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.

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 decline³ 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. 3 In addition, small colony variants 5,10 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 SCCmec elements described to date. 15,16 The epidemiology of MRSA is SCCmec type-specific, with hospital-associated MRSA (HA-MRSA) strains being more often SCCmec type I, II, and III, whereas community-associated MRSA (CA-MRSA) strains tend to carry SCCmec 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 (SCCmec II strains) and one-third CA-MRSA (SCCmec 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, Dassenbrook 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 [FEV1] 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. 6,27 *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 other virulence factors, 30–33 *P. aeruginosa* will also overproduce exopolysaccharides such as alginate which confers mucoid 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. 38,39 Chronically infected patients may require combination therapy due to the development of resistance. 40

**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*
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, and the ability to form biofilms. Antimicrobial resistance may occur due to the presence of multidrug efflux pumps, β-lactamases, aminoglycoside-modifying enzymes, and reduced outer membrane permeability.

The prevalence of S. maltophilia has been shown to vary from 12% to as high as 30% in CF populations. Previously identified risk factors for acquisition include antibiotic use, in particular following the use of antipseudomonal agents. 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 FEV₁ (ppFEV₁) or decreased 3-year survival. 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; 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.

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 fibronecint, thereby facilitating initial attachment and invasion of the respiratory tract. Biofilm formation as well as intrinsic resistance to several classes of antimicrobials through the expression of efflux pumps, β-lactamases, and aminoglycoside-modifying enzymes 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. Prevalence of Achromobacter infections varies greatly and has been reported between 3 and 30%. Acquisition is thought to occur mostly from the environment, although patient-to-patient transmission has been previously described.

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. 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. 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. Currently, no consensus data exist on optimal treatment strategies for initial acquisition, treatment during pulmonary exacerbation, or for chronic suppression of Achromobacter infections.
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 $9 \times 10^7$ colony forming unit (CFU)/mL of sputum. Some of the main anaerobic bacteria found in the CF airways include Prevotella, Veillonella, Fusobacterium, Propionibacterium, and Actinomyces. 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. 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, 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.

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.

**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. These molecular assays allow for rapid, highly sensitive and relatively cost-effective identification of viruses in the respiratory tract. 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.

The overall prevalence of viral infections during pulmonary exacerbations in individuals with CF is estimated to be between 13 and 60%. 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, although many other viruses including human metapneumovirus, picornavirus, coronavirus, and coxsackie/echovirus have also been described. Viral infections are detected more frequently in children than in adults with CF. In children, RSV is associated with significant respiratory morbidity. Rates of hospitalization due to RSV-related admissions to hospital compared with healthy children. In infants who have CF disease, RSV is associated with significant respiratory morbidity. Rates of hospitalization due to RSV-related admissions to hospital compared with healthy children.

**Fungal Infections**

Several different yeasts and filamentous fungi can be recovered from the respiratory tract secretions of CF patients. Direct microscopic examination of specimens using fungal stains can reveal yeast cells, pseudohyphae, or hyphae and some media can be used to improve the recovery of fungi from clinical specimens. 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. The most common filamentous fungi recovered from CF airways are Aspergillus species with prevalence rates up to
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. 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 RNA 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.

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 *Actinomycetes*. 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. Zemanick et al also corroborated these main findings, with classic CF pathogens found more commonly in adults. 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.

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 mortality and morbidity, even in the era of treatment that corrects and/or potentiates CFTR channel function. 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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Microbiology of CF Airway Disease

Blanchard, Waters

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