Chapter 5
Bacterial Infections and the Respiratory Microbiome

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Introduction

Cystic fibrosis (CF) is a genetic, multisystem disease due to defects in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, an anion channel responsible for chloride and bicarbonate trafficking [1, 2]. Although this channel is expressed in many tissues, its impaired function in airway epithelial cells leads to hyperviscous mucous secretions impeding effective mucociliary clearance. Impaired clearance of inhaled microorganisms results in the establishment of chronic infection, triggering an overexaggerated inflammatory response [3]. The resulting release of inflammatory cytokines and enzymes causes pulmonary damage in the form of bronchiectasis, further impairing mucociliary action, forming a vicious cycle. Subsequent respiratory failure remains the leading cause of death in individuals with CF [4].

The epidemiology of bacterial pulmonary infections in CF follows a typical pattern (Fig. 5.1) [5]. The airways of young children are typically colonized early in life with organisms such as *Staphylococcus aureus* and *Haemophilus influenzae.* Over time, these bacteria are gradually replaced with opportunistic, Gram-negative organisms such as *Pseudomonas aeruginosa* (most commonly), *Stenotrophomonas maltophilia*, *Burkholderia cepacia* complex, and *Achromobacter* species. More recently, there has also been increasing identification of infection due to nontuberculous

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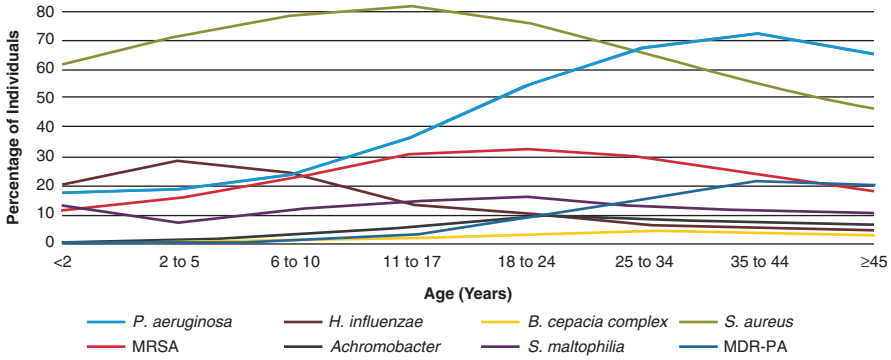

Fig. 5.1 Prevalence of respiratory microorganisms by age cohort in cystic fibrosis patients in 2017. (Cystic Fibrosis Foundation Patient Registry, 2017 Annual Data Report, Bethesda, Maryland ©2018 Cystic Fibrosis Foundation)

mycobacterial species in patients with CF. Furthermore, although much of the focus has traditionally been on bacterial pulmonary infection, the role of fungal and viral infections of CF airways have increasingly been studied as well.

The aim of this chapter is to review the microbiological diagnosis and epidemiology of CF airway infections. Fungal and atypical mycobacterial infections are covered in more detail in Chaps. 5 and 6, respectively. Infection prevention and control recommendations [6] as well as treatment guidelines for these infections [7] are outside the scope of this chapter and are extensively reviewed elsewhere.

Cystic Fibrosis Airway Microbiome

Much of what is known about the microbiology of airway infection in CF has been learned through the use of in vitro culture of respiratory specimens from affected individuals. While early studies (1940–1970) focused on the recovery in culture of known human pathogenic bacteria (e.g., *S. aureus* and *H. influenzae*), refined bacterial taxonomy and the use of selective culture media in subsequent studies (1970–2000) led to the recognition that several opportunistic bacterial species, in particular *P. aeruginosa*, are also involved in CF airway infection. In the early 2000s, so-called "culture-independent" methods of profiling mixed bacterial communities began to be applied to the study of CF airway microbiology [8]. These methods, which rely on amplification and analysis of bacterial DNA to identify species present in a particular site (or biologic specimen), demonstrated that the airways of persons with CF most often harbor rich microbial communities that are poorly reflected in selective culture. In addition to providing a less biased and sensitive means of detecting microbial species, culture-independent analyses also allow an estimation of the relative abundances of the species in a polymicrobial

community. Applying these methods to CF respiratory specimens has shown that beyond the species typically associated with infection in CF based on routine culture, a variety of "nonpathogenic" bacteria, especially anaerobic species, are both prevalent and often present in relatively high abundances [9].

Studies of the CF airway microbiome using culture-independent methods have challenged several tenets of CF microbiology conventional wisdom [10]. For example, the commonly held beliefs that exacerbation of pulmonary symptoms results from an increase in the density of *P. aeruginosa* or reflects the acquisition of a "new" pathogen by an individual with CF have not been borne out by DNA sequence-based analyses. Another unexpected finding, demonstrated in several studies of the CF airway microbiome, is that the diversity of the bacterial communities in airways *decreases* with advancing patient age and lung disease progression [11]. That is, at younger ages, during the early stages of lung disease, CF airways typically harbor rich polymicrobial communities that include high relative abundances of anaerobic species derived from the oropharynx. As lung disease progresses with age, and as the administration of antimicrobials accelerates, airway bacterial communities become increasingly less diverse. With advanced lung disease, these communities often constrict markedly, becoming dominated by a single species (most often *P. aeruginosa*, but occasionally *B. cepacia* complex or an *Achromobacter* species).

Perhaps the most important development reshaping current views of CF microbiology is the increasing appreciation that myriad interspecies interactions – as well as complex interactions with the human host – likely have profound effects on lung disease and clinical response to therapy. In this sense, the polymicrobial community in CF airways, including bacteria, viruses, and fungi, may be considered the "pathogenic unit." The structure and activity of this microbial consortium in the context of the host's response to infection is the major determinant of CF respiratory disease.

While studies of the CF microbiome hold promise to translate into novel therapies and improved management of patients, our understanding of this complex ecology requires additional investigation. What has been learned about the epidemiology of CF airway infections, as described in the following sections, provides a foundation for these studies.

Diagnostics

Airway Sampling

The identification of bacterial organisms in the airways is dependent on the type of respiratory tract specimen sent for culture. Spontaneously expectorated sputum is the most common sample used for bacterial culture in both adults and children of sufficient age. In children who are too young to expectorate, oropharyngeal swabs (or "cough swabs") are used and have been shown, in the case of *P. aeruginosa* infection, to have a good negative predictive value but poor positive predictive value

compared to lower respiratory tract sampling [12, 13]. Specimens from bronchoalveolar lavage (BAL) may also be used for bacterial culture and have a higher sensitivity for detecting *P. aeruginosa* in the lower airway compared to oropharyngeal swabs [14]. However, this type of airway sampling is invasive and has not been shown to result in a lower prevalence of *P. aeruginosa* infection or less structural lung disease when compared to the use of oropharyngeal swabs in the diagnosis and management of CF pulmonary infections [15].

Bacterial Identification

Once a respiratory tract sample is sent for bacterial culture, it is then plated on selective media to enhance the growth of specific, well-known CF pathogens [16]. These media include mannitol salt agar, using sodium chloride as a selective agent and phenol red as an indicator of mannitol utilization, to recover *S. aureus* and *B. cepacia* selective agar (BCSA) and chocolate agar (incubated anaerobically) to recover *H. influenzae* [17]. Special sputum processing is required to improve recovery of nontuberculous mycobacteria by decontaminating sputum with N-acetyl-L-cysteine and sodium hydroxide followed by oxalic acid [18]. Identification of bacterial growth has traditionally been done using microscopy and biochemical testing. However, the introduction of matrix-assisted desorption ionization-time of flight mass spectrometry (MALDI-TOF) in the clinical microbiology laboratory has significantly accelerated the bacterial identification process [19]. Molecular methods using polymerase chain reaction (PCR) can also be used to speciate certain bacterial organisms such as *B. cepacia* complex (by amplification and sequencing of the *recA* gene) [20] and *Achromobacter* species (by *nrdA* gene sequencing) [21].

Antimicrobial Susceptibility Testing

Once a bacterial organism has been identified, antimicrobial susceptibility testing may be done to guide antibiotic therapy. Microbroth dilution assay is often considered the gold standard for antimicrobial susceptibility testing of CF bacterial isolates; however, agar-based diffusion assays, such as antibiotic disks or E-tests (antibiotic impregnated strips), are also accurate and comparable to the reference method [17]. In contrast, commercial automated microbroth dilution assays, such as Microscan and Vitek, have unacceptably high error rates when compared with reference broth dilution assay for antimicrobial susceptibility testing of CF bacterial isolates [22]. The minimum inhibitory concentration (MIC) obtained for each drug is interpreted according to interpretive criteria outlined by the Clinical Laboratory Standards Institute, which sets breakpoints for determining resistance [23]. There are no interpretive criteria for many antibiotics for multidrug-resistant CF organisms, such as *B. cepacia* complex, *S. maltophilia*, and *Achromobacter* spp.

Bacterial Infections

Staphylococcus aureus (Including MRSA)

S. aureus is a Gram-positive coccus that is nonmotile, non-spore-forming, and coagulase positive. It is a frequent colonizer of the skin and mucosa of humans and animals and, in contrast to most other CF pathogens, is well known to cause infections in non-CF individuals as well [24]. It has a host of virulence factors including Staphylococcal protein A (SpA) (a potent IgG binder) and more than 30 adhesin and toxin genes [25]. Antimicrobial resistance occurs through the expression of penicillinases, drug efflux pumps, and ribosome modification (mediated by the *erm* gene), for example [26]. Upon exposure to antibiotics such as trimethoprim-sulfamethoxazole, *S. aureus* may also grow as small colony variants [27]. Small colony variants are slow-growing secondary to metabolic defects and are associated with resistance to many antibiotics and persistence of infection [28]. Methicillin-resistant *S. aureus* (MRSA) is resistant to methicillin through the expression of an altered penicillin-binding protein (PBP2a) encoded by the *mecA* gene [29].

S. aureus is an early colonizer and the most frequent bacterial organism isolated from CF patients [5]. The prevalence of infection peaks in childhood with approximately 70% of CF children ages 11–17 having *S. aureus*-positive respiratory tract culture. As the reservoir for *S. aureus* are humans/animals, initial infection is thought to occur from direct contact, with family members, for example [30]. As *S. aureus* is an early colonizer in CF, it is often difficult to measure its impact on clinical outcomes as children under 6 may not reproducibly perform pulmonary function testing. In a multicenter observational study of CF patients (average age 16 yrs) with *S. aureus* infection, a higher density of *S. aureus* on throat swab was associated with accelerated lung function decline [31]. Similarly, in CF children undergoing bronchoalveolar lavage, those with *S. aureus* infection had higher levels of pulmonary inflammation (as measured by neutrophil counts, IL-8, and neutrophil elastase levels in bronchoalveolar lavage fluid) compared to those without *S. aureus* infection [32].

The prevalence of MRSA infection in CF is considerably less than the prevalence of methicillin-susceptible *S. aureus* (MSSA) infection, and ranges from 5% to 26% depending on the country of origin [5, 33]. Acquisition is through direct contact and patient to patient transmission of MRSA infection has been well described [34]. MRSA strains can be either hospital-acquired MRSA (HA-MRSA) or community-acquired MRSA and are distinguished by the type of *mecA* gene they carry [35]. Studies have reported that approximately 14% of CF patients with MRSA infection harbor CA-MRSA, while the remainder harbor HA-MRSA strains [36]. The impact of MRSA infection on clinical outcomes in individuals with CF has been well defined. Using data from the US CF Patient Registry, investigators showed that chronic MRSA infection (3 or more positive cultures in the preceding year) in CF patients ages 8–21 years was associated with increased lung function decline (as measured by forced expiratory volume in 1 second [FEV_1]) compared to those

without chronic infection [37]. Furthermore, after adjusting for other markers of disease severity, MRSA infection was independently associated with an earlier time to death in CF patients [38]. MRSA infection has also been demonstrated to be a risk factor for failing to recover baseline lung function following antibiotic treatment for a pulmonary exacerbation [39].

Pseudomonas aeruginosa

P. aeruginosa is a non-lactose-fermenting Gram-negative bacterium, commonly found in water sources such as rivers, lakes, and municipal water systems [24]. It has both flagella, conferring motility, and pili, important for initial attachment to airway epithelial cells [40, 41]. *P. aeruginosa* expresses three main exopolysaccharides, alginate, Pel, and Psl, which are important in the establishment and maintenance of a biofilm structure (see below) [42]. It is an aerobe but can survive under anaerobic conditions and grows selectively at 42 ° C [16]. It is intrinsically resistant to many ß-lactam antibiotics and can acquire resistance through either chromosomal mutation or horizontal gene transfer. Some of the antimicrobial resistance mechanisms in *P. aeruginosa* include the expression of aminoglycoside-modifying enzymes, multidrug efflux pumps, and carbapenamase production, to name a few [43].

Approximately 40% of individuals with CF have *P. aeruginosa* infection [5, 33]. *P. aeruginosa* has a predilection for causing pulmonary infection in individuals with CF and is considered a criteria in the diagnosis of CF disease [44]. *P. aeruginosa* infection may be a consequence of impaired mucociliary clearance but others have suggested that *P. aeruginosa* flagella may bind directly to defective CFTR protein [4, 45]. Children with CF are likely most often initially colonized with *P. aeruginosa* strains acquired from their environment [46]. Genotypic studies of incident CF *P. aeruginosa* isolates have found sharing of strains with those isolated from environmental sources, suggesting a mode of initial acquisition [47]. However, patient to patient transmission of *P. aeruginosa* infection has been documented in infants identified through newborn screening who attended an unsegregated CF clinic, prior to the implementation of current infection prevention guidelines [48]. Once *P. aeruginosa* enters the CF airways through inhalation, it can establish chronic infection. Randomized controlled trials have demonstrated that antibiotic treatment, with inhaled tobramycin, for example, improves the clearance rate of incident *P. aeruginosa* compared to no treatment [49, 50]. Thus, it is standard of care to treat first time *P. aeruginosa* infection with antibiotic therapy with the goal of eradication.

Without eradication, *P. aeruginosa* will adapt itself to the environment of the CF airway [51]. After initial colonization, *P. aeruginosa* will downregulate its acute virulence factors, such as flagella, to evade detection and clearance by cells of the immune system [52]. Furthermore, it will upregulate genes involved in quorum sensing (*las/rhl* genes) leading to biofilm formation [53]. *P. aeruginosa* biofilms are microbial communities encased in a matrix composed primarily of its exopolysaccharides: alginate, Psl, and Pel [54]. Biofilms are antibiotic resistant due to impairment of antibiotic diffusion through the matrix as well as slowed bacterial growth [55].

Additional bacterial adaptations permitting the establishment of chronic infection include the development of persister cells (metabolically dormant cells) and alginate hyperproduction (also known as mucoidy status) impairing neutrophil phagocytosis [56–58].

Chronic *P. aeruginosa* infection is associated with increased pulmonary inflammation as *P. aeruginosa* stimulates IL-8 production by airway epithelial cells, leading to neutrophil recruitment and neutrophil elastase release [4, 59]. Sputum neutrophil elastase levels have been shown to most closely correlate with the development of bronchiectasis in CF patients [60]. CF patients with chronic *P. aeruginosa* infection will thus experience worse clinical outcomes in the form of accelerated lung function decline and earlier death [61, 62]. Supporting this fact, studies using the US CF Patient Registry have demonstrated that treatment of chronic *P. aeruginosa* infection (with inhaled tobramycin) is associated with prolonged survival in individuals with CF [63].

While *P. aeruginosa* is appropriately considered the major opportunistic bacterial pathogen in CF, its role in lung disease within the broader context of the airway microbiome is the subject of current investigation. For example, the commonly held belief that exacerbation of pulmonary symptoms results from an increase in the density of *P. aeruginosa* has not been borne out by DNA-sequence-based analyses. In fact, it appears that *P. aeruginosa* density *decreases* with exacerbation, while the relative abundance of anaerobic species increases [64, 65]. Further, recent studies have suggested that myriad interspecies interactions likely play critical roles in influencing *P. aeruginosa* virulence in vivo [66]. Interactions between *P. aeruginosa* and *S. aureus*, in particular, are of considerable interest and hold promise to advance our understanding of how complex polymicrobial communities in CF airways act in concert to impact lung disease [67–70].

Burkholderia cepacia *Complex*

The *Burkholderia cepacia* complex is comprised of over 23 *Burkholderia* species, with *Burkholderia multivorans* and *Burkholderia cenocepacia* being the most common species isolated from CF patients [71]. *Burkholderia gladioli* is a closely related species that is the third most common *Burkholderia* species isolated in CF, but it is not part of the *B. cepacia* complex. *Burkholderia* are aerobic, Gram-negative bacteria that are motile due to the presence of flagella. Like *P. aeruginosa*, *B. cepacia* complex are frequently found in moist environments and in the soil, including in plant material [72]. *B. cepacia* complex species exhibit a number of different virulence factors including pili that facilitate epithelial cell attachment, extracellular proteases resulting in tissue damage, quorum sensing genes facilitating biofilm formation, and a type III secretion system promoting cellular invasion [73–77]. *B. cepacia* complex are intrinsically resistant to a number of different antimicrobial classes including aminoglycosides due to efflux pumps and penicillin derivatives secondary to inducible chromosomally encoded ß-lactamases [78, 79].

The prevalence of *B. cepacia* complex infection in CF patients is approximately 3–5% according to American and Canadian Registry data [5, 33]. The distribution of infection by species has changed over the last several decades, shifting from a predominance of *B. cenocepacia* to *B. multivorans* in many CF populations [80]. This is likely due to the recognition of patient to patient transmission of *B. cenocepacia*, leading to changes in infection control practices and subsequent decline in new *B. cenocepacia* infections [17, 81, 82]. The majority of new *B. cepacia* complex infections are now most commonly acquired from environmental sources, although the potential for patient to patient transmission still exists [83]. Infection with *B. cepacia* complex has been associated with worse lung function and is a risk factor for earlier death [84–86]. Infection with *B. cenocepacia* in particular has been associated with decreased survival in CF patients post lung transplantation compared to CF patients without *B. cepacia* complex infection or with infection with other *Burkholderia* species [87]. *B. cenocepacia* has the ability to angio-invade leading to necrotizing pneumonia and sepsis, known as "cepacia syndrome" [88]. Although this has been most commonly described with *B. cenocepacia*, it can occur with other *Burkholderia* species as well, such as *B. dolosa* [89]. CF patients with cepacia syndrome have high markers of inflammation, commonly develop diffuse pulmonary infiltrates, and are often bacteremic. The associated mortality can be as high as 80% [78].

Stenotrophomonas maltophilia

In addition to *P. aeruginosa* and *B. cepacia* complex, there are a number of other non-lactose-fermenting Gram-negative bacilli that are known to infect the airways of CF patients. One of the most common is *Stenotrophomonas maltophilia*. *S. maltophilia* is an aerobic, motile bacteria commonly found in water supplies and known to cause infections in immunocompromised individuals as well as CF pulmonary infections [90]. *S. maltophilia* produces a variety of extracellular enzymes, such as alkaline serine proteases, which cause tissue necrosis. Their outer membrane lipopolysaccharides are also a potent inducer of cytokine-mediated inflammation [91]. *S. maltophilia* can form biofilms as well and are inherently resistant to a number different classes of antimicrobials [92, 93]. Resistance occurs through the expression of multidrug efflux pumps, ß-lactamases, aminoglycoside-modifying enzymes, and reduced outer membrane permeability [94].

The prevalence of *S. maltophilia* infection in the CF population ranges from 12% to 30% [5, 33, 95, 96]. Initial infection is likely from environmental sources with little evidence of patient to patient transmission. Risk factors for acquisition include intravenous and oral antibiotic use [97]. Epidemiologic studies using the CF Foundation Patient Registry found that CF patients with *S. maltophilia* infection were older and had worse lung function compared to those without *S. maltophilia*, but after controlling for confounders, they did not have a steeper FEV_1 decline or worse short-term survival (3 years) [98, 99]. However, studies focusing on CF

patients with chronic *S. maltophilia* infection (2 or more positive cultures in preceding year) have noted that it is an independent risk factor for pulmonary exacerbations treated with intravenous antibiotics as well as a risk factor for death or lung transplantation [100, 101]. In a multicenter study examining survival post lung transplantation, infection with multidrug-resistant (MDR) organisms including *S. maltophilia* (but excluding *B. cepacia* complex) was associated with worse survival in CF patients compared to those without MDR infection, although many MDR organisms were included in the analyses [102].

Achromobacter Species

Achromobacter species are Gram-negative, non-sporulating straight rods. The *Achromobacter* genus has undergone many taxonomic reclassifications [103, 104]. Currently, a total of 23 species are identified within the *Achromobacter* genus. Within CF populations, *A. xylosoxidans* and *A. ruhlandii* are the two most common *Achromobacter* species identified, accounting for 42% and 23% of infections, respectively [21]. *Achromobacter* species are generally aerobic and non-fermentative, with growth occurring between 25 and 37 °C. They are widely distributed in the environment, particularly in water and soil. In addition to being motile due to the presence of flagella, certain strains of *Achromobacter* have the ability to bind to mucin, collagen, and fibronectin, which may facilitate initial infection in the airways [105, 106]. As with the other aforementioned Gram-negative organisms, *Achromobacter* species can also form biofilms and are intrinsically resistant to several classes of antimicrobials through the expression of efflux pumps, ß-lactamases, and aminoglycoside-modifying enzymes [107–109]. They are generally resistant to narrow-spectrum penicillins, cephalosporins such as cefotaxime and ceftriaxone, aztreonam, and aminoglycosides.

The average prevalence of *Achromobacter* infection is approximately 5–6% although rates as high as 29% have been reported in certain CF centers [5, 33, 110, 111]. Most patients harbor unique strains acquired from the environment, although cross-contamination between patients has rarely been described [111–114]. Epidemiologic studies that have examined the effect of *Achromobacter* infection on clinical outcomes in CF have generally been limited by small sample size. The study by De Baets et al. demonstrated that CF individuals with chronic *Achromobacter* infection had lower lung function and more pulmonary exacerbations, than age-, gender-, and *P. aeruginosa*–matched controls, representing a sicker patient population [110]. Only one study, using serum antibodies to *Achromobacter* to define chronic infection, showed worsening lung function upon the development of chronic *Achromobacter* infection [115]. In a registry-based epidemiologic study of over 1000 CF patients, chronic *Achromobacter* infection (defined as 2 or more positive cultures in the previous 12 months) was associated with a twofold increased risk of death or transplant compared to those with no history of *Achromobacter* infection, even after adjusting for other known confounders [116].

Anaerobes

The bacterial species discussed in the preceding sections are aerobic organisms, although some can survive under anaerobic conditions. With the advent of molecular methods of identification, there has been an increasing recognition of the presence and potential role of anaerobic bacteria in CF lung disease [117, 118]. An anaerobe is an organism that requires reduced oxygen for growth, failing to grow on the surface of solid media in 10% CO_2 in air [119]. Anaerobes can be both Gram-positive and Gram-negative and are part of the normal human microbiota of many mucosal surfaces including the upper airways, the gastrointestinal tract, and the female genital tract. They can cause serious infections such as brain abscesses, sinusitis, necrotizing pneumonia, liver abscess, and bacteremia, to name a few [24]. A number of virulence factors are associated with pathogenic anaerobes including capsular polysaccharide, hemolysins, proteases, and lipopolysaccharides [120]. Anaerobes that commonly cause human infection (e.g., *Bacteroides fragilis*, *Prevotella melaninogenica*, *Fusobacterium nucleatum*) are generally aerotolerant (tolerating 2–8% oxygen) [121].

Respiratory specimens obtained from CF airways are not routinely cultured under anaerobic conditions, and the culture and identification of anaerobic species can be both difficult and time-consuming [16]. Thus, the general prevalence of anaerobic infections in CF patients is not known. However, studies that have specifically cultured CF sputum samples under anaerobic conditions have identified obligate anaerobes in approximately 60–90% of samples, predominantly in the genera *Prevotella, Veillonella, Propionibacterium,* and *Peptostreptococcus* [122–124]. Culture-independent microbial detection methods, such as 16S-rRNA-based analysis, generally have shown higher prevalence rates of anaerobes in CF respiratory specimens and have identified a vast diversity of anaerobic species in CF. In a study of CF children undergoing bronchoscopic alveolar lavage, Harris et al. found 65 different anaerobic species in BAL samples by rRNA sequence analysis [125]. Given the fact that the upper airways are colonized by anaerobic bacteria, identifying lower airway infection by anaerobes (rather than contamination passing through the oral cavity) can be difficult. Furthermore, the association between the presence of anaerobic organisms and CF lung disease is somewhat controversial [126]. In vitro studies have demonstrated that anaerobic bacteria can produce short-chain fatty acids that mediate the release of pro-inflammatory cytokines from human bronchial epithelial cells [127]. These pro-inflammatory cytokines lead to neutrophil recruitment into CF lungs [128]. Anaerobes can also interact with other organisms within the CF airways, increasing the virulence of *P. aeruginosa*, for example, and producing extended-spectrum ß-lactamases conferring antimicrobial resistance to *P. aeruginosa* [129, 130]. In contrast, both cross-sectional and longitudinal studies have noted an association between anaerobes and lower pulmonary inflammation as well as better lung function [118, 124, 131–134]. This association has been demonstrated using culture-based methods (increased bacterial density) as well as molecular-based methods (increased relative abundance). Higher loads of

anaerobes in airway secretions may represent greater microbial community diversity in CF patients with milder lung disease who have received less antimicrobial therapy.

Viral Infections

In addition to bacterial organisms, a significant number of CF patients will harbor viruses in their airways [135]. Viruses are not detected by traditional culturing techniques of sputum and, thus, both the correct specimen and test must be requested in order to diagnose a viral infection [24]. In the past, viral culture and serologic testing (acute and convalescent serology) were used to identify viral infection, but these methods are expensive and relatively insensitive and time-consuming [136]. The introduction of direct immunofluorescence for viruses in respiratory tract samples improved the sensitivity and turnaround time of testing. The advent of molecular methods, based on polymerase chain reaction (PCR), led to more reliable, rapid, and cost-effective viral diagnostics [137].

The overall prevalence of viral infections during exacerbations in individuals with CF is estimated to be between 13% and 60% [135, 138]. Respiratory syncytial virus (RSV) and influenza A and B virus are the most common viruses identified in CF patients. However, sensitive multiplex molecular diagnostics assays have detected a great diversity of respiratory viruses in people with CF including rhinovirus, human metapneumovirus, picornavirus, coronavirus, and coxsackie/echovirus [139–143]. Although viruses are detected more frequently in CF children, a significant proportion of adults with CF also develop respiratory viral infections [144]. CF and non-CF children (including infants) are equally likely to acquire viral infections; however, CF children are more likely to suffer viral-related morbidity [136, 139, 145, 146]. Viral infections in both adults and children with CF are associated with an increased risk of pulmonary exacerbation [142, 147]. These viral-associated exacerbations are characterized by greater drops in lung function at the time of presentation, higher markers of systemic inflammation, longer duration of intravenous antibiotic therapy, and poorer lung function response [148, 149]. RSV in particular has been associated with failure to recover to baseline FEV_1 following antibiotic treatment for a pulmonary exacerbation. Infants with CF who are infected with RSV not only have a higher rate of respiratory exacerbations but also prolonged hospitalizations and prolonged symptoms over the ensuing 2 years [150]. Similarly, influenza virus infection has also been associated with significant morbidity in children with CF, specifically an increased risk of hospital admission [151]. A large epidemiologic study using the CF Foundation Patient Registry determined that there was an estimated excess of 2.1% of total exacerbations during the influenza season [152]. The exact mechanism through which viral infections lead to worse lung function in CF patients is not fully understood but may involve interactions with bacteria such as *P. aeruginosa*. Epidemiologic studies have demonstrated

that new *P. aeruginosa* infection occurs more commonly in the winter season and is often preceded by a viral infection [153]. RSV has been shown to enhance *P. aeruginosa* adherence to epithelial cells [154]. In addition, RSV infection of airway epithelium induces antiviral IFN signaling leading to enhanced *P. aeruginosa* biofilm growth [155]. Less is known about other viral-induced mechanisms of CF lung disease.

Summary

In summary, the microbiology of CF pulmonary infections is complex, characterized by a polymicrobial environment and changing with age and varying lung disease severity. Infection with opportunistic, environmental Gram-negative organisms is typical in CF due to defective mucociliary clearance and contributes to the ongoing inflammation and tissue destruction within the lung. Our understanding of the CF pulmonary microbiome continues to evolve and has the potential to inform the infectious disease management of CF patients in the future.

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