in stable COPD include Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria while predominant genera include *Haemophilus*, *Moraxella*, *Streptococcus, Veillonella, Pseudomonas* and *Prevotella*.[2,16,18] Important microbial alteration occurs with COPD treatments, including during and following AECOPD. Long-term inhaled corticosteroid (ICS) use increases Proteobacteria *(Haemophilus* and *Streptococcus*) particularly in patients with low (<2%) blood eosinophils, while an increased Bacteroidetes (*Prevotella)* is observed in patients using monotherapy with long acting beta-agonists (LABAs).[19] During AECOPD, significant inter-individual variation in microbiomes is reported with increases in Proteobacteria *(Haemophilus, Moraxella)* and decreases in Firmicutes *(Veillonella)* and Bacteroides *(Prevotella)*.[13,14,18,20,21] In addition, a small single centre study reports predominance of Ascomycota and the genera *Candida, Phialosimplex, Aspergillus*, *Penicillium*, *Cladosporium* and *Eutypella* during AECOPD although further research is necessary to confirm this.[22] Microbial dysbiosis between baseline and exacerbation events is critical and influenced by exacerbation symptoms (CAT score) and severity.[20] Three specific COPD exacerbation phenotypes are described: bacterial, viral and eosinophilic, each with variation in microbiome composition.[14,23,24] Higher Proteobacterial load in bacterial and viral-induced exacerbations is further accompanied by increases in sputum production, systemic and airway neutrophils, airway neutrophil extracellular traps (NETs) and pro-inflammatory cytokines.[20,24-27] In contrast, eosinophilic exacerbations associate with increased Firmicutes and/or Bacteroidetes.[20,28] Treatments administered during AECOPD further influences the microbiome: decreased Proteobacteria with an increased overall diversity is observed following antibiotics while an increased Proteobacteria with reduced diversity is associated with systemic corticosteroid treatment.[28,29] Interestingly, such treatment-associated effects persist until full recovery. Taken together, these data suggest the microbiome is intimately linked to COPD pathogenesis and with exacerbations, however, is also potentially modifiable by existing COPD therapies which can alter disease trajectory in individual patients.

Some advocate for bronchoscopy-derived sampling (including bronchoalveolar lavage (BAL) and biopsies) as better representative of the lower airway, however, no universally employed standardised sampling methodologies are in widespread use. Studies using bronchoscopic-derived specimens have employed different practices between centres, and, with inherent sample variation (upper, middle or lower lobe), site of bronchoscope introduction (nasal vs. oral), bronchoscopic technique (protected brush, bronchial washing and/

or BAL) and contamination controls (sheathed brush, lack of suction prior to wedging, saline equipment controls, two bronchoscope procedures and/or supraglottic washes).[2,5,30-34] As a more invasive procedure, use of bronchoscopy is more challenging to apply in longitudinal and large-scale studies as repetitive sampling during times of clinical instability (eg, AECOPD) are often required. Nevertheless, the predominant bacteria observed using such specimens are comparable to those in sputum, highlighting the usefulness of the latter.[2,5,31,32,34,35] As observed with sputum studies, increased Proteobacteria including *Haemophilus* and *Pseudomonas* with decreased Bacteroidetes (*Prevotella*) are reported in COPD when bronchial brushings and BAL is used.[2,32] Decreased alpha-diversity with increased disease severity is also observed with invasive specimens, again comparable to that reported in sputum.[31,32]

Lung tissue is infrequently used in COPD microbiome studies and restricted to lung transplant cohorts with severe COPD. Comparing severe COPD with healthy controls, Sze et al (2015) report increases in Proteobacteria and Actinobacteria with a reduced diversity that correlates with emphysematous destruction.[36] Importantly, the dominant phyla mirrored those found in sputum, BAL and brushings.[36,37] Studies employing lung tissue also illustrates that an altered COPD microbiome modulates host immunity with observed increases in neutrophil, eosinophil and B-cell infiltration.[36] Significantly, microbiome composition differs between sampled lung regions, even within the same host, most evident in severe COPD. This suggests that consideration of anatomical influence during sampling should be accounted for when interpreting airway bacterial communities in COPD.[35]

Despite detectable variation in the COPD microbiome with different sampling strategies, the most dominant taxa remain comparable[9,10] (Figure 1). Stable COPD is characterised by increased Proteobacteria and reduced Bacteroidetes compared with healthy controls, with further alteration during AECOPD and following treatment. Contrasting bacterial diversity between sampling sources is best exemplified by Pragman et al (2018) and Cabrera-Rubio et al (2012) which illustrate a reduced oral and airway microbial diversity compared to the bronchial mucosa and BAL. Importantly, however, Mika et al (2018) report a lack of such difference when protected brush specimens obtained from different parts of the airway (including the pharynx, trachea, main and lobar bronchi) were compared.[5,9,31] Differing sampling techniques, size and location may account for the observed variation. While bronchoscopic sampling likely best characterises the lower airway, its invasive nature precludes widespread use and accessibility for large-scale and longitudinal studies. Sputum, however, has been widely employed for COPD microbiome assessment despite concerns about oral 'contamination'. Sputum does appear to be a good airway surrogate, if appropriately sampled, sometimes induced, and acts as a clinically relevant, cost-effective and non-invasive alternate to bronchoscopy.[38]

Contamination may also be encountered during the microbiome sequencing process, largely attributed to the sensitivity associated with sequencing. Contamination can occur at any step or be introduced by the researcher, environment, sample crossover, DNA extraction kits or various reagents used.[39,40] Faulty aseptic precautions, contaminated reagents, differences in polymerase chain reaction (PCR) and library

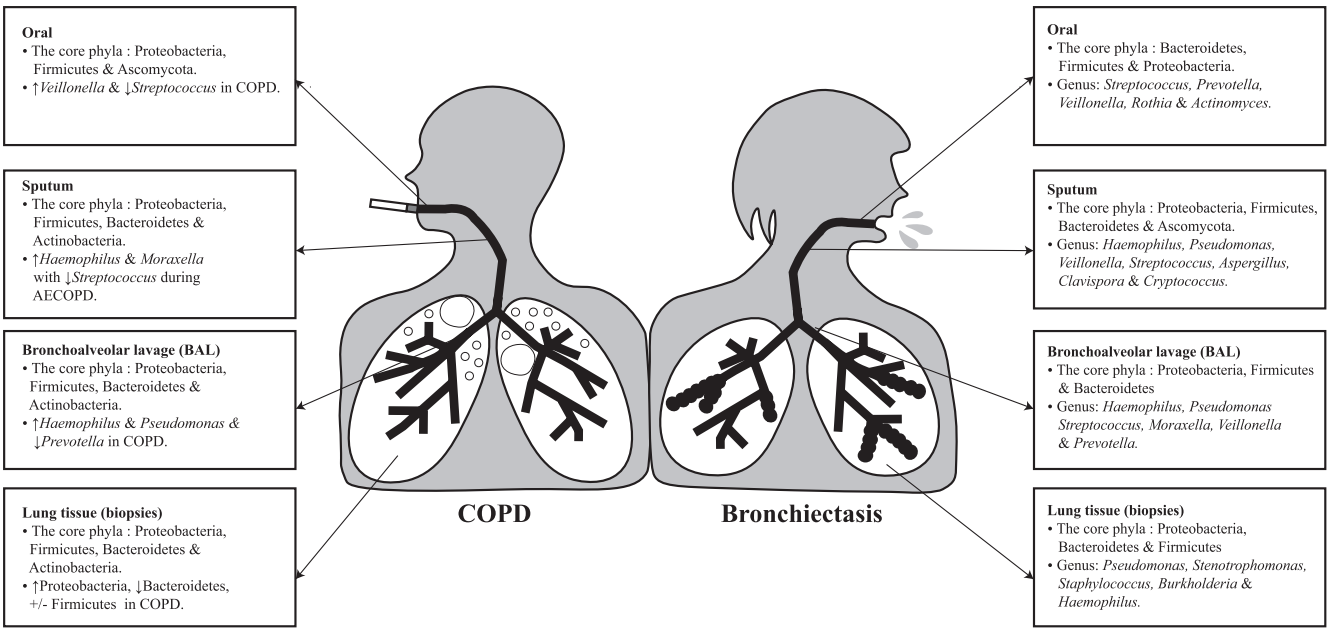

FIGURE 1 Microbiome composition using different sampling approaches in chronic obstructive pulmonary disease (COPD) and bronchiectasis. ↑, increased; ↓, decreased; AECOPD, acute exacerbation of COPD

preparation protocols are all common sources of undesirable background signals.[41] It is therefore imperative that adequate controls (both positive and negative) are incorporated into study design and reported by publication.[42] Extra care must be taken particularly with low-biomass samples as contamination can form the large proportion of detected microbial loads.[42] While no standardised method of assessing, analysing and/or interpreting control samples is routine, they must be included in experimental design and be accounted for in data analysis.[43]

2.2 | The microbiome and clinical outcomes in COPD

Despite an increasing number of studies over the past decade, the precise role of the microbiome in COPD pathogenesis remains unclear. To best exploit and translate microbiome findings from COPD into clinical practice, a more detailed understanding of its precise role in COPD phenotypes and outcomes are required.

An AECOPD represents a heterogeneous event with multiple aetiologies and varied clinical presentation including types and extent of airway inflammation. This results in variable responses in individual patients to guideline-directed treatment. Currently, sputum culture is used to guide antibiotic treatment decisions in AECOPD, despite its limitation in differentiating acute infection from chronic colonisation. Sputum culture does importantly correlate with dominant taxa identified by microbiome sequencing, however, sequencing provides additional information on diversity, composition and variability which importantly are associated with clinical outcomes.[32,44] Of note however, the detection of an organism by sequencing alone cannot confirm its viability. Millares et al (2014) assessed the sputum microbiome in COPD and compared *Pseudomonas* colonised and non-colonised patients.[44] Interestingly, microbiome composition was comparable with the exception of the expected higher *Pseudomonas* genus abundance in the colonised group.[44] During AECOPD, however, microbiome composition was similar in both groups.[44] These data suggest that drivers of exacerbations remain similar regardless of *Pseudomonas* colonisation status and that sequence-based microbial profiling may assist in guiding appropriate antibiotic choices above and beyond culture-based methodologies.

The frequent COPD exacerbator (FE) (≥2 exacerbations/year) is a key phenotype as it is complicated by higher mortality and healthcare utilisation.[45] Increased abundance of *Haemophilus, Moraxella* and *Pseudomonas* are described in COPD FE while non-FEs appear to associate with increased *Actinomyces*.[12,14,17,46] The stability of the COPD microbiome through longitudinal assessment remains inconsistent, with high variability reported in the AERIS study as compared to COPDMAP which found lower variability of beta-diversity in FE.[14,20] During AECOPD, a decreased alpha diversity associates with higher mortality and abundance of *Staphylococcus,* however, an absence of *Veillonella* is also a poor prognostic indicator.[47] Similarly, in the stable COPD state, a lower microbiome diversity with increased Proteobacteria and predominant *Haemophilus* are predictors of poorer survival.[17]

Besides association to survival, the COPD microbiome has been assessed in relation to radiological phenotype. Using quantitative computed tomography (QCT) to define emphysema and other airway features characteristic of COPD, Engel et al (2017) report increased *Streptococcus* in patients with radiological emphysema and COPD-related airway features, whilst a higher *Prevotella* is seen when such features are absent.[33] COPD patients often suffer from persistent symptoms, and, this associates with poorer quality of life, more exacerbations and higher mortality. In a small single centre study, changes to microbiome composition were observed in symptomatic COPD patients (SGRQ > 25) with an increased relative abundance of *Neisseria* when compared to those with milder symptoms.[48] Furthermore, *Neisseria* negatively correlates with SGRQ score.[48] During AECOPD, an increased microbial dysbiosis between the stable and exacerbation state is associated with higher symptom (CAT score) burden. Taken together, these studies suggest an important prognostic value to the COPD microbiome which can be linked to clinical outcome in FEs, those with poorest survival and who are most symptomatic (Figure 2). Table S1 summarises the microbiome studies that have been performed in COPD from 2013 to date.

2.3 | Specimen type and microbiome composition in bronchiectasis

When compared to COPD, fewer studies in bronchiectasis have been performed. In the upper airway and using the BLESS (Bronchiectasis and Low-dose Erythromycin Study) cohort, Choo et al (2018) illustrate dominance of *Streptococcus, Prevotella, Veillonella, Rothia* and *Actinomyces* using oropharyngeal swabs.[49] The richness and diversity of the oral microbiome remains stable with long-term use of low-dose erythromycin, with the exception of decreased *Actinomyces*.[49]

By far, sputum is the most used specimen for studying the bronchiectasis microbiome. Dominant genera include *Haemophilus, Pseudomonas, Veillonella* and *Streptococcus*.[50-52] *Haemophilus*-dominant microbiomes appear to associate with less severe disease, in contrast to *Pseudomonas* which associates with more severe disease and poorer lung function.[51,53] The key association of *Pseudomonas* with disease severity in bronchiectasis is further supported through sequencing of lung tissue obtained

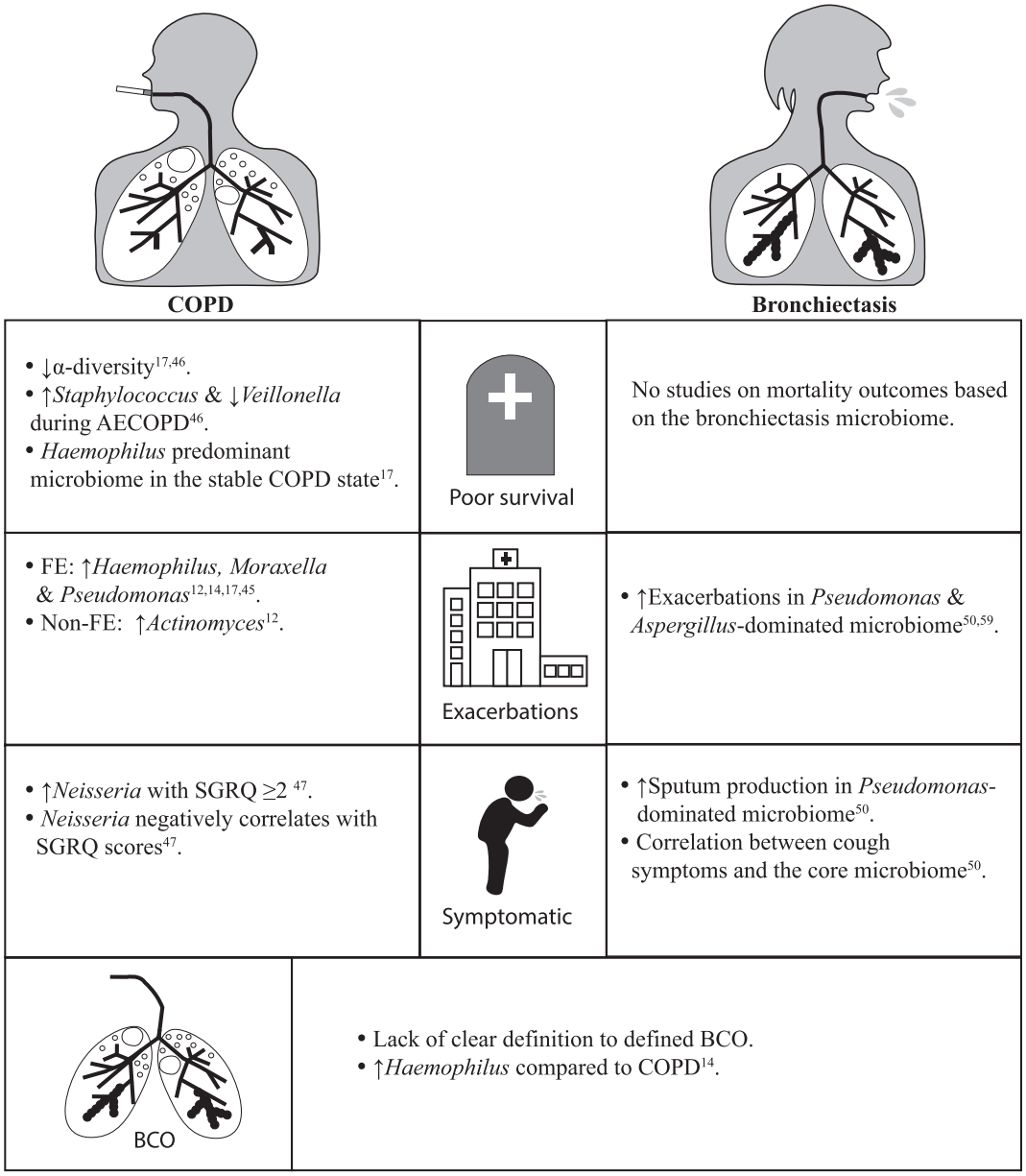

FIGURE 2 Microbiome profile and clinical outcomes in COPD, bronchiectasis and bronchiectasis- COPD overlap (BCO). ↑, increased; ↓, decreased; BCO, bronchiectasis-COPD overlap; FE, frequent exacerbator; SGRQ, St. George's Respiratory Questionnaire

from severe bronchiectasis where *Pseudomonas* is the commonest microbe identified in the excised bronchiectasis lung.[54] The presence of *Pseudomonas* in bronchiectasis is further associated with neutrophilic inflammation and increased sputum neutrophil elastase.[55] Apart from bacteria, fungi also play key roles in the cause and consequences associated with bronchiectasis.[56-59] The reported CAMEB (Cohort of Asian and Matched European Bronchiectasis) studies characterise airway mycobiome profiles in 'matched' patients recruited in both Asia and Europe and identify *Aspergillus, Cryptococcus* and *Clavispora* as the commonest genera compared to healthy individuals.[60,61] Comparing mycobiome composition between CF and non-CF bronchiectasis, Cuthbertson et al (2020) reported lower diversity with increased fungal burden in CF, with a predominance of *Candida* in both groups.[62,63]

In general, respiratory microbiome studies report that sputum has greater resemblance to that of the upper airway while BAL better represents the lower airway, however, this does not appear the case in bronchiectasis. Limited variability is observed in profiles obtained by sputum and BAL, respectively, in microbial richness, diversity and abundance.[64] The dominant genera detected in BAL were largely similar to that in sputum with a predominance of *Haemophilus, Pseudomonas, Streptococcus Moraxella, Veillonella* and *Prevotella*[64,65] (Figure 1). Moreover, paired sputum and BAL samples obtained from patients with bronchiectasis reveal no significant differences to microbiome profiles based on sampling methodology alone.[64]

2.4 | The microbiome and clinical outcomes in bronchiectasis

Next-generation sequencing offer advantages over traditional culture in characterising and comparing the lower airway microbiome at different stages of bronchiectasis. Additionally, patients may be classified into distinct endophenotypes offering a potential for precision-based therapeutic approaches where disease is heterogeneous.[66-70] Attempts to stratify bronchiectasis based on symptoms, inflammation and the airway microbiome have been undertaken. A significant correlation between cough and bacterial communities are reported in the BLESS cohort indicative that cough symptoms may be predictive of disease progression associated with the presence of specific microbial taxa.[64] In the same work, a positive correlation between lung function (FEV_1) and bacterial diversity is observed. *Pseudomonas*-dominant microbiomes associate with poor clinical outcome, and an activation of host inflammatory responses with increased CRP, IL-1β and IL-8.[51,71] This appears consistent with findings in COPD that illustrate lower microbial diversity in association with severe disease.[15,16] Positive correlation is further reported between radiological severity (using chest high-resolution computed tomography (HRCT)) and decreased diversity although studies remain limited by sample size.[72]

The role, if any, of the airway microbiome in bronchiectasis exacerbations remains poorly understood. The microbiome in bronchiectasis exacerbations is highly individualised and exacerbations are complex events not solely explained by bacterial overgrowth but include interaction with the surrounding environment and/or co-infection.[52,67] Bacterial microbiomes appear relatively stable by longitudinal sampling and comparisons between the baseline, exacerbation and post-exacerbation state in bronchiectasis reveals little change. No significant difference is detectable in bacterial load, α and/or β-diversity through longitudinal assessment.[50,65,73] *Pseudomonas aeruginosa* and *Haemophilus influenzae* remain the dominant bronchiectasis taxa and chronic infection with these microbes persist over time unrelated to antibiotic treatment.[74,75] Their relative abundance and bacterial load are linked to airway neutrophilic inflammation and patients with *Pseudomonas*-dominant microbiomes appear to more frequently exacerbate compared to those with *Haemophilus*-dominant profiles[51,71] (Figure 2). Chronic *P aeruginosa* infection in bronchiectasis associates with poor clinical outcome, higher exacerbations, more sputum, worse lung function and a higher need for recurrent antibiotics.[76-82] Long-term antibiotic use alters microbiome composition and decreases exacerbation frequency particularly in *Pseudomonas*-dominant patients.[83] Anaerobic bacterial genera, like that observed in CF, are detected in bronchiectasis.[84] *Prevotella* and *Veillonella* are in the core bronchiectasis microbiome but any association with clinical outcome including exacerbations remains undefined.[51,64,85] While these genera are rarely detected by culture-based methods, their role in bronchiectasis pathogenesis should not be underestimated and requires future study. The currently available microbiome studies in bronchiectasis from 2013 to date are summarised in Table S2.

In addition to bacteria, the fungal mycobiome has roles in bronchiectasis with a reported increased exacerbation frequency in those *Aspergillus*-dominant.[60,61] This is further demonstrated by qPCR with an increased exacerbation related to airway *Aspergillus terreus*. Distinct mycobiome profiles associate with the various *Aspergillus*-associated disease states and 'immunoallertypes' of sensitisation are described in association with clinical outcomes.[60] A fungal driven pro-inflammatory group is associated with poorer lung function and increase disease severity compared to a house-dust mite chemokine-dominant group.[60,61]

The role of the virome in bronchiectasis is less well studied and remains uncertain. Respiratory viruses have been identified in the stable and exacerbation states with associated airway and systemic inflammation.[86-90] Further study is now necessary to better understand the role of microbial interactions between bacteria, fungi and viruses in the development and progression of bronchiectasis.

2.5 | The microbiome in bronchiectasis-COPD overlap (BCO)

Bronchiectasis and COPD commonly coexist and this clinical state is associated with greater disease severity.[91] Spirometry, used to define COPD, is employed as a severity indicator in bronchiectasis and the presence of radiological features of bronchiectasis in COPD is associated with poorer prognosis. The existence of overlap syndromes in airways disease remains an emerging area and a lack of formal definition describing BCO continues to exist despite its recognition in clinical practice. Further, the radiological criteria used to define bronchiectasis in COPD remains challenging.[92] A number of asymptomatic healthy individuals also demonstrate the presence of radiological bronchiectasis.[93] Challenges to defining BCO result in variable reported prevalence and while microbiome alteration is reported in COPD and bronchiectasis, the role of microbiome in BCO remains unclear and understudied.[92,94,95] In the AERIS COPD study, microbiome composition in the BCO subgroup appears different with increased *Haemophilus* overall but no difference during exacerbations.[14] Further work is necessary to define BCO and the role of the microbiome in this clinical state.

2.6 | The metagenomics revolution in microbiome analyses

Metagenomics involves unbiased sequencing of all extracted DNA. Other than revealing the composition and

diversity of the microbiome (achieved by targeted amplicon sequencing), metagenomics additionally provides functional annotation revealing-specific genes and microbial pathways that influence the host and the respective disease state. Metagenomics therefore can significantly deepen our understanding in both health and disease where it has been used to isolate and annotate antibiotic-resistance genes (ARGs), exemplified by Mac Aogain et al (2020), who demonstrate a core macrolide resistome within the host microbiome in patients with COPD and bronchiectasis.[96-98] Metagenomics also provides a broader view and application across disciplines.[99] Shotgun metagenomics has uncovered changes to microbial pathways in the stable and exacerbation state in severe COPD.[100] While no change to microbiomes is detected between these states, altered functional metabolic pathways occur, providing additional evidence of active microbial metabolism during exacerbations, a finding unattainable by targeted amplicon sequencing alone. Although metagenomics is an emerging and robust tool, it does possess limitations, for instance, requiring high-quality and quantity DNA sampling for successful sequencing and generally demonstrating a lower fungal abundance relative to bacterial. It is also more costly and time-consuming compared to targeted amplicon sequencing precluding its widespread used.

Overall, while traditional clinical diagnosis of infection uses microbiology to cultivate bacteria for identification, sequencing approaches (targeted amplicon and metagenomics) provide additional useful information particularly on host-microbe interaction and affected functional pathways, however, their clinical translation remains limited.[101,102] This is largely due to a lack of standardised methodology, the labour intensive process, concerns over cost and the complexity of the bioinformatics analysis.[75,103] Translating findings from metagenomic-based studies remains an important area as it provides strong potential for more precise diagnostics and information to inform clinical management.

2.7 | Respiratory models to study the airway microbiome

Various immortalised respiratory cell lines including 16HBE14o-, HBE1, A549 and Calu-3 can be used for microbiome studies. These are commercially available and easy to maintain, however, huge discrepancies in their genetic and phenotypic characteristics compared to that offered by primary airway cultures result in poorer prediction of in situ outcomes.[104-106] Recently, Charles et al have developed an immortalised well-differentiated air–liquid interface (ALI) nasal epithelial cell model that supports colonisation with nasal microbiomes. This model is physiologically relevant and useful for exploration of host-microbe interaction.[107] Airway organoids hold great promise and can be prepared from healthy volunteers or by sampling individuals with chronic respiratory disease. This serves as a novel, more advanced and sophisticated model system to determine the effect of the lung microbiome in situ.[108,109] Apart from in vitro models, humanised mice also provide useful information in understanding the impact of the microbiome in healthy and diseased states, however, their anatomy, physiology and microbial composition particularly of the respiratory system remains different to humans.[110,111]

3 | CONCLUSIONS AND FUTURE DIRECTIONS

Next-generation sequencing has evolved significantly with an increasing number of microbiome studies now available in COPD and bronchiectasis. Various sampling methods are employed to characterise airway microbiomes with minimal observed differences. Microbes play a significant role in the pathogenesis and progression of COPD and bronchiectasis, particularly *Haemophilus* and *Pseudomonas*. Microbial dysbiosis associates with more severe disease. Microbial change associates with activation of the host-immune and inflammatory response. Despite our increasing knowledge on airway microbiomes in COPD and bronchiectasis, their specific role in pathogenesis is unclear and usefulness in diagnosis and prognostication remains to be evaluated. Our current understanding of airway microbiomes remains nascent, and further study is necessary using novel model systems such as airway organoids. Greater focus should be placed on longitudinal and functional microbiome assessment, as these are critical if we are to achieve its potential for precision-medicine. These are exciting times for microbiome researchers as the potential to add clinical value remains strong, especially if reproducible and robust biomarkers or therapeutic targets can be identified for COPD and bronchiectasis.

CONFLICT OF INTEREST

All authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Conception: Tiew, Chotirmall
Literature review: Tiew, Jaggi, Chan, Chotirmall
Figures and manuscript writing: Tiew, Jaggi, Chan, Chotirmall

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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