Microbiology of Cystic Fibrosis Airway Disease

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Abstract

Although survival of individuals with cystic fibrosis (CF) has been continuously improving for the past 40 years, respiratory failure secondary to recurrent pulmonary infections remains the leading cause of mortality in this patient population. Certain pathogens such as *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, and species of the *Burkholderia cepacia* complex continue to be associated with poorer clinical outcomes including accelerated lung function decline and increased mortality. In addition, other organisms such as anaerobes, viruses, and fungi are increasingly recognized as potential contributors to disease progression. Culture-independent molecular methods are also being used for diagnostic purposes and to examine the interaction of microorganisms in the CF airway. Given the importance of CF airway infections, ongoing initiatives to promote understanding of the epidemiology, clinical course, and treatment options for these infections are needed.

Keywords
- cystic fibrosis
- microbiology
- bacteria
- fungi
- viruses
- microbiome

Cystic fibrosis (CF) is a hereditary and fatal disease that is caused by mutations of the CF transmembrane conductance regulator (CFTR) gene on chromosome 7, which encodes the CFTR protein. This protein functions as an anion channel that is responsible for negatively charged chloride ion transport across cells in the body.[1] This protein is present in various organs of the body, including the respiratory tract, the gastrointestinal tract, the liver, the pancreas as well as the male reproductive tract. In the airways, impaired function of this protein leads to increased mucus thickness, which fails to be cleared by the mucociliary system. This in turn leads to chronic infection of the respiratory tract and subsequent unregulated inflammation.[2] Inflammatory cytokines and secreted products accumulate, leading to lung damage and bronchiectasis. Airway infections are associated with progressive lung function decline[3] and ultimately, with respiratory failure, which is the leading cause of mortality in CF.[4,5]

Individuals with CF develop recurrent infections during their lifetime and the organisms identified in their respiratory tract differ over time based on age.[6] *Staphylococcus aureus* is commonly found in younger children, whereas *Pseudomonas aeruginosa, Achromobacter* spp., *Stenotrophomonas maltophilia*, and species of the *Burkholderia cepacia* complex (Bcc) become more prevalent in older children and adults. Although these bacteria are considered classic CF pathogens, the importance and the pathogenicity of mycobacteria, fungi, and viruses are increasingly being recognized.

The aim of this review is to summarize the epidemiology and pathogenesis of the most common bacterial, viral, and fungal species infecting the airways of CF patients. Mycobacterial infections will be covered in the article written by Drs. Richards and Olivier.

Bacterial Infections

Staphylococcus aureus

Staphylococcus aureus is commonly detected early on in life in the respiratory tract of children with CF. *Staphylococcus aureus* is the most prevalent organism in children with CF in the United States and reaches its highest prevalence between the ages of 11 and 17 years, with infection in up to 80% of patients in that age group.[6] *Staphylococcus aureus* is a gram-positive coccus which typically grows in aerobic conditions, but can also grow as a facultative anaerobe.[7] It is usually considered a commensal on human skin and can be commonly isolated from anterior nares and skin creases. Key virulence factors in

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S. aureus include the leukocytolytic toxin Panton–Valentine leukocidin, which has been associated with necrotizing lung infections.[8] In addition, small colony variants[9,10] and biofilm formation[11,12] may contribute to increased antimicrobial resistance and accelerate lung disease. Although the pathogenicity of methicillin-sensitive *S. aureus* (MSSA) has been questioned, coinfection with other pathogens such as *P. aeruginosa* may be associated with worsened clinical outcomes including more severe lung disease.[13]

Methicillin-resistant *S. aureus* (MRSA) infection tends to occur more commonly in young adults[6] rather than in children. Methicillin resistance is due to the presence of an altered penicillin binding protein, which is encoded by the *mecA* gene belonging to the Staphylococcal Cassette Chromosome (SCC).[14] There have been at least 12 types of SCC*mec* elements described to date.[15,16] The epidemiology of MRSA is SCC*mec* type-specific, with hospital-associated MRSA (HA-MRSA) strains being more often SCC*mec* type I, II, and III, whereas community-associated MRSA (CA-MRSA) strains tend to carry SCC*mec* type IV or V.[17] Additionally, *mecA*-negative MRSA (also known as borderline oxacillin-resistant *S. aureus* or BORSA) is described in CF with β−lactam resistance through various potential mechanisms, including (1) hyper β−lactamase enzyme production,[18] (2) plasmid-mediated, inducible methicillinase,[19] or (3) modification of the penicillin-binding protein genes.[20] Initial epidemiological studies in children with CF demonstrated that about two-thirds of MRSA infections were HA-MRSA (SCC*mec* II strains) and one-third CA-MRSA (SCC*mec* IV strains)[21]; however, *SCCmec* IV strains have been increasing in recent years.[22] The prevalence of MRSA-positive cultures has increased about threefold between 2002 and 2017 in individuals with CF living in the United States.[6] Chronic MRSA infection is of particular significance. It has been associated with several negative clinical outcomes, including accelerated decline in lung function, increased hospitalization, and earlier mortality in patients with CF. Ren et al noted significantly lower lung function in MRSA-infected individuals with CF compared with those with predominant MSSA-positive respiratory tract cultures.[23] Individuals with CF who are MRSA positive have a higher rate of hospitalization and increased use of oral, inhaled, and intravenous antibiotics, compared with MRSA-negative patients.[23] Furthermore, Dasenbrook et al reported that the rate of lung function decline was greater in patients with MRSA compared with MRSA-negative patients in patients aged 8 to 21 years (MRSA-positive patients had a forced expiratory volume in 1 second [FEV_1] decline of 2.06% predicted/year compared with 1.44% predicted/year in those without MRSA; difference—0.62% predicted/year, 95% confidence interval [CI]: − 0.70 to − 0.54; $p = 0.001$).[24]

In summary, although both MSSA and MRSA are common pathogens in the CF airways, MRSA in particular is associated with detrimental outcomes in patients with CF.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is an important gram-negative pathogen in patients with CF. It is a non-lactose fermenter commonly found in freshwater, which grows at an optimal temperature for growth of 42°C.[25] *Pseudomonas aeruginosa* has several virulence factors associated with infection of the host, including flagella which makes it a motile organism, as well as pili which facilitate attachment to epithelial cells in the respiratory tract.[26,27] *Pseudomonas aeruginosa* expresses three main exopolysaccharides: alginate, Pel, and Psl, which are important in the establishment and maintenance of a biofilm structure.[28] It grows mainly as an aerobe but can also survive under anaerobic conditions. *Pseudomonas aeruginosa* is intrinsically resistant to some β-lactam antibiotics and can acquire antimicrobial resistance via either chromosomal mutation or horizontal gene transfer.[29]

As per the CF Foundation Patient Registry Annual Report, the percentage of individuals with a positive culture for *P. aeruginosa* has declined over time, with the largest decrease observed among individuals younger than 18 years (47.0 percent had a positive culture in 1997 compared with 27.5 percent in 2017).[6] The decrease in *P. aeruginosa* infection prevalence may be due to early antibiotic eradication treatment of incident infections. In 2017, 44.6% of individuals with CF in the United States. were culture positive for *P. aeruginosa*.[6]

Pseudomonas aeruginosa is often initially acquired from environmental sources. Once the bacteria establish themselves in the CF airways, they undergo adaptive changes such as decreasing motility by downregulating flagellum expression. In addition to downregulating of other virulence factors,[30–33] *P. aeruginosa* will also overproduce exopolysaccharides such as alginate which confers mucoidy status.[33] Chronic infection, which is often monoclonal before undergoing adaptive diversification of clonal variants, has been associated with accelerated lung function decline and earlier mortality.[34] To prevent these poor outcomes, initial and new-onset *P. aeruginosa* infections are usually aggressively treated in an attempt to eradicate the organism from the airways.[35–37] However, eradication failure remains a problem in this patient population[38]; chronic phenotype of the isolate such as mucoid status is a risk factors for eradication failure.[39]

Burkholderia cepacia Complex

The Bcc includes over 20 species of nonfermenting gram-negative bacilli, which can be acquired from the environment or transmitted from person to person.[40]

Burkholderia species grow under aerobic conditions. This organism is frequently found in the environment, especially soil and potted plants.[41] It is considered to be a highly virulent organism, with factors such as pili facilitating epithelial cell attachment, extracellular proteases resulting in tissue damage, quorum sensing genes facilitating biofilm formation, and a type III secretion system promoting cellular invasion.[42–46] As previously mentioned, Bcc species are intrinsically resistant to several different antimicrobial classes including aminoglycosides due to efflux pumps and β-lactams via inducible chromosomally encoded β-lactamases.[47,48]

The epidemiology of Bcc infections in CF has been extensively examined given the potential for transmission between patients.[49,50] In 2017, 2.4 percent of individuals with CF in the CF Foundation Patient Registry Annual Report were culture positive for Bcc.[6] In early epidemiological studies, *Burkholderia*

cenocepacia was initially described as the most common Bcc organism in individuals with CF[42,51] and this species has been linked to several epidemic strains worldwide.[52–56] In particular, the *B. cenocepacia* ET-12 strain (ET-12Bc) has caused one of the largest epidemics in CF individuals in Canada and the United Kingdom[55] since the 1980s and has been associated with very poor clinical outcomes. The epidemiology of Bcc infections in CF has changed over the last several decades, however, as *Burkholderia multivorans* is becoming more common than *B. cenocepacia*.[57–59] This is thought to be due to implementation and reinforcement of infection control and prevention measures lowering *B. cenocepacia*[60] acquisition rates, whereas *B. multivorans* may be more often acquired from the environment. *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 Bcc.

Burkholderia cenocepacia is of particular importance in CF because it has been associated with poor clinical outcomes including accelerated lung function decline[61] as well as increased mortality both before and after lung transplantation.[62,63] In addition, *B. cenocepacia*,[42,64] as well as other species such as *B. multivorans*,[65] has been linked to cepacia syndrome, a clinical entity characterized by necrotizing pneumonia and sepsis with near-total fatality rates. Therefore, infection with Bcc species remains an important concern in the CF population due to the significant associated morbidity and mortality.

Stenotrophomonas maltophilia

Stenotrophomonas species are gram-negative rods and obligate aerobes. They are nonfermenting, oxidase-negative organisms that can be found in water sources in the environment. Although four species of *Stenotrophomonas* exist, *S. maltophilia* is the most common one identified in human hosts. *Stenotrophomonas maltophilia* virulence factors include extracellular enzymes (such as alkaline serine proteases), outer membrane lipopolysaccharides,[66] and the ability to form biofilms.[67,68] Antimicrobial resistance may occur due to the presence of multidrug efflux pumps, β-lactamases, aminoglycoside-modifying enzymes, and reduced outer membrane permeability.[69]

The prevalence of *S. maltophilia* has been shown to vary from 12% to as high as 30% in CF populations.[70–73] Previously identified risk factors for acquisition include antibiotic use,[74] in particular following the use of antipseudomonal agents.[75,76] Initial infection is thought to be due to acquisition from environmental sources rather than person-to-person transmission.

Previous studies have described that individuals with CF who are infected with *S. maltophilia* infection tend to be older and have lower baseline lung function compared with patients without *S. maltophilia*. However, in these studies, *S. maltophilia*–positive individuals did not have more rapidly declining percent predicted FEV_1 ($ppFEV_1$) or decreased 3-year survival.[77,78] However, chronic *S. maltophilia* infection (defined as two or more positive cultures in the year prior) has been described as a significant risk factor for pulmonary exacerbations treated with intravenous antibiotics[79]; it is not, however, associated with a higher risk of failing to recover baseline lung function following an exacerbation event. In addition, registry-based studies have shown that patients with chronic *S. maltophilia* have a three times higher risk of death or lung transplantation compared with those without *S. maltophilia* infection.[80,81]

Achromobacter Species

Achromobacter species are gram-negative, catalase-positive, oxidase-positive, nonsporulating rods. Up to 23 species are now known within the *Achromobacter* genus to date. *Achromobacter* species tend to grow under aerobic, nonfermentative conditions and at an optimal temperature of 25 to 37°C. They are environmental organisms, commonly found in soil and water. *Achromobacter* species are motile due to the presence of flagella, and can exhibit binding factors to mucin, collagen, and fibronectin, thereby facilitating initial attachment and invasion of the respiratory tract.[82,83] Biofilm formation as well as intrinsic resistance to several classes of antimicrobials through the expression of efflux pumps, β-lactamases, and aminoglycoside-modifying enzymes[84–86] is also expressed by this group of pathogens.

Achromobacter xylosoxidans is the most common *Achromobacter* species identified in individuals with CF, accounting for 42% of *Achromobacter* respiratory tract infections.[87] Prevalence of *Achromobacter* infections varies greatly and has been reported between 3 and 30%.[72,73,88,89] Acquisition is thought to occur mostly from the environment, although patient-to-patient transmission has been previously described.[89–92]

Published data regarding the risk factors for initial infection and clinical impact of *Achromobacter* infection are limited and include studies with small sample sizes. Risk factors for chronic infection include older age and chronic *P. aeruginosa* infection.[88,93] Of note, patients with chronic *Achromobacter* infection had lower lung function and more pulmonary exacerbations than age, gender, and *P. aeruginosa* matched controls in one of the main observational studies assessing clinical outcomes in patients with *Achromobacter* infection.[88] In a large epidemiologic study using the Toronto CF Database, chronic *Achromobacter* infection (defined as two or more positive cultures in the previous 12 months) was associated with a twofold increase in the risk of death or lung transplantation compared with patients with no history of *Achromobacter* infection.[94] Currently, no consensus data exist on optimal treatment strategies for initial acquisition, treatment during pulmonary exacerbation, or for chronic suppression of *Achromobacter* infections.

Anaerobes

Anaerobes are a group of gram-positive and gram-negative organisms which require reduced oxygen for survival.[95] They are commonly found in various mucosal surfaces of the human body including the upper airways, the gastrointestinal tract, and the female genital tract. They have been associated with invasive suppurative infections of the brain, sinuses, lung, liver, and blood vessels.[25] Capsular polysaccharide, hemolysins, proteases, and lipopolysaccharides are virulence factors associated with pathogenic anaerobes.[96]

Due to the technical difficulties of isolating and identifying anaerobes in culture-dependent methods, the prevalence of anaerobic infections in patients with CF is not well known. Recently, culture-independent methods have helped identify that anaerobic bacteria are found in abundant quantities in sputum and bronchoalveolar lavage fluid of individuals with CF, with a density estimated between 10^4 and 9×10^7 colony forming unit (CFU)/mL of sputum.[97–100] Some of the main anaerobic bacteria found in the CF airways include *Prevotella*, *Veillonella*, *Fusobacterium*, *Propionibacterium*, and *Actinomyces*.[99] However, the role of anaerobes in CF lung disease remains controversial. In recent years, studies have described the association between the detection of anaerobes and diminished clinical response to systemic antimicrobials with lung function decline.[99,101–105] One of the major limitations in the study of anaerobes in CF lung disease is the risk of contamination of lower airway samples by oropharyngeal secretions during collection,[101,106,107] although recent studies have tried to address this concern. Anaerobes may interact with other organisms present in the CF airways, increasing the virulence of *P. aeruginosa* and transferring extended-spectrum β-lactamases to *P. aeruginosa* for example.[108,109]

In contrast, the potential beneficial role of anaerobes has also been described in studies using both culture-dependent and culture-independent methods. Patients exposed to antimicrobial therapy may experience a decrease in the relative abundance of anaerobes, with subsequent increased inflammation and decreased lung function. Therefore, reducing microbial community diversity with regard to anaerobes may be playing a role in CF lung disease progression.[110–115]

Viral Infections

The role of viruses in CF airway disease has increasingly been recognized in recent years, due to ongoing advances in molecular detection, using methods such as polymerase chain reaction.[25] These molecular assays allow for rapid, highly sensitive and relatively cost-effective identification of viruses in the respiratory tract.[116] Viral culture and serology used to be the main methods of detection in the past, but these techniques were limited due to high cost, labor intensity, and lack of sensitivity.[117]

The overall prevalence of viral infections during pulmonary exacerbations in individuals with CF is estimated to be between 13 and 60%.[118,119] However, viral infections may be underreported due to infrequent use of viral swabs and the limited number of respiratory viruses detected in a given assay. The most commonly identified viruses in CF patients are respiratory syncytial virus (RSV), human rhinovirus, influenza types A and B, and parainfluenza virus,[120–122] although many other viruses including human metapneumovirus, picornavirus, coronavirus, and coxsackie/echovirus have also been described.[120,121,123–125] Viral infections are detected more frequently in children than in adults with CF.[126] In addition, children with CF are more likely to experience significant morbidity associated with viral infections compared with children without CF.[117,123,127,128] The increased severity of viral infections in individuals with CF compared with non-CF populations has been linked to reduced innate antiviral response, whereby CF individuals may not mount a sufficient interferon response or adequately express certain interferon-stimulated genes, as compared with non-CF controls.[129]

Viral infections increase the risk of pulmonary exacerbations in both children and adults with CF,[124,130] as well as increased inflammatory markers, leading to longer duration of intravenous antibiotic therapy and greater drops in lung function.[131,132]

RSV is of particular importance in CF, as it is frequently encountered in both children and adults with CF and can result in severe symptoms. Symptoms may include rhinosinusitis, cough, fever, and acute otitis media; RSV infection can also progress to lower airway disease with bronchiolitis, pneumonia, and exacerbation of chronic airway disease.[133] Recent studies have highlighted that children with CF have increased RSV-related admissions to hospital compared with healthy children.[134] In infants who have CF disease, RSV is associated with significant respiratory morbidity.[135] Increased rates of pulmonary exacerbations, longer stay in hospital as well as prolonged lower airway disease in the 2 years following the initial respiratory infection have been described in these patients.[135] Similarly, influenza virus infection has also been associated with significant morbidity in children with CF, with studies describing an increased risk of admission to hospital for pulmonary exacerbations associated with influenza infection compared with those without.[136,137]

A potential mechanism for these worsened clinical outcomes in individuals with CF who contract respiratory viral infections may be due to the interaction of viruses with bacterial species in the airways and a subsequent change in microbial community composition. Viral infections have been linked to both new acquisition of *P. aeruginosa* in previously culture-negative patients[138] and conversion from intermittent to chronic *P. aeruginosa* infection in patients with CF.[117,120,138,139] RSV infection has also been linked to increased *P. aeruginosa* biofilm formation, through dysregulation of the iron homeostasis in the CF airway epithelium.[140] Similarly, a study has previously shown that identification of both rhinovirus and *S. aureus* is among the most frequent viral/bacterial coinfection in children.[141] In summary, viral infections are an important component of the CF airway microbial community and contribute to CF lung disease.

Fungal Infections

Several different yeasts and filamentous fungi can be recovered from the respiratory tract secretions of CF patients.[142] Direct microscopic examination of specimens using fungal stains can reveal yeast cells, pseudohyphae, or hyphae and several media can be used to improve the recovery of fungi from clinical specimens.[95] Fungal growth can take as long as 4 weeks depending on the species. Identification of fungal isolates can be done using microscopic examination, biochemical testing, DNA sequence analysis, or matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.[143]

The most common filamentous fungi recovered from CF airways are *Aspergillus* species with prevalence rates up to

78%.[144] Often, the recovery of *Aspergillus* species in CF sputum represents asymptomatic colonization but can represent allergic bronchopulmonary aspergillosis (ABPA). ABPA is characterized by asthma-like symptoms, a positive *Aspergillus* skin test and an elevated serum IgE.[145] Episodes of ABPA can lead to decline in pulmonary function and are typically treated with systemic steroids.[146,147] Occasionally, *Aspergillus* can cause a bronchitis associated with increased pulmonary inflammation. In a study of over 200 children with CF, chronic *Aspergillus fumigatus* infection was found to be an independent risk factor for pulmonary exacerbation treated with intravenous antibiotics.[148] Although patients with persistent *A. fumigatus* infection had lower ppFEV$_1$ during the course of the study compared with those uninfected, there was a significant interaction between *A. fumigatus* and *P. aeruginosa* on lung function. Interventional studies of itraconazole treatment of CF patients chronically infected with *Aspergillus* species did not demonstrate any benefit in terms of lung function or occurrence of pulmonary exacerbation compared with placebo-treated patients.[149] Invasive pulmonary aspergillosis occurs rarely in immunocompetent individuals with CF pretransplant.[150]

Scedosporium species are saprophytic filamentous fungi that are much less commonly found in CF patients but can also cause serious invasive disease in immunocompromised conditions.[151] Scedosporiosis infections can involve the lung, bone, eyes, blood vessels, and central nervous system.[152]

Exophiala (Wangiella) dermatitidis can also be recovered from CF respiratory specimens.[153] It grows as a black yeast at 37°C and as a filamentous fungus at room temperature. Anecdotal reports describe clinical decline in CF patients who harbor *E. dermatitidis* in their sputum.[154]

Finally, *Candida* species are the most frequently isolated yeast from CF airways. Its prevalence ranges as high as 80%, which is not surprising given that it is a normal colonizer of the oropharynx.[144] Although studies have suggested that chronic infection with *Candida* spp. is associated with worse clinical outcomes, these investigations have not controlled for potential contamination of expectorated sputum samples by *Candida* species present in the oral cavity.[155]

The CF Microbiome

With the advent of culture-independent molecular methods of microbial detection, our understanding of microbial diversity and the interactions of microbial communities in the CF airways has significantly expanded.[102] These newer techniques not only allow the identification of microorganisms, but also the estimation of relative abundances of microbial communities in the CF airways. Methods such as 16S ribosomal ribonucleic acid (rRNA) gene sequencing of respiratory tract specimens have characterized the polymicrobial nature of lower airway infections in CF, including the coexistence of classic CF pathogens with both aerobic and anaerobic bacteria in the lower airways that were previously considered oropharyngeal contaminants.[156–159]

In a recent study of 269 children and adults with CF, 16S rRNA sequencing was used to investigate the lower airway microbiota. Despite significant interindividual variability in community structure and composition, the core microbiota included *Streptococcus*, *Prevotella*, *Rothia*, *Veillonella*, and *Actinomyces*. However, when classic CF pathogens such as *Pseudomonas*, *Burkholderia*, *Stenotrophomonas*, or *Achromobacter* were found to be present, they tended to dominate the microbial community within individuals.[156] Zemanick et al also corroborated these main findings, with classic CF pathogens found more commonly in adults.[111] Both Coburn et al and Zhao et al have described a decrease in both microbial diversity and lung function as age increases and lung disease progresses.[156,160] Overall, these findings suggest that the microbiome of CF airways changes across ages and disease stages. In addition, recent studies based on 16S rRNA sequencing have highlighted the potential significance of anaerobes, whereby the relative abundance of anaerobic taxa in respiratory tract specimens of individuals with CF was dominant during pulmonary exacerbations.[161,162]

In summary, many studies of the CF microbiome have recently documented a diversity much more complex than that described by conventional culture alone, with changes in relative abundance and structure of microbial communities in response to age, disease progression, and acute clinical events. Further studies are needed to understand how these changes impact clinical outcomes and are affected by therapeutic interventions.

Conclusions

Infections of the lower respiratory tract remain a significant contributor to CF morbidity and mortality, even in the era of treatment that corrects and/or potentiates CFTR channel function.[163] Pathogens such as MRSA, *P. aeruginosa*, and species of the Bcc continue to have significant clinical impacts on lung function and mortality rates in individuals with CF. Advances in molecular technology will help our understanding of the microbial communities and their interactions in the CF airways. Due to the ongoing impact of pulmonary infections on CF patient survival, novel eradication strategies and effective chronic suppressive treatments are needed.

Conflict of Interest
None declared.

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