Infection and the microbiome in bronchiectasis

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Exploring the nexus of infection and microbiome dynamics offers new avenues in understanding and treating bronchiectasis. https://bit.ly/3UtqoUl

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Abstract

Bronchiectasis is marked by bronchial dilatation, recurrent infections and significant morbidity, underpinned by a complex interplay between microbial dysbiosis and immune dysregulation. The identification of distinct endophenotypes have refined our understanding of its pathogenesis, including its heterogeneous disease mechanisms that influence treatment and prognosis responses. Next-generation sequencing (NGS) has revolutionised the way we view airway microbiology, allowing insights into the "unculturable". Understanding the bronchiectasis microbiome through targeted amplicon sequencing and/or shotgun metagenomics has provided key information on the interplay of the microbiome and host immunity, a central feature of disease progression. The rapid increase in translational and clinical studies in bronchiectasis now provides scope for the application of precision medicine and a better understanding of the efficacy of interventions aimed at restoring microbial balance and/or modulating immune responses. Holistic integration of these insights is driving an evolving paradigm shift in our understanding of bronchiectasis, which includes the critical role of the microbiome and its unique interplay with clinical, inflammatory, immunological and metabolic factors. Here, we review the current state of infection and the microbiome in bronchiectasis and provide views on the future directions in this field.

Introduction

Bronchiectasis, characterised by irreversible and progressive bronchial dilatation, presents complex challenges due to chronic and recurrent infection, inflammation and structural damage [1]. This poses significant challenges in its management, primarily due to its complex aetiology involving microbial infection, immune dysfunction and inflammation [2]. Acknowledging disease heterogeneity as a key feature in the lack of available evidence-based therapeutics is now being increasingly addressed by advanced multiomics technologies that provide a deeper understanding of disease pathogenesis, including the role of infection and the microbiome. Here, we review the current state of the literature in relation to infection and the microbiome in bronchiectasis. We based this review on relevant PubMed/Medline articles identified using the primary search terms "infection" and "microbiome" together with "bronchiectasis" (search query: "bronchiectasis AND (infection OR microbiome)"). This synthesis underscores the urgent need for more effective, timely and targeted interventions in this field.

Infections in bronchiectasis

Infection and its related inflammation remain key pathophysiological and clinical aspects in bronchiectasis and play a crucial role in the primary development and secondary progressive process in this disease state (figure 1)

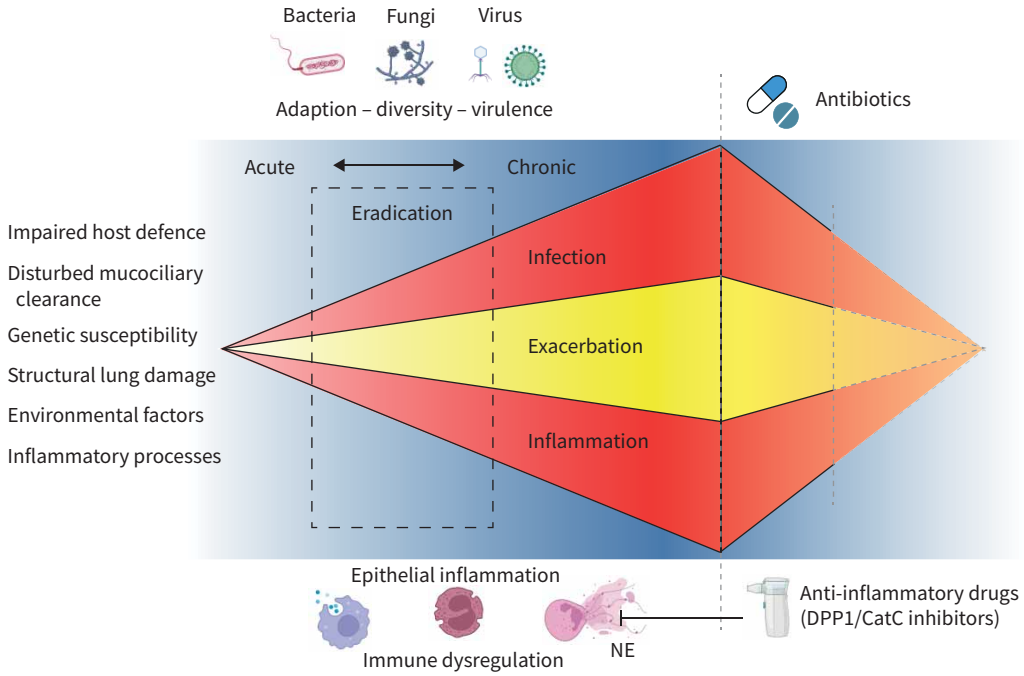

FIGURE 1 Overview of bronchiectasis pathogenesis. A complex interaction between infection and inflammation results in a self-perpetuating cycle initially triggered by various conditions (indicated on the left). This cycle is primarily driven by bacterial infections, with growing evidence of significant contributions from viruses and fungi. The progression from acute to chronic infection hinges on factors such as pathogen virulence, adaptability and the selective pressures within the host environment. Identifying the exact stage of infection (acute or chronic) is crucial, as it influences the effectiveness of eradication efforts (broken rectangle). Central to this process is an excessive (usually neutrophilic) inflammatory response that leads to further tissue damage and impairs mucociliary clearance. This infection-driven inflammation can increase the likelihood of acute exacerbations. Therapeutic strategies aim to mitigate both infection and inflammation. This is achieved through conventional antibiotics and newer pharmacological interventions targeting neutrophilic inflammation, such as dipeptidyl peptidase 1 (DPP1)/cathepsin C (CatC) inhibitors. Despite these efforts, the structural lung damage and the conditions present at the onset of infection and inflammation preclude a reversal to the pre-disease state. Figure created with BioRender.com.

[3]. The ability to distinguish between acute events, pulmonary exacerbations and chronic conditions, associated with persistent microbial colonisation, is crucial in understanding the role infections play in bronchiectasis. An acute deterioration in respiratory symptoms, termed an "exacerbation", often indicates an infectious episode, although it is important to note that changes in the lung microbiome are not always evident during such episodes [4, 5]. While the term "colonisation" traditionally suggests a benign presence of micro-organisms, in the context of bronchiectasis, it can be associated with negative outcomes [6, 7]. Exacerbations are characterised by an acute worsening of respiratory symptoms, which may not always coincide with the detection of pathogens in cultures, underscoring the complex interactions between host immunity and microbial presence in the lungs [8–14]. Thus, defining exacerbations remains challenging because of their inherent heterogeneous nature characterised by a fluctuating course of symptoms, coupled with varied patient perceptions of severity. Consensus definitions have been established for use in clinical practice and trials [4, 15, 16]. The underlying pathophysiological mechanisms leading to an exacerbation are not fully understood; however, in recent years, increasing evidence suggests that inflammatory processes (neutrophilic and/or eosinophilic inflammation) and environmental factors play key roles [17–20]. Long-held assumptions presume that all exacerbations in bronchiectasis are infective (*i.e.* new pathogen, increased bacterial load from an existing pathogen or altered bacterial virulence) and hence are primarily treated with antibiotics [15, 21]. However, such views are evolving and have been questioned in the light of more recent studies, as described below.

Bacterial infection in bronchiectasis

Bacterial infections remain key drivers of exacerbations, with relevant but less understood contributions from viral or fungal infection [7, 22–25]. Several studies have demonstrated a strong association between

bacterial infection, increased bacterial sputum load and the heightened risk of exacerbation, while antibiotic therapy has been shown to effectively reduce inflammation, thereby mitigating the severity of exacerbations and improving patient outcomes [6, 7, 17, 18, 26, 27]. Bacteria commonly associated with exacerbations in bronchiectasis include Pseudomonas (*P.*) aeruginosa, *Haemophilus* (*H.*) *influenzae*, *Staphylococcus* (*Sta.*) *aureus* and, less commonly, *Stenotrophomonas maltophilia* (figure 2a) [28–32]. Additionally, the role of nontuberculous mycobacteria (NTM) in bronchiectasis is increasingly recognised, indicating a potential sub-phenotype of the disease that warrants further study and understanding [1, 33].

Viral infection in bronchiectasis

Several studies now contribute to a growing evidence base supporting viral infection as an important trigger of bronchiectasis exacerbations. Two studies have demonstrated that viruses are more frequently detected during exacerbations (20.9–49.0%) than in the stable state (11.4–18.9%) [23, 24], with the

a)

Microbial pathogen	RIBRON – Spain (n=1912)	KMBARC – Korea (n=598)	EMBARC – Europe (n=16963)	BRR – USA (n=1826)	EMBARC – India (n=2195)
***P. aeruginosa* (%)**	40.4	11.0	25.1	33.0	13.7
***H. influenzae* (%)**	18.9	1.5	23.6	8.0	0.5
***M. catarrhalis* (%)**	5.4	0.5	5.4	1.0	1.0
***Enterbacteriales* (%)**	5.3	3.9	15.9	–	9.8
***Sta. aureus* (%)**	7.6	0.7	8.6	12.0	2.3
***Str. pneumoniae* (%)**	5.1	–	8.5	3.0	0.8
***Ste. maltophilia* (%)**	2.4	–	2.6	5.0	–
***A. fumigatus* (%)**	0.7	–	3.2	19.0	–
Other/unknown	14.2	82.4	7.1	19.0	71.9

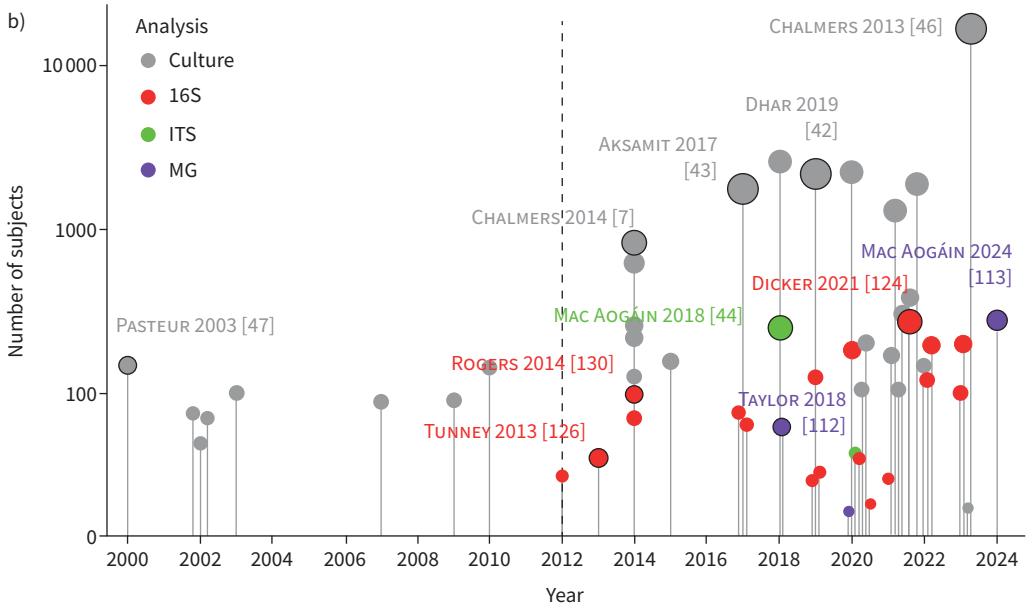

FIGURE 2 Increasing scale of culture-based and airway microbiome studies in bronchiectasis. a) Table illustrating prevalence of key bacterial taxa from several major bronchiectasis registries. b) Visual timeline of microbiological and microbiome research outputs in bronchiectasis (2000–2024) comparing culture-based and sequencing-based (microbiome) studies. Each point on the chart indicates an individual study with the size of the circle and the length of the associated vertical lines reflecting study size. Points on the chart are colour-coded by the type of analysis conducted (grey: culture based; red: bacterial 16S rRNA analysis; green: fungal internal transcribed spacer (ITS) analysis; purple: metagenomics (MG)). Selected studies specifically discussed in the review are indicated by black borders surrounding dots and have accompanying citations. A broken line demarcates the beginning of the "microbiome era" in bronchiectasis. The complete list of studies illustrated in the figure is detailed in supplementary appendix 1. *A.*: *Aspergillus*; *H.*: *Haemophilus*; *P.*: *Pseudomonas*; *Sta.*: *Staphylococcus*; *Ste.*: *Stenotrophomonas*; *Str*: *Streptococcus*.

following viruses detected at highest frequency: coronavirus, rhinovirus, influenza A/B, respiratory syncytial virus B and human metapneumovirus [23, 24, 34]. Two studies from Australia and Europe investigated viruses in stable bronchiectasis and while the Australian study (n=27) showed a higher frequency of viruses in sputum during winter *versus* summer months (92% and 33%, respectively), the European study (n=219) did not detect any seasonal differences (13.5% in winter *versus* 12.3% in summer) [35, 36]. Furthermore, there appears to be links between viral upper respiratory tract infections (URTIs) and bronchiectasis exacerbations, as viral detection rates in sputum in patients experiencing exacerbations following URTIs remain higher compared to individuals either without URTI or subsequent exacerbation [37]. Some studies have proposed that detection of the Epstein–Barr virus in bronchiectasis confers a shorter time to exacerbation; however, this relationship requires validation [38]. During the coronavirus disease 2019 pandemic, studies reported a decrease in bronchiectasis exacerbations, most likely attributed to enhanced social hygiene and shielding through social distancing, resulting in reduced viral transmission [25, 39, 40]. Taken together, it appears that viruses play a relevant role in bronchiectasis exacerbations; however, their role in stable disease remains to be clarified.

Fungal infection in bronchiectasis

The role of fungi in the development of acute infection in bronchiectasis remains unclear. Other than *Candida albicans*, *Aspergillus* (*A.*) *fumigatus* represents the most common isolated fungi with significant pathogenic potential [41]. The prevalence rates of *A. fumigatus* in bronchiectasis are uncertain, with a European registry study illustrating it in 3% (279 out of 9226) of stable samples and 3.2% (393 out of 12152) of all stable and exacerbation samples [42]. Among patients within the US Bronchiectasis Registry, 60% (1087/1826) had at least one fungal culture, with *A. fumigatus* detected in 16% of those without NTM [43]. Current molecular and/or serological approaches illustrate that fungal detection and/or sensitisation may be seen in stable bronchiectasis. *A. fumigatus* and *A. terreus* are associated with more severe disease, with the latter associated with exacerbations [44]. The CAMEB (Cohort of Asian and Matched European Bronchiectasis) study, including patients from Asia and Europe, and excluding clinical allergic bronchopulmonary aspergillosis (ABPA) demonstrated serological ABPA in 18% (n=224) [45]. The most common fungal-associated aetiology in bronchiectasis is ABPA [42, 46, 47], which itself may lead to acute deterioration in individual patients. While emerging evidence suggests that fungi influence bronchiectasis, our understanding of their specific role in acute infection and on disease course remains insufficient.

Chronic infection in bronchiectasis

Chronic (bacterial) infection adversely impacts the progression of bronchiectasis, leading to a higher risk of exacerbations, hospitalisations, increased mortality, poorer lung function and reduced quality of life [6, 7, 22, 29, 45, 48]. Between research studies and clinical guidelines, the term “chronic infection” has not been uniformly defined [5, 49, 50]. An international expert group recently proposed that it be defined as “two positive cultures of the same microorganism, at least three months apart, in the context of clinically relevant bronchiectasis” [7]. Additionally, the same authors recommend avoiding the term “chronic colonisation” to allow clear emphasis on the negative impact of chronic bacterial infection on disease course [7]. Culture-based methods have commonly identified *P. aeruginosa*, *H. influenzae*, *Enterobacteriales*, *Sta. aureus*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* as most abundant in bronchiectasis [43, 46, 48]. Chronic infections occur in 45–72% of cases, with *P. aeruginosa* and *H. influenzae* being most frequent (figure 2a) [6, 7, 22, 48].

Chronic infection in bronchiectasis involves complex immune dysregulation (figure 1), primarily characterised by neutrophil dominance, but an increasing recognition of a role for Th2-dominant (eosinophilic) inflammation [20]. It is important to consider the bi-directionality of this association; while inflammation may result from persistent microbial presence, it could equally contribute to an inability to effectively clear infections. This cyclical relationship complicates the determination of initial causality in chronic airway conditions, where both microbial colonisation and immune dysregulation represent parallel pathways of disease progression. A recent study demonstrated that different patient clusters may be defined based on airway and systemic inflammation with those exhibiting more pronounced neutrophilic inflammation demonstrating an increased risk for exacerbations and altered microbiomes [51]. Neutrophilic inflammation further correlates with airway bacterial load [17, 52], which triggers further recruitment of neutrophils to the airway, primarily driven by C-X-C motif ligand 8, interleukin (IL)-1β, IL-8, tumour necrosis factor-α and leukotriene B4 [53–55]. These processes activate host defence mechanisms, including phagocytosis, formation of neutrophil extracellular traps (NETs), neutrophil degranulation, production of reactive oxygen species and release of pro-inflammatory cytokines [18, 55, 56]. The serine protease neutrophil elastase (NE) has been associated with increased exacerbations, decreased lung function and disease severity in bronchiectasis [52, 57, 58]. NETs, web-like structures composed of DNA, histones and

serine proteases (primarily NE), serve to contain and neutralise pathogens. Their overabundance is central to the development and progression of bronchiectasis and chronicity of infection in relation to NETs and are linked to increased disease severity, exacerbations and mortality in bronchiectasis [18]. Interestingly, *P. aeruginosa*, *H. influenzae* and *Sta. aureus* may induce and degrade NETs, providing a survival advantage for microbes and thus contributing to chronic infection [59–61]. Other host factors which contribute to chronic infection in bronchiectasis include impaired neutrophil phagocytosis and disrupted mucociliary clearance, both associated with high NE activity [54, 62–65]. Given the close association between neutrophilic inflammation and bacterial infection, targeting neutrophils has emerged as a promising therapy for frequent exacerbators with bronchiectasis; an approach being currently explored in clinical trials (figure 1) [17, 19, 52, 66, 67].

P. aeruginosa *in bronchiectasis*

The Gram-negative bacterium *P. aeruginosa* has major clinical relevance in bronchiectasis owing to its frequent detection and related pathogenicity. Registry data revealed its presence in 11–40% of patients, with notable geographical variation, even within continents, including an increased incidence in Southern Europe [7, 46, 48, 68]. The true prevalence of chronic infection with *P. aeruginosa* remains uncertain, with reported rates ranging widely between 8 and 32% across jurisdictions [7, 22, 48, 49]. Prior analysis of >10 000 individuals recorded in the European Bronchiectasis Registry revealed several independent risk factors for a new *P. aeruginosa* infection, including poorer lung function, history of frequent exacerbations, specific radiology, use of inhaled corticosteroids (ICS), macrolide therapy, low body mass index and increased sputum production [69]. Further studies on smaller populations have also confirmed elevated risks of new infection in patients with aetiologies such as COPD or primary ciliary dyskinesia, as well as prior infection with *H. influenzae* and in those in older age groups [70–72]. Numerous studies demonstrate that patients with chronic *P. aeruginosa* infection experience significant adverse outcomes, including poor and accelerated decline in lung function, increased disease severity, more pronounced radiological findings, poorer quality of life, and higher frequencies of exacerbation and hospitalisation with greater risk of mortality. Such findings underscore the impact that *P. aeruginosa* infection has on individuals with bronchiectasis, highlighting the clinical need for effective identification, eradication and therapy [6, 29, 48, 49, 73–76]. Here, eradication is defined as an absence of *P. aeruginosa* in respiratory cultures after targeted antibiotic treatment, coupled with clinical improvement and sustained nonrecurrence over a specified follow-up period. National and international guidelines recommend eradication in early infection, despite the uncertainty of natural progression between individual patients and the varied efficacy of early antipseudomonal antimicrobial therapy, especially in asymptomatic patients [15, 21, 50]. Retrospective studies on *P. aeruginosa* eradication in bronchiectasis, employing varying treatment regimens including oral, inhaled and intravenous antibiotics, have reported initial eradication rates ranging from 52 to 80%, which decrease to 36–60% after 1 year, highlighting the relevance of prompt identification and clinical action [71, 77, 78]. Prospective studies using tobramycin and colistin for eradication report initial success rates of 90.9 and 61.2% at 1 and 3 months, respectively, with rates dropping to 76.5 and 40.3% after 12 months [79, 80]. It remains unclear whether such longitudinal change in eradication success indicate treatment-related suppression of infection or if re-infection occurs following successful eradication. There remains an important research gap related to the long-term effects of *P. aeruginosa* eradication in bronchiectasis and how, if at all, this affects future disease course.

The microbiome in bronchiectasis

NGS approaches to assess microbiomes

Targeted amplicon sequencing

In the last 25 years, there has been an explosion of NGS technologies that offer fresh possibilities for investigating the role of bacterial, viral and fungal communities in the setting of acute and/or chronic infection in bronchiectasis. The technological advantages and limitations of the different methodologies have been extensively reviewed elsewhere [81, 82], with recommendations and guidelines for best practice now accessible [83, 84]. This review will therefore focus on how such NGS approaches have been applied to evaluate the microbiome in bronchiectasis.

The most frequently used methods to investigate bacterial microbiota in bronchiectasis have been amplicon-based approaches, targeting and selectively amplifying specific regions of the 16S rRNA gene. Typically, one to two hypervariable regions of the 16S rRNA gene (present in all prokaryotes including nonculturable organisms [85]) are amplified by PCR and sequenced. Sequencing two (rather than one) hypervariable regions allows for improved taxonomic classification but remains restricted to classifying bacteria to the genus level, with biases in identification of certain taxa possible depending on the primers and 16S rRNA region selected [86, 87]. It has been proposed that using an intergenic spacer region between the 16S and 23S rRNA gene subunits as the amplicon of choice may resolve taxonomy to

subspecies level with greater confidence [88]; however, more recently, synthetic long-read sequencing methodologies have been developed including PacBio Single Molecule Real Time circular consensus sequences, Oxford Nanopore or Loop synthetic long-read sequencing [89–92]. These latter approaches enable classification to species or strain level and may provide insight into functional differences within bacterial populations [93]. Such methods exhibit higher throughput than second-generation sequencing but come with increased costs and bioinformatics requirements [94].

In terms of defining fungal taxonomy, several genetic markers are traditionally used, including the fungal 18S and 28S rRNA genes, the associated long spacer region, translation elongation factor 1-alpha, RNA polymerase II (RPB1 and RPB2) and beta-tubulin [95, 96]. For comprehensive profiling of fungi in complex ecosystems, the ITS (internal transcribed spacer) regions have emerged as markers of choice. The preference for ITS regions is attributed to species-specific variation, extensive databases and relative standardisation, although careful interpretation of sequences obtained are required [96–98]. Standardisation of fungal mycobiome sequencing has yet to reach the same level as bacterial 16S rRNA gene sequencing [97]. Challenges persist in terms of optimising detection sensitivity, achieving broad taxonomic coverage and developing efficient DNA extraction techniques [99]. Overcoming such challenges is hampered by the limited availability of fungal genomes in the public domain, leading to identification biases towards specific fungal taxa [98–101]. While the technical feasibility of dual ITS1 and ITS2 sequencing *via* targeted ITS shotgun metagenomics has been demonstrated, selective sequencing of either ITS1 or ITS2 has gained prominence due its relative simplicity, lower cost and less complex wet and dry lab protocols [44, 99]. The choice of specific primer sets targeting alternate ITS1 and ITS2 regions is also a key consideration, impacting the levels of detection and range of coverage in mycobiome studies. Moreover, a preference for the ITS2 region for analysis of commensal microbes has been advanced given the apparent biases associated with ITS1 [101]. Fungal ITS analysis has significantly benefited from years of bioinformatic refinement of bacterial 16S rRNA gene sequencing analysis. Specialised pipelines and tools tailored for fungal ITS data processing now include QIIME2 q2-ITSxpress plugin, DADA2 adapted for ITS sequences, the UNITE Pipeline and FUNGuild for taxonomic and functional annotation [102–105], all of which offer robust solutions for quality filtering, taxonomic classification, diversity analysis and functional annotation, enabling a comprehensive exploration of fungal communities within microbiome studies.

Metagenomics

The application of metagenomic whole genome sequencing (WGS) to airway microbiome samples now represents an attractive alternative to targeted amplicon sequencing. This approach offers assessment of the entire genomic content (bacteria, fungi and viruses) within a sample, allowing for comprehensive analysis of microbial diversity and functional potential, including antibiotic resistance genes, while minimising primer bias [106]. Metagenomics enables identification of novel micro-organisms and functions and allows a layered approach to analysis [107, 108]. Taxonomic identification is facilitated by rapid classification methods and mirrors the insights attainable with 16S rRNA, with added robust species-level resolution [109]. Functional microbiome analysis is possible and has yielded insight into the functional composition of the bronchiectasis microbiome; revealing its distinct profile and composition as correlated with exacerbation [107, 110]. A particularly noteworthy aspect of the metagenomic approach is the rich array of antimicrobial resistance genes identifiable in the bronchiectasis airway termed the “resistome” [110–112]. The presence of bacteriophages within the bronchiectasis microbiome has been investigated by this approach but has yet to reveal clear clinical association [107, 113, 114]. While WGS metagenomics offers kingdom-agnostic analysis, investigation of low-abundance taxa such as fungi may necessitate deeper sequencing rendering greater cost and time required compared to alternative targeted approaches. Limitations exist even with metagenomics and the field is still evolving toward methodological consensus, complicated by emerging novel technologies for sequencing and advancing bioinformatic approaches [115, 116]. Meta-transcriptomics offers even deeper analysis into the metabolic states associated with the bronchiectasis microbiome and has been employed in several reported studies, highlighting its ability to simultaneously capture bacterial, viral and fungal profiles in tandem with functional characterisation and host response, affording additional view of the airway microbiome [117, 118].

The bacteriome in bronchiectasis

Almost a decade since Rogers *et al.* [119] set out key research questions related to respiratory microbiota, knowledge of the bacteriome in bronchiectasis has significantly advanced, both during disease stability and exacerbations. This has been extensively reviewed elsewhere [120–122]. The stable sputum bacteriome in bronchiectasis is dominated by the phyla Pseudomonadota (was Proteobacteria) and Bacillota (formerly Firmicutes) whilst, at the genus level, *Pseudomonas*, *Haemophilus*, *Streptococcus*, *Veillonella*, *Prevotella*, *Stenotrophomonas* and *Neisseria* are typically seen alongside less abundant taxa including *Rothia*, *Staphylococcus*, *Enterobacteriaceae* and *Leptotrichia* [123–127]. Patients with more severe disease appear

to have a more unbalanced or "dysbiotic" bacteriome, often dominated by *Pseudomonas*, *Streptococcus* or *Haemophilus* [124, 128, 129]. Early studies have often been small (<100 patients) (figure 2b), cross-sectional and focused on clinical subsets of bronchiectasis largely dominated by Caucasian subjects [58, 130, 131]. More recent studies have included cohorts of patients from different geographic regions including Asia and the Middle East [20, 44, 107, 128, 131–133]. Longitudinal studies with follow-up to 16 years have shown that individual bacterial profiles from sputum or bronchoalveolar lavage (BAL), during stable disease and exacerbations, generally remain unchanged over time [124–126, 134, 135].

Our understanding of the bronchiectasis bacteriome in upper and lower airway niches is limited. One study, with 35 paired sputum and nasopharyngeal (NP) swabs found statistically significant differences between these niches; where Bacillota and *Streptococcus* were more prevalent in sputum and Bacillota, Actinobacteriota, *Corynebacterium* and *Dolosigranulum* predominant in NP swabs [131]. Choo *et al.* [136] investigated the oropharyngeal (OP) microbiota in 84 bronchiectasis patients and showed that core OP microbiota were largely comparable to sputum but with additional genera including as *Gemella*, *Oribacterium* and *Campylobacter*. Three studies have utilised BAL, revealing little difference between the bacteriomes obtained from sputum and BAL [123, 134, 137]. Only a single study to date has investigated multiple body sites such as the stomach or oral cavity alongside the respiratory bacteriome in bronchiectasis [138]. Comparisons of bacteria with clinical characteristics reveal that the bacteriome is altered in patients with more severe disease (determined by FACED score (forced expiratory volume in 1 s, age, chronic *P. aeruginosa* colonisation, radiological extension and dyspnoea) or the bronchiectasis severity index) with lower alpha diversity and increased abundance of taxa such as *Pseudomonas* being associated with more severe scores and the abundance of taxa such as *Rothia* or *Haemophilus* associating with milder disease [124, 128, 139]. Importantly, associations between alpha diversity and lung function are less clear, with contradictory results currently reported [123–125, 135]. A large (n=281) observational cohort, including both cross-sectional and longitudinal sampling in bronchiectasis demonstrated that patients with a stable bacteriome dominated by *Pseudomonas* or *Stenotrophomonas* were at increased risk of mortality or severe exacerbations, indicating the potential for microbiome related disease stratification [124]. Furthermore, emerging work links bacteriome profiles to inflammatory patterns and disease endophenotypes (figure 3) [18, 20, 51, 58, 140, 141]. The chronicity of bacterial infection or airway colonisation in bronchiectasis leads to the development of aberrant neutrophilic immune responses such as

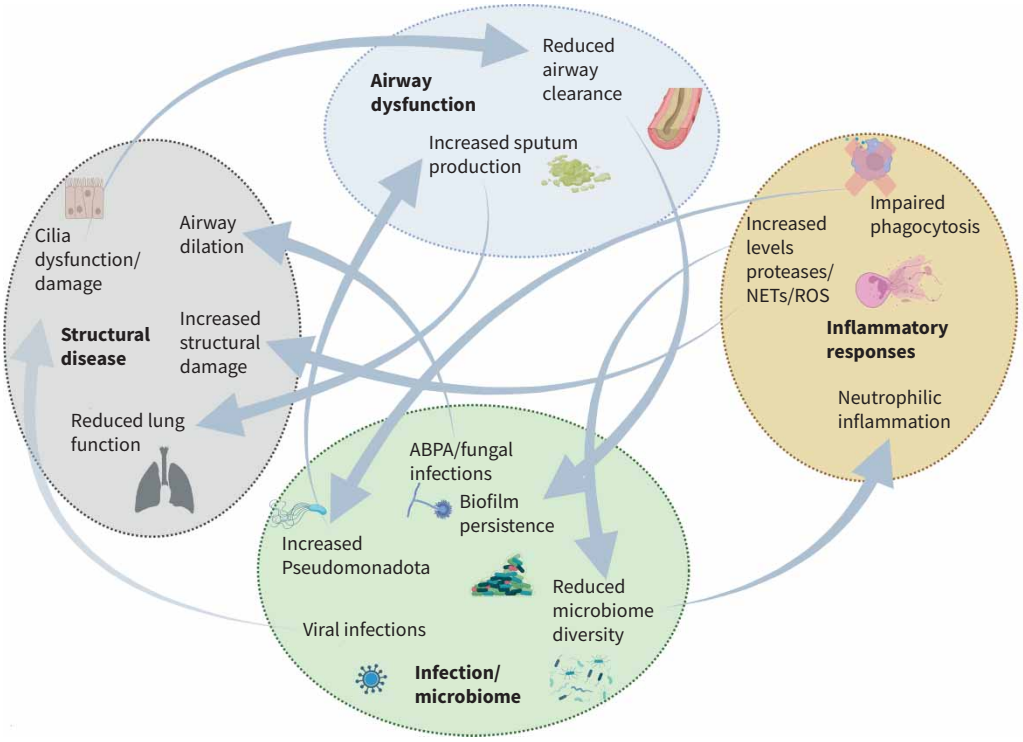

FIGURE 3 Microbiome and microbial interactions in the vicious vortex of bronchiectasis pathogenesis, adapted from the vicious vortex hypothesis of Flume *et al.* [3]. NET: neutrophil extracellular trap; ROS: reactive oxygen species. Figure created with BioRender.com.

those described in previous sections of this review [17, 52]. Comparable patterns are observed when comparing bacteriomes with inflammatory markers including increased levels of neutrophil-associated proteins such as NE, NETs, MPO (myeloperoxidase) or ELANE (elastase, neutrophil expressed) detected in individuals with Pseudomonadota- or *Pseudomonas*-dominated bacteriomes that predispose to increased sputum production and persistence of bacteria-related biofilms [18, 51, 58, 140, 141]. NTM are increasingly recognised in bronchiectasis, inducing cavitary, infiltrative and nodular lung diseases, particularly in patients with underlying pulmonary abnormalities [1, 33, 142]. The role of NTM, including species such as *Mycobacterium avium* complex, the most prevalent NTM, followed by *Mycobacterium abscessus* and *Mycobacterium chelonae*, is crucial, particularly given the significant challenges in their detection [43, 143]. Bronchiectasis exhibits distinct microbiome features associated with NTM infection, including decreased alpha diversity, enrichment with oral commensals and a distinct pro-inflammatory immune profile [143]. Furthermore, the interaction between NTM and the host immune system, particularly impaired interferon-γ and granulocyte–macrophage colony-stimulating factor production coupled with associations with upper airway taxa and T-helper (Th) 17 cytokines, highlights the complex microbiological landscape in the airways and the associated treatment challenges [143]. The correlation between NTM infection and increased risk of fungal colonisation by *A. fumigatus* represents an important example of inter-kingdom interaction in this setting [144, 145].

The mycobiome in bronchiectasis

Fungi are ubiquitous constituents of the air microbiome and have been detected in healthy and diseased airways [146, 147]. Though often challenging to identify and clinically characterise, they contribute significantly to airway immunopathology and remain central in allergic respiratory disease [148, 149]. Host pattern recognition receptors such as Toll-like receptors and C-type lectin receptors are central to host detection of fungal pathogen-associated molecular patterns, such as β-glucans, chitin and galactomannan [150, 151]. In this context, the mycobiome has been identified as a priority area for bronchiectasis research [21, 44, 45, 107, 138, 152, 153]. This adds to existing literature describing the mycobiome in cystic fibrosis (CF), where fungal colonisation, sensitisation and ABPA are closely correlated with clinical outcome [153–160]. Recent work in bronchiectasis highlights the increased sensitisation response, corroborating early studies describing atopy associated with non-CF disease [45]. Sensitisation in bronchiectasis and the presence of poly-sensitisation to multiple fungal and nonfungal allergens are closely correlated with poorer lung function and disease severity, while multiplex analysis of host airway immunology identified “immunoallertypes” including a fungal-driven and proinflammatory profile, seen in a subgroup of patients with bronchiectasis, where it was associated with worse clinical outcome [45]. Intriguingly, high sensitisation levels in COPD have also been linked to high-risk airway mycobiome profiles, while environmental metagenomics now highlights several airborne fungal taxa (and allergens) that trigger sensitisation responses and poorer clinical outcomes [161, 162]. While such environmental metagenomic analysis and its disease correlates are yet to be characterised in bronchiectasis, the characterisation of distinct mycobiome profiles associating with serological ABPA or sensitisation including that in bronchiectasis–COPD overlap clearly suggests a role for environmental allergens in bronchiectasis including those of fungal origin [152]. In light of such findings, a continued exploration of the airway mycobiome and associated environmental sources of fungal allergens is warranted in bronchiectasis and may offer fresh avenues for disease stratification and targeted intervention.

The virome in bronchiectasis

DNA-based microbiome analytics do not allow evaluation of RNA viruses, key organisms within the bronchiectasis microbiome. Consequently, viruses including influenza, respiratory syncytial virus, coronaviruses and rhinovirus have been overlooked by many existing studies. In the clinical setting, targeted detection of specific viruses by reverse transcriptase PCR is common and roles in other respiratory disease states recognised. Nevertheless, holistic RNA-based assessment of the virome is less commonly performed and lacks thorough application and study in bronchiectasis. While several methods related to metatranscriptomics have been proposed, these analyses face challenges including RNA degradation, reverse transcription biases, inherent complexity related to respiratory viruses, host RNA contamination and bioinformatic challenges [163, 164]. Addressing such factors necessitates a tailored experimental and analytical approach to accurately study the respiratory virome. Furthermore, metatranscriptomics will only capture RNA virus profiles, while DNA viruses (and phages) may play important roles and, therefore, to ensure a holistic assessment of the virome, analysis of both RNA and DNA viral species are necessary [165]. While it remains a challenge to apply in clinical settings, virome analysis is likely to reveal important components related to bronchiectasis pathogenesis, although type and timing of sample collection is important given the dynamics related to viral burden and host response [164, 166]. Notably, viral infection commonly precedes bacterial infection which, in turn, can influence the microbiome, highlighting the relevance of considering viral–bacterial interaction. In an important demonstration of this phenomenon,

MOLYNEAUX *et al.* [167] highlighted the role of rhinovirus infection in enhancing bacterial burden, noting an increase in *H. influenzae* within the existing microbiota. This finding points to complex interactions between viral infections and bacterial dynamics, suggesting that viruses may influence the exacerbation of underlying respiratory conditions associated with alterations in the composition of respiratory microbiota. These insights underscore the need for integrated viral and bacterial management in respiratory diseases [167]. Indeed, integration of targeted viral identification within microbiome and mycobiome data has already highlighted the relevance of such interaction, particularly in association with exacerbation [107]. The occurrence of specific bacteriophage profiles associated with clinical outcomes in bronchiectasis is yet to be elucidated and a key unmet research need in relation to identifying phage patterns and clinical outcomes continues to exist [107, 113]. Bacteriophages may reshape microbiomes and lead to altered community structures with relevant diagnostic and therapeutic implications for bronchiectasis [168, 169].

Inter-kingdom analysis and multiomic approaches in bronchiectasis

Inter-kingdom analyses related to the airway microbiome has informed mechanisms related to exacerbations in CF and bronchiectasis [107, 170]. Networks highlighting microbial interactions may even more accurately reflect clinical variation, offering a novel framework for understanding bronchiectasis and exacerbations [171, 172]. Microbial networks are significantly altered in bronchiectasis; observed interactions between bacteria, viruses and fungi offer gains in predicting exacerbation as compared to analysis of microbial relative abundance profiles in isolation [107]. The holistic capture of the inter-kingdom "interactome" provides a framework to assess the intricate and often unpredictable behaviour of pathogens in clinical settings. This approach recognises that interactions are not static but dynamic, embedded in a multidimensional space where simplistic, reductionist clustering of microbiome data can mislead or obscure clinically significant patterns. Exploring interaction profiles of specific pathogens can uncover synergistic or antagonistic relationships that may critically influence disease progression or therapeutic responses [107, 108]. This strategy is particularly relevant in chronic diseases, where understanding shifts in microbial communities could inform targeted therapies, potentially leading to more effective and personalised treatment strategies [107, 108, 122]. Furthermore, analysis of gut–lung microbiota (including bacteria and fungi) further highlights the importance of network interaction dynamics and their association with bronchiectasis phenotypes [138]. Increasing application of such "multi-biome" approaches, including longitudinal studies, will likely provide additional insight into potential interaction dynamics that correlate with health, disease and predictions of treatment response. Interestingly, it has been recently observed that particular bronchiectasis "resistotypes" are identifiable by metagenomics and correlate with clinical phenotypes [113]. The relationship between mobile genetic elements, plasmids and the airway resistome remains to be studied in bronchiectasis and may uncover further insight into bacteriome–resistome correlates of clinical relevance [173].

Microbiomes for diagnosis, stratification and prognostication in bronchiectasis

Large bronchiectasis patient registries, with associated biobanks, are now established globally (figure 2a). These have enabled, for the first time, microbiome studies to be combined with clinical data in large numbers of patients [43, 174, 175]. This undoubtedly will lead to more comprehensive, multiomic studies, replete with longitudinal follow-up and representation of more diverse patient populations and disease phenotypes. Differences in the bronchiectasis microbiome can be leveraged to stratify patients and compare clinical outcomes and disease characteristics [18, 42, 124, 129]. Importantly, however, existing studies are often comparative, with patients grouped by microbiome population medians or tertiles. Such approaches preclude the use of microbiome data as clinical, diagnostic and/or prognostication tools. Individual NGS microbiome data, however, may be utilised alongside traditional microbiology as part of enhanced clinical monitoring of "at-risk" patients, particularly for cases where standard culture has failed to identify potential causative agents related to exacerbations and/or to monitor the effectiveness of eradication therapies or novel treatments [113, 176, 177]. One of the biggest challenges to developing novel therapeutics for bronchiectasis has often been the failure of reproducible randomised controlled trials, such as the RESPIRE or ORBIT trials of dry powder or inhaled ciprofloxacin [9, 10, 178–180]. In nonalcoholic fatty liver disease, microbiome network analysis has been employed to identify responders or nonresponders to novel interventions [181]. The same approach should be considered in bronchiectasis.

Can we target the microbiome for therapeutic benefit?

Focus has traditionally been on symptom and infection control, with minimal attention on how treatments affect the microbiome in bronchiectasis. Use of macrolides and other antibiotics are central for managing infections and reducing exacerbations, yet these treatments alter resident microbial composition and affect resistance patterns, highlighting the complex interaction between microbial communities and therapeutic intervention [113, 130, 182, 183]. This emphasises the need to consider the therapeutic-related effects on the microbiome, as they may influence disease progression and future response to treatments. More recent

emphasis has shifted towards targeted microbial therapies, such as narrow-spectrum antibiotics and bacteriophage therapy, aiming to address pathogens while preserving beneficial "commensal" bacteria. Such strategies represent a move towards precision medicine, by attempting to minimise the drawbacks of broad-spectrum antibiotics and target therapy at the individual level [184, 185]. Additionally, interconnections between gut and lung microbiomes, alongside environmental factors, suggest that a comprehensive treatment approach incorporating lifestyle, diet, comorbidities and the patient's living environment should be considered [186, 187]. This broader view appears essential for developing fresh therapeutic options for bronchiectasis that considers the complex interplay between microbiomes, immunity and the surrounding environment [122]. ICS have limited and poorly understood impacts on the respiratory microbiome in bronchiectasis [188]. Recent work has indicated the potential benefits of ICS in patients with a predominantly eosinophilic bronchiectasis phenotype, with microbiome composition related to this inflammatory endotype, suggesting the potential for microbiome-targeted approaches [20]. Biologics represent an additional potential treatment approach in bronchiectasis, especially with agents that target eosinophilic inflammation and Th2-mediated responses. These therapies offer a route to modulate the immune–microbiome axis and individualise therapy [56, 189, 190]. This is an exciting era for bronchiectasis, one where targeting the microbiome represents a fresh opportunity to realise precision medicine and develop more effective, targeted treatments that consider infection, inflammation and immunity (table 1) [122].

Conclusions and future directions

The intricate interplay between microbiome, immune response and host factors in bronchiectasis emphasises the need for targeted therapeutic strategies that address such complexity. To advance the management of bronchiectasis, future research must prioritise a better understanding of the airway microbiome and the precise role of commensals and/or pathobionts in maintaining respiratory health [132, 191, 192]. Such work entails exploring the symbiotic relationship between host and microbial communities. Crosstalk between the microbiome and other host features such as genetics, inflammation and immunity represents another crucial avenue for investigation [51, 193]. Understanding these interactions will uncover mechanisms of disease, exacerbation and risks associated with progression, which in turn will guide the development of novel therapeutics aimed at disrupting these pathological processes. The application of multiomics in bronchiectasis research holds great promise. By integrating microbiome data with that from genomics, transcriptomics, proteomics and metabolomics, a more holistic view of disease will be gained and novel biomarkers to detect, monitor and tailor treatment can be developed. Such approaches must be applied at scale in large and carefully curated global biobanks which must be maintained and supported. Identifying early or "at-risk" microbial features to arrest disease progression should be prioritised [192, 194] and the future successes for discovering novel interventions for bronchiectasis are grounded in the integration of multiomics approaches with microbiology and NGS, allowing an improved understanding of host–microbe dynamics. Employing such a strategy promises to transform the bronchiectasis landscape and move beyond the traditional symptomatic care to one addressing fundamental mechanisms that drive disease [122].

TABLE 1 Current and emerging stratification and therapeutic targeting of the microbiome in bronchiectasis

Current methods	Emerging/future methods
Stratification based on clinical phenotypes, *e.g.* frequent exacerbators	Microbiome as an integrated biomarker and treatable trait
Stratification based on disease severity (BSI, FACED)	Ecological metrics (α-/β-diversity), graph-theoretic measures (degree, betweenness centrality and modularity)
Broad antimicrobial therapy based on microbial culture results	Evaluations of pathogens and associated commensal–pathobiont interaction networks to inform antimicrobial therapies
Use of azithromycin, doxycycline, fluoroquinolones (pathogen-centric) for pathogen eradication	Targeted antimicrobial eradication programmes, bacteriophage therapy, antibody–drug conjugates, narrow-spectrum microbiome-targeting
Broad anti-inflammatory therapy, *e.g.* inhaled corticosteroids	Precision therapeutics, *e.g.* anti-IL5 and DPP1 inhibitors
Focus on the lung as an independent biome	Holistic integration and appreciation of multi-site "axes", *e.g.* gut–lung and oral–lung
One size fits all treatments based on the "vicious vortex" model	Multimodal precision interventions integrating ongoing assessments of the microbiome into the vicious vortex
Stratification of patients into microbiome types defined in relation to other patients' microbiomes	Personalised individual prognostic/diagnostic microbiome results

BSI: bronchiectasis severity index; DDP1: dipeptidyl peptidase 1; FACED: forced expiratory volume in 1 s, age, chronic *Pseudomonas aeruginosa* colonisation, radiological extension and dyspnoea; IL: interleukin.

Points for clinical practice

- Integrating advanced multiomics technologies to understand the complex interplay of infection and the microbiome in bronchiectasis will bring new perspectives to disease management.
- The diversity of microbial dynamics should be considered when diagnosing and managing bronchiectasis, emphasising personalised treatment plans that address specific microbial imbalances and immune responses.
- The application of available genomic technologies, such as targeted amplicon sequencing and shotgun metagenomics, could substantially improve therapeutic outcomes by enabling tailored microbial management strategies.

Questions for future research

To advance bronchiectasis management, future research must harness the complex interactions between microbes, immune responses, host genetics and the exposome. Increased understanding of the microbiome and its role in health, including both symbiotic and pathogenic relationships, is crucial. Future research must reveal mechanisms driving disease exacerbation and progression, guiding the development of novel, targeted and preventative therapies. Employing multiomics approaches, integrating genomic, transcriptomic, proteomic and metabolomic data, will enrich our perspective on disease. While this brings analytical, interpretive and translational challenges, it holds the potential to uncover novel biomarkers and refine treatment strategies. Implementing these methods in large, well-maintained global biobanks is vital for validating biomarkers and therapeutic targets across diverse populations. These efforts aim to shift bronchiectasis management from symptomatic treatment to a focus on fundamental disease mechanisms and diverse clinical endophenotypes.

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