

Microbial Epidemiology of the Cystic Fibrosis Airways: Past, Present, and Future

Christina S. Thornton, MD, PhD¹ Michael D. Parkins, MD, MSc, FRCPC^{1,2}

¹Department of Medicine, Cumming School of Medicine, University of Calgary, Alberta, Canada

²Department of Microbiology, Immunology and Infectious Diseases, University of Calgary, Alberta, Canada

Semin Respir Crit Care Med 2023;44:269–286.

Address for correspondence Michael D. Parkins, MD, MSc, FRCPC, Departments of Medicine and Microbiology, Immunology & Infectious Diseases, Southern Alberta Adult Cystic Fibrosis Clinic, Division of Infectious Diseases, Calgary Zone, Alberta Health Services, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4N1 (e-mail: mdparkin@ucalgary.ca).

Abstract

Progressive obstructive lung disease secondary to chronic airway infection, coupled with impaired host immunity, is the leading cause of morbidity and mortality in cystic fibrosis (CF). Classical pathogens found in the airways of persons with CF (pwCF) include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, the *Burkholderia cepacia* complex, *Achromobacter* species, and *Haemophilus influenzae*. While traditional respiratory-tract surveillance culturing has focused on this limited range of pathogens, the use of both comprehensive culture and culture-independent molecular approaches have demonstrated complex highly personalized microbial communities. Loss of bacterial community diversity and richness, counteracted with relative increases in dominant taxa by traditional CF pathogens such as *Burkholderia* or *Pseudomonas*, have long been considered the hallmark of disease progression. Acquisition of these classic pathogens is viewed as a harbinger of advanced disease and postulated to be driven in part by recurrent and frequent antibiotic exposure driven by frequent acute pulmonary exacerbations. Recently, CF transmembrane conductance regulator (CFTR) modulators, small molecules designed to potentiate or restore diminished protein levels/function, have been successfully developed and have profoundly influenced disease course. Despite the multitude of clinical benefits, structural lung damage and consequent chronic airway infection persist in pwCF. In this article, we review the microbial epidemiology of pwCF, focus on our evolving understanding of these infections in the era of modulators, and identify future challenges in infection surveillance and clinical management.

Keywords

- ▶ cystic fibrosis
- ▶ epidemiology
- ▶ microbiology
- ▶ microbiome
- ▶ CFTR modulators

Cystic fibrosis (CF) is the most common, fatal genetic disease among the Caucasian population with an estimated prevalence of $\geq 100,000$ people worldwide.¹ Abnormal CF transmembrane conductance regulator (CFTR) function results in altered sodium and bicarbonate transport across epithelial surfaces with sequelae of multiorgan involvement. Thick tenacious mucus in the lungs results in compromised mucociliary clearance and predisposes to chronic bacterial infections, felt to be key drivers of progressive and irreversible airway damage.² Moreover, persons with CF (pwCF) demon-

strate periods of recurrent cycles of increasing respiratory symptoms and reduction in lung function, known as pulmonary exacerbations (PEX), interspersed between periods of relative clinical stability.³ Importantly, 25% of individuals fail to recover baseline lung function from these episodes despite aggressive antimicrobial therapy.^{4,5} The advent of highly effective modulator therapy (HEMT) has dramatically improved both the respiratory and overall well-being of many pwCF; however, structural lung damage with consequent infections persists. Airway infections have consistently been

article published online
January 9, 2023

Issue Theme Cystic Fibrosis; Guest
Editors: Andrew M. Jones, BSc, MD, FRCP
(UK), and Siobhain Mulrennan, MBChB,
MRCP (UK), MD, FRACP

© 2023. Thieme. All rights reserved.
Thieme Medical Publishers, Inc.,
333 Seventh Avenue, 18th Floor,
New York, NY 10001, USA

DOI <https://doi.org/10.1055/s-0042-1758732>.
ISSN 1069-3424.

identified as a top research priority topic by both CF community members and clinicians with a specific goal of improving “respiratory microorganism detection and treatment.”⁶ In this review, we evaluate the historical and evolving landscape of CF airway infections with particular focus on the era of HEMT. As other reviews in this series focus on nontuberculous mycobacteria, fungi, and viral infections, we direct the reader to those individual aforementioned sections.

Traditional Landscape of Cystic Fibrosis Airway Infections: the Classics

Microbial proliferation in the CF airways, as a consequence of dysfunctional mucociliary clearance, is dynamic with inhaled and aspirated microbes immigrating to the lower airways where they have the opportunity to adapt to the surrounding microenvironment, compete with resident microflora, where they may progress to chronic airway infections (►Fig. 1).^{7–9} In turn, these chronic airway infections lead to persistent host inflammation which then potentiates a vicious cycle of structural lung damage, airflow obstruction, and remodeling, and deteriorating respiratory function.

In the absence of life-saving lung transplantation, progressive respiratory disease is the leading cause of death among pwCF.^{10–12} The historic view of CF airways was one of evolution through age and disease-stage with the characteristic presence of *Haemophilus influenzae* and *Staphylococcus aureus* during infancy and early childhood, eventually supplanted by Gram-negative bacteria, including *Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex (►Fig. 2).^{2,13} These classic pathogens have long been considered as hallmarks of progressive lung disease—where they increase in prevalence and abundance with advancing disease severity and their presence is associated with accelerated lung function decline.¹⁴ The eventual domination of airway communities by these classical pathogens (acquired from either natural environments or patient-patient spread) is postulated to be driven in part by recurrent and frequent antibiotic exposure in response to acute PEX.^{15–17} However, as we will explore further in this review, the evolving landscape of airway microbiology created through intense study has offered new insight into these complex communities (►Fig. 3). Importantly, our review of each individual pathogen is by necessity general—and the reader is directed to several detailed reviews for further reading.^{15,18–23}

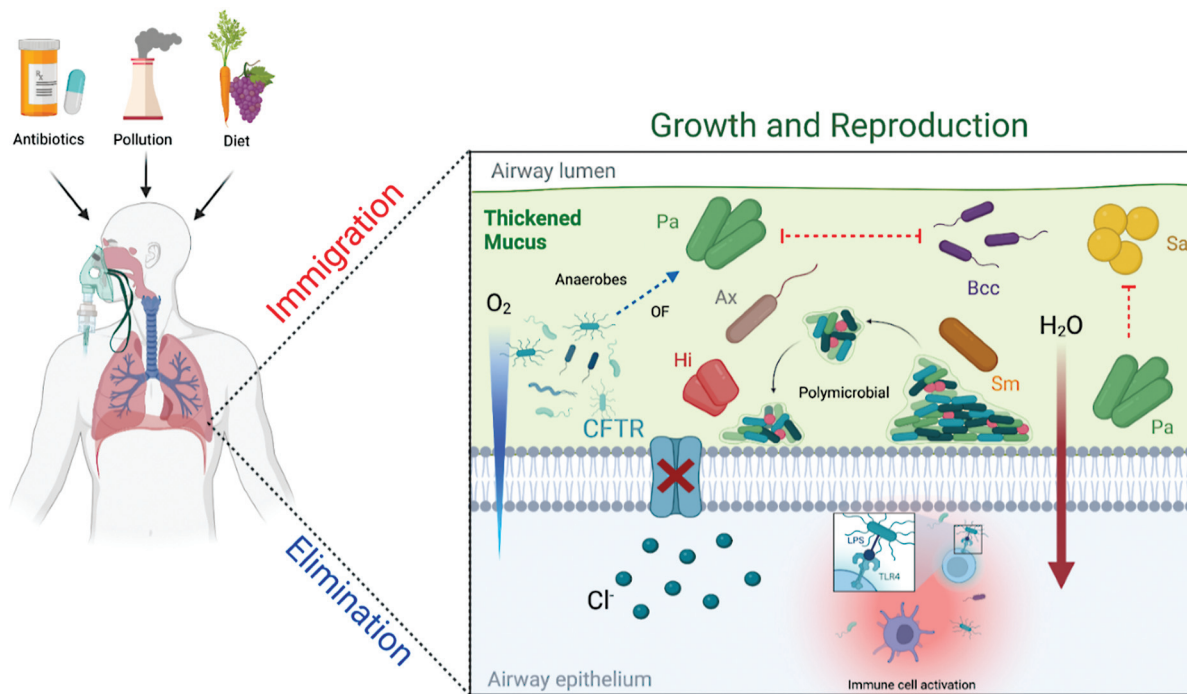


Fig. 1 Microbiology of the Cystic Fibrosis Airways. Microaspirated microbiota shapes early colonizing communities through a balance of microbial *immigration* (movement of microbes into a new environment), *growth and reproduction* (influenced by factors for regional growth conditions including: (1) environmental (i.e., nutrient availability, temperature, pH, and oxygen tension); (2) host (i.e., concentration and activation of inflammatory cells); and (3) bacterial (i.e., local microbial composition/competition) and subsequent *elimination* (movement of microbes out of an environment (i.e. through cough and adjunctive airways clearance measures, antimicrobial therapies, and host immune defenses)). In CF microbial elimination, as a function of mucociliary clearance and host defense is critically impaired. Classic pathogens that infect CF airways include *Staphylococcus aureus* (Sa), *Pseudomonas aeruginosa* (Pa), the *Burkholderia cepacia* complex (Bcc), *Haemophilus influenzae* (Hi), *Stenotrophomonas maltophilia* (Sm), and *Achromobacter spp* (Ax). Nonclassical taxa such as anaerobes and oropharyngeal flora (OF) also reside within the microbiome. Development of aggregates and polymicrobial biofilms contribute to longevity of chronic infections and antimicrobial resistance. Microbe–microbe interactions within this community are numerous with examples including synergy (blue arrows, i.e. upregulation of pathogen virulence by otherwise commensal bacteria) and inhibition (red dashed lines, i.e., competition and growth inhibition of *S. aureus* by *P. aeruginosa*). Taken together, this complex milieu contributes to the upregulation of host proinflammatory mechanisms and perpetuates vicious cycles of inflammation and infection. Figure created with BioRender.

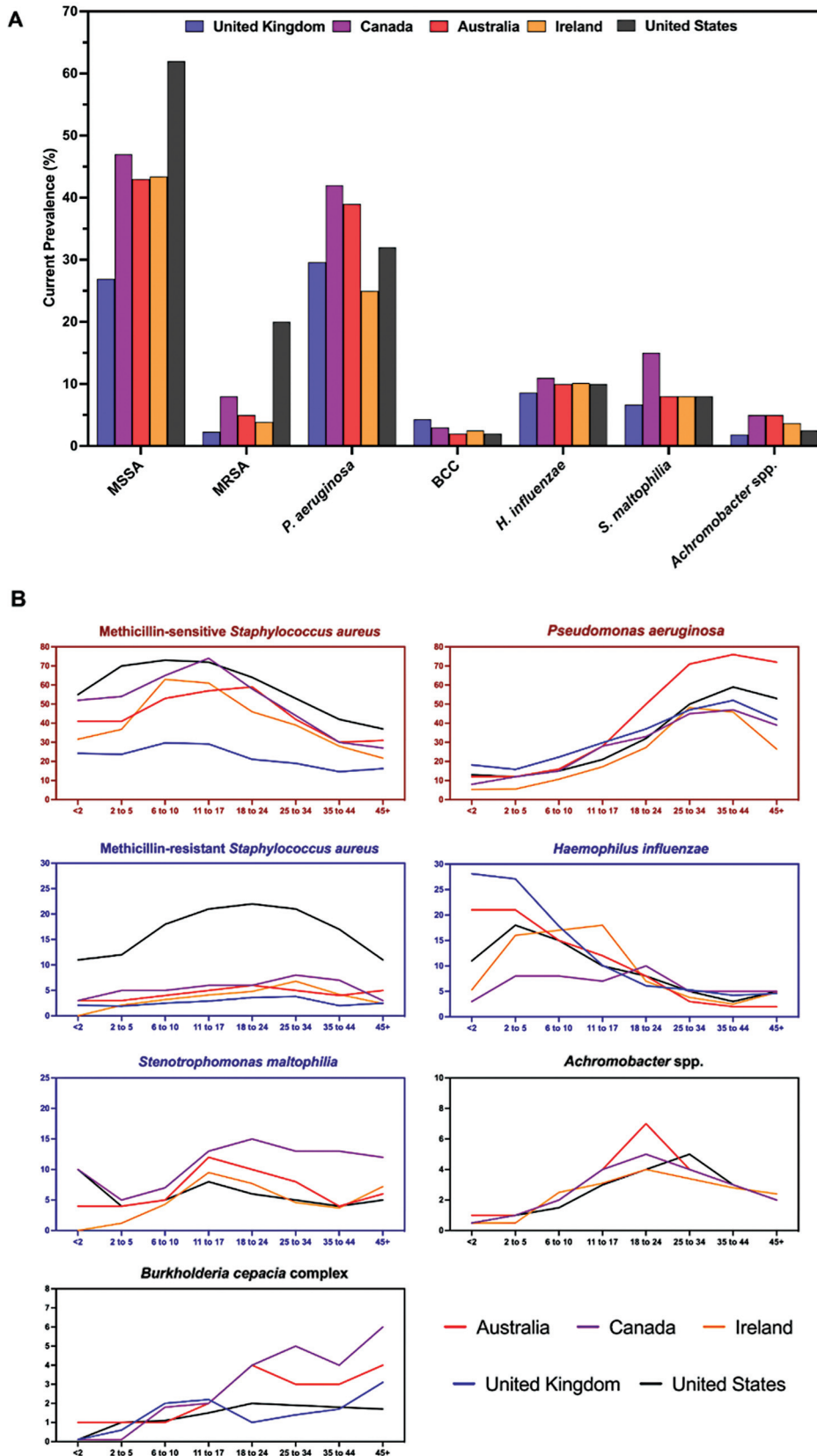


Fig. 2 Epidemiology of cystic fibrosis airways pathogens—a global perspective. (A) Overall prevalence of classical pathogens by any positive culture in 2020 as reported by patient registry data from the United Kingdom,²¹¹ Canada,²¹² Australia,²¹³ Ireland,²¹⁴ and the United States.²¹⁵ (B) Prevalence (y-axis) of CF pathogens by any positive culture stratified by age groups (x-axis) by country. Minor corrections for age ranges were made in order to compare to other regions. All data were used with permission by each respective CF registry.

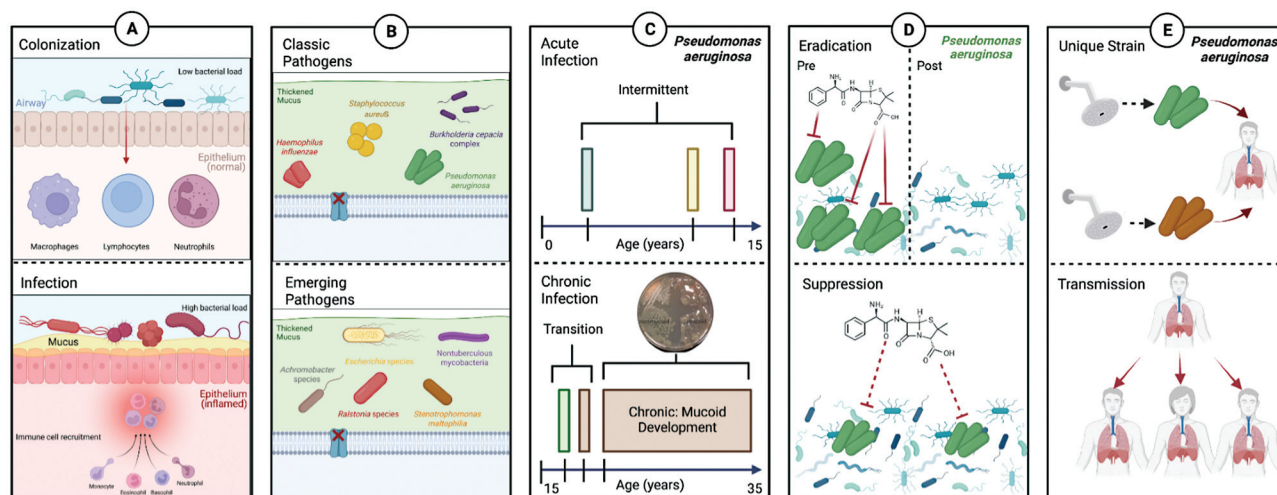


Fig. 3 Depictions of microbial concepts in the CF airway. (A) Within CF airways, organisms can cause *infection* (i.e., microorganism that directly or indirectly causes or can cause, disease) or *colonization* (i.e., microbes that reside within the lung without causing harm). (B) Pathogens in CF airways can be viewed as *classic* (i.e., well-established organisms that cause CF progression and are tracked in national registries) or *emerging* (i.e., microorganisms not traditionally associated with CF, increasingly being recovered). (C) The natural history of infection—in particular with *P. aeruginosa*—includes both *acute* (i.e., acquisition of intermittent unique strains) and the eventual development of *chronic* (i.e., persistence of one *P. aeruginosa* strain, often associated with mucoid conversion) status. (D) Antibiotics can be used to achieve *eradication* (i.e., early and intensive use of antibiotics in response to a newly identified pathogen to eliminate it from airways, thus preventing or delaying the establishment of chronic infection) or *suppression* (i.e., intermittent use to alleviate or control symptoms rather than eliminate chronic infection—which is not possible once established). (E) Strains of *P. aeruginosa* may be *unique* (i.e., those acquired through environmental exposures and not shared among other individuals) and/or *epidemic* (i.e., a clonal strain that exists among a local CF population at a high prevalence and has been conclusively demonstrated to be spread from CF person-to-person). Figure created with BioRender.

Staphylococcus aureus

S. aureus, a gram positive, is often the earliest cultured pathogen in pwCF and reaches its highest prevalence relatively early in life (50% of infants less than 2 years of age and up to 80% in early adolescence).^{24,25} Acquisition of *S. aureus* early in life is often attributed to high nasal colonization, estimated to be up to 30% in the general population.²⁵ *S. aureus* has recently become the most prevalent pathogen in pwCF due to a number of factors including the efficacy of early eradication interventions directed against other pathogens. Ecological pressure including regions of relative hypoxia secondary to mucus plugging, host immune defenses, availability of nutrients, antibiotic therapy, and competition with other pathogens results in the need for rapid adaptation to enable survival. Such mechanisms include aggregate and biofilm formation,²⁶ in which both host immune response (i.e., host antibodies and macrophages) and antibacterial therapy poorly penetrate, thereby ensuring persistence.²⁷ Infection with *S. aureus* has adverse clinical outcomes including a greater degree of airway inflammation and lower lung function.^{28–31} Given the importance of this pathogen in younger pwCF, prophylactic therapy followed by suppressive strategies following acquisition with narrow-spectrum anti-*Staphylococcal* therapies has been assessed in multiple contexts,^{32–35} but its routine use is regionally dependent.

Methicillin-resistant *S. aureus* (MRSA) emerged shortly after the introduction of methicillin as a semisynthetic penicillinase-resistant antibiotic in the 1960s.²⁵ The MRSA phenotype results from the horizontal gene transfer of *mecA*, encoding the alternative penicillin-binding protein-2a which

has low affinity for methicillin and the isoxazolyl penicillins (and by inference all other beta-lactams, other than the fifth generation cephalosporins).³⁶ While in the minority compared to methicillin-sensitive *S. aureus* (MSSA), the prevalence of MRSA ranges from 10 to 25% of pwCF globally—but with significant variation by country (►Fig. 2). MRSA infection is of particular clinical concern as its presence is associated with accelerated lung function decline³⁷ and excess-associated mortality.^{24,38,39}

S. aureus phenotypic divergence has been shown to influence clinical outcomes. Small colony variant (SCV) transformation is another such adaptation disproportionate in CF that is characterized by mutations in metabolic genes causing nutritional growth deficiencies (making their identification in the laboratory more challenging) but conferring a survival advantage including resistance to antibiotic pressure and host defenses. SCVs are prevalent (found in 8.1–24% of pwCF) and disproportionately observed in higher numbers among MRSA—attributed to higher sulfonamide treatments.⁴⁰ SCVs add a further layer of complexity for clinicians as they are frequently found within host cells and are thereby relatively protected from the effects of many antibiotics—making management difficult (►Table 1).^{41–43} Consequently, SCVs have been associated with chronic infection, worse lung function, antibiotic resistance, and proliferation in the presence of other pathogens including *P. aeruginosa*.^{42,44–46} Notably, in a recent large multicenter-longitudinal study of CF children, across multivariate models including *P. aeruginosa* and MRSA, only SCVs were consistently associated with worse clinical outcomes.⁴⁰

Table 1 Commonly used antibacterial drugs and their relative spectrum of activity against classical CF pathogens^a

Route of delivery	Antibiotic	<i>Staphylococcus aureus</i>				CF-relevant gram negatives				
		MSSA ^b	MSSA SCV	MRSA ^b	MRSA SCV	Hi	Pa	Bcc	Sm	A
Nebulized	Tobramycin	High	Moderate	Minimal	Minimal	High	Negligible	Negligible	None	None
	Colistin	High	High	High	High	High	High	High	High	High
	Levofloxacin	High	High	Moderate	Minimal	High	High	High	High	High
	Aztreonam	High	High	High	High	High	High	High	High	High
Oral	Minocycline	High	High	High	High	Moderate	High	High	High	High
	Doxycycline ^c	High	High	High	High	Moderate	High	High	High	High
	1 st -Cephalexin	High	Moderate	High	High	Moderate	High	High	High	High
	2 nd -Cefuroxime	High	Moderate	High	High	High	High	High	High	High
	3 rd -Cefixime	High	Moderate	High	High	High	High	High	High	High
	Amoxicillin-clavulanic acid	High	Moderate	High	High	High	High	High	High	High
	Trimethoprim-sulfamethoxazole ^c	High	Moderate	High	High	High	High	High	High	High
	Ciprofloxacin ^c	High	High	High	High	High	High	High	High	High
	Levofloxacin ^c	High	High	High	High	High	High	High	High	High
	Moxifloxacin ^c	High	High	High	High	High	High	High	High	High
Linezolid ^c	High	High	High	High	High	High	High	High	High	
Parenteral	Tobramycin	High	High	Moderate	Minimal	High	High	High	High	High
	Amikacin	High	High	Moderate	Minimal	High	High	High	High	High
	Colistin	High	High	High	High	High	High	High	High	High
	1 st -Cefazolin	High	Moderate	High	High	Moderate	High	High	High	High
	2 nd -Cefuroxime	High	Moderate	High	High	High	High	High	High	High
	3 rd -Ceftazidime	High	Moderate	High	High	High	High	High	High	High
	3 rd -Ceftriaxone	High	Moderate	High	High	High	High	High	High	High
	4 th -Cefepime	High	Moderate	High	High	High	High	High	High	High
	5 th -Ceftobiprole	High	Moderate	High	High	High	High	High	High	High
	5 th -Ceftaroline	High	Moderate	High	High	High	High	High	High	High
	Piperacillin-tazobactam	High	Moderate	High	High	Moderate	High	High	High	High
	Ceftolozane-tazobactam	High	Moderate	High	High	High	High	High	High	High
	Ceftazidime-avibactam	High	Moderate	High	High	High	High	High	High	High
	Cefiderocol	High	Moderate	High	High	High	High	High	High	High
	Ertapenem	High	Moderate	High	High	High	High	High	High	High
	Meropenem	High	Moderate	High	High	High	High	High	High	High
	Meropenem-vaborbactam	High	Moderate	High	High	High	High	High	High	High
Imipenem-cilastatin-relebactam	High	Moderate	High	High	High	High	High	High	High	
Ticarcillin-clavulanic acid	High	Moderate	High	High	High	High	High	High	High	
Vancomycin	High	Moderate	High	High	High	High	High	High	High	
Drug potency legend		High	Moderate	Minimal	Negligible	None				

Abbreviations: A, *Achromobacter. spp.*; Bcc, *Burkholderia cepacia complex*; Hi, *Haemophilus influenzae*; MRSA, *Methicillin-resistant Staphylococcus aureus*; MSSA, *Methicillin-sensitive Staphylococcus aureus*; Pa, *Pseudomonas aeruginosa*; SCV, small-colony variant; Sm, *Stenotrophomonas maltophilia*. Note: Green boxes denote sensitivity, yellow boxes denote intermediate activity, and red boxes denote lack of efficacy.

^aDrugs are organized by class and activity is inferred from references.^{48,97,215}

^bRefers to regular growth.

^cAgent with extensive bioavailability with oral route generally preferred.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

The role of chronic MSSA prophylaxis and/or suppression is unclear with a lack of consensus around whether prevention of colonization is an effective and safe measure to reduce the acquisition of early lung infection. Indeed, a Cochrane review found that while fewer children were positive for *S. aureus* when commenced with therapy early in infancy, the clinical impact was unclear with no significant differences in lung function, hospital admissions, or nutrition when compared to those without prophylaxis.³³ Moreover, given the association with an increased prevalence of *P. aeruginosa* following chronic suppressive anti-staphylococcal therapy,^{32,47} the U.S. CF Foundation guidelines recommend against its routine use.⁴⁸ In contrast, given the disproportionate impact of chronic MRSA on CF clinical outcomes, eradication attempts may be warranted to prevent persistent infection. Two placebo-controlled randomized trials have been conducted to evaluate the efficacy of MRSA early eradication using decolonization protocols (nasal, skin, and/or oral) with mupirocin/chlorhexidine and 14 to 21 days of systemic trimethoprim-sulfamethoxazole in combination with rifampin (with alternate regimens allowed in the case of resistance or intolerance). Muhlebach and colleagues used a 14-day antibiotic protocol and showed significantly lower MRSA positivity in the treated (26%) compared to the placebo (82%) arm at the primary endpoint of 28 days ($p < 0.001$).⁴⁹ Moreover, this effect was sustained with 54% of subjects in the MRSA-negative treatment arm after 12 weeks, compared to 10% in the control group. In contrast, Neri et al identified a trend that did not meet significance, in the prevalence of chronic MRSA negativity (defined as three negative cultures) between treated and control arms after 6 months.⁵⁰ Taken further, the Persistent MRSA Eradication Protocol was a randomized-control trial evaluating a cohort of 29 adult pwCF with chronic (rather than new MRSA) colonization to evaluate the potential of nebulized vancomycin or placebo in addition to oral antibiotics (rifampin and trimethoprim/sulfamethoxazole or doxycycline) at clearing infection.^{51,52} There were no significant differences in MRSA culture negativity at 1 or 3 months, lung function, symptom scores, or absolute density of MRSA between those receiving nebulized vancomycin or placebo.⁵² Finally, a Cochrane systematic review evaluating the evidence of eradication of MRSA colonization in general (non-CF) populations evaluated six clinical trials and concluded not only insufficient data around eradication efficacy but also a high rate of adverse events associated with treatment (20%).⁵³ Taken together, these data demonstrate that eradication does decrease rates of persistence and reduce exacerbation frequency—but have not confirmed slower lung disease progression, likely in part due to recruitment challenges.^{49,54} This is particularly relevant in MRSA-related outcomes as early CF studies that were not adequately powered initially failed to demonstrate MRSA's significant potential for harm.⁵⁵

Haemophilus influenzae

H. influenzae, a gram-negative coccobacillus, is commonly found in the upper respiratory tract of healthy and ill individuals alike—but recovery from the lower airways in pwCF is thought to indicate disease as its presence is associated with

local inflammation.^{56,57} *H. influenzae* is the second most prevalent species (approximately 28%) in the first 5 years of life of pwCF—with similar rates into early adolescence before declining to approximately 10% in adulthood.⁵⁸ *H. influenzae* possesses several adherence factors that contribute to bacterial colonization, persistence, and biofilm formation.⁵⁹ Nonencapsulated *H. influenzae* are most commonly identified in pwCF including those associated with PEX.^{15,60} Unique relative to other CF pathogens, strains of *H. influenzae* causing infections in pwCF are usually transient in airways with persistent infection over years by a single strain occurring only in a minority of cases.⁶¹ *H. influenzae* from pwCF have the ability to become resistant to several classes of antibiotics (► **Table 1**), driven in part by hypermutability phenotypic adaptation.^{61,62} While the clinical implications of *H. influenzae* isolation are less clear, a retrospective longitudinal study of 349 patients over 15 years (1998–2012) indicated a doubling in prevalence (8–16%, $p < 0.0001$; mean age 7.6 years) over the study period, suggesting this is likely becoming a greater cause of infection in younger pwCF.⁶³ Given the high turnover of *H. influenzae* strains within the CF airways, studies examining the potential impact of early confirmed culture-directed therapy are lacking, and the practice of treatment (vs. observation) is currently clinician dependent.

Pseudomonas aeruginosa

P. aeruginosa, a gram-negative bacillus, is historically the most prevalent (approximately 60–80%) pathogen in adult pwCF. The increasing prevalence of *P. aeruginosa* in adolescence and young adulthood is felt to be driven by both bacterial exposure and recurrent antibiotic courses from the eradication of early airway colonizers.⁶⁴ Using data from several national CF patient registries (► **Fig. 2**), a significant rise in *P. aeruginosa* prevalence can be observed between those 6 to 10 years and those 18 to 24. Concurrently, the prevalence of both *H. influenzae* and *S. aureus* decreases in parallel with increases in *P. aeruginosa*, further depicting dynamic longitudinal shifts among the classical pathogens.⁶⁵

P. aeruginosa is ubiquitous in the local soil and aquatic environment, thus ensuring exposure from a range of sources.⁶⁶ Historically, it was perceived that *P. aeruginosa* strains were unique to each individual CF patient and that for transmission to occur, repeated and close contact was required (i.e., such as that exhibited in the same household or between siblings).⁶⁶ However, our understanding of transmission has expanded with the recognition of several epidemic strains passed exclusively from pwCF to pwCF (i.e., the transcontinental Liverpool epidemic strain), many of whose infection is associated with worse outcomes.^{66,67} Once acquired chronically, pwCF typically harbor strains that rapidly evolve through several adaptive mechanisms, including the acquisition of mutations. One such example is the transition from nonmucoid to mucoid phenotype, which is associated with fitness advantages and consequent accelerated clinical deterioration.^{68,69}

The natural history of incident *P. aeruginosa* acquisition is such that infections may evolve into chronic infection whereby a specific strain recovered by culture is observed with

increasing frequency before eventually being identified.⁷⁰ While multiple definitions of chronic infection exist,⁷¹ the most commonly used is the Leeds criteria⁷² (i.e., isolation of *P. aeruginosa* in >50% of sputum cultures over a 12-month period). Development of chronic *P. aeruginosa* has significant adverse clinical impacts including worse baseline ppFEV₁,^{66,73–75} accelerated lung function decline,^{74,76,77} worse radiographic and symptom scores,^{78,79} lower nutritional status,⁸⁰ and faster progression to end-stage lung disease and death^{77,81–84} compared to those pwCF without *P. aeruginosa*. Moreover, children colonized with *P. aeruginosa* have on average a 10-year reduced survival compared to those patients without *P. aeruginosa* infection.⁸⁵

Traditional dogma once held that *P. aeruginosa* chronic airway infection was an inevitable consequence of disease progression; however, it is now understood that early and aggressive antibacterial eradication protocols may abrogate *P. aeruginosa*'s potential to cause chronic infection until further into adulthood (and potentially indefinitely).^{66,86} A wide range of *P. aeruginosa* early eradication protocols have been developed and assessed for efficacy relative to historical controls. Because of this compelling evidence base, early eradication—in one form or another—has become the standard of care without a placebo-controlled randomized study having ever been performed.^{87–89} Indeed, early eradications are largely credited with the tremendous drop in *P. aeruginosa* prevalence observed in recent cohorts (►Fig. 2). Accordingly, guidelines recommend serial airway surveillance and prompt eradication in response to new *P. aeruginosa* acquisition with inhaled antibiotic regimens that include tobramycin, colistin, and/or aztreonam (►Table 1); however, optimal regimens are continually being explored.⁹⁰ The Early *Pseudomonas* Infection Control (EPIC) study found no additional benefit to oral ciprofloxacin in addition to nebulized high-dose tobramycin relative to tobramycin alone over 28 days.⁸⁸ Similarly, extending treatment with tobramycin over 56 days conferred no additional benefit in the ELITE study.⁸⁷ The recent Trial of Optimal Therapy for *Pseudomonas* Eradication in Cystic Fibrosis (TORPEDO-CF) sought to evaluate the addition of 14 days of ceftazidime/tobramycin or 12 weeks of oral ciprofloxacin in combination with 12 weeks of nebulized colistin. Although there were fewer hospitalized patients in the intravenous group during follow-up, the authors' concluded outcomes were not different.⁹¹ While antipseudomonal prophylaxis is not currently recommended following successful eradication (given the observation that secondary cycled prophylactic antipseudomonal antibiotic therapy offers no advantage over culture-based treatment), clinical uncertainty exists with the repeat isolation of *P. aeruginosa*—where this may represent a new infection or initial failure of eradication.⁶⁶ When possible, management strategies may be informed with the use of isolate genotyping to discern incident versus persistent strain status.⁶⁶ Novel, incremental eradication strategies that seek to sequentially manage initial failures with increasingly aggressive regimens are increasingly being invoked.^{92,93}

While prevention of chronic infection is felt to ameliorate adverse clinical outcomes, the evidence this provides a

lasting clinical benefit is less convincing. Mayer-Hamblett and colleagues evaluated long-term efficacy over 5 years in a cohort of pediatric subjects in the EPIC study who received eradication therapy for newly acquired *P. aeruginosa*. While those who achieved sustained eradication throughout the trial had a reduced risk of developing chronic *P. aeruginosa*, mucoid phenotype detection, and less antipseudomonal antibiotics, there was no significant association in the rate of PEX or lung-function decline—suggesting microbiologic outcomes may not directly translate to clinical response.⁹⁴ Alternatively, this may demonstrate the durability of chronic suppressive antipseudomonal therapies. Indeed, many of the gains achieved in CF outcomes prior to the HEMT era directly relate to the potency and efficacy of antipseudomonal suppression developed through dedicated clinical studies.^{95–98} A discussion on the strategies, regimens, and drugs used for the acute and longitudinal management of chronic *P. aeruginosa* is well beyond the scope of this review. Indeed, lessons derived from *P. aeruginosa* have now been applied to other pathogens in pwCF and other chronic lung diseases (i.e., non-CF bronchiectasis, NCFB).

Burkholderia cepacia Complex

The *B. cepacia* complex (Bcc) consists of over 20 closely related species¹⁵ with the two most commonly identified in CF being *B. cenocepacia* and *B. multivorans*. While these species are lumped together based on bacteriologic features, the clinical impact of these different species is often profoundly different.³¹ While the prevalence of Bcc is generally reported as 5 to 10% of pwCF (►Fig. 2), a recent Canadian study demonstrated profound epidemiological shifts within this range, with a significant decline in the limited numbers of epidemic *B. cenocepacia* strains more recently being replaced with the increasing prevalence of nonclonal isolates of *B. multivorans* and other species.⁹⁹ *B. cenocepacia*, in particular, have been associated with more severe lung disease including rapid respiratory decline and increased overall mortality.¹⁰⁰ While uncommon and very rare in recent years, rapidly progressive necrotizing pneumonia with or without refractory bacteremia termed “cepacia syndrome” from (predominately) *B. cenocepacia* has been associated with near-uniform fatality.¹¹ Those pwCF with Bcc infections prior to lung transplant are more likely to result in rapid decline and experience higher mortality following lung transplant, with infection often precluding transplant listing in all but the most experienced centers.¹⁰¹ Recognized even prior to *P. aeruginosa*, patient-to-patient transmission of epidemic clones of *B. cenocepacia* (in particular ET-12) and *B. dolosa* has been one of the driving forces behind the advancement of infection and prevention control measures to prevent the spread.^{102–104} Given the adverse outcomes associated with many Bcc chronic infections, there is great interest in extrapolating early eradication learnings. Owing to low incidence, currently, there are no uniform practice guidelines around eradication in response to early isolation.^{31,105,106} Antibiotic therapy for Bcc is even more complicated than *P. aeruginosa* and limited by the high level

of intrinsic and easily acquired antibiotic resistance, leaving few therapeutic options (–Table 1).

Stenotrophomonas maltophilia

Stenotrophomonas maltophilia is a gram-negative bacteria with inherent multidrug resistance that has increasingly emerged over the last few decades as pwCF are living longer—with prevalence rates ranging from 8 to 14% of adults.¹⁰⁷ Infection with *S. maltophilia* disproportionately occurs in those with more advanced disease and those who experience more exaggerated rates of lung function decline.^{108,109} While some speculate that the presence of *S. maltophilia* may simply represent colonization without directly impacting long-term lung function or survival,^{108,110} a large cross-sectional cohort study of pwCF using serum antibody levels demonstrated that chronic infection was an independent risk factor for PEx requiring hospitalization.¹¹¹ Notably, the association of chronic *S. maltophilia* infection as a risk factor for hospitalization for PEx remained when adjusted for other clinical factors including baseline ppFEV₁, disease stage, age, and presence of *P. aeruginosa*. Moreover, there were increased unadjusted rates of mortality and lung transplantation among those with *S. maltophilia* infection although the effect was not significant after adjustment using a time-varying model.¹¹² More recently, a single-center cohort study identified pwCF and incident acquisition of *S. maltophilia* to have a worsening mean annual decline in ppFEV₁ (–2.14 vs. –1.67/year) and increased hospitalization rates.¹¹³ A recent Cochrane review found no available randomized control trials in either the setting of acute PEx or chronic infection to evaluate the effectiveness of antibiotic therapy against *S. maltophilia* in pwCF.¹⁰⁷ There are currently no consensus guidance statements regarding treatment or eradication strategies. Given this lack of evidence, the current mainstay of management is determined by clinical experience with the field uniformly acknowledging a desperate need for pragmatic trials.

***Achromobacter* species**

Achromobacter spp. are aerobic, nonfermenting gram-negative bacilli frequently recovered from environmental reservoirs.¹¹⁴ Variations in center-to-center reported prevalence are significant and likely reflect varying ability to correctly identify and distinguish from other pathogens such as *P. aeruginosa*, infection control, and antibiotic usage.^{115,116} While infections were historically attributed exclusively to *Achromobacter xylosoxidans*, we now recognize the diversity of *Achromobacter* species in pwCF—with many species as prevalent as *A. xylosoxidans*.¹¹⁷ Similar to *P. aeruginosa*, *Achromobacter* spp possess several intrinsic characteristics enabling survival and potentially contributing to disease progression including highly dynamic genomes, hypermutation, intrinsic multidrug resistance, and capability to produce biofilms.¹¹⁸ The clinical significance of *Achromobacter*, in particular *A. xylosoxidans*, has been debated with some early studies demonstrating no differences in lung function between those colonized and not.^{114,119} More recently,

others have demonstrated pwCF colonized with *A. xylosoxidans* exhibit accelerated decline in pulmonary function^{115,120} with decline in ppFEV₁ at rates similar to colonization with *P. aeruginosa*.¹²¹ While most studies evaluating *Achromobacter* have been done in adults, Sunman et al observed similar findings in children with CF with significantly greater ppFEV₁ decline (–9.07 vs. –1.18/year, $p=0.0043$) and number of PEx (4 vs. 3, $p=0.0001$) in the infected compared to uninfected group, respectively.¹²² Similar to *P. aeruginosa* and Bcc, clonality and potential transmission of *Achromobacter* between pwCF have been suspected.¹¹⁴ Currently, there is no evidence to provide guidance on whether eradication may abrogate chronic infection and no standard eradication protocols exist. The role of additional inhaled antibiotics in systemic therapy in *Achromobacter* eradication is not definitively established, although one study showed 56% of patients who received inhalation therapy (ceftazidime, colistin, or tobramycin) remained colonization-free after 3 years compared to 13% of patients without treatment.¹²³ Early studies evaluating cefiderocol, a newer generation parenteral siderophore-linked cephalosporin,¹²⁴ have shown some promise toward eradication in small cohorts^{125,126} but long-term efficacy and dosage in pwCF remain unknown.

Other Cystic Fibrosis Pathogens

While the classical pathogens detailed above and reported in national data registries remain the most common among pwCF (–Fig. 2), a range of organisms previously rarely observed (or recognized) have increasingly been reported (i.e., *Chryseobacterium*, *Inquilinus*, *Pandoraea*, and *Ralstonia* species).¹⁵ Given the rarity of these species in clinical medicine, they have garnered considerable attention. In contrast, members of the *Enterobacteriales* (i.e., *Escherichia coli* and *Serratia marcescens*) are commonly found in single-center studies but comprehensive efforts to understand their prevalence in large datasets remain lacking.¹²⁷ Hector et al found changing epidemiology patterns of classic pathogens over the last decade across Europe including decreases in *P. aeruginosa* and Bcc with subsequent increases in NTM, *S. aureus* and *S. maltophilia*.¹²⁸ Changes in prevalence are multifactorial and likely reflect improvements in both clinical care and infection control practice patterns.^{105,129} Taken together, whether these truly represent “emerging pathogens” only time and development of longitudinal studies will tell.

Culture-Independent Techniques and the Evolving Landscape of Cystic Fibrosis Airway Infections

The adoption of culture-independent molecular analysis over the last two decades has demonstrated complex microbial communities within the airways of pwCF well beyond those recovered through traditional aerobic culture using a limited number of semiselective media.¹⁵ This relative “shift” in the understanding of infection in CF, from single host-pathogen relationship to complex polymicrobial

community complete with interspecies competition and differential host response, has moved to the forefront of CF microbiology. Moreover, understanding the nuance between colonization and infection is crucial as historically this distinction was made based on the ability to provoke an inflammatory host response, and new data demonstrate the complexity of indirect pathogenesis and potential for antagonism. Pioneering work by Rogers et al¹³⁰ and Harris et al¹³¹ first recognized complex communities in CF-derived sputum far beyond that identified through traditional aerobic culture. Following these studies, a wide range of investigators using next-generation sequencing have worked to expand our understanding of the CF airways. A relative “core community” (present in high abundance across a large proportion of patients) including *Streptococcus*, *Prevotella*, *Veillonella*, *Neisseria*, and *Porphyromonas*, has been detected in the majority of adult studies in addition to the classic pathogens of *Pseudomonas* and *Staphylococcus*.¹³² However, the breadth of diversity is much greater. Culture-independent methods have identified 50 to 200 unique operational taxonomic units in individual CF samples (with the majority representing satellite organisms—present in low abundance and/or in a minority of individuals). Accordingly, it is now widely accepted that the CF lung is home to diverse bacterial, fungal, and viral taxa and that these polymicrobial communities are highly individuated to each patient^{17,133–142} such that the same community makeup persist even in pwCF postlung transplantation.¹⁴³

Not surprisingly, the microbial community in advanced CF disease is particularly skewed with a small number of dominant pathogens that clonally expand to occupy a sizable proportion of the surrounding niche.^{144–146} In contrast, diversity, a measure of species richness and evenness of a community, is often maintained in patients with stable respiratory function.¹⁷ Multiple studies have established that a pattern of decreasing microbial diversity is associated with subsequent deterioration in lung function over time^{147–153} counteracted with relative increases in dominant taxa by traditional CF pathogens.^{17,134,154,155} For instance, as *P. aeruginosa* colonization becomes chronic in late adolescence and early adulthood, community richness and diversity are lost and this associates with disease progression.^{17,156} Finally, as in any ecosystem, microbes found in the CF lung are linked by dynamic and complex webs of interactions with dysbiosis allowing new organisms to proliferate and existing populations to expand.¹⁵⁷

The CF lung represents a unique ecological niche where mucosal hyperviscosity and ongoing bacterial proliferation further propagate the development of a heterogenous oxygen gradient,¹⁵⁸ in part due to diffuse areas of bronchiectasis and mucus plugging.^{159,160} Consequently, anaerobic bacteria are now recognized as part of the core CF microbiota including *Prevotella*, *Veillonella*, *Fusobacterium*, *Peptostreptococcus*, and *Porphyromonas*.^{161–163} Furthermore, many CF pathogens may also function as facultative anaerobes given hypoxic regions within the airways. While ample studies have clearly established that these organisms can colonize the airways of patients at cell densities comparable to classical CF patho-

gens,^{161,164–166} if and how they might contribute to disease progression remains controversial. A large cross-sectional cohort analysis by Zemanick et al identified subjects with a high proportion of anaerobes within sputum experienced reduced inflammation and improved lung function compared to those subjects with *P. aeruginosa*.¹⁶⁷ Using extended bacterial culture methods to assess sputum and bronchoalveolar lavage specimens from a large cohort of pwCF, Muhlebach et al sought to better delineate this relationship. Ultimately, both the presence and relative abundance of anaerobes were associated with milder disease, including improved lung function.¹⁶² While beneficial roles of anaerobes have been postulated, negative associations have more frequently been reported with several studies demonstrating increased abundance correlating to PEx.^{147–150,168,169}

The lower airways should be considered from a polymicrobial perspective with microbe–microbe and microbe–host interactions. Several animals and *in vitro* studies have demonstrated microbial interactions contributing to a greater degree and persistence of infection. For example, the increased virulence potential of *P. aeruginosa* in response to the presence of previously considered benign commensal microbiota is partially mediated by the general bacterial signaling molecules AI-2^{170,171} and 2,3-butanediol.¹⁷² Microbial metabolites produced locally in the airways or from the gut, such as small chain fatty acids, can affect host responses although there are contradictory data around the beneficial or harmful response.¹⁷³ Although most studies to date have sought interactions that increase the virulence of CF pathogens, it is expected that antagonistic interactions may occur such as *in vitro* data demonstrating that several commensal isolates of *S. mitis* and *S. oralis* derived from patient sputum can reduce epithelial cell proinflammatory responses to *P. aeruginosa*.¹⁷⁴ Recently, *Rothia mucilaginosa*, commonly found in the oropharyngeal and lower respiratory tract in CF, was found to have an inhibitory effect on pathogen (i.e., *P. aeruginosa*, and *S. aureus*) induced pro-inflammatory responses, both *in vitro* and *in vivo*.¹⁷⁵

How Cystic Fibrosis Transmembrane Conductance Regulator Modulators Can Affect Infections: Real-World Evidence

The successful introduction of HEMT to a large fraction of the CF population (estimated >90% in some countries)¹⁷⁶ has been a pivotal milestone in the fight to control and mitigate CF. However, as airway infections persist (as described below), a great many questions have arisen. While still in its infancy, several real-world studies provide some early insight into these issues (→ Fig. 4).

Ivacaftor, targeting the Gly551Asp gating mutation to increase channel time, was the first available HEMT released in 2012 but available only to a fraction of pwCF.⁶⁵ Several outcome measures including ppFEV₁, body mass index, and quality of life showed remarkable improvement in clinical trials for both pediatric (STRIVE) and adult (ENVISION) cohorts. Given these changes, how do CF pathogens and microbial communities differ following therapy? Heltsh

Modulator Therapy and the Cystic Fibrosis Airway

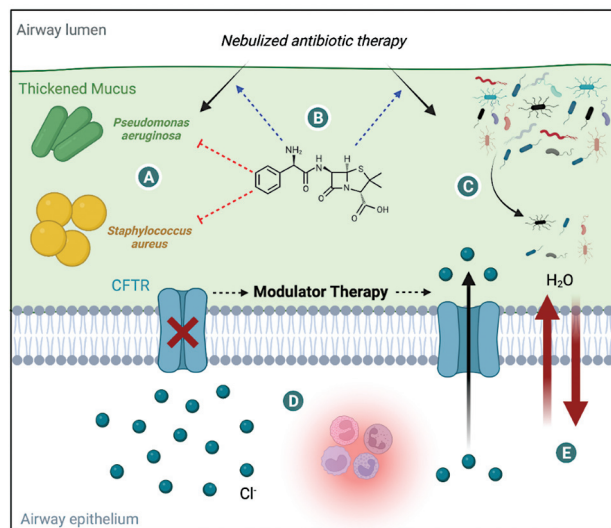


Fig. 4 Mechanisms by which HEMT is proposed to impact microbial communities in the CF airways. (A), Modulators may have direct antimicrobial properties against pathogens, such as killing of *Staphylococcus aureus* and *P. aeruginosa*. (B) CFTR modulators may synergize with traditional antibiotic therapy, such as those delivered by chronic nebulization, to disproportionately kill pathogens. (C) Therapy with CFTR modulators leads to decreased bioburden and relative restructuring of microbiome constituents. (D) Anti-inflammatory properties, whether direct through the modulator therapy itself or indirect through mechanisms described previously reduce host inflammation. (E) Improved mucus hydration and subsequent mucociliary clearance may improve bacterial clearance and therefore reduce bioburden within the airways. Figure created with BioRender.

et al evaluated *P. aeruginosa* persistence by culture in 151 pwCF and found that 29% of those positive in the year prior to ivacaftor initiation were culture negative in the year following, while 88% of those that were *P. aeruginosa* free in the year prior remained negative,¹⁷⁷ confirming earlier observations of *P. aeruginosa* burden reduction after 6 months of therapy.¹⁷⁸ Similarly, a retrospective cohort of 275 pwCF on ivacaftor showed a significant long-term decline in the relative abundance of all classical pathogens, except Bcc.¹⁷⁹ Given most adult pwCF exhibit chronic airway infections, what is the sustainability of microbial changes observed with HEMT? To address this, Hisert et al assessed a cohort of 12 pwCF chronically infected with *P. aeruginosa*, Bcc, and/or *S. aureus* over 2 years of ivacaftor treatment.¹⁸⁰ All subjects exhibited reduced *P. aeruginosa* culture abundance in sputum within the first year, with 10-fold declines in colony forming units (CFUs) seen as early as the first week following therapy. However, CFUs rebounded by 210 days and none of the subjects exhibited persistently negative cultures for *P. aeruginosa* during the study period—indicating eradication did not occur. Moreover, each subject continued to be infected with the same strain of *P. aeruginosa* preivacaftor initiation.

Given the changes observed in cultured pathogens, speculation around whether the microbiome changes have also arisen. Harris et al used 16S rRNA sequencing to measure

total and specific bacterial load from sputum in 31 pwCF pre- and postivacaftor initiation.¹⁸¹ While ppFEV₁ improved over the 6-month study period, there were no significant changes in bacterial load or diversity following treatment. Einarsson et al investigated a cohort of pwCF followed prospectively for 12 months with serial sputum samples pre- and postivacaftor.¹⁸² Extended-culture methodology demonstrated higher densities of obligate anaerobic bacteria, and greater richness and diversity posttreatment; however, no significant difference in bioburden was observed by qPCR for either total bacterial 16S rRNA or *P. aeruginosa*. Culture-independent approaches confirmed significant increases in richness and a trend toward increased diversity ($p = 0.07$) following treatment. Moreover, improvement in lung function, richness and diversity displayed an inverse correlation with inflammatory markers. While this relative shift toward a “healthier” lung microbiome was observed postivacaftor, the authors note overall community composition changes were modest.

Approved in 2019, elexacaftor tezacaftor ivacaftor (ETI) is relevant to many pwCF as it is effective for all those with at least one copy of the F508del *CFTR* mutation (and other specific rare mutations),¹⁸³ accounting for $\geq 90\%$ of individuals.¹⁸⁴ ETI has demonstrated impressive multifaceted clinical benefits including a median 13% absolute improvement in ppFEV₁ and approximately 60% reduction in PEx compared to placebo. Moreover, ETI is superior when compared to other modulators such as tezacaftor/ivacaftor in head-to-head studies.¹⁸⁵ The first microbiome study completed by Sosinski et al found that diversity and evenness increased in 24 pwCF (with ≥ 1 F508del mutation) pre- and post-ETI therapy but with no specific microbial taxa changes apart from the log-ratio of pathogens to anaerobes, indicating modest community restructuring.¹⁸⁶ Consistent with almost all other longitudinal studies, the microbiome structure was more similar within an individual pre- and posttreatment than between subjects after-modulator initiation.^{133,187}

Given the findings described above, questions have been raised around the durability of HEMT-related effects in airway microbiology. To address this, the upcoming PROMISE (NCT04038047), a large U.S. multidisciplinary prospective study assessing the broad impacts of long-term ETI therapy in pwCF ≥ 6 years aims to clarify some of these questions raised by evaluating both culture-dependent and independent measures of pathogen/microbiome-constituent abundance.¹⁸⁸ PROMISE will examine nearly 250 subjects with collections of sputum at 1-, 3-, 6-, 12-, and 24-month time points with the goal to define changes in pathogen density over time by utilizing 16S rRNA gene sequencing and pathogen-targeted quantitative PCR to determine pathogen persistence and individual microbiomes changes over time.

How Does Partial Correction of Cystic Fibrosis Transmembrane Conductance Regulator Dysfunction Impact Cystic Fibrosis Airway Infections?

CFTR modulators have the potential to exert an impact on airway infections through several mechanisms (\rightarrow Fig. 4).¹⁸⁹

Table 2 Common airway sampling modalities for cystic fibrosis bacterial surveillance and their associated features

Method	Definition	Advantages	Disadvantages	Proposed uses
Bronchoalveolar lavage	<ul style="list-style-type: none"> Fluid instilled into and recollected from the lungs during bronchoscopy 	<ul style="list-style-type: none"> Established gold standard method for obtaining lower airway samples High sensitivity and specificity to detect lower airway pathogens 	<ul style="list-style-type: none"> Invasive No proven benefit relative to noninvasive methods Time and resource consuming Difficult to perform longitudinally 	<ul style="list-style-type: none"> Infants and critically ill pwCF unable to expectorate
Expectorated sputum	<ul style="list-style-type: none"> Spontaneous expectoration of sputum 	<ul style="list-style-type: none"> Easily acquired and noninvasive. Beneficial for longitudinal sampling Equivocal efficacy to detect colonization of major pathogens (i.e., <i>P. aeruginosa</i>) when compared to BAL 	<ul style="list-style-type: none"> Pediatric pwCF and those on HEMT may not spontaneously expectorate 	<ul style="list-style-type: none"> Standard of care for those children and adults able to expectorate (i.e., with moderate-to-severe lung disease)
Induced sputum	<ul style="list-style-type: none"> Sputum obtained following inhalation of hypertonic saline 	<ul style="list-style-type: none"> More effective at identifying bacterial pathogens when compared to oropharyngeal sampling (i.e., swabs) Improved pathogen detection in nonexpectorating pwCF 	<ul style="list-style-type: none"> May be associated with bronchospasm Time and resource consuming in clinic Wide sensitivity and specificity 	<ul style="list-style-type: none"> Those unable to naturally expectorate such as those with mild lung disease or receiving HEMT for airway surveillance
Cough	<ul style="list-style-type: none"> Swab placed into the posterior pharynx, during or immediately after patient cough, without direct contact with the oropharyngeal mucosa 	<ul style="list-style-type: none"> Sensitivity may increase following airway clearance to detect pathogens Convenient and easy to collect 	<ul style="list-style-type: none"> Poor concordance between cough swabs and sputum to identify pathogens 	<ul style="list-style-type: none"> Serial sampling for those unable to expectorate such as children and infants
Oropharyngeal	<ul style="list-style-type: none"> Swab of the posterior oropharyngeal wall 	<ul style="list-style-type: none"> In pediatric pwCF, high specificity and NPV for pathogen detection Convenient and easy to collect 	<ul style="list-style-type: none"> Higher prevalence of airway pathogens in adults shifts to lower NPV and higher PPV 	<ul style="list-style-type: none"> Those unable to expectorate such as infants and children, can be an alternative to cough swabs
Nasal	<ul style="list-style-type: none"> Swab of the nasopharyngeal wall 	<ul style="list-style-type: none"> Convenient and easy to collect 	<ul style="list-style-type: none"> Low sensitivity and wide-ranging predictive values in detecting lower airway pathogens 	<ul style="list-style-type: none"> Not routinely recommended

Abbreviations: BAL, bronchoalveolar lavage; HEMT, highly effective cystic fibrosis transmembrane conductance regular modulator therapy; NPV, negative predictive value; PPV, positive predictive value; pwCF, person with cystic fibrosis.

First, improved mucus hydration and subsequent mucociliary clearance may improve bacterial clearance and therefore reduce bioburden within the airways.^{178,180,190} Despite this, structural lung disease including irreversible bronchiectasis will continue to persist—potentially creating a phenotype more in keeping with NCFB. Notably, while infection abounds in NCFB, rates are lower and respiratory deterioration slower.¹⁹¹ Second, growing evidence suggests CFTR modulators may have intrinsic, albeit weak, and antimicrobial properties. Ivacaftor, a G551D potentiator, contains a quinoline ring in its structure of which derivatives often have antibacterial activity through attenuation of DNA replication.¹⁹² In a small study, Schneider et al demonstrated Ivacaftor as a weak inhibitor of DNA gyrase and topoisomerase IV.¹⁹³ Direct inhibition in a dose-dependent fashion by ivacaftor was observed against *S. aureus* and to a lesser extent, *P. aeruginosa*.¹⁹⁴ Modulators may act synergistically with traditional antibiotic therapy. Ivacaftor/lumacaftor, in combination with polymyxin B, was over 100-fold more potent than either one in isolation.¹⁹³ Ivacaftor has also been shown to be synergistic with tobramycin against *S. aureus*¹⁹⁵ and ciprofloxacin against *P. aeruginosa*.¹⁹⁶ Finally, recent preliminary *in vitro* studies evaluating the effects of ETI demonstrated potentiation of neutrophilic antimicrobial mechanisms critical for host anti-inflammatory response, identifying yet another mechanism towards potential mitigation of chronic infection.¹⁹⁷

Airway Infection Surveillance in the Age of Highly Effective Modulator Therapy

Infection surveillance and microbiome analysis in pwCF have traditionally utilized sputum; however, a significant proportion of patients, particularly those with milder disease or of younger age ranges are unable to expectorate sputum spontaneously. With a marked reduction in sputum production from pwCF following HEMT, the need to consider new noninvasive, sputum-independent sampling methods to both diagnose and track CF lung infections is critical (→Table 2). In particular, sputum induction with inhaled hypertonic saline is a promising accessible technique with good bacteriologic correlation by BAL in both children^{198–200} and adults.²⁰¹ Recently, interest in the identification of volatile organic compounds (VOCs) through exhaled breath condensate (EBC) to facilitate rapid, noninvasive, and direct analysis of ventilated lung to diagnose infections and enable microbiota analysis has gained popularity. EBCs are a biological matrix comprised of aerosolized particles from the airway lining and water-soluble volatiles.²⁰² Bacteria produce a broad spectrum of highly specific secondary metabolites including VOCs that can allow rapid and accurate identification.²⁰³ Examination of VOCs released by classic CF pathogens including *P. aeruginosa*^{204,205} and *S. aureus*²⁰⁶ reveals the presence of numerous compounds, with unique metabolic profiles. This was further highlighted in a study of 1,099 VOCs from 105 CF children and healthy controls.²⁰⁷ Moreover, utilizing a panel of 22 VOCs, investigators were able to discern CF subjects from controls with 100% certainty

and were further able to discriminate those with *Pseudomonas* colonization.²⁰⁸ While EBCs are an attractive biomarker target, lack of consistency and standardization raises concerns over the multitude of confounders including varying collection containers, preconcentration methods, and phase of breath-sample obtained.²⁰⁹

Conclusions and Considerations About the Future of Cystic Fibrosis Airway Infections

The use of HEMT has revolutionized the care of many pwCF and is one of the most successful large-scale examples of personalized therapy. While the benefits of HEMT in pwCF have been clearly demonstrated, airway infection persists and infection control measures to prevent patient–patient spread will be required indefinitely. Moreover, the reduced respiratory symptoms associated with HEMT come with the paradoxical effect that attenuates the ability to assess for airway infections easily and longitudinally (from spontaneously expectorated sputum) for the vast majority of individuals, thus limiting our understanding of the natural history of CF infections. Understanding the impact of CFTR modulators on the prevalence and incidence of airway infection is an important and emerging area of research. If effective, early intervention with CFTR modulators from infancy may aid in the conservation of microbial diversity; providing less opportunity for the development of chronic airway infections with classical pathogens,¹³⁸ thereby reducing treatment burden, which could further significantly improve the quality of life of pwCF. While HEMTs are now available to an increasing number of pwCF, they are not yet available for those with rarer mutations and in particular for those with class I mutations. Thus, ongoing attempts to surveil for incident infection with new airway pathogens and controlling chronic airway infections will continue to remain a cornerstone of care for clinicians in the care of pwCF.

Conflict of Interest

None declared.

References

- 1 Shteinberg M, Haq IJ, Polineni D, Davies JC. Cystic fibrosis. *Lancet* 2021;397(10290):2195–2211
- 2 Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 2003;168(08):918–951
- 3 Fuchs HJ, Borowitz DS, Christiansen DH, et al; The Pulmozyme Study Group. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. *N Engl J Med* 1994;331(10):637–642
- 4 Sanders DB, Bittner RC, Rosenfeld M, Hoffman LR, Redding GJ, Goss CH. Failure to recover to baseline pulmonary function after cystic fibrosis pulmonary exacerbation. *Am J Respir Crit Care Med* 2010;182(05):627–632
- 5 Waters V, Stanojevic S, Atenafu EG, et al. Effect of pulmonary exacerbations on long-term lung function decline in cystic fibrosis. *Eur Respir J* 2012;40(01):61–66
- 6 Saiman L. Improving outcomes of infections in cystic fibrosis in the era of CFTR modulator therapy. *Pediatr Pulmonol* 2019;54 (Suppl 3):S18–S26

- 7 Bergeron C, Cantin AM. Cystic fibrosis: pathophysiology of lung disease. *Semin Respir Crit Care Med* 2019;40(06):715–726
- 8 O'Brien S, Fothergill JL. The role of multispecies social interactions in shaping *Pseudomonas aeruginosa* pathogenicity in the cystic fibrosis lung. *FEMS Microbiol Lett* 2017;364(15):fnx128
- 9 Bottery MJ, Matthews JL, Wood AJ, Johansen HK, Pitchford JW, Friman VP. Inter-species interactions alter antibiotic efficacy in bacterial communities. *ISME J* 2022;16(03):812–821
- 10 Sibley CD, Rabin H, Surette MG. Cystic fibrosis: a polymicrobial infectious disease. *Future Microbiol* 2006;1(01):53–61
- 11 Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. *Clin Microbiol Rev* 2002;15(02):194–222
- 12 Smith JJ, Travis SM, Greenberg EP, Welsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. *Cell* 1996;85(02):229–236
- 13 Hoppe JE, Sagel SD. Shifting landscape of airway infection in early cystic fibrosis. *Am J Respir Crit Care Med* 2019;200(05):528–529
- 14 Bevivino A, Bacci G, Drevinek P, Nelson MT, Hoffman L, Mengoni A. Deciphering the ecology of cystic fibrosis bacterial communities: towards systems-level integration. *Trends Mol Med* 2019;25(12):1110–1122
- 15 Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev* 2010;23(02):299–323
- 16 Millar FA, Simmonds NJ, Hodson ME. Trends in pathogens colonising the respiratory tract of adult patients with cystic fibrosis, 1985–2005. *J Cyst Fibros* 2009;8(06):386–391
- 17 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(15):5809–5814
- 18 Hurley MN. *Staphylococcus aureus* in cystic fibrosis: problem bug or an innocent bystander? *Breathe (Sheff)* 2018;14(02):87–90
- 19 Goss CH, Muhlebach MS. Review: *Staphylococcus aureus* and MRSA in cystic fibrosis. *J Cyst Fibros* 2011;10(05):298–306
- 20 Malhotra S, Hayes D Jr, Wozniak DJ. Cystic fibrosis and *Pseudomonas aeruginosa*: the host-microbe interface. *Clin Microbiol Rev* 2019;32(03):e00138-18
- 21 Rossi E, La Rosa R, Bartell JA, et al. *Pseudomonas aeruginosa* adaptation and evolution in patients with cystic fibrosis. *Nat Rev Microbiol* 2021;19(05):331–342
- 22 Scoffone VC, Chiarelli LR, Trespidi G, Mentasti M, Riccardi G, Buroni S. *Burkholderia cenocepacia* infections in cystic fibrosis patients: drug resistance and therapeutic approaches. *Front Microbiol* 2017;8:1592
- 23 Lipuma JJ. Update on the *Burkholderia cepacia* complex. *Curr Opin Pulm Med* 2005;11(06):528–533
- 24 Rumpf C, Lange J, Schwartbeck B, Kahl BC. *Staphylococcus aureus* and cystic fibrosis—a close relationship. What can we learn from sequencing studies? *Pathogens* 2021;10(09):1177
- 25 Akil N, Muhlebach MS. Biology and management of methicillin resistant *Staphylococcus aureus* in cystic fibrosis. *Pediatr Pulmonol* 2018;53(53):S64–S74
- 26 Høiby N. Understanding bacterial biofilms in patients with cystic fibrosis: current and innovative approaches to potential therapies. *J Cyst Fibros* 2002;1(04):249–254
- 27 Waters EM, Rowe SE, O'Gara JP, Conlon BP. Convergence of *Staphylococcus aureus* persister and biofilm research: can biofilms be defined as communities of adherent persister cells? *PLoS Pathog* 2016;12(12):e01006012
- 28 Gangell C, Gard S, Douglas T, et al; AREST CF. Inflammatory responses to individual microorganisms in the lungs of children with cystic fibrosis. *Clin Infect Dis* 2011;53(05):425–432
- 29 Sagel SD, Gibson RL, Emerson J, et al; Inhaled Tobramycin in Young Children Study Group Cystic Fibrosis Foundation Therapeutics Development Network. Impact of *Pseudomonas* and *Staphylococcus* infection on inflammation and clinical status in young children with cystic fibrosis. *J Pediatr* 2009;154(02):183–188
- 30 Kerem E, Viviani L, Zolin A, et al; ECFS Patient Registry Steering Group. Factors associated with FEV1 decline in cystic fibrosis: analysis of the ECFS patient registry. *Eur Respir J* 2014;43(01):125–133
- 31 Zemanick ET, Hoffman LR. Cystic fibrosis: microbiology and host response. *Pediatr Clin North Am* 2016;63(04):617–636
- 32 Stutman HR, Lieberman JM, Nussbaum E, Marks MI. Antibiotic prophylaxis in infants and young children with cystic fibrosis: a randomized controlled trial. *J Pediatr* 2002;140(03):299–305
- 33 Smyth AR, Rosenfeld M. Prophylactic anti-staphylococcal antibiotics for cystic fibrosis. *Cochrane Database Syst Rev* 2017;4:CD001912
- 34 Hurley MN, Fogarty A, McKeever TM, Goss CH, Rosenfeld M, Smyth AR. Early respiratory bacterial detection and antistaphylococcal antibiotic prophylaxis in young children with cystic fibrosis. *Ann Am Thorac Soc* 2018;15(01):42–48
- 35 Westphal C, Görlich D, Kampmeier S, et al; Staphylococcal CF Study Group. Antibiotic treatment and age are associated with *Staphylococcus aureus* carriage profiles during persistence in the airways of cystic fibrosis patients. *Front Microbiol* 2020;11:230
- 36 Chambers HF. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clin Microbiol Rev* 1997;10(04):781–791
- 37 Dasenbrook EC, Merlo CA, Diener-West M, Lechtzin N, Boyle MP. Persistent methicillin-resistant *Staphylococcus aureus* and rate of FEV1 decline in cystic fibrosis. *Am J Respir Crit Care Med* 2008;178(08):814–821
- 38 Esposito S, Pennoni G, Mencarini V, Palladino N, Peccini L, Principi N. Antimicrobial treatment of *Staphylococcus aureus* in patients with cystic fibrosis. *Front Pharmacol* 2019;10:849
- 39 Dasenbrook EC. Update on methicillin-resistant *Staphylococcus aureus* in cystic fibrosis. *Curr Opin Pulm Med* 2011;17(06):437–441
- 40 Wolter DJ, Onchiri FM, Emerson J, et al; SCVSA study group. Prevalence and clinical associations of *Staphylococcus aureus* small-colony variant respiratory infection in children with cystic fibrosis (SCVSA): a multicentre, observational study. *Lancet Respir Med* 2019;7(12):1027–1038
- 41 Yagci S, Hascelik G, Dogru D, Ozcelik U, Sener B. Prevalence and genetic diversity of *Staphylococcus aureus* small-colony variants in cystic fibrosis patients. *Clin Microbiol Infect* 2013;19(01):77–84
- 42 Besier S, Smaczny C, von Mallinckrodt C, et al. Prevalence and clinical significance of *Staphylococcus aureus* small-colony variants in cystic fibrosis lung disease. *J Clin Microbiol* 2007;45(01):168–172
- 43 Besier S, Ludwig A, Ohlsen K, Brade V, Wichelhaus TA. Molecular analysis of the thymidine-auxotrophic small colony variant phenotype of *Staphylococcus aureus*. *Int J Med Microbiol* 2007;297(04):217–225
- 44 Schneider M, Mühlemann K, Droz S, Couzinet S, Casaulta C, Zimmerli S. Clinical characteristics associated with isolation of small-colony variants of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from respiratory secretions of patients with cystic fibrosis. *J Clin Microbiol* 2008;46(05):1832–1834
- 45 Wolter DJ, Emerson JC, McNamara S, et al. *Staphylococcus aureus* small-colony variants are independently associated with worse lung disease in children with cystic fibrosis. *Clin Infect Dis* 2013;57(03):384–391
- 46 Junge S, Görlich D, den Reijer M, et al. Factors associated with worse lung function in cystic fibrosis patients with persistent *Staphylococcus aureus*. *PLoS One* 2016;11(11):e0166220
- 47 Ratjen F, Comes G, Paul K, Posselt HG, Wagner TO, Harms KGerman Board of the European Registry for Cystic Fibrosis (ERCF) Effect of continuous antistaphylococcal therapy on the rate of *P. aeruginosa* acquisition in patients with cystic fibrosis. *Pediatr Pulmonol* 2001;31(01):13–16
- 48 Mogayzel PJ Jr, Naureckas ET, Robinson KA, et al; Pulmonary Clinical Practice Guidelines Committee. Cystic fibrosis

- pulmonary guidelines. Chronic medications for maintenance of lung health. *Am J Respir Crit Care Med* 2013;187(07):680–689
- 49 Muhlebach MS, Beckett V, Popowitch E, et al; STAR-too study team. Microbiological efficacy of early MRSA treatment in cystic fibrosis in a randomised controlled trial. *Thorax* 2017;72(04):318–326
 - 50 Neri S, Campana S, Dolce D, et al. Early antibiotic treatment for MRSA eradication in cystic fibrosis patients: a multicenter RCT. *Pediatr Pulmonol* 2016;51:309
 - 51 Jennings MT, Boyle MP, Weaver D, Callahan KA, Dasenbrook EC. Eradication strategy for persistent methicillin-resistant *Staphylococcus aureus* infection in individuals with cystic fibrosis—the PMEP trial: study protocol for a randomized controlled trial. *Trials* 2014;15:223
 - 52 Dezube R, Jennings MT, Rykiel M, et al. Eradication of persistent methicillin-resistant *Staphylococcus aureus* infection in cystic fibrosis. *J Cyst Fibros* 2019;18(03):357–363
 - 53 Loeb MB, Main C, Eady A, Walker-Dilks C. Antimicrobial drugs for treating methicillin-resistant *Staphylococcus aureus* colonization. *Cochrane Database Syst Rev* 2003;(04):CD003340
 - 54 Dolce D, Neri S, Grisotto L, et al. Methicillin-resistant *Staphylococcus aureus* eradication in cystic fibrosis patients: a randomized multicenter study. *PLoS One* 2019;14(03):e0213497
 - 55 Miall LS, McGinley NT, Brownlee KG, Conway SP. Methicillin resistant *Staphylococcus aureus* (MRSA) infection in cystic fibrosis. *Arch Dis Child* 2001;84(02):160–162
 - 56 Rosenfeld M, Gibson RL, McNamara S, et al. Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis. *Pediatr Pulmonol* 2001;32(05):356–366
 - 57 Saliu F, Rizzo G, Bragonzi A, et al. Chronic infection by nontypeable *Haemophilus influenzae* fuels airway inflammation. *ERJ Open Res* 2021;7(01):00614–2020
 - 58 Cystic Fibrosis Foundation. Cystic Fibrosis Foundation Patient Registry 2019 Annual Data Report. Bethesda, MD: Cystic Fibrosis Foundation; 2020
 - 59 Allen JL, Panitch H, Rubenstein R. Cystic Fibrosis. *Lung Biology in Health and Diseases*. Vol. 242. New York, NY: Informa Healthcare; 2010:242
 - 60 Murphy TF, Bakaletz LO, Smeesters PR. Microbial interactions in the respiratory tract. *Pediatr Infect Dis J* 2009;28(10, suppl):S121–S126
 - 61 Román F, Cantón R, Pérez-Vázquez M, Baquero F, Campos J. Dynamics of long-term colonization of respiratory tract by *Haemophilus influenzae* in cystic fibrosis patients shows a marked increase in hypermutable strains. *J Clin Microbiol* 2004;42(04):1450–1459
 - 62 Watson ME Jr., Burns JL, Smith AL. Hypermutable *Haemophilus influenzae* with mutations in *mutS* are found in cystic fibrosis sputum. *Microbiology (Reading)* 2004;150(pt. 9):2947–2958
 - 63 Ebbing R, Robertson CF, Robinson PJ. *Haemophilus influenzae* and *Haemophilus parainfluenza* in cystic fibrosis: 15 years experience. *J Med Microbiol Diagn* 2015;S5(004): doi:10.4172/2161-0703.S5-004
 - 64 Frayman KB, Armstrong DS, Carzino R, et al. The lower airway microbiota in early cystic fibrosis lung disease: a longitudinal analysis. *Thorax* 2017;72(12):1104–1112
 - 65 Manos J. Current and emerging therapies to combat cystic fibrosis lung infections. *Microorganisms* 2021;9(09):1874
 - 66 Parkins MD, Somayaji R, Waters VJ. Epidemiology, biology, and impact of clonal *Pseudomonas aeruginosa* infections in cystic fibrosis. *Clin Microbiol Rev* 2018;31(04):e00019-18
 - 67 Cheng K, Smyth RL, Govan JR, et al. Spread of beta-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. *Lancet* 1996;348(9028):639–642
 - 68 Davies JC. *Pseudomonas aeruginosa* in cystic fibrosis: pathogenesis and persistence. *Paediatr Respir Rev* 2002;3(02):128–134
 - 69 Pritt B, O'Brien L, Winn W. Mucoid *Pseudomonas* in cystic fibrosis. *Am J Clin Pathol* 2007;128(01):32–34
 - 70 Folkesson A, Jelsbak L, Yang L, et al. Adaptation of *Pseudomonas aeruginosa* to the cystic fibrosis airway: an evolutionary perspective. *Nat Rev Microbiol* 2012;10(12):841–851
 - 71 Rosenfeld M, Faino AV, Onchiri F, et al. Comparing encounter-based and annualized chronic *Pseudomonas* infection definitions in cystic fibrosis. *J Cyst Fibros* 2022;21(01):40–44
 - 72 Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients. *J Cyst Fibros* 2003;2(01):29–34
 - 73 Kerem E, Corey M, Gold R, Levison H. Pulmonary function and clinical course in patients with cystic fibrosis after pulmonary colonization with *Pseudomonas aeruginosa*. *J Pediatr* 1990;116(05):714–719
 - 74 Konstan MW, Morgan WJ, Butler SM, et al; Scientific Advisory Group and the Investigators and Coordinators of the Epidemiologic Study of Cystic Fibrosis. Risk factors for rate of decline in forced expiratory volume in one second in children and adolescents with cystic fibrosis. *J Pediatr* 2007;151(02):134–139, 139.e1
 - 75 Navarro J, Rainisio M, Harms HK, et al; European Epidemiologic Registry of Cystic Fibrosis. Factors associated with poor pulmonary function: cross-sectional analysis of data from the ERCF. *Eur Respir J* 2001;18(02):298–305
 - 76 Kosorok MR, Zeng L, West SE, et al. Acceleration of lung disease in children with cystic fibrosis after *Pseudomonas aeruginosa* acquisition. *Pediatr Pulmonol* 2001;32(04):277–287
 - 77 Nixon GM, Armstrong DS, Carzino R, et al. Clinical outcome after early *Pseudomonas aeruginosa* infection in cystic fibrosis. *J Pediatr* 2001;138(05):699–704
 - 78 Sawicki GS, Rasouliyan L, McMullen AH, et al. Longitudinal assessment of health-related quality of life in an observational cohort of patients with cystic fibrosis. *Pediatr Pulmonol* 2011;46(01):36–44
 - 79 West SE, Zeng L, Lee BL, et al. Respiratory infections with *Pseudomonas aeruginosa* in children with cystic fibrosis: early detection by serology and assessment of risk factors. *JAMA* 2002;287(22):2958–2967
 - 80 Pamukcu A, Bush A, Buchdahl R. Effects of *Pseudomonas aeruginosa* colonization on lung function and anthropometric variables in children with cystic fibrosis. *Pediatr Pulmonol* 1995;19(01):10–15
 - 81 Courtney JM, Bradley J, Mccaughan J, et al. Predictors of mortality in adults with cystic fibrosis. *Pediatr Pulmonol* 2007;42(06):525–532
 - 82 Demko CA, Byard PJ, Davis PB. Gender differences in cystic fibrosis: *Pseudomonas aeruginosa* infection. *J Clin Epidemiol* 1995;48(08):1041–1049
 - 83 Hudson VL, Wielinski CL, Regelman WE. Prognostic implications of initial oropharyngeal bacterial flora in patients with cystic fibrosis diagnosed before the age of two years. *J Pediatr* 1993;122(06):854–860
 - 84 Somayaji R, Lam JC, Surette MG, et al. Long-term clinical outcomes of 'Prairie Epidemic Strain' *Pseudomonas aeruginosa* infection in adults with cystic fibrosis. *Thorax* 2017;72(04):333–339
 - 85 Saiman L, Siegel J. Infection control in cystic fibrosis. *Clin Microbiol Rev* 2004;17(01):57–71
 - 86 Jones AM. Eradication therapy for early *Pseudomonas aeruginosa* infection in CF: many questions still unanswered. *Eur Respir J* 2005;26(03):373–375
 - 87 Ratjen F, Munck A, Kho P, Angyalosi GELITE Study Group. Treatment of early *Pseudomonas aeruginosa* infection in patients with cystic fibrosis: the ELITE trial. *Thorax* 2010;65(04):286–291
 - 88 Treggiari MM, Retsch-Bogart G, Mayer-Hamblett N, et al; Early *Pseudomonas* Infection Control (EPIC) Investigators. Comparative efficacy and safety of 4 randomized regimens to treat

- early *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *Arch Pediatr Adolesc Med* 2011;165(09):847–856
- 89 Tiddens HA, De Boeck K, Clancy JP, et al; ALPINE study investigators. Open label study of inhaled aztreonam for *Pseudomonas* eradication in children with cystic fibrosis: the ALPINE study. *J Cyst Fibros* 2015;14(01):111–119
 - 90 Mogayzel PJ Jr., Naureckas ET, Robinson KA, et al; Cystic Fibrosis Foundation Pulmonary Clinical Practice Guidelines Committee. Cystic Fibrosis Foundation pulmonary guideline. pharmacologic approaches to prevention and eradication of initial *Pseudomonas aeruginosa* infection. *Ann Am Thorac Soc* 2014;11(10):1640–1650
 - 91 Langton Hewer SC, Smyth AR, Brown M, et al. Intravenous or oral antibiotic treatment in adults and children with cystic fibrosis and *Pseudomonas aeruginosa* infection: the TORPEDO-CF RCT. *Health Technol Assess* 2021;25(65):1–128
 - 92 Green HD, Jones AM. Managing pulmonary infection in adults with cystic fibrosis: adult cystic fibrosis series. *Chest* 2022;162(01):66–75
 - 93 Blanchard AC, Horton E, Stanojevic S, Taylor L, Waters V, Ratjen F. Effectiveness of a stepwise *Pseudomonas aeruginosa* eradication protocol in children with cystic fibrosis. *J Cyst Fibros* 2017;16(03):395–400
 - 94 Mayer-Hamblett N, Kloster M, Rosenfeld M, et al. Impact of sustained eradication of new *Pseudomonas aeruginosa* infection on long-term outcomes in cystic fibrosis. *Clin Infect Dis* 2015;61(05):707–715
 - 95 Somayaji R, Parkins MD, Shah A, et al; Antimicrobial Resistance in Cystic Fibrosis International Working Group. Antimicrobial susceptibility testing (AST) and associated clinical outcomes in individuals with cystic fibrosis: a systematic review. *J Cyst Fibros* 2019;18(02):236–243
 - 96 Castellani C, Duff AJA, Bell SC, et al. ECFS best practice guidelines: the 2018 revision. *J Cyst Fibros* 2018;17(02):153–178
 - 97 Flume PA, Mogayzel PJ Jr., Robinson KA, et al; Clinical Practice Guidelines for Pulmonary Therapies Committee. Cystic fibrosis pulmonary guidelines: treatment of pulmonary exacerbations. *Am J Respir Crit Care Med* 2009;180(09):802–808
 - 98 Flume PA, O'Sullivan BP, Robinson KA, et al; Cystic Fibrosis Foundation, Pulmonary Therapies Committee. Cystic fibrosis pulmonary guidelines: chronic medications for maintenance of lung health. *Am J Respir Crit Care Med* 2007;176(10):957–969
 - 99 Zlosnik JEA, Henry DA, Hird TJ, et al. Epidemiology of *Burkholderia* infections in people with cystic fibrosis in Canada between 2000 and 2017. *Ann Am Thorac Soc* 2020;17(12):1549–1557
 - 100 Zlosnik JE, Zhou G, Brant R, et al. *Burkholderia* species infections in patients with cystic fibrosis in British Columbia, Canada. 30 years' experience. *Ann Am Thorac Soc* 2015;12(01):70–78
 - 101 Canada CF. Canadian Cystic Fibrosis Patient Data Registry Report. 2009:2–8
 - 102 Govan JR, Brown PH, Maddison J, et al. Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. *Lancet* 1993;342(8862):15–19
 - 103 LiPuma JJ, Dasen SE, Nielson DW, Stern RC, Stull TL. Person-to-person transmission of *Pseudomonas cepacia* between patients with cystic fibrosis. *Lancet* 1990;336(8723):1094–1096[pii]
 - 104 LiPuma JJ, Mortensen JE, Dasen SE, et al. Ribotype analysis of *Pseudomonas cepacia* from cystic fibrosis treatment centers. *J Pediatr* 1988;113(05):859–862
 - 105 Crull MR, Somayaji R, Ramos KJ, et al. Changing rates of chronic *Pseudomonas aeruginosa* infections in cystic fibrosis: a population-based cohort study. *Clin Infect Dis* 2018;67(07):1089–1095
 - 106 Gilligan PH. Infections in patients with cystic fibrosis: diagnostic microbiology update. *Clin Lab Med* 2014;34(02):197–217
 - 107 Amin R, Jahnke N, Waters V. Antibiotic treatment for *Stenotrophomonas maltophilia* in people with cystic fibrosis. *Cochrane Database Syst Rev* 2020;3:CD009249
 - 108 Goss CH, Mayer-Hamblett N, Aitken ML, Rubenfeld GD, Ramsey BW. Association between *Stenotrophomonas maltophilia* and lung function in cystic fibrosis. *Thorax* 2004;59(11):955–959
 - 109 Berdah L, Taytard J, Leyronnas S, Clement A, Boelle PY, Corvol H. *Stenotrophomonas maltophilia*: a marker of lung disease severity. *Pediatr Pulmonol* 2018;53(04):426–430
 - 110 Goss CH, Otto K, Aitken ML, Rubenfeld GD. Detecting *Stenotrophomonas maltophilia* does not reduce survival of patients with cystic fibrosis. *Am J Respir Crit Care Med* 2002;166(03):356–361
 - 111 Waters V, Yau Y, Prasad S, et al. *Stenotrophomonas maltophilia* in cystic fibrosis: serologic response and effect on lung disease. *Am J Respir Crit Care Med* 2011;183(05):635–640
 - 112 Waters V, Atenafu EG, Lu A, Yau Y, Tullis E, Ratjen F. Chronic *Stenotrophomonas maltophilia* infection and mortality or lung transplantation in cystic fibrosis patients. *J Cyst Fibros* 2013;12(05):482–486
 - 113 Barsky EE, Williams KA, Priebe GP, Sawicki GS. Incident *Stenotrophomonas maltophilia* infection and lung function decline in cystic fibrosis. *Pediatr Pulmonol* 2017;52(10):1276–1282
 - 114 Lambiase A, Catania MR, Del Pezzo M, et al. *Achromobacter xylosoxidans* respiratory tract infection in cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis* 2011;30(08):973–980
 - 115 Tetart M, Walle F, Kyheng M, et al. Impact of *Achromobacter xylosoxidans* isolation on the respiratory function of adult patients with cystic fibrosis. *ERJ Open Res* 2019;5(04):00051–2019
 - 116 Parkins MD, Floto RA. Emerging bacterial pathogens and changing concepts of bacterial pathogenesis in cystic fibrosis. *J Cyst Fibros* 2015;14(03):293–304
 - 117 Edwards BD, Greysson-Wong J, Somayaji R, et al. Prevalence and outcomes of *Achromobacter* species infections in adults with cystic fibrosis: a North American cohort study. *J Clin Microbiol* 2017;55(07):2074–2085
 - 118 Cabak A, Hovold G, Petersson AC, Ramstedt M, Pählman LI. Activity of airway antimicrobial peptides against cystic fibrosis pathogens. *Pathog Dis* 2020;78(07):ftaa048
 - 119 De Baets F, Schelstraete P, Van Daele S, Haerynck F, Vaneechoutte M. *Achromobacter xylosoxidans* in cystic fibrosis: prevalence and clinical relevance. *J Cyst Fibros* 2007;6(01):75–78
 - 120 Recio R, Brañas P, Martínez MT, Chaves F, Orellana MA. Effect of respiratory *Achromobacter* spp. infection on pulmonary function in patients with cystic fibrosis. *J Med Microbiol* 2018;67(07):952–956
 - 121 Hansen CR, Pressler T, Nielsen KG, Jensen PO, Bjarnsholt T, Høiby N. Inflammation in *Achromobacter xylosoxidans* infected cystic fibrosis patients. *J Cyst Fibros* 2010;9(01):51–58
 - 122 Sunman B, Emiralioglu N, Hazirolan G, et al. Impact of *Achromobacter* spp. isolation on clinical outcomes in children with cystic fibrosis. *Pediatr Pulmonol* 2022;57(03):658–666
 - 123 Wang M, Ridderberg W, Hansen CR, et al. Early treatment with inhaled antibiotics postpones next occurrence of *Achromobacter* in cystic fibrosis. *J Cyst Fibros* 2013;12(06):638–643
 - 124 Zhanel GG, Golden AR, Zelenitsky S, et al. Cefiderocol: a siderophore cephalosporin with activity against carbapenem-resistant and multidrug-resistant gram-negative bacilli. *Drugs* 2019;79(03):271–289
 - 125 Warner NC, Bartelt LA, Lachiewicz AM, et al. Cefiderocol for the treatment of adult and pediatric patients with cystic fibrosis and *Achromobacter xylosoxidans* infections. *Clin Infect Dis* 2021;73(07):e1754–e1757
 - 126 Gainey AB, Burch AK, Brownstein MJ, et al. Combining bacteriophages with cefiderocol and meropenem/vaborbactam to treat a pan-drug resistant *Achromobacter species* infection in a pediatric cystic fibrosis patient. *Pediatr Pulmonol* 2020;55(11):2990–2994
 - 127 Edwards BD, Somayaji R, Greysson-Wong J, et al. Clinical outcomes associated with *Escherichia coli* infections in adults with cystic fibrosis: a cohort study. *Open Forum Infect Dis* 2019;7(01):ofz476

- 128 Hector A, Kirn T, Ralhan A, et al. Microbial colonization and lung function in adolescents with cystic fibrosis. *J Cyst Fibros* 2016;15(03):340–349
- 129 Hatziaiorou E, Orenti A, Drevinek P, Kashirskaya N, Mei-Zahav M, De Boeck KEFSPR. Electronic address: ECFS-Patient.Registry@uz.kuleuven.ac.be ECFSPR. Changing epidemiology of the respiratory bacteriology of patients with cystic fibrosis—data from the European cystic fibrosis society patient registry. *J Cyst Fibros* 2020;19(03):376–383
- 130 Rogers GB, Carroll MP, Serisier DJ, Hockey PM, Jones G, Bruce KD. Characterization of bacterial community diversity in cystic fibrosis lung infections by use of 16s ribosomal DNA terminal restriction fragment length polymorphism profiling. *J Clin Microbiol* 2004;42(11):5176–5183
- 131 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(51):20529–20533
- 132 van der Gast CJ, Walker AW, Stressmann FA, et al. Partitioning core and satellite taxa from within cystic fibrosis lung bacterial communities. *ISME J* 2011;5(05):780–791
- 133 Heirali A, Thornton C, Acosta N, et al. Sputum microbiota in adults with CF associates with response to inhaled tobramycin. *Thorax* 2020;75(12):1058–1064
- 134 Zemanick ET, Wagner BD, Robertson CE, et al. Airway microbiota across age and disease spectrum in cystic fibrosis. *Eur Respir J* 2017;50(05):1700832
- 135 Price KE, Hampton TH, Gifford AH, et al. Unique microbial communities persist in individual cystic fibrosis patients throughout a clinical exacerbation. *Microbiome* 2013;1(01):27
- 136 Acosta N, Whelan FJ, Somayaji R, et al. The evolving cystic fibrosis microbiome: a comparative cohort study spanning 16 years. *Ann Am Thorac Soc* 2017;14(08):1288–1297
- 137 Hahn A, Whiteson K, Davis TJ, et al. Longitudinal associations of the cystic fibrosis airway microbiome and volatile metabolites: a case study. *Front Cell Infect Microbiol* 2020;10:174
- 138 Whelan FJ, Heirali AA, Rossi L, Rabin HR, Parkins MD, Surette MG. Longitudinal sampling of the lung microbiota in individuals with cystic fibrosis. *PLoS One* 2017;12(03):e0172811
- 139 Raghuvanshi R, Vasco K, Vázquez-Baeza Y, et al. High-resolution longitudinal dynamics of the cystic fibrosis sputum microbiome and metabolome through antibiotic therapy. *mSystems* 2020;5(03):e00292-20
- 140 Caverly LJ, Lu J, Carmody LA, et al. Measures of cystic fibrosis airway microbiota during periods of clinical stability. *Ann Am Thorac Soc* 2019;16(12):1534–1542
- 141 Acosta N, Thornton CS, Surette MG, et al. Azithromycin and the microbiota of cystic fibrosis sputum. *BMC Microbiol* 2021;21(01):96
- 142 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
- 143 Syed SA, Whelan FJ, Waddell B, Rabin HR, Parkins MD, Surette MG. Reemergence of lower-airway microbiota in lung transplant patients with cystic fibrosis. *Ann Am Thorac Soc* 2016;13(12):2132–2142
- 144 Feigelman R, Kahlert CR, Baty F, et al. Sputum DNA sequencing in cystic fibrosis: non-invasive access to the lung microbiome and to pathogen details. *Microbiome* 2017;5(01):20
- 145 Coutinho CP, Dos Santos SC, Madeira A, Mira NP, Moreira AS, Sá-Correia I. Long-term colonization of the cystic fibrosis lung by *Burkholderia cepacia* complex bacteria: epidemiology, clonal variation, and genome-wide expression alterations. *Front Cell Infect Microbiol* 2011;1:12
- 146 Cramer N, Wiehlmann L, Tümmler B. Clonal epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis. *Int J Med Microbiol* 2010;300(08):526–533
- 147 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(03):e0194060
- 148 Carmody LA, Zhao J, Schloss PD, et al. Changes in cystic fibrosis airway microbiota at pulmonary exacerbation. *Ann Am Thorac Soc* 2013;10(03):179–187
- 149 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(04):1052
- 150 Layeghifard M, Li H, Wang PW, et al. Microbiome networks and change-point analysis reveal key community changes associated with cystic fibrosis pulmonary exacerbations. *NPJ Biofilms Microbiomes* 2019;5(01):4
- 151 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(12):e82432
- 152 Smith DJ, Badrick AC, Zakrzewski M, et al. Pyrosequencing reveals transient cystic fibrosis lung microbiome changes with intravenous antibiotics. *Eur Respir J* 2014;44(04):922–930
- 153 Acosta N, Heirali A, Somayaji R, et al. Sputum microbiota is predictive of long-term clinical outcomes in young adults with cystic fibrosis. *Thorax* 2018;73(11):1016–1025
- 154 Coburn B, Wang PW, Caballero JD, et al. Lung microbiota across age and disease stage in cystic fibrosis. *Sci Rep* 2015;5:10241
- 155 Cuthbertson L, Walker AW, Oliver AE, et al. Lung function and microbiota diversity in cystic fibrosis. *Microbiome* 2020;8(01):45
- 156 Cuthbertson L, Rogers GB, Walker AW, et al. Respiratory microbiota resistance and resilience to pulmonary exacerbation and subsequent antimicrobial intervention. *ISME J* 2016;10(05):1081–1091
- 157 Gannon AD, Darch SE. Same game, different players: emerging pathogens of the CF lung. *MBio* 2021;12(01):e01217-20
- 158 Cowley ES, Kopf SH, LaRiviere A, Ziebis W, Newman DK. Pediatric cystic fibrosis sputum can be chemically dynamic, anoxic, and extremely reduced due to hydrogen sulfide formation. *MBio* 2015;6(04):e00767
- 159 Worlitzsch D, Tarran R, Ulrich M, et al. Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J Clin Invest* 2002;109(03):317–325
- 160 Yoon SS, Hennigan RF, Hilliard GM, et al. *Pseudomonas aeruginosa* anaerobic respiration in biofilms: relationships to cystic fibrosis pathogenesis. *Dev Cell* 2002;3(04):593–603
- 161 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(09):995–1001
- 162 Muhlebach MS, Hatch JE, Einarsson GG, et al. Anaerobic bacteria cultured from cystic fibrosis airways correlate to milder disease: a multisite study. *Eur Respir J* 2018;52(01):1800242
- 163 Worlitzsch D, Rintelen C, Böhm K, et al. Antibiotic-resistant obligate anaerobes during exacerbations of cystic fibrosis patients. *Clin Microbiol Infect* 2009;15(05):454–460
- 164 Bittar F, Richet H, Dubus JC, et al. Molecular detection of multiple emerging pathogens in sputa from cystic fibrosis patients. *PLoS One* 2008;3(08):e2908
- 165 Field TR, Sibley CD, Parkins MD, Rabin HR, Surette MG. The genus *Prevotella* in cystic fibrosis airways. *Anaerobe* 2010;16(04):337–344
- 166 Sibley CD, Grinwis ME, Field TR, et al. Culture enriched molecular profiling of the cystic fibrosis airway microbiome. *PLoS One* 2011;6(07):e22702
- 167 Zemanick ET, Harris JK, Wagner BD, et al. Inflammation and airway microbiota during cystic fibrosis pulmonary exacerbations. *PLoS One* 2013;8(04):e62917
- 168 Sibley CD, Parkins MD, Rabin HR, Duan K, Norgaard JC, Surette MG. A polymicrobial perspective of pulmonary infections

- exposes an enigmatic pathogen in cystic fibrosis patients. *Proc Natl Acad Sci U S A* 2008;105(39):15070–15075
- 169 Caverly LJ, LiPuma JJ. Good cop, bad cop: anaerobes in cystic fibrosis airways. *Eur Respir J* 2018;52(01):1801146
 - 170 Duan K, Dammel C, Stein J, Rabin H, Surette MG. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. *Mol Microbiol* 2003;50(05):1477–1491
 - 171 Sibley CD, Duan K, Fischer C, et al. Discerning the complexity of community interactions using a *Drosophila* model of polymicrobial infections. *PLoS Pathog* 2008;4(10):e1000184
 - 172 Nguyen M, Sharma A, Wu W, et al. The fermentation product 2,3-butanediol alters *P. aeruginosa* clearance, cytokine response and the lung microbiome. *ISME J* 2016;10(12):2978–2983
 - 173 Price CE, O'Toole GA. The gut-lung axis in cystic fibrosis. *J Bacteriol* 2021;203(20):e0031121
 - 174 Tony-Odigie A, Wilke L, Boutin S, Dalpke AH, Yi B. Commensal Bacteria in the cystic fibrosis airway microbiome reduce *P. aeruginosa* induced inflammation. *Front Cell Infect Microbiol* 2022;12:824101
 - 175 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(02):e00151-18
 - 176 Lopes-Pacheco M. CFTR Modulators: the changing face of cystic fibrosis in the era of precision medicine. *Front Pharmacol* 2020;10:1662
 - 177 Heltshe SL, Mayer-Hamblett N, Burns JL, et al; GOAL (the G551D Observation-AL) Investigators of the Cystic Fibrosis Foundation Therapeutics Development Network. *Pseudomonas aeruginosa* in cystic fibrosis patients with G551D-CFTR treated with ivacaftor. *Clin Infect Dis* 2015;60(05):703–712
 - 178 Rowe SM, Heltshe SL, Gonska T, et al; GOAL Investigators of the Cystic Fibrosis Foundation Therapeutics Development Network. Clinical mechanism of the cystic fibrosis transmembrane conductance regulator potentiator ivacaftor in G551D-mediated cystic fibrosis. *Am J Respir Crit Care Med* 2014;190(02):175–184
 - 179 Frost FJ, Nazareth DS, Charman SC, Winstanley C, Walshaw MJ. Ivacaftor is associated with reduced lung infection by key cystic fibrosis pathogens. a cohort study using national registry data. *Ann Am Thorac Soc* 2019;16(11):1375–1382
 - 180 Hisert KB, Heltshe SL, Pope C, et al. Restoring cystic fibrosis transmembrane conductance regulator function reduces airway bacteria and inflammation in people with cystic fibrosis and chronic lung infections. *Am J Respir Crit Care Med* 2017;195(12):1617–1628
 - 181 Harris JK, Wagner BD, Zemanick ET, et al. Changes in airway microbiome and inflammation with ivacaftor treatment in patients with cystic fibrosis and the G551D mutation. *Ann Am Thorac Soc* 2020;17(02):212–220
 - 182 Einarsson GG, Ronan NJ, Mooney D, et al. Extended-culture and culture-independent molecular analysis of the airway microbiota in cystic fibrosis following CFTR modulation with ivacaftor. *J Cyst Fibros* 2021;20(05):747–753
 - 183 Taylor-Cousar JL, Mall MA, Ramsey BW, McKone EF, Tullis E, Marigowda G, Mckee CM, Waltz D, Moskowitz SM, Savage J, Xuan F, Rowe SM. Clinical development of triple-combination CFTR modulators for cystic fibrosis patients with one or two F508del alleles. *ERJ Open Res* 2019;5(02):00082–2019
 - 184 Cuevas-Ocaña S, Laselva O, Avolio J, Nenna R. The era of CFTR modulators: improvements made and remaining challenges. *Breathe (Sheff)* 2020;16(02):200016
 - 185 Heijerman HGM, McKone EF, Downey DG, et al; VX17-445-103 Trial Group. Efficacy and safety of the elxacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. *Lancet* 2019;394(10212):1940–1948
 - 186 Sosinski LM, H CM, Neugebauer KA, et al. A restructuring of microbiome niche space is associated with elxacaftor-tezacaftor-ivacaftor therapy in the cystic fibrosis lung. *J Cyst Fibros* 2021 (e-pub ahead of print). Doi: 10.1016/j.jcf.2021.11.003
 - 187 Heirali AA, Workentine ML, Acosta N, et al. The effects of inhaled aztreonam on the cystic fibrosis lung microbiome. *Microbiome* 2017;5(01):51
 - 188 Nichols DP, Donaldson SH, Frederick CA, et al. PROMISE: Working with the CF community to understand emerging clinical and research needs for those treated with highly effective CFTR modulator therapy. *J Cyst Fibros* 2021;20(02):205–212
 - 189 Rogers GB, Taylor SL, Hoffman LR, Burr LD. The impact of CFTR modulator therapies on CF airway microbiology. *J Cyst Fibros* 2020;19(03):359–364
 - 190 Donaldson SH, Pilewski JM, Griese M, et al; VX11-661-101 Study Group. Tezacaftor/ivacaftor in subjects with cystic fibrosis and F508del/F508del-CFTR or F508del/G551D-CFTR. *Am J Respir Crit Care Med* 2018;197(02):214–224
 - 191 Woo TE, Lim R, Surette MG, et al. Epidemiology and natural history of *Pseudomonas aeruginosa* airway infections in non-cystic fibrosis bronchiectasis. *ERJ Open Res* 2018;4(02):00162–2017
 - 192 Heeb S, Fletcher MP, Chhabra SR, Diggle SP, Williams P, Cámara M. Quinolones: from antibiotics to autoinducers. *FEMS Microbiol Rev* 2011;35(02):247–274
 - 193 Schneider EK, Azad MA, Han ML, et al. An “Unlikely” pair: the antimicrobial synergy of polymyxin b in combination with the cystic fibrosis transmembrane conductance regulator drugs KALYDECO and ORKAMBI. *ACS Infect Dis* 2016;2(07):478–488
 - 194 Reznikov LR, Abou Alaiwa MH, Dohrn CL, et al. Antibacterial properties of the CFTR potentiator ivacaftor. *J Cyst Fibros* 2014;13(05):515–519
 - 195 Payne JE, Dubois AV, Ingram RJ, et al. Activity of innate antimicrobial peptides and ivacaftor against clinical cystic fibrosis respiratory pathogens. *Int J Antimicrob Agents* 2017;50(03):427–435
 - 196 Cho DY, Lim DJ, Mackey C, et al. Ivacaftor, a cystic fibrosis transmembrane conductance regulator potentiator, enhances ciprofloxacin activity against *Pseudomonas aeruginosa*. *Am J Rhinol Allergy* 2019;33(02):129–136
 - 197 Robledo F, Kopp B, Partida-Sanchez S. 493: Effects of elxacaftor/tezacaftor/ivacaftor on antimicrobial functions of CF neutrophils. *J Cyst Fibros* 2021;20:S233
 - 198 Blau H, Linnane B, Carzino R, et al. Induced sputum compared to bronchoalveolar lavage in young, non-expectorating cystic fibrosis children. *J Cyst Fibros* 2014;13(01):106–110
 - 199 Sagel SD, Sontag MK, Wagener JS, Kapsner RK, Osberg I, Accurso FJ. Induced sputum inflammatory measures correlate with lung function in children with cystic fibrosis. *J Pediatr* 2002;141(06):811–817
 - 200 Ronchetti K, Tame JD, Paisey C, et al. The CF-sputum induction trial (CF-SpIT) to assess lower airway bacterial sampling in young children with cystic fibrosis: a prospective internally controlled interventional trial. *Lancet Respir Med* 2018;6(06):461–471
 - 201 Ordoñez CL, Henig NR, Mayer-Hamblett N, et al. Inflammatory and microbiologic markers in induced sputum after intravenous antibiotics in cystic fibrosis. *Am J Respir Crit Care Med* 2003;168(12):1471–1475
 - 202 Davis MD, Montpetit AJ. Exhaled breath condensate: an update. *Immunol Allergy Clin North Am* 2018;38(04):667–678
 - 203 Sethi S, Nanda R, Chakraborty T. Clinical application of volatile organic compound analysis for detecting infectious diseases. *Clin Microbiol Rev* 2013;26(03):462–475
 - 204 Labows JN, McGinley KJ, Webster GF, Leyden JJ. Headspace analysis of volatile metabolites of *Pseudomonas aeruginosa* and related species by gas chromatography-mass spectrometry. *J Clin Microbiol* 1980;12(04):521–526
 - 205 Shestivska V, Nemeč A, Dřevínek P, Sovová K, Dryahina K, Spaněl P. Quantification of methyl thiocyanate in the headspace of

- Pseudomonas aeruginosa* cultures and in the breath of cystic fibrosis patients by selected ion flow tube mass spectrometry. *Rapid Commun Mass Spectrom* 2011;25(17):2459–2467
- 206 Filipiak W, Sponring A, Baur MM, et al. Molecular analysis of volatile metabolites released specifically by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *BMC Microbiol* 2012;12:113
- 207 Robroeks CM, van Berkel JJ, Dallinga JW, et al. Metabolomics of volatile organic compounds in cystic fibrosis patients and controls. *Pediatr Res* 2010;68(01):75–80
- 208 Robroeks CM, Roozeboom MH, de Jong PA, et al. Structural lung changes, lung function, and non-invasive inflammatory markers in cystic fibrosis. *Pediatr Allergy Immunol* 2010;21(03):493–500
- 209 Lawal O, Ahmed WM, Nijssen TME, Goodacre R, Fowler SJ. Exhaled breath analysis: a review of 'breath-taking' methods for off-line analysis. *Metabolomics* 2017;13(10):110
- 210 Chmiel JF, Aksamit TR, Chotirmall SH, et al. Antibiotic management of lung infections in cystic fibrosis. I. The microbiome, methicillin-resistant *Staphylococcus aureus*, gram-negative bacteria, and multiple infections. *Ann Am Thorac Soc* 2014;11(07):1120–1129
- 211 Cystic Fibrosis Trust: UK Cystic Fibrosis Registry 2020 Annual Data Report; 2020
- 212 Cystic Fibrosis Canada: The Canadian Cystic Fibrosis Registry 2020 Annual Data Report; 2020
- 213 Cystic Fibrosis Australia: Australian Cystic Fibrosis Data Registry Annual Report; 2020
- 214 Cystic Fibrosis Ireland: Annual Report of Cystic Fibrosis Registry of Ireland; 2020
- 215 Cystic Fibrosis Foundation. Cystic Fibrosis Foundation Patient Registry: 2019 Annual Data Report. Bethesda, MD: Cystic Fibrosis Foundation; 2019