HHS Public Access

Author manuscript

Expert Rev Respir Med. Author manuscript; available in PMC 2019 October 01.

Published in final edited form as:

Expert Rev Respir Med. 2018 October ; 12(10): 857–865. doi:10.1080/17476348.2018.1513331.

Cystic fibrosis respiratory microbiota: unraveling complexity to inform clinical practice

Lindsay J. Caverly and **John J. LiPuma***

Department of Pediatrics and Communicable Diseases, University of Michigan, 8200 MSRB3, 1150 W. Medical Center Dr., Ann Arbor, MI, 48109

Abstract

Introduction: Cystic fibrosis (CF) lung disease is characterized by chronic cycles of pulmonary infection, inflammation, and mucus obstruction, beginning early in life, and eventually leading to progressive lung damage and early mortality. During the past ~15 years, culture-independent analyses of CF respiratory samples have identified diverse bacterial communities in CF airways, and relationships between respiratory microbiota and clinical outcomes.

Areas covered: This article reviews recent advances in our understanding of the relationships between respiratory microbiota and CF lung disease. The article focuses on measures of airway bacterial community diversity and estimates of the relative abundance of anaerobic species. Finally, this paper will review the opportunities for advancing patient care suggested by these studies and highlight some of the ongoing challenges and unmet needs in translating this knowledge into clinical practice.

Expert Commentary: Culture-independent analyses of respiratory microbiota have suggested new strategies for advancing CF care, but have also highlighted challenges in understanding the complexity of CF respiratory infections. Development of more sophisticated models and analytic approaches to better account for this complexity are needed to elucidate mechanistic links between CF respiratory microbiota and clinical outcomes, and to ultimately translate this knowledge into better patient care.

Keywords

Cystic fibrosis; microbiome; metabolomics; anaerobes; exacerbation

*Corresponding author: John J. LiPuma, Department of Pediatrics and Communicable Diseases, University of Michigan, 8200 MSRB3, 1150 W. Medical Center Dr., Ann Arbor, MI, 48109, Phone: 734-763-3643, Fax: 734-764-4279, jlipuma@med.umich.edu.

Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Reference annotations

*Of interest

**Of considerable interest

1. Background

1.1 Importance of airway infection in CF lung disease

Cystic fibrosis (CF) is an autosomal recessive disease in which mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein result in abnormal transepithelial ion transport, leading to multi-organ disease. The life-limiting nature of CF is primarily due to lung disease, in which cycles of airway mucus obstruction, infection, and inflammation begin early in life and lead to progressive lung damage and respiratory failure. While the recent development of drugs that directly modulate CFTR activity has opened new avenues for treatment, efforts to mitigate CF-related morbidity and mortality continue to rely primarily on treatment of the downstream effects of the CFTR defect. For CF lung disease, this involves treatment of airway mucus obstruction and infection through mucus clearance, and the use of mucolytics and antibiotics.

Antibiotic selection is typically based on the results of *in vitro* bacterial culture of respiratory specimens: oropharyngeal (OP) or nasopharyngeal (NP) swabs, expectorated sputum, or, less commonly, bronchoalveolar lavage fluid (BALF). By convention, such cultures are intended to identify a relatively small set of known human pathogens prevalent in CF airways. Infections with *Staphylococcus aureus* and *Haemophilus influenzae* are most prevalent in infants and young children, followed by increasing rates of infection with *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Achromobacter* spp., *Burkholderia* spp., and nontuberculous mycobacteria (*Mycobacterium avium* complex and *Mycobacterium abscessus* complex) throughout adolescence and young adulthood(1,2).

1.2 Limitations of culture-based understanding of CF airway infections

Antibiotic therapy directed towards CF pathogens identified by bacterial culture has played a major role in the steadily improving outcomes and life-spans for persons with CF over the past ~ 50 years. However, several lines of evidence highlight knowledge gaps in this culture-based understanding of CF airway infections. Despite intensive antibiotic therapy directed against bacteria recovered in culture, chronic infection and inflammation still lead to progressive lung function decline and early mortality in the majority of patients(2). In ~25% of CF pulmonary exacerbations, patients do not recover their baseline lung function despite intravenous antibiotic treatment directed against bacteria recovered in culture(3). Similarly, in the setting of pulmonary exacerbation, clinical response to antibiotic therapy correlates poorly with *in vitro* antibiotic susceptibility testing of bacteria recovered in culture(4). Finally, pulmonary exacerbations can occur in the absence of identifiable CF pathogens in respiratory cultures (“culture-negative exacerbations”)(5).

1.3 Culture-independent studies of CF airway infections

Culture-independent studies of CF respiratory microbiota, which involve analyses of bacterial DNA directly in clinical samples without the need for recovery of viable bacteria in culture, offer opportunities to address the knowledge gaps described above. Early culture-independent studies revealed that CF respiratory microbiota comprise complex bacterial communities that include bacteria not routinely identified by culture, many of which are obligate and facultative anaerobes. The most prevalent and abundant anaerobic genera

identified in CF respiratory specimens are consistent across patient ages and sampling methods, and include the obligate anaerobes *Actinomyces, Fusobacterium, Porphyromonas, Prevotella, Veillonella,* and the facultative anaerobes *Gemella, Granulicatella, Rothia,* and *Streptococcus*(6–10). These genera are similar to those found in BALF samples of healthy adults using both culture(11) and culture-independent methods(12,13), supporting the presence of anaerobes in the lower airways (i.e., not merely representing salivary contamination of specimens).

In addition to being prevalent in CF airways, anaerobic genera collectively can comprise a significant proportion of the relative abundance of the CF respiratory microbiota(7–10,14–24). In a recent study using DNA sequence analysis of 945 CF sputum samples(10), anaerobic genera collectively had a median relative abundance of 37%, and in a third of the samples comprised at least 50% of the community. The relative abundances of individual anaerobic species, however, tend to be low (<20%)(10). In contrast, traditional CF pathogens, when present, tend to have higher individual relative abundances compared to anaerobes.

The tendency of CF pathogens to dominate bacterial communities when present, compared to the lower relative abundances of individual anaerobic taxa, is reflected in measures of bacterial community diversity, which include measures of the number (richness) and/or distribution (evenness) of taxa. In the same study of 945 CF sputum samples described above(10), anaerobic genera were relatively more abundant in samples with higher overall community diversity. In contrast, samples containing traditional CF pathogens had lower community diversity(10). Higher bacterial community diversity is thus, to a large degree, reflective of higher relative abundance of anaerobes.

In early culture-independent studies comparing respiratory microbiota to clinical outcomes, both bacterial community diversity and the relative abundance of anaerobes were consistently associated with patient age, lung function, and pulmonary exacerbations(25). However, the relationships observed between community diversity, anaerobes, and clinical outcomes can appear to be discordant. For example, higher relative abundance of anaerobes, and higher measures of bacterial community diversity, are associated with younger age, and milder lung disease(6,26). In contrast, higher relative abundance of anaerobes and higher community diversity have also been observed coincident with the onset of pulmonary exacerbation(27).

1.4 Applications of culture-independent analyses to clinical practice

These seemingly discordant findings complicate the application of culture-independent profiling to CF clinical care and raise many questions. How should measures of anaerobe relative abundance and/or community diversity be used to guide treatment? In the long term, should antibiotic management aim to preserve community diversity and high relative abundance of anaerobes? What are the driving forces behind the observed associations between anaerobes, community diversity, age, and lung disease? Can respiratory microbiota be manipulated to alter the course of lung function decline over time? In the short term, in the setting of pulmonary exacerbation, should anaerobes be targeted with antibiotic

treatment? Can measures of anaerobe relative abundance or community diversity serve as predictive markers for pulmonary exacerbation?

In the following sections, we will summarize recent culture-independent studies of CF airway microbiota with particular emphasis on the relationships between anaerobes, diversity, and long term and short term clinical outcomes. We will discuss how recent studies have further revealed the complex system of CF airway infections and lung disease, with relevant components including clinical variables (e.g. age, clinical state, disease stage, disease aggressiveness, etc.) (Table 1), microbial community structure, microbial metabolic activities, microbial interactions, and host-pathogen interactions. Through unraveling these components of system complexity, we have advanced our understanding of the relationships between respiratory microbiota and CF lung health and disease, and ultimately moved closer towards our goal of applying this knowledge to advance CF clinical care.

2. Anaerobes and diversity relative to long term outcomes

2.1 Anaerobes and diversity decrease with advancing age and lung disease stage

An early finding of culture-independent studies of CF respiratory microbiota was the association between patient age and anaerobe-dominated bacterial communities. Cross-sectional studies indicated that during the first decade of life bacterial communities are highly diverse and comprised primarily of anaerobic genera(6,8). Starting in the second decade of life, communities experience progressive decreases in the relative abundances of anaerobes and community diversity, and corresponding increases in the relative abundances of typical CF pathogens (*Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Achromobacter* spp., *Burkholderia* spp)(6,26–29). Using a cross-sectional set of sputum samples from 296 persons with CF ranging in age from 4 to 64 years, Coburn and colleagues(7) identified a similar pattern of maximal diversity in the first decade of life, followed by decreasing anaerobe relative abundance and community diversity over the second decade of life. This study corroborated the previously observed inverse relationships between diverse, anaerobe-dominated communities and patient age in a large cohort that included a higher percentage of pediatric subjects than previous studies.

The observed decreases in anaerobe relative abundance and community diversity with advancing patient age is of interest in that these changes occur in parallel with the characteristic progression of CF lung disease during adolescence and early adulthood. Several early studies demonstrated relationships between diverse, anaerobe-dominated communities and lung disease *stage*, defined as early (percent predicted forced expiratory volume in one second [ppFEV1] >70%), intermediate (ppFEV1 between 40% and 70%), and advanced (ppFEV1 <40%)(26). These relationships have been observed in more recent cross-sectional studies in large patient cohorts(7,30).

2.2 Anaerobes and diversity decrease with more aggressive lung disease

In addition to these cross-sectional studies, longitudinal analyses also showed relationships between diverse, anaerobe-dominated communities and rate of change in lung function over time relative to age, or disease *aggressiveness* (mild, moderate, or severe)(31). In an early

study, Zhao and colleagues(26) used 16S rRNA gene sequencing to analyze sputum samples collected over 10 years from six age-matched adult male subjects with CF. In this small cohort, bacterial community diversity remained relatively stable over time in subjects with stable lung function. Bacterial community diversity, however, decreased over time in subjects with increased lung function decline (i.e., more aggressive disease), largely driven by antibiotic use(26).

More recently, Carmody and colleagues(30) performed 16S rRNA gene sequencing on sputum samples collected over periods of at least five years from 24 subjects. To control for differences in respiratory microbiota related to different disease stages, only samples collected during intermediate disease stage were included. In this cohort, and similar to prior findings(26), patients with mild disease aggressiveness had more diverse communities and a higher proportion of anaerobic genera compared to patients with moderate or severe disease aggressiveness. Similarly, the relative abundance of anaerobes was positively associated with lung function. Collectively, these longitudinal studies demonstrate that disease aggressiveness, along with age and lung disease stage, is a relevant clinical variable to consider in studies of CF respiratory microbiota. Although the causal relationships between respiratory microbiota and lung function decline over the long term remain unclear, studies of respiratory microbiota in relation to age, lung disease stage, and disease aggressiveness suggest the potential for clinical benefit from preserving diverse, anaerobe-dominated communities.

2.3 Anaerobes and diversity in the first years of life

Studies of respiratory microbiota in relation to age, lung disease stage, and disease aggressiveness have primarily relied on analyses of sputum samples from older children and adults. Studies employing sputum samples typically exclude infants and younger children who are unable to expectorate sputum. An understanding of the dynamics of airway microbiota during the first few years of life, a period in which airway infection, inflammation, bronchiectasis, and lung function abnormalities are known to occur (32), is important to our efforts to preserve lung health in CF.

Investigators in three recent studies performed 16S rRNA gene sequencing on large numbers of (primarily cross-sectional) banked BALF samples collected from infants and young children with CF in Australia(16,18) and the United States(9), and demonstrated that relationships between diverse, anaerobe dominated communities and age begin in the first years of life. In all three studies, bacterial community diversity was highest in infants, then *decreased* over the first few years of life(9,16,18). These findings are in contrast to the cross-sectional studies of older children and adults discussed above, in which a decrease in community diversity was observed beginning in the second decade of life(6,7). Possible factors accounting for these discrepancies include different patient populations (infants and toddlers, versus older children), small sample sizes in the earlier studies, different sampling methods (i.e., BALF vs sputum or OP swab), and/or the limitations of cross-sectional studies.

While these recent studies were also primarily cross-sectional, a subset of the children (N=27) studied by Frayman and colleagues(16) had repeated BALF sampling. While

decreasing diversity over time was seen in the majority, certain children had increasing diversity over time. This between-subject variability in community dynamics emphasizes the importance of longitudinal studies of CF respiratory microbiota, suggests the presence of identifiable factors that influence diversity dynamics, and could potentially be employed to modulate community for clinical benefit(16).

In yet another study(15) of BALF samples from infants and young children, lower community diversity was associated with younger age. While this finding is seemingly at odds with the inverse relationship between age and diversity identified in the studies discussed above(9,16,18), the lower community diversity observed in this study may be confounded by the disproportionate use of prophylactic antibiotics in the youngest subjects(15). Further identification of factors that drive age-related changes in diversity (e.g. antibiotic use), and their relationships to lung disease offers opportunities for insights into early determinants of CF lung disease, and the development of novel therapeutic strategies.

Recent studies also demonstrate that the relationships between diverse, anaerobe-dominated communities and lung disease stage also begin in the first years of life. In a small study of BALF sampling of infants with CF, the relative abundance of *Streptococcus* was positively associated with infant pulmonary function tests (PFTs)(33). Lung function testing with infant PFTs, however, is not routinely performed in all centers, and is known to be imperfectly sensitive to early lung damage(34). Given the limitations of using infant PFTs to gauge disease stage, several studies have investigated relationships between respiratory microbiota and markers of inflammation as surrogate outcomes to identify early lung disease.

Consistent with data from older children and adults regarding respiratory microbiota and disease stage, anaerobes and diversity are inversely correlated with lung disease (as measured by airway inflammation) in the first years of life. In infant BALF, this has included an inverse association between bacterial community diversity and airway neutrophil elastase(16). BALF communities dominated by *Streptococcus* were found to have lower levels of IL-8 and neutrophil elastase than those dominated by CF pathogens(16). Zemanick and colleagues(17) demonstrated that greater similarities between the oral (i.e., OP swab) and lower respiratory (i.e, sputum) microbiomes were associated with lower levels of lower respiratory tract inflammation(17). That is, diverse, anaerobe-dominated communities were associated with less respiratory tract inflammation, a finding again consistent with those seen in older children and adults. While the causal relationships between respiratory microbiota and lung disease and respiratory tract inflammation in the first years of life remain unclear, these data suggest the potential for clinical benefit from preserving diverse, anaerobe-dominated communities early in life.

The studies above demonstrate associations between diverse, anaerobe-dominated communities and better lung health in infants and young children with CF. However, recent data also suggest associations between diverse, anaerobe-dominated communities and negative markers of lung health CF. In a recent, largely cross-sectional study, Muhlebach and colleagues(18) analyzed 53 BALF samples from 46 children ages 3.5 months to 5 years with 16S rRNA gene sequencing. In this cohort, children under one year of age had BALF

microbiota that was indistinguishable from the environmental control samples. Children between the ages of 1 and 2 years had BALF predominantly composed of anaerobes typically associated with the oral cavity (e.g. *Streptococcus*, *Prevotella*, and *Veillonella*), and also had higher levels of inflammatory markers (cell counts and neutrophils) and total bacterial load (16S qPCR) than younger infants. Further increases in age to 3–5 years was associated with increases in relative abundances of CF pathogens and increased bronchiectasis, as well as trends towards further increases in inflammatory markers and total bacterial load. These findings underscore prior conclusions that diverse, anaerobe dominated communities are associated with less disease than CF pathogen-dominated communities, but also illustrate the potential for anaerobe-associated disease (i.e., increased inflammation and total bacterial load).

3. Anaerobes and diversity relative to short term outcomes

3.1 Anaerobes and diversity related to pulmonary exacerbation

Culture-independent studies of changes in respiratory microbiota associated with CF pulmonary exacerbations (i.e., disease *state*(26)) have further illustrated the potential for anaerobe-associated disease. Conventionally, pulmonary exacerbations were thought to result from proliferation of the CF pathogens identified in culture. Early culture-independent studies challenged this perspective by identifying associations between other microbiota and pulmonary exacerbation. For example, in a study comparing sputum samples collected at baseline to those collected during pulmonary exacerbation, communities dominated by *P. aeruginosa* experienced a *decrease* in the relative abundance of *P. aeruginosa* (27). This decrease was accompanied by increases in community diversity and the relative abundance of anaerobic species(27). Other studies have also identified anaerobes associated with pulmonary exacerbation, including species in the *Streptococcus milleri* group(35,36), and *Rothia* spp.(37,38).

Based on these data, a *climax-attack* model of CF exacerbations has been proposed(39–41). This model describes two functional microbial communities in CF airways. The “climax” community is associated with baseline/stable clinical states, while the “attack” community dominates during exacerbation of symptoms. Although these communities are not defined taxonomically, climax communities are composed primarily of typical CF pathogens, whereas attack communities are associated with anaerobic genera. While these functional communities exist in a stable configuration during periods of clinical stability, anaerobic fermentation periodically increases, lowering local pH, and resulting in community instability and pulmonary exacerbation(37,39–41).

Supporting the proposed climax-attack model of CF, other studies have provided evidence for associations between anaerobes and pulmonary exacerbation. In a recent study by Carmody and colleagues(30), samples collected at exacerbation had higher cumulative relative abundance of anaerobic genera and higher levels of community diversity compared to samples obtained during periods of baseline health. In another study, based on a single sample collected from 78 persons with CF(42), anaerobic species (*Streptococcus pneumoniae* and *Rothia mucilaginosa*) were associated with greater rate of lung function

decline in the year preceding sample collection. Products of anaerobic glycolysis (e.g., pyruvate and lactate) have also been shown to be elevated at times of exacerbation(43).

While these studies do not demonstrate a causal relationship between anaerobes and pulmonary exacerbation, other studies have identified potential mechanisms by which anaerobes may exacerbate CF lung disease through interactions with CF pathogens. Anaerobic genera including *Prevotella*, *Veillonella*, *Streptococcus*, and *Fusobacterium* are capable of degrading mucins in CF sputum to generate amino acids and short chain fatty acids, which then stimulate *P. aeruginosa* growth in a co-culture model(44). Short chain fatty acid production by anaerobes has also been shown to result in a dose-dependent increase in IL-8 when applied to CF epithelial cells(45). *P. aeruginosa* virulence factor production is also stimulated by co-culture with oral *Streptococci* species(46). In a recent study, *P. aeruginosa* appeared to use substrates produced by *Rothia* to generate primary metabolites in vitro, providing further evidence that anaerobic species impact the growth, and potentially the virulence, of *P. aeruginosa* in CF airways(47).

3.2 Clinical variables relevant to respiratory microbiota and pulmonary exacerbations

These relationships between diversity, anaerobe relative abundance, and pulmonary exacerbation have suggested potential clinical applications. Should anaerobes be targeted for antibiotic treatment in the setting of pulmonary exacerbation? Can measures of anaerobes or community diversity serve as predictive markers for pulmonary exacerbation? The answers to these and other questions remain unclear, in part due to inconsistencies in the data regarding respiratory microbiota and pulmonary exacerbation. For example, multiple large, cross-sectional studies, have not identified consistent, microbiological features of pulmonary exacerbation(7,9,27), suggesting that the determinants of pulmonary exacerbation are likely multifactorial. This, in turn, suggests the need to take relevant clinical variables into account when considering changes in respiratory microbiota relative to pulmonary exacerbation.

Differences in respiratory microbiota based on patient age and lung disease stage also need to be considered(6,26). Insofar as bacterial community structure typically changes relative to patient age and disease stage (i.e., diversity decreases with increasing age and lung disease), analyses of community dynamics relative to pulmonary exacerbation must take these features into account. The need to control for these features in studies of pulmonary exacerbation was illustrated in a recent large, cross-sectional study comparing samples obtained at baseline to those collected during pulmonary exacerbation(48). While, in general, samples collected at exacerbation had more diverse communities and higher relative abundances of anaerobes, these findings were true *only* for samples collected from patients in early and intermediate disease stages. In contrast, samples from older patients in late disease stage, whose bacterial communities tend to have low diversity with dominance by a typical CF pathogen, did not have identifiable changes between baseline and exacerbation samples.

Another factor likely contributing to the lack of identification of a consistent change in respiratory microbiota with pulmonary exacerbation is the interpatient differences observed in baseline bacterial communities. This was demonstrated in an early study by Carmody and colleagues(27), in which 34 paired baseline and exacerbation sputum samples from 28 adults

with CF were analyzed. Significant interpatient differences in changes in respiratory microbiota from baseline to exacerbation were observed. For example, communities dominated by *Pseudomonas* had different changes in diversity between baseline and exacerbation than communities dominated by other species(27). Communities dominated by *Pseudomonas* have been shown to have a *decrease* in relative abundance of *Pseudomonas* between baseline and exacerbation samples(27), whereas an *increase* in the abundance of *Burkholderia multivorans* (using genus-specific qPCR) has been identified prior to exacerbation onset in a separate study(49). These data demonstrate that the dominant CF pathogen is a relevant variable in considering changes in respiratory microbiota between baseline and exacerbation.

Several groups have controlled for interpatient variability in baseline respiratory microbiota by using longitudinal study designs to identify intrapatient changes coincident with pulmonary exacerbation. Carmody and colleagues(50) performed 16S rRNA gene sequencing on daily sputum samples from four persons with CF, starting at a period of baseline and going through to the onset of exacerbation. In this cohort, a decrease in the relative abundance of dominant taxa (with a corresponding increase in the nondominant taxa, i.e. largely anaerobes) occurred before symptom onset in some, but not all, exacerbations(50). More recently, Whelan and colleagues(51) collected three times weekly sputum samples from six subjects for one year, and similarly identified subject-specific changes in community composition of respiratory microbiota during periods of clinical stability prior to some exacerbations, but also did not identify universal indicators of exacerbation either between or within subjects(51). While communities that share certain common features (e.g., dominant taxa, baseline diversity) may also share more common dynamics between baseline clinical stability and exacerbation, the lack of unifying, within-subject changes in respiratory microbiota with pulmonary exacerbation in these longitudinal studies emphasizes the complex nature of CF lung disease, and the likelihood that additional variables are relevant to the goal of untangling changes in CF respiratory microbiota associated with exacerbation.

4. System complexity

In the prior sections we have discussed relationships between CF respiratory microbiota and several relevant variables, including age, disease stage, disease state, and disease aggressiveness, and features of baseline bacterial communities. In recent years, the complexity of CF respiratory microbiology has been further elucidated, and additional relevant variables have been identified. The complexity of this system presents a challenge to efforts to identify meaningful microbiologic features that can translate to changes in clinical practice. Better accounting of the variables in this complex system will be needed to reconcile the discordant findings described above. In the following sections we discuss some of these additional features of system complexity, including antibiotic use, regional disease heterogeneity, bacterial interactions, unique characteristics of CF sputum, and relationships between lung and gut microbiota.

4.1 Antibiotics

Early culture-independent studies identified antibiotic use as a critical variable impacting respiratory microbiota. Antibiotic use affects respiratory microbiota both in the short term, with episodic therapy of pulmonary exacerbations(26), and in the long term, in the form of cumulative antibiotic pressure being a driver of decreases in diversity(7,26,52). Changes in respiratory microbiota related to antibiotic pressure also vary based on age and disease stage. In infancy, antibiotic use may result in shifts to new bacterial community compositions (i.e., communities do not return to the pre-antibiotic community composition), including increased bacterial community diversity(53), and increased relative abundance of gram negative genera (e.g., *Burkholderia* spp. and *Enterobacteriaceae*)(54). Prophylactic antibiotic use in infants is associated with lower diversity(15). In early stage lung disease, bacterial communities may be temporarily perturbed with episodic antibiotic treatment, but are generally resilient, returning to their prior structure following therapy(21,28,55,56). Eventually, in advanced stage disease, CF bacterial communities become dominated by typical CF pathogens, and relatively resistant to antibiotic perturbation(27–29).

Less well studied are the effects of maintenance (chronic suppressive) antibiotics, and the role that these play in baseline dynamics of respiratory microbiota. In a recent study of subjects using ivacaftor versus placebo(57), changes in respiratory microbiota based on ivacaftor usage were not identified. However, a subgroup analysis that only included subjects with a stable maintenance antibiotic regimen during the study period identified significant reductions in total bacterial load associated with ivacaftor treatment. This study emphasizes the need to consider changes in maintenance antibiotics in studies of respiratory microbiota relative to clinical outcomes.

4.2 Regional disease heterogeneity

Recent studies have also expanded our understanding of other unique features of the CF respiratory system that impact bacterial communities, including the regional heterogeneity of airway disease typical in CF. Hogan and colleagues(58) investigated the influence of regional lung damage on airway microbiota in 9 adults with CF and early or intermediate lung disease (FEV1 > 50% predicted)(58). The bacterial communities detected in BALF samples from multiple lobes per lung did not differ from each other relative to the degree of lung damage as determined by CT scan. Inter-subject differences in microbiota were greater than intra-subject differences between different lung regions. Within individual subjects, communities measured in sputum samples correlated well with those measured in BALF(58). These data support the use of sputum samples as representative of lower airway microbiota, even in the setting of regional disease heterogeneity.

4.3 Microbial interactions

Bacterial interactions within airway microbiota likely have bearing on relationships between respiratory microbiota and clinical outcomes. Interactions between *P. aeruginosa* and other community members are perhaps the best characterized. The majority of persons with CF will have *S. aureus* as the primary pathogen recovered in respiratory culture during childhood, and will acquire chronic *P. aeruginosa* infection at some point during adolescence or early adulthood(2). As acquisition of chronic *P. aeruginosa* infection is associated with

increased rates of lung function decline, the mechanisms by which *P. aeruginosa* outcompetes *S. aureus* and other community members have been the subject of much interest. Using a co-culture model on CF bronchial epithelial cells, Filkins and colleagues(59) recently demonstrated that *P. aeruginosa* preferentially consumes *S. aureus*-produced lactate as a carbon source, then over time produces metabolites to reduce *S. aureus* viability.

Recent data suggest that microbial interactions likely have bearing on response to antibiotic therapy, and may contribute to the observed discordance between *in vivo* antibiotic susceptibility testing results and clinical response(4). In a recent study, 76% of *Prevotella* isolates from persons with CF produced extended spectrum beta lactamases (ESBL)(60). In a co-culture model, ESBL-producing *Prevotella* isolates protected *P. aeruginosa* from killing by ceftazidime(60). Jorth and colleagues(61) recently demonstrated significant regional diversity of virulence traits and antibiotic resistance phenotypes in *P. aeruginosa* isolates obtained from CF lung explants(61). This study suggests that regional differences in lung disease likely exert differing selective pressures on bacterial communities, and highlight the limitations of *in vitro* susceptibility testing of a single isolate recovered from respiratory culture in predicting the range of antibiotic susceptibilities of the entire population.

4.4 Unique characteristics of CF sputum

An important determinant of the efficacy of antibiotic killing of bacteria is bacterial growth rate. Kopf and colleagues(62) labelled sputum with heavy water, and demonstrated that *S. aureus* growth rate in sputum was consistently lower than growth in culture media *in vitro* (2–100 times slower in sputum). Using longitudinally collected samples from subjects during treatment for pulmonary exacerbation, they observed variability of *S. aureus* growth rate during the treatment course, including initial slowing of bacterial growth followed by increased growth rate while still on antibiotics(62). To identify factors of CF sputum that could influence microbial growth rates, Cowley and colleagues(63) measured the chemical composition of 48 freshly expectorated sputum samples from 22 children with CF. Through modelling of oxygen dynamics, the investigators concluded that CF sputum is spatially heterogeneous, with a thin oxygen-replete outer layer, a hypoxic zone, and an anoxic core(63). These and other studies represent advances in our understanding of how CF bacterial communities adapt and survive *in vivo*, and underscore the continued need to take these distinctive features of CF respiratory microbiota into account in future work to develop new strategies to treat CF infections.

4.5 Relationships between gut and respiratory microbiota in infants with CF

The final component of system complexity that relates to respiratory microbiota and clinical outcomes extends beyond the respiratory system to the gut. Studies that have investigated the relationships between respiratory and gut microbiomes, and clinical outcomes in children with CF have made several important observations, including: (1) gut colonization with certain genera precede detection of the same genera in the respiratory tract(20); (2) changes in the gut microbiome (e.g., a decrease in *Parabacteroidetes*) occurred prior to onset of respiratory *P. aeruginosa* infection(19); (3) gut microbiome composition was associated with pulmonary exacerbation(19); and (4) changes in diet (i.e., breast feeding, introduction

of solid foods) resulted in changes in respiratory microbiota(20). Together these studies identify connections between respiratory and gut microbiota early in life in CF that could ultimately be leveraged for future diagnostic or therapeutic applications. Additionally, identification of the temporal relationships between gut and respiratory microbiota (i.e., gut microbiota features change prior to respiratory microbiota features) represents a step towards identifying determinants of relevant changes in respiratory microbiota and clinical outcomes.

4.6 Potential impact on clinical practice

These and other studies have highlighted the relevance of the system complexity of CF respiratory infections in developing microbiota-based applications to clinical practice. Relevant components of system complexity include bacterial metabolic activities, bacterial interactions, the chemical properties of CF sputum, and gut microbiota. Unanswered questions include the following: Are there bacterial metabolites that can serve as biomarkers of pulmonary exacerbation or future lung damage? Can exhaled breath condensate be useful as a noninvasive means of microbiota sampling, either in the clinics (in particular for young patients who cannot produce sputum), or as a device to use for home monitoring for exacerbations? Can pathways of bacterial metabolism or bacterial interactions be interrupted to reduce harmful infections? How can we use culture- independent data and knowledge of the chemical properties of CF sputum to maximize *in vivo* efficacy of antibiotic therapy? Can gut microbiota be used for early detection of pulmonary exacerbation? Can dietary interventions modulate gut microbiota for clinical benefit?

5. Expert commentary and five-year view

Culture-independent analyses are expected to have multiple future applications to routine clinical practice. An additional area of future application is clinical diagnostics. As culture-independent methods continue to develop it is likely that more rapid, sensitive, and specific characterization of clinically relevant features of CF respiratory microbiota will become available. The development of point-of-care devices to assess key elements of the CF airway microbiome could enable earlier, more effective treatment of pulmonary exacerbations or eradication of new pathogens. Potential future applications of culture-independent techniques to clinical diagnostics include portable devices for real-time DNA sequencing, breath gas analysis devices, and platforms for performing rapid multi-omic evaluations(64).

A particular challenge in modeling the complexity of CF infections is accounting for the impacts of the varied acute and chronic therapies provided to patients. Chief among these is the role of antibiotics on measures of CF respiratory microbiota(26,53,56,65–66). More robust models of antibiotic use that incorporate route of delivery, spectrum of activity, pharmacokinetic, and pharmacodynamic properties are needed to better understand antibiotic-related influences on CF respiratory microbiota and their bearing on clinical outcomes. The impact of other CF therapies, including CFTR modulator drugs, on CF respiratory microbiota similarly requires detailed study(67).

Culture-independent analyses of CF respiratory microbiota have highlighted the complexity of CF microbiology and the challenges inherent in designing and analyzing experiments that

adequately take this complexity into account. It is clear that multiple features are relevant to understanding the complexity of CF lung disease, including clinical variables (age, lung disease stage, disease aggressiveness, disease state, and antibiotic use), the structure and metabolic activities of CF respiratory microbiota, bacterial interactions, the chemical composition of CF sputum, regional heterogeneity of CF lung disease, gut microbiota, and the interpatient variability of these factors. Further development of more sophisticated models and analytic approaches to better identify mechanistic links between CF respiratory microbiota and clinical outcomes, and how to modulate microbiota for clinical benefit, are needed to ultimately lead to new strategies to treat CF infections.

Acknowledgments

Funding

This manuscript has received financial support from the Cystic Fibrosis Foundation (CAVERL17A0 and LIPUMA15P0) and the National Institutes of Health, National Heart, Lung, and Blood Institute (1 R01 HL 136647-01 And K23HL136934).

References

1. Salsgiver EL, Fink AK, Knapp EA, et al. Changing Epidemiology of the Respiratory Bacteriology of Patients with Cystic Fibrosis. Chest 2016;149:390–400 [PubMed: 26203598]
2. Cystic Fibrosis Foundation. Patient Registry Annual Data Report 2016
3. Sanders DB, Bittner RC, Rosenfeld M, et al. Failure to recover to baseline pulmonary function after cystic fibrosis pulmonary exacerbation. Am J Respir Crit Care Med 2010;182:627–32 [PubMed: 20463179]
4. Smith AL, Fiel SB, Mayer-Hamblett N, et al. Susceptibility Testing of Pseudomonas aeruginosa Isolates and Clinical Response to Parenteral Antibiotic Administration Lack of Association in Cystic Fibrosis. Chest 2003;123:1495–502 [PubMed: 12740266]
5. Zemanick ET, Wagner BD, Harris JK, et al. Pulmonary exacerbations in cystic fibrosis with negative bacterial cultures. Pediatr Pulmonol 2010;45:569–77 [PubMed: 20503282]
6. Cox MJ, Allgaier M, Taylor B, et al. Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. PLoS One 2010;5:e11044 [PubMed: 20585638]
7. Coburn B, Wang PW, Diaz Caballero J, et al. Lung microbiota across age and disease stage in cystic fibrosis. Sci Rep 2015;5:10241 [PubMed: 25974282]
8. Harris JK, De Groote MA, Sagel SD, et al. Molecular identification of bacteria in bronchoalveolar lavage fluid from children with cystic fibrosis. Proc Natl Acad Sci U S A 2007;104:20529–33 [PubMed: 18077362]
9. Zemanick ET, Wagner BD, Robertson CE, et al. Airway microbiota across age and disease spectrum in cystic fibrosis. Eur Respir J 2017;50:pii: 1700832
10. Mahboubi MA, Carmody LA, Foster BK, et al. Culture-based and culture-independent bacteriologic analysis of cystic fibrosis respiratory specimens. J Clin Microbiol 2015;54:613–9 [PubMed: 26699705]
11. Venkataraman A, Bassis CM, Beck JM, et al. Application of a neutral community model to assess structuring of the human lung microbiome. MBio 2015;6
12. Dickson RP, Erb-Downward JR, Freeman CM, et al. Bacterial topography of the healthy human lower respiratory tract. MBio 2017;8:pii: e02287–16
13. Bassis CM, Erb-Downward JR, Dickson RP, et al. Analysis of the upper respiratory tract microbiotas as the source of the lung and gastric microbiotas in healthy individuals. MBio 2015;6:e00037 [PubMed: 25736890]
14. Brown PS, Pope CE, Marsh RL, et al. Directly sampling the lung of a young child with cystic fibrosis reveals diverse microbiota. Ann Am Thorac Soc 2014;11:1049–55 [PubMed: 25072206]

15. Pittman JE, Wylie KM, Akers K, et al. Association of Antibiotics, Airway Microbiome and Inflammation in Infants with Cystic Fibrosis. Ann Am Thorac Soc 2017;14:1548–55 [PubMed: 28708417]

16. Frayman KB, Armstrong DS, Carzino R, et al. The lower airway microbiota in early cystic fibrosis lung disease: a longitudinal analysis. Thorax 2017;72:1104–12 [PubMed: 28280235] *Large study of lower airway microbiota in infants and young children with CF, demonstrating decreasing diversity with advancing age in the first years of life.

17. Zemanick ET, Wagner BD, Robertson CE, et al. Assessment of airway microbiota and inflammation in cystic fibrosis using multiple sampling methods. Ann Am Thorac Soc 2015;12:221–9 [PubMed: 25474078]

18. Muhlebach MS, Zorn BT, Esther CR, et al. Initial acquisition and succession of the cystic fibrosis lung microbiome is associated with disease progression in infants and preschool children. PLoS Pathog 2018;14:e1006798 [PubMed: 29346420] *Study of lower airways of infants and young children with CF, identified increased inflammation and total bacterial load associated with acquisition of an anaerobe-dominated airway microbiome.

19. Hoen AG, Li J, Moulton LA, et al. Associations between gut microbial colonization in early life and respiratory outcomes in cystic fibrosis. J Pediatr 2015;167:138–147 [PubMed: 25818499]

20. Madan JC, Koestler DC, Stanton BA, et al. Serial analysis of the gut and respiratory microbiome in cystic fibrosis in infancy: interaction between intestinal and respiratory tracts and impact of nutritional exposures. MBio 2012;3*Identified connections between gut and respiratory microbiome in infants with CF.

21. Tunney MM, Klem ER, Fodor AA, et al. Use of culture and molecular analysis to determine the effect of antibiotic treatment on microbial community diversity and abundance during exacerbation in patients with cystic fibrosis. Thorax 2011;66:579–84 [PubMed: 21270069]

22. Worlitzsch D, Rintelen C, Bohm K, et al. Antibiotic-resistant obligate anaerobes during exacerbations of cystic fibrosis patients. Clin Microbiol Infect 2009;15:454–60 [PubMed: 19196263]

23. Tunney MM, Field TR, Moriarty TF, et al. Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. Am J Respir Crit Care Med 2008;177:995–1001 [PubMed: 18263800]

24. Rogers GB, Hart CA, Mason JR, et al. Bacterial diversity in cases of lung infection in cystic fibrosis patients: 16S ribosomal DNA (rDNA) length heterogeneity PCR and 16S rDNA terminal restriction fragment length polymorphism profiling. J Clin Microbiol 2003;41:3548–58 [PubMed: 12904354]

25. Caverly LJ, Zhao J, LiPuma JJ. Cystic fibrosis lung microbiome: Opportunities to reconsider management of airway infection. Pediatr Pulmonol 2015;50:S31–8 [PubMed: 26335953]

26. Zhao J, Schloss PD, Kalikin LM, et al. Decade-long bacterial community dynamics in cystic fibrosis airways. Proc Natl Acad Sci U S A 2012;109:5809–14 [PubMed: 22451929]

27. Carmody LA, Zhao J, Schloss PD, et al. Changes in cystic fibrosis airway microbiota at pulmonary exacerbation. Ann Am Thorac Soc 2013;10:179–87 [PubMed: 23802813]

28. Fodor AA, Klem ER, Gilpin DF, et al. The adult cystic fibrosis airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations. PLoS One 2012;7:e45001 [PubMed: 23049765]

29. Deschaght P, Schelstraete P, Van Simaey L, et al. Is the improvement of CF patients, hospitalized for pulmonary exacerbation, correlated to a decrease in bacterial load? PLoS One 2013;8:e79010 [PubMed: 24312174]

30. Carmody LA, Caverly LJ, Foster BK, et al. Fluctuations in airway bacterial communities associated with clinical states and disease stages in cystic fibrosis. PLoS One 2018;13:e0194060 [PubMed: 29522532] **Recent study demonstrating relevance of accounting for disease stage and aggressiveness in studying differences in respiratory microbiota between baseline and exacerbation.

31. Konstan MW, Wagener JS, VanDevanter DR. Characterizing aggressiveness and predicting future progression of CF lung disease. J Cyst Fibros 2009;8:S15–S19 [PubMed: 19460682]

32. Ranganathan SC, Hall GL, Sly PD, et al. Early lung disease in infants and pre-school children with cystic fibrosis: What have we learnt and what should we do about it? Am J Respir Crit Care Med 2017;195:1567–75 [PubMed: 27911585]
33. Laguna TA, Wagner BD, Williams CB, et al. Airway Microbiota in Bronchoalveolar Lavage Fluid from Clinically Well Infants with Cystic Fibrosis. PLoS One 2016;11:e0167649 [PubMed: 27930727]
34. Davis SD, Ratjen F, Brumback LC, et al. Infant lung function tests as endpoints in the ISIS multicenter clinical trial in cystic fibrosis. J Cyst Fibros 2016;15:386–91 [PubMed: 26547590]
35. Sibley CD, Parkins MD, Rabin HR, et al. A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients. Proc Natl Acad Sci U S A 2008;105:15070–5 [PubMed: 18812504]
36. Filkins LM, Hampton TH, Gifford AH, et al. Prevalence of streptococci and increased polymicrobial diversity associated with cystic fibrosis patient stability. J Bacteriol 2012;194:4709–17 [PubMed: 22753064]
37. Whiteson KL, Meinardi S, Lim YW, et al. Breath gas metabolites and bacterial metagenomes from cystic fibrosis airways indicate active pH neutral 2,3-butanedione fermentation. ISME J 2014;8:1247–58 [PubMed: 24401860]
38. Lim YW, Schmieder R, Haynes M, et al. Mechanistic model of Rothia mucilaginosa adaptation toward persistence in the CF lung, based on a genome reconstructed from metagenomic data. PLoS One 2013;8:e64285 [PubMed: 23737977]
39. Quinn RA, Whiteson K, Lim YW, et al. Ecological networking of cystic fibrosis lung infections. NPJ Biofilms Microbiomes 2016;2
40. Conrad D, Haynes M, Salamon P, et al. Cystic fibrosis therapy: A community ecology perspective. Am J Respir Cell Mol Biol 2013;48:150–6 [PubMed: 23103995]
41. Quinn RA, Whiteson K, Lim YW, et al. A Winogradsky-based culture system shows an association between microbial fermentation and cystic fibrosis exacerbation. ISME J 2015;9:1052
42. Paganin P, Fiscarelli EV, Tuccio V, et al. Changes in cystic fibrosis airway microbial community associated with a severe decline in lung function. PLoS One 2015;10:1–19
43. Twomey KB, Alston M, An SQ, et al. Microbiota and metabolite profiling reveal specific alterations in bacterial community structure and environment in the cystic fibrosis airway during exacerbation. PLoS One 2013;8:e82432 [PubMed: 24358183]
44. Flynn JM, Niccum D, Dunitz JM, Hunter RC. Evidence and Role for Bacterial Mucin Degradation in Cystic Fibrosis Airway Disease. PLoS Pathog 2016;12:1–21*Identification of potential mechanism through which anaerobes contribute to pathogenesis of CF lung disease.
45. Mirković B, Murray MA, Lavelle GM, et al. The Role of Short-Chain Fatty Acids, Produced by Anaerobic Bacteria, in the Cystic Fibrosis Airway. Am J Respir Crit Care Med 2015;192:1314–24 [PubMed: 26266556] *Identification of potential mechanism through which anaerobes contribute to pathogenesis of CF lung disease.
46. Whiley RA, Fleming EV., Makhija R, Waite RD. Environment and colonisation sequence are key parameters driving cooperation and competition between Pseudomonas aeruginosa cystic fibrosis strains and oral commensal streptococci. PLoS One 2015;10:1–14
47. Gao B, Gallagher T, Zhang Y, et al. Tracking Polymicrobial Metabolism in Cystic Fibrosis Airways: Pseudomonas aeruginosa Metabolism and Physiology Are Influenced by Rothia mucilaginosa-Derived Metabolites. mSphere 2018;3:e00151–18 [PubMed: 29695623]
48. Carmody LA, Caverly LJ, Foster BK, et al. Fluctuations in airway bacterial communities associated with clinical states and disease stages in cystic fibrosis. PLoS One 2018;13
49. Stokell JR, Gharaibeh RZ, Hamp TJ, et al. Analysis of changes in diversity and abundance of the microbial community in a cystic fibrosis patient over a multiyear period. J Clin Microbiol 2015;53:237–47 [PubMed: 25392361]
50. Carmody LA, Zhao J, Kalikin LM, et al. The daily dynamics of cystic fibrosis airway microbiota during clinical stability and at exacerbation. Microbiome 2015;3:12 [PubMed: 25834733]
51. Whelan FJ, Heirali AA, Rossi L, et al. Longitudinal sampling of the lung microbiota in individuals with cystic fibrosis. PLoS One 2017;12:e0172811 [PubMed: 28253277]

52. Zhao J, Murray S, LiPuma JJ. Modeling the impact of antibiotic exposure on human microbiota. Sci Rep 2014;4:4345 [PubMed: 24614401]
53. Mika M, Korten I, Qi W, et al. The nasal microbiota in infants with cystic fibrosis in the first year of life: a prospective cohort study. Lancet Respir Med 2016;4:627–35 [PubMed: 27180018]
54. Prevaes SMPJ, de Winter-de Groot KM, Janssens HM, et al. Development of the Nasopharyngeal Microbiota in Infants with Cystic Fibrosis. Am J Respir Crit Care Med 2016;193:504–15 [PubMed: 26492486]
55. Stressmann FA, Rogers GB, van der Gast CJ, et al. Long-term cultivation-independent microbial diversity analysis demonstrates that bacterial communities infecting the adult cystic fibrosis lung show stability and resilience. Thorax 2012;67:867–73 [PubMed: 22707521]
56. Smith DJ, Badrick AC, Zakrzewski M, et al. Pyrosequencing reveals transient cystic fibrosis lung microbiome changes with intravenous antibiotics. Eur Respir J 2014;44:922–30 [PubMed: 25034564]
57. Peleg AY, Choo JM, Langan KM, et al. Antibiotic exposure and interpersonal variance mask the effect of ivacaftor on respiratory microbiota composition. J Cyst Fibros 2018;17:50–6 [PubMed: 29042177] *Illustration of how failure to account for maintenance antibiotic use can mask microbiome signals between groups.
58. Hogan DA, Willger SD, Dolben EL, et al. Analysis of Lung Microbiota in Bronchoalveolar Lavage, Protected Brush and Sputum Samples from Subjects with Mild-To-Moderate Cystic Fibrosis Lung Disease. PLoS One 2016;11:e0149998 [PubMed: 26943329]
59. Filkins LM, Graber JA, Olson DG, et al. Coculture of Staphylococcus aureus with Pseudomonas aeruginosa Drives S. aureus towards Fermentative Metabolism and Reduced Viability in a Cystic Fibrosis Model. J Bacteriol 2015;197:2252–64 [PubMed: 25917910]
60. Sherrard LJ, McGrath SJ, McIlreavey L, et al. Production of extended-spectrum beta-lactamases and the potential indirect pathogenic role of Prevotella isolates from the cystic fibrosis respiratory microbiota. Int J Antimicrob Agents 2015;47:140–5 [PubMed: 26774156] *Illustration of potential contribution by anaerobes to antimicrobial resistance in CF airway microbiota.
61. Jorth P, Staudinger BJ, Wu X, et al. Regional Isolation Drives Bacterial Diversification within Cystic Fibrosis Lungs. Cell Host Microbe 2015;18:307–19 [PubMed: 26299432] **Identifies relationships between regional disease heterogeneity and CF pathogen diversification, including variation in antimicrobial susceptibility profiles.
62. Kopf SH, Sessions AL, Cowley ES, et al. Trace incorporation of heavy water reveals slow and heterogeneous pathogen growth rates in cystic fibrosis sputum. Proc Natl Acad Sci U S A 2016;113:E110–6 [PubMed: 26715741]
63. Cowley ES, Kopf SH, LaRiviere A, et al. Pediatric Cystic Fibrosis Sputum Can Be Chemically Dynamic, Anoxic, and Extremely Reduced Due to Hydrogen Sulfide Formation. MBio 2015;6:e00767 [PubMed: 26220964] *Identification of chemical heterogeneity within CF sputum samples, and relevance to microbiota.
64. Quinn RA, Navas-Molina JA, Hyde ER, et al. From Sample to Multi-Omics Conclusions in under 48 Hours. mSystems 2016;1:pii: e00038–16
65. Prevaes SMPJ, De Winter-De Groot KM, Janssens HM, et al. Development of the nasopharyngeal microbiota in infants with cystic fibrosis. Am J Respir Crit Care Med 2016;193:504–15 [PubMed: 26492486]
66. Heirali AA, Workentine ML, Acosta N, et al. The effects of inhaled aztreonam on the cystic fibrosis lung microbiome. Microbiome 2017;5
67. Bernarde C, Keravec M, Mounier J, et al. Impact of the CFTR-Potentiator ivacaftor on airway microbiota in cystic fibrosis patients carrying a G551D mutation. PLoS One 2015;10:1–18

Key issues

- Initial culture-independent studies of CF respiratory microbiota identified diverse bacterial communities, including genera not routinely identified by clinical laboratory culture.
- Multiple clinical variables have bearing on respiratory microbiota: clinical state, lung disease stage, disease aggressiveness, antibiotic use, and inter-subject variability.
- Over time, diverse respiratory bacterial communities containing anaerobes typically found in oral microbiota are replaced by less diverse communities dominated by typical CF pathogens.
- Less diverse bacterial communities have been associated with poorer clinical outcomes, including pulmonary exacerbations and lung function decline, starting in childhood.
- Universal changes in respiratory microbiota associated with pulmonary exacerbation have not been identified, but common motifs of within-subject changes have been suggested, including changes in the dominant pathogen and alterations in baseline community stability.
- Relationships between the presence, relative abundance, and metabolic activities of anaerobic genera and pulmonary exacerbations have been identified.
- Multiple features of respiratory microbiota have been identified that likely influence efficacy of antibiotic therapy, including bacterial interactions, regional lung disease heterogeneity, and the chemical composition of CF sputum.
- Further development of more sophisticated models and analytic approaches to links between CF respiratory microbiota and lung disease are needed.

Table 1:

Terminology used to describe acute and chronic lung health conditions in persons with CF

Category	Definition	Terminology	References
Clinical State	Describes an individual's current clinical status with respect to baseline health, exacerbation and episodic antibiotic use	Mutually exclusive states including Baseline, Exacerbation, Treatment, Recovery	(26, 31)
Disease Stage	Describes an individual's level of pulmonary disease, based on lung function ($ppFEV_1$)	Early ($ppFEV_1$ >70) Intermediate ($ppFEV_1$ 40–70) Advanced ($ppFEV_1$ <40)	(26)
Disease Aggressiveness Phenotype	Describes the rate of decline in lung function relative to an individual's age, *independent* of lung disease stage	Mild, Moderate, Severe	(30, 31)