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Infections in Patients with Cystic Fibrosis

Diagnostic Microbiology Update

Peter H. Gilligan, PhD, DABMM

KEYWORDS

• Infection • Cystic fibrosis • Diagnostic microbiology • Update

KEY POINTS

- Cystic fibrosis is the most important genetic disease in Caucasians. Patients with this disease die prematurely primarily as a result of chronic lung infection. *Staphylococcus aureus* and mucoid *Pseudomonas aeruginosa* continue to be the key pulmonary pathogens.
- Survival has improved in patients with CF in part because of aggressive antimicrobial management. An unintended consequence of this therapy has been the emergence of 2 multidrug-resistant environmental bacteria: the *Burkholderia cepacia* group and nontuberculous mycobacteria.
- *Burkholderia cenocepacia*, a species within the *Burkholderia cepacia* complex, is associated with high mortality and is a contraindication for lung transplantation. The key nontuberculous mycobacterial pathogen, *Mycobacterium abscessus*, is not so virulent and is not a lung transplantation contraindication. Both present an infection control challenge, because they can be spread from person to person.
- Improving genomic and proteomic technologies are allowing better identification of bacteria and fungi found in the CF lung and to detect viral agents that may be associated with pulmonary exacerbations. Chronic rhinovirus infections are of particular interest.
- Microbiome studies have identified 2 groups of bacteria that may play a role in chronic CF lung infections: anaerobic bacteria and *Streptococcus angionsus* group organisms. Microbiome studies also show that as the diversity of organisms decline, perhaps as a result of aggressive antimicrobial therapy, an apex predator, etc., *Pseudomonas aeruginosa,* may emerge in many patients with CF.

INTRODUCTION, EPIDEMIOLOGY, AND CLINICAL PRESENTATION

Cystic fibrosis (CF) is the most common autosomal-recessive genetic disease that occurs in non-Hispanic Caucasians populations, although other racial groups may have this disease as well.[1] Affected individuals have mutation in the CF transmembrane

Pathology-Laboratory Medicine and Microbiology-Immunology, Clinical Microbiology-Immunology Laboratories, UNC Health Care, UNC Hospitals, UNC School Medicine, Room 1035, CB 7600, Chapel Hill, NC 27516, USA
E-mail address: gilliganncphd@gmail.com

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conductance gene (CFTR), a membrane protein involved in sodium and chloride transport in epithelial cells.[2] The resulting dysregulation in electrolyte transport leads to depletion in airway surface liquid on bronchial epithelial cell surfaces. As a result, patients with CF have thick, dry, tenacious mucus, which impairs mucociliary clearance of particulates, especially bacteria and fungal conidia, from the airways. This environment is ideal for the growth of a limited number of organisms, primarily those that thrive in natural environments such as water. This thickened mucus provides an ideal niche for the establishment of chronic infection. It is this chronic infection that results in the premature death that in seen in CF.[3]

More than 1800 CFTR mutations have been associated with CF.[4] The most common mutation is F508del, which is found in ~85% of people in the United States; approximately 47% are homozygous for this mutated gene.[4] Further carrier rate for mutated CFTR genes is estimated to range from 1/25 for non-Hispanic Caucasians to 1/61 for African Americans to 1/94 for Asian Americans.[1] CF is seen most frequently in North America, Northern Europe, Australia, New Zealand, Brazil, and Argentina. It is estimated that 1 in 3500 live births result in clinical disease.[4]

Currently life expectancy in US patients with CF is approximately 38 years, significantly less than that of the general population.[4] Cardiopulmonary failure secondary to chronic lung disease is responsible for 85% of premature deaths in CF. The airways of patients with CF become infected in infancy. This situation begins periods of chronic infection and lung inflammation with accompanying cough, which is a lifelong reality in patients with CF. A hallmark of chronic infection and airway inflammation is periods of pulmonary exacerbations. Pulmonary exacerbations are characterized by worsening symptoms, including increased cough and sputum production, hemoptysis, shortness of breath, increased respiratory rate, loss of appetite, weight loss, increased neutrophil counts, and declining pulmonary function.[5] The events that trigger these pulmonary exacerbations are not clearly understood, although viral infections and perhaps changes in the microbiome may be important.[6,7] Exacerbations are characterized by the recruitment of neutrophils, cytokine release, and high level of neutrophil-derived elastases in the bronchi and bronchioles, causing significant lung disease.[8] Antimicrobial therapy has been shown to be effective in treating exacerbations symptomatically.[9] However, over time, lung function deteriorates and becomes so low, that it is no longer compatible with life (**Fig. 1**). Only lung transplantation can successfully reverse this disease course.[8]

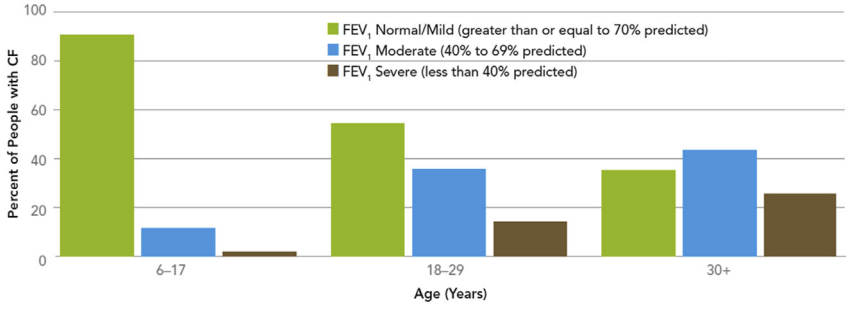


Fig. 1. Lung function by age group, 2011. FEV_1, forced expiratory volume in 1 second. (*From* Cystic Fibrosis Foundation. Cystic Fibrosis Foundation patient registry 2011 annual data report. 2012.)

MICROBIOLOGY, DIAGNOSTIC CONSIDERATIONS, AND SUSCEPTIBILITY TESTING OF AGENTS OF CHRONIC INFECTION

Over the past 4 decades, our understanding of the complex nature of chronic lung infections has greatly expanded. Over the past 3 decades, there has been more than a doubling in life expectancy in the population with CF.[4] Three factors have been central to this improvement:

a. More effective antimicrobial therapy and treatment strategies, with early eradication of *Pseudomonas aeruginosa* being a key strategy
b. Improvement in airway clearance techniques
c. Improvements in infection control techniques to prevent the spread of organisms highly virulent to patients with CF, especially *Burkholderia cenocepacia*[2,9]

With the use of broader-spectrum antimicrobials, we are seeing a plethora of emerging highly resistant bacteria and fungi in the CF airways. Our understanding of the role of these organisms in chronic infection and inflammation is poorly delineated (**Box 1**). Over the past decades, new technologies (**Fig. 2**) have been developed and applied to this understanding. These technologies include nucleic acid amplification techniques (NAATs) for direct organism detection, including multiplex NAAT for viruses; the use of DNA sequence analysis for organism identification; molecularly based epidemiologic techniques, including pulsed field gel electrophoresis (PFGE), multilocus sequence type, whole genome sequencing; and matrix-assisted laser desorption ionization–time of flight mass spectroscopy (MALDI-TOF MS).

Perhaps the most exciting development in the application of new technologies is the use of direct sequencing technique to study the CF microbiome.[5,10–13] These studies hold the promise for new understanding of the disease process, with accompanying improved treatment strategies.

STAPHYLOCOCCUS AUREUS

Before the antimicrobial era, few children with CF survived past the age of 2 years. The children died primarily from failure to thrive, coupled with bacterial pneumonia. The organism found in the lungs at autopsy in these children was almost always *Staphylococcus aureus*.[14] With the advent of antistaphylococcal antimicrobial therapy, mortality caused by *S aureus* decreased substantially, but it continues to be the predominant CF pathogen from birth to adolescence, with 80% of children infected in their preadolescence and early adolescence.[4] Improvements in antistaphylococcal therapy, especially the use of oral agents such as oral cephalosporins and trimethoprim-sulfamethoxazole (TMP-SMX), are likely to have contributed to the improved life expectancy seen in patients with CF.[3,15] However this effective antistaphylococcal therapy is not without its costs.

Three antimicrobial resistance issues have arisen, which must be considered in the care of patients with CF. First, the use of oral TMP-SMX has been associated with the emergence of small colony variants (SCV) of *S aureus*.[16–18] The resistance mechanism in these organisms is their ability to use thymidine obtained from the environment, bypassing the folic acid synthesis pathway, the target of TMP-SMX. Such organisms are considered to be thymidine dependent. Such organisms are not as fit as wild-type organism and grow slowly, producing small, streptococcallike colonies on most enrichment media.[16] Another SCV that is seen in *S aureus* are strains with defect in electron transport. These strains are resistant to aminoglycosides. These mutants cannot generate sufficient proton motor force to drive transport of these large, highly charged molecules into the bacterial cytoplasm.[17] SCV are less susceptible to

Box 1
Pathogenic potential of commonly recovered organisms from chronic CF airway infections or pulmonary exacerbations

- Known
  - *Pseudomonas aeruginosa*
  - *Staphylococcus aureus*
    - Methicillin resistant
    - Small colony variant
  - *Burkholderia multivorans*
  - *Burkholderia cenocepacia*
  - *Burkholderia dolosa*
  - *Aspergillus* spp
  - *Scedosporium* spp
  - *Mycobacterium abscessus*
  - Influenza virus
  - Respiratory syncytial virus
- Possible/likely
  - *Haemophilus influenzae*
  - *Mycobacterium avium* complex
  - Anaerobic bacteria especially *Prevotella* spp
  - *Streptococcus anginosus* group
  - Respiratory viruses other than influenza virus and respiratory syncytial virus
- Unknown
  - *Nocardia* spp
  - *Pandoraea* spp
  - Other members of the *Burkholderia cepacia* complex
  - *Inquilinus* spp
  - *Trichosporon* spp
- Unlikely
  - *Stenotrophomonas maltophilia*
  - *Achromobacter* spp
  - *Ralstonia* spp
  - *Burkholderia gladioli*
  - *Streptococcus pneumoniae*
  - *Candida albicans*

antimicrobials, especially β-lactams, because of their slow growth rate, making them more challenging to treat and resulting in a greater decline in pulmonary function.[17,19]

The second resistance problem in patients with CF is the problem of methicillin-resistant *S aureus* (MRSA). It is now recognized that approximately 20% to 25% of patients with CF have MRSA (**Fig. 3**).[4,20] It has also been recognized that patients with CF

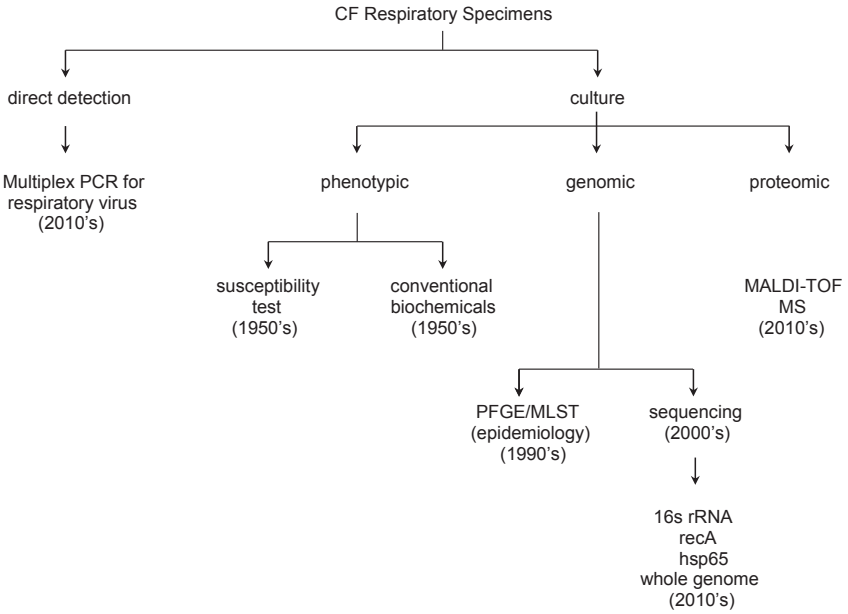
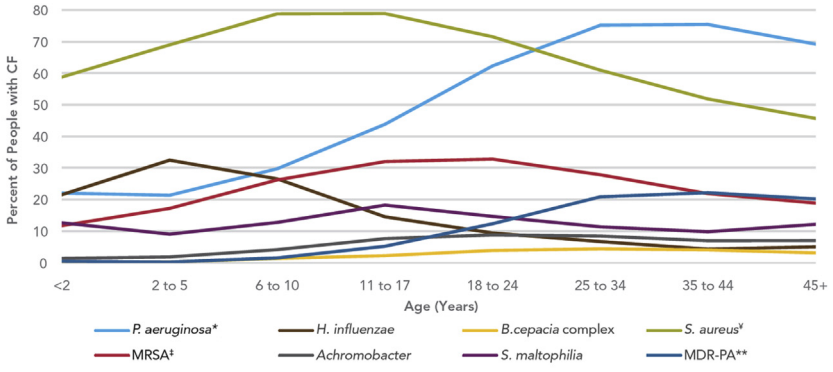


Fig. 2. CF lung infection diagnostics 2014.

with MRSA have a higher mortality than those patients with CF without this organism.[21] Most CF MRSA isolates can be divided into 2 molecular types based on staphylococcal chromosomal cassette (SCC) *mecA* type and whether they have the gene for Panton-Valentine leukocidin (*pvl*). Approximately 70% are SCC*mecA* type II *pvl* negative and 17% are SCC*mecA* type IV *pvl* positive; the other 13% are SCC*mecA* type IV *pvl* negative.[20] SCC*mecA* type IV, *pvl* positive MRSA isolates are also referred to as community-acquired (CA)-MRSA strains. Initial studies of respiratory infections in healthy adults caused by CA-MRSA suggested that this organism caused severe,



*P. aeruginosa *includes people with MDR-PA.*
***MDR-PA is multi-drug resistant* Pseudomonas aeruginosa (P. aeruginosa).*
*¥ S. aureus *includes people with MRSA.*
‡ MRSA is methicillin-resistant Staphylococcus aureus (S. aureus).

Fig. 3. Germs found in the lungs of people with CF by age, 2012. (*From* Cystic Fibrosis Foundation. Cystic Fibrosis Foundation patient registry 2012 annual data report. 2013.)

necrotizing pneumonia, with significant morbidity and mortality in excess of 50% in some series.[22] We were concerned that this organism would be a problem in patients with CF as well. In a survey performed between 2005 and 2007, we found that approximately 3% of patients with CF in our institution had CA-MRSA recovered from their respiratory specimen and that approximately 1% of patients in our center were chronically infected. Further, no episodes of necrotizing pneumonia were seen in our population of patients with CF during that period.[23]

The third antimicrobial resistance issue we have observed is vancomycin-intermediate *S aureus* (VISA). The mechanism of resistance is believed to be a thickened cell wall, which prevents vancomycin from reaching the site of peptidoglycan synthesis.[24] We have screened *S aureus* isolates for the VISA phenotype (a vancomycin minimum inhibitory concentration [MIC] of 4–8 μg/mL) for the past 5 years by the use of vancomycin E-test or the Vitek 2 susceptibility testing (Biomerieux, Durham, NC). We have tested in excess of 1500 isolates from more than 700 patients with CF (Miller M and Gilligan P, 2013, unpublished data). We have found VISA in only 1 patient. This patient had been chronically infected with MRSA for more than 10 years and had received repeated courses of vancomycin. Over time, her pulmonary function declined. With the emergence of VISA, she was treated with multiple courses of linezolid and ceftaroline. Neither was successful in eradicating the organism. Because of declining lung function, she received a double lung transplant. On her most recent bronchoscopy culture 9 months after transplant, she had positive *S aureus* infection culture; the organism was both vancomycin and oxacillin susceptible, but the VISA strain has not been recovered from multiple bronchoscopy specimens obtained during the posttransplant period (Miller M and Gilligan P, 2013, unpublished data).

Laboratory detection and identification of *S aureus* from patients with CF is straightforward. Because these patients often are infected with other organisms, especially mucoid *P aeruginosa*, selective media for the recovery should be used.[25] Two media, mannitol salts agar (**Fig. 4**) and chromogenic *S aureus* agar, are effective in the recovery of SCV and wild-type strains.[16,26] SCV *S aureus* have typical morphology on mannitol salts agar, whereas on chromogenic media, these organisms may grow

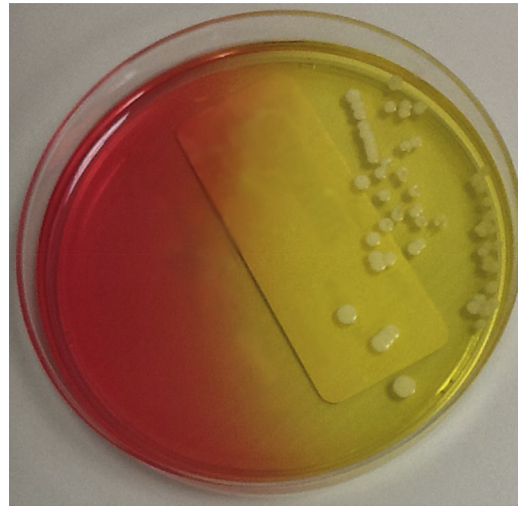


Fig. 4. *Staphylococcus aureus* on mannitol salt agar.

more slowly and not give typical phenotypic reactions.[16,26] Organism identification is straightforward, with catalase and latex agglutination or coagulase testing being highly accurate.[27] MALDI-TOF MS works well for identification of *S aureus* from patients with CF, although its ability to speciate SCV has not been reported.[28] Susceptibility testing is straightforward, except for the detection of VISA strains. For those isolates, the most accurate method is an MIC-based method. Because E-test MICs tend to run higher than more conventional agar or broth dilution methods, all *S aureus* isolates with a vancomycin MIC of 4 μg/mL or greater should be confirmed by a conventional MIC method.[29]

PSEUDOMONAS AERUGINOSA

The key pathogen in chronic lung disease is an unusual morphotype of *P aeruginosa*, referred to as mucoid. The organism is typically obtained from the patient's environment, although patient-to-patient spread of virulent clones does occur.[30] The pathogenesis of *P aeruginosa* is dependent on the transition of the organism from a motile, virulent, nonmucoid organism to a nonmotile, comparatively avirulent, mucoid organism, which has adapted to living in the CF airways. The transition is believed to be caused by mutations that inactivate the *mucA* gene, which regulates alginate production. Alginate-producing organisms grow in a biofilm within the mucous layer in bronchioles of the CF lung. These organisms may be observed as microcolonies when the mucous layer is observed microscopically.[31] On agar plates, these organisms appear as mucoid colonies (**Fig. 5**). In the lung, these organisms are resistant to mechanical clearance, phagocytosis, and antimicrobial therapy.[3,31] Although they are comparatively avirulent, they induce a chronic inflammatory response, which is believed to be responsible for the lung damage that results in the patient's demise.[31] This disease process takes place over a period of years to decades.

In the United States, 60% to 75% of adults are infected with this organism.[4] The longer a patient can remain free of chronic infection with *P aeruginosa,* the longer the life expectancy is. A highly aggressively antimicrobial therapeutic approach, first used in Danish patients with CF, for eradicating early infection with *P aeruginosa* has been shown to lengthen the time that patients with CF remain *P aeruginosa* free. As a result, Danish patients with CF have perhaps the longest life expectancy of any CF populations in the world, at 42 years.[9]

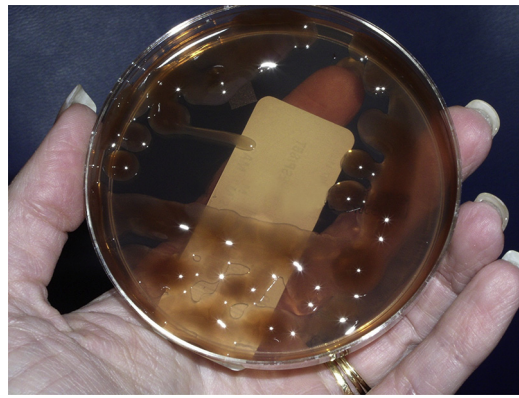


Fig. 5. Mucoid *Pseudomonas aeruginosa* on MacConkey agar.

Laboratory diagnosis of infections with this organism is easily accomplished. It is readily recovered on both nonselective agar (chocolate) or selective agar (MacConkey). Selective agar specific for *P aeruginosa* is not required. In addition, identification is easily accomplished. The organism is oxidase positive, mucoid, and produces green (pyoverdin) or blue-green (pyocyanin) pigments. These characteristics identify at least 90% of CF isolates.[25] MALDI-TOF MS has been shown to accurately identify both mucoid and nonmucoid strains of *P aeruginosa*.[28,32]

Susceptibility testing of *P aeruginosa* from patients with CF can be challenging. Antimicrobial susceptibility results using automated susceptibility systems correlate poorly with reference methods and are not recommended. Rather, disk diffusion or E-tests are recommended.[33] Paradoxically, it is unlikely that conventional susceptibility testing accurately predicts in vivo response.[34] The reasons for this situation are complex but can be explained in part by comparing susceptibility of *P aeruginosa* with antimicrobials when the organism is grown in a conventional manner or planktonically versus a biofilm mode of growth. When *P aeruginosa* is grown as a biofilm, it is 8 to 32 times more resistant to all classes of β-lactams than when it is grown planktonically.[35] This difference can be explained in part by reduced growth rates of mucoid colonies and by the possibility that these antimicrobials cannot penetrate to the cell through the alginate layer. There is little difference between the MICs for aminoglcyosides or fluoroquinolones grown under these 2 conditions.[35] Further complicating interpretation of these results is a growing body of literature that suggests that *P aeruginosa* may grow under anaerobic conditions behind mucous plugs or in the thickened mucus of the airway.[36] Tobramycin is not active under anaerobic conditions, because of poor transport into bacteria. Aerosolized tobramycin therapy, a key strategy in early eradication of *P aeruginosa*,[9,31] may act only against organisms in the airway that are growing in the superficial layer of mucus. Chronic *P aeruginosa* infection may be established only when the organism is growing as a biofilm in the anoxic portion of the mucous layer. Further complexity in susceptibility testing is caused by the widespread use of aerosolized antimicrobials, tobramycin, colistin, and aztreonam, used to prevent the establishment of chronic *P aeruginosa* or to suppress the growth of *P aeruginosa* in those patients chronically infected.[37] Because antimicrobial susceptibility is based on achievable serum drug concentrations, susceptibility testing does not accurately predict resistance in patients receiving aerosolized antimicrobials, which reach airway levels at least 10 to 25 times greater than serum levels.

There are 2 additional problems that confront laboratorians when determining antimicrobial susceptibility of *P aeruginosa.* Technically, susceptibility testing of mucoid *P aeruginosa* is poorly reproducible and operator dependent.[38] In the late stages of chronic *P aeruginosa* infection, after many rounds of intravenous and aerosolized antimicrobial therapy, the organism may become resistant to all classes of antimicrobial.[34] One of the strategies was to perform multicombinational bactericidal testing (MCBT).[39] In this method, 2-drug, 3-drug, and 4-drug combinations at serum achievable concentrations are placed in a microtiter well, inoculated with a bacteria, incubated overnight, and then subcultured to determine which combinations were bactericidal. The idea behind this method was to give a rationale for choosing antimicrobial combinations that would be used to treat these infections. However, when a randomized, double-blind, controlled clinical trial was performed to compare MCBT with conventional susceptibility, outcomes were similar for the 2 groups.[40] Although the study was technically well performed, it was flawed because the patients studied were infected with organisms that had some level of susceptibility to antimicrobials and would not have needed MCBT. Based on this study, MCBT has been abandoned by the CF community.

BURKHOLDERIA CEPACIA COMPLEX AND RELATED ORGANISMS

In the early 1980s, a significant new pathogen, then called *Pseudomonas cepacia*, was recognized in lung infections in patients with CF cared for at 3 different major CF centers.[41–43] In approximately 20% of patients with CF, the organism caused a fatal illness called the cepacia syndrome. In this syndrome, the patients had respiratory infections, with hallmarks of rapid decline in pulmonary function and frequent bacteremia, a highly unusual finding in patients with CF.[41,42] Subsequently, *P cepacia* was reclassified as belonging to the genus *Burkholderia*.[44] Vandamme and colleagues[45] showed that the organisms identified as *Burkholderia cepacia* represented multiple species of bacteria, which they called genomovars. The *B cepacia* complex is now recognized to consistent of 18 different species.[46,47] The species within this complex that is believed to be primarily responsible for the cepacia syndrome is *B cenocepacia*, although both *B multivorans* and *B dolosa* have also been associated with it.[46] *B cenocepacia* or *B multivorans* are recovered from 80% of the *B cepacia* complex infections seen in patients with CF.[46] The molecular epidemiology of *B cenocepacia* is reasonably well understood. Three different *B cenocepacia* clones, designated ET12,[42] PHDC,[43] and Midwest,[44] were associated with the initial description of *B cepacia* as a pathogen in patients with CF.[46] All 3 of these clones can be spread from person to person, although many patients have unique clones, which they likely acquired from their environment.[48] It has been shown that the ET-12 strain has been spread from Toronto to Europe and that it is the predominant strain in Canada.[46,49] The organism has an unusual appendage called a cable pilin, which is believed to be responsible for its high transmissibility.[50]

Because *B cepacia* and *B multivorans* were associated with accelerated lung function decline and high mortality and were often panresistant to antimicrobials, lung transplantation was believed to be a life-saving therapy in those patients. Early molecular epidemiology studies using PFGE showed that patients infected with *B cepacia* complex after transplant had the same highly antimicrobial-resistant strain of *B cepacia* complex before transplant.[51] It was subsequently observed that patients with CF chronically infected with *B cenocepacia* who received lung transplant had a higher mortality at years 1, 3, and 5 years after transplant than those who were infected with other bacteria.[52,53] Early in the posttransplant period, some patients infected with *B cenocepacia* had a sepsis with positive blood cultures. Some of the transplants were further complicated by wound dehiscence. Posttransplant patients with *B cenocepacia* sepsis had a grim prognosis, with few survivors. Bronchiolitis obliterans was an additional problem seen in this patient population.[52] Other studies also showed increased risk of death after transplant in patients with CF infected before transplant with *B cenocepacia*.[53,54] As result, most centers no longer transplant patients with CF who are infected with *B cenocepacia.*

One of the difficulties in recovering *B cepacia* from respiratory specimens of patients with CF is that the organism grows slowly compared with mucoid *P aeruginosa.* Consequently, the growth of *B cepacia* may be obscured on most media. Three different groups developed isolation media for the recovery of *B cepacia* complex organism. *B cepacia* selective agar (BCSA)[55] was superior for *B cepacia* recovery compared with *P cepacia* agar[56] and oxidation-fermentation polymyxin-bacitracin-lactose agar.[57] All 3 media contain polymixin as a selective agent. Not only were *B cepacia* complex organisms isolated on these media from CF respiratory specimens but also a large group of environmental glucose nonfermenters, which were similar to *B cepacia*, were also recovered. These organisms were often phenotypically difficult to separate from *B cepacia* using commercially available kits, resulting in

misidentification of non-*B cepacia* isolates as *B cepacia* complex ones.[58,59] Alternatively, some isolates were relatively inert biochemically, making them difficult to identify using commercially available phenotypic methods.[58,59]

Misidentification of non-*B cepacia* organisms as *B cepacia* has grim consequences for the patient with CF. In addition to no longer being candidates for lung transplantation in most centers, the patients are completely segregated from other children and adults with CF. They are likely to have a different clinic day, must always be on isolation when hospitalized, meaning no trips to the playroom to interact with other children, may no longer attend any CF sponsored social function or meetings, or participate in camps with other patients with CF.[60] The result is social isolation for these individuals and potential exclusion from a life-saving therapy. These draconian measures have been successful in blunting the spread of this organism among patients with CF.[60] Perhaps just as importantly, misidentifying *B cepacia* complex organism as some other species or genera results in failure to isolate patients with a potentially deadly organism, which can be spread to both patients with CF and those who do not have CF.[49,61]

Burkholderia gladioli was the first organism recovered on *B cepacia*–selective media and recognized to be misidentified as *B cepacia* using phenotypic means.[59,62] By the early part of this century, it was recognized that 16s ribosomal RNA (rRNA) sequencing was superior to phenotypic methods for differentiating *B cepacia* complex organisms from other genera growing on *B cepacia*–selective media such as *B gladioli*.[63] Our experience with 16s rRNA sequencing over the past 6 years is shown in **Table 1**. With the exception of the unusual *P aeruginosa* phenotypes identified by sequencing, the rest of the organisms identified were recovered on BCSA, the *B cepacia* selective medium that we use. Many more non-*B cepacia* complex organisms requiring identification grow on BCSA. Almost all the organisms listed in **Table 1** are recovered primarily from patients with CF. Most of them, including *Ralstonia*, *Chyseobacterium*, *Inquilinus*, and *Pandoraea*, are infrequently recovered and are believed to be of limited clinical importance. One group is reported in our laboratory as gram-negative rods,

Table 1
Organisms identified by 16S rRNA sequencing from patients with CF at UNC Hospitals from Jan 2008 to Nov 2013

Achromobacter	67
Burkholderia cepacia complex[a]	64
Burkholderia gladioli	63
Chryseobacterium	38
Gram-negative rods not *Burkholderia cepacia complex, Ralstonia* or *Pandoraea*[b]	35
Ralstonia spp	32
Pandoraea	12
Pseudomonas aeruginosa	10
Pseudomonas not *aeruginosa*	10
Acinetobacter spp	4
Inquilinus	2

[a] Isolates speciated by recA sequencing at *Burkholderia cepacia* Reference Laboratory and Repository, University of Michigan.
[b] Includes isolates of *Bordetella bronchiseptica, Comamonas, Herbaspirillium, Elizabethkingia,* and *Cupriavidus*.

not *Burkholderia*, *Ralstonia*, or *Pandoraea.* Included in this group are *Bordetella* spp, including *Bordetella broncoseptica*, *Comamonas*, *Herbaspirillium*, *Elizabethkingia*, and *Cupriavidus.* These organisms have not been associated with pulmonary exacerbation in patients with CF.

Although there are few published data suggesting a role for *B gladioli* in patients with CF,[64] the exception seems to be in lung transplant recipients, who have decreased survival compared with those transplant recipients who do not have *Burkholderia*.[53]

Both *Stenotrophomonas* and *Achromobacter* spp are recovered with frequency in patients with CF, especially older patients who have received many rounds of antipseudomonal antimicrobials. These species are the subject of the next section of this article.

One of the problems with 16s rRNA sequence identification is that it cannot accurately differentiate the members of the *B cepacia* complex. Because there seem to be differences in virulence among the different species within the complex, accurate speciation is important.[46] Three species are recognized as being pathogenic in patients with CF and being able to be spread from person to person: *B cenocepacia*, *B multivorans*, and *B dolosa*.[46] Other species seen with some degree of frequency in our patient population include *B vietnamienesis* and *B cepacia*. In our experience, the other 13 species within the complex are rarely if ever isolated. Accurate speciation of isolates of the *B cepacia* complex can be accomplished by sequencing of the *recA* gene.[65]

The requirement to use 2 sequencing steps to accurately identify isolates belonging to the *B cepacia* complex is time consuming and expensive, requiring days to obtain an accurate answer. As a result, alternative identification methods have been sought. The most promising of these is MALDI-TOF MS. We[66] and others[28,65,67] have shown that MALDI-TOF accurately identifies organisms to the *B cepacia* complex and can differentiate them readily from other genera of organisms that grow on BCSA, most importantly *B gladioli.* Preliminary studies suggest that the Bruker system more accurately identifies *B cenocepacia* isolates than the Vitek system.[65,67] Both systems accurately speciate *B multivorans* and *B vietnamienesis.* The Bruker system misidentifies *B contaminans* primarily as *B cepacia* but may also misidentify it as *B cenocepacia* or *B multivorans.* With database improvements, it is likely that the 2 major CF pathogens, *B cenocepacia* and *B multivorans*, will be accurately identified by both systems.

B cepacia complex is one of the most antimicrobial-resistant group of organisms. These organisms are intrinsically resistant to both colistin and aminoglycosides.[68] Clinical and Laboratory Standards Institute susceptibility breakpoints exist for ceftazidime, ticarcillin-clavulanic acid, TMP-SMX, meropenem. minocycline, and fluoroquinolones.[69] Although a large percentage of isolates may be initially susceptible to these agents, resistance develops over time, and many strains become resistant to all these antimicrobial agents.[68] Further complicating treatment is the lack of evidence that any specific antimicrobial treatment regimen is effective in treating *B cepacia* complex pulmonary exacerbations.[70]

STENOTROPHOMONAS AND *ACHROMOBACTER*

Both *Stenotrophomonas* and *Achromobacter* are glucose nonfermenting rods, which are found more frequently in patients with CF than are *B cepacia* complex organisms. *Stenotrophomonas* is found in approximately 10% to 20% of patients with CF, whereas *Achromobacter* is found in between 5% and 10%.[4] The incidence of these organisms increases with age and likely reflects patients who have had multiple rounds of antipseudomonal antimicrobial therapy.

The clinical significance of both of these organisms has been debated. One study suggested that patients who had precipitating antibodies to *Achromobacter* antigens

have a more rapid decline in lung function.[71] Other studies do not support the notion that *Achromobacter* is pathogenic.[72,73] The story is similar with *Stenotrophmonas.* Patients may be more likely to have poor pulmonary function and may require lung transplantation. What is not clear is whether this organism represents a marker of severe lung disease or is the cause of this disease.[74,75]

Three different approaches have been used to identify these 2 organism: conventional phenotypic methods, 16s rRNA sequencing, and MALDI-TOF MS. Phenotypic methods work reasonably well for these organisms, although we have greater confidence in the use of the VITEK 2 for *Stenotrophomonas maltophilia* compared with *Achromobacter* spp.[76] MADLI-TOF MS accurately identifies both species.[66,67]

Both organisms are resistant to aminoglycosides and may develop resistance to colistin.[77] In addition, *Stenotrophomonas* produces a metallo-β-lactamase, which confers resistance to carbapenems.[78] The organism may be initially susceptible to levofloxacin and TMP-SMX but can develop resistance over time.[78] The initial susceptibility of *Achromobacter* is similar to that of *B cepacia* complex, with susceptibility to ceftazidime, meropenem, fluoroquinolones, and TMP-SMX being common.[79] However, drug resistance can develop over time and panresistant organisms can be seen.[80]

MYCOBACTERIUM

Over the past 25 years, mycobacteria are being recognized as an increasingly important agent of chronic lung infection in patients with CF.[81] Although we have been culturing patients with CF for *Mycobacterium* for more than 25 years in our institution, we have never recovered *Mycobacterium tuberculosis* from them.[82] The mycobacterial species most frequently associated with these infections are the nontuberculous mycobacteria, *M avium* complex and *M abscessus* complex.[82,83] There is an increasing body of evidence that both *M avium* complex and *M abscessus* complex can cause chronic infection in patients with CF and may be responsible for decline in pulmonary function, although it is not as dramatic as *B cepacia* or *P aeruginosa*.[81,84,85] In addition, the patient population infected with *M avium* complex seems to be older and does not have as severe disease as those infected with the *M abscessus* group, who seem to be younger and to have more severe lung disease.[85] Recent studies using whole genome sequencing have suggested that the *M abscessus* group show the potential for spread from person to person in CF centers, making it another target for infection prevention.[86]

Attempting to recover nontuberculous mycobacteria from a CF respiratory specimen is challenging for the laboratory. Approximately 50% to 70% of specimens contain *P aeruginosa*, which is resistant to 0.25% *N*-acetyl cysteine-1% sodium hydroxide decontamination, the standard method used in the culture respiratory specimens for mycobacteria. Contamination rates between 35% and 70% may be seen using this decontamination method in CF respiratory specimens. When a second step is added, 2.5% oxalic acid, the contamination rate is reduced to 3% to 5%, although low numbers of *M avium* complex may not be recovered.[87,88] Chlorohexidine alone has been shown to be an effective means of decontaminating CF respiratory specimens for the recovery of nontuberculous mycobacteria.[89]

A useful observation concerning the recovery of *M abscessus* group organisms is the observation that this group of organisms can be recovered on medium that is routinely used for bacterial isolation, specifically BCSA. *M abscessus* group clinical isolates were recovered on BCSA from 65% to 75% of infected individuals, whereas the organism was recovered from 85% using mycobacterial-specific culture methods. Twenty-five percent of patients with CF with *M abscessus* complex had it first

recovered on routine culture. In addition, incubation of BCSA plates for 14 days greatly enhanced recovery compared with 5-day incubation.[90] As a result, we routinely examine BCSA plates for the presence of rapidly growing *Mycobacterium*.

Phenotypic identification of nontuberculous mycobacteria is challenging and not particularly accurate. As a result, we have used 16S rRNA sequence analysis for identification of these organisms. A 16S rRNA sequence works well for identification of *M avium* complex, but *M abscessus* complex isolates cannot be distinguished from *M chelonae*.[91] As a result, a second target is needed. Both *rpoB* and *hsp65* have been used for this purpose, with both being useful for differentiating *M abscessus* complex from *M chelonae*.[91] Whole genome sequencing has shown that in 1 center, *M abscessus* subspecies *massilense* was predominant and there was evidence of person-to-person spread.[86] Whole genome sequencing of *M abscessus* complex isolates from more than 100 patients with CF in our center showed *M abscessus* subspecies *abscessus* to be predominant (Grogono, Floto, Gilligan unpublished).

Antimicrobial resistance is a major problem in nontuberculous mycobacteria treatment. Anti–*M tuberculosis* therapy is ineffective against these organisms. Further complicating treatment is the lack of randomized, controlled trials of antimicrobial treatment against this group of organisms.[92] Susceptibility testing using broth dilution is helpful in guiding antimicrobial choice, because susceptibility is not predictable.[81] Broth MIC susceptibility tests of macrolides against the *M abscessus* group should be incubated for 14 days to detect inducible macrolide resistance. Clarithromycin seems to be a more potent inducer of this resistance than azithromycin, making azithromycin central to the treatment of *M abscessus* infections.[93]

A potential life-saving therapy for patients with CF infected with severe *M abscessus* is lung transplantation. However, based on anecdotal experience and case reports, *M abscessus* complex infection has been a contraindication for this life-saving procedure.[81] This strategy seems logical, given some of the similarities between *B cepacia* and *M abscessus.* Both organisms are environment organisms, which are highly drug resistant, making them difficult to treat, able to be spread from person to person, and associated with wound dehiscence after transplant. A recent single-center study with 13 patients with CF with *M abscessus* receiving double lung transplant showed the same survival rate as the control population, suggesting that this organism is not a contraindication to lung transplantation.[94]

FUNGI

Fungi have long been recognized as having a role in CF lung disease. In particular, 2 fungi, *Aspergillus fumigatus* and *Scedosporium apiospermum*, have been shown to be long-term colonizers in patients with CF.[95] Both organisms are most frequently associated with allergic bronchopulmonary disease, characterized by subacute clinical disease with eosinophilia, increased IgE levels, skin test reactivity in the case of *Aspergillus*, presence of serum IgE specific for the *Aspergillus* when it is the cause, and chest imaging changes that are not attributable to bacterial agents.[95] In addition, post–lung transplant infections are well described for both organisms, although colonization with these organisms is not a contraindication for lung transplantation.[96,97] *Candida* spp are found in as many as 70% of patients with CF, especially when selective media are used. Because patients with CF receive antibacterials from an early age, the presence of these organisms colonizing the airways is not surprising. The clinical significance of *Candida* in patients with CF is not clearly understood, but most clinicians discount these organisms as not significant.[98]

NAAT allows the detection of *Pneumocystis jirovecii* in the airway of patients with CF. In a prospective study, 12.5% of adults with CF were found to have *Pneumocystis jirovecii* in sputum. Lung function in these patients was better than in those without *Pneumocystis jirovecii.* These patients were more likely to have mild disease and be free of *P aeruginosa*.[99]

A fungal agent that has recently emerged in patients with CF is *Trichosporon*. With the increasing use of antifungal agents to treat disease associated with *Aspergillus* and *Scedosporium*, *Trichosporon,* which is resistant to the antifungals amphotericin and the echinocandins, may find a niche in the CF airway.[100] Fungemia caused by *Trichosporon* has been reported in 2 patients with CF, 1 of whom was a double lung transplant recipient.[101,102] Without using a selective fungal medium, we found it in 0.2% of our patients with CF,[103] and these patients tend to have more rapid decline in lung function. Whether this situation is directly attributable to *Trichosporon* infections is unknown.

Isolation of *Aspergillus, Scedosporium*, and *Trichosporon* can all be accomplished with media used for bacterial isolation. Because of its highly selective nature, BCSA is particularly useful for the isolation of fungi. Conventional techniques are sufficient for identification of these organisms. Susceptibility testing is not recommended.

RESPIRATORY VIRUSES

For many years, viral agents have been considered to play a prime role in the initial steps of establishing chronic lung infections.[15] However, almost all the data to support this theory were based on serologic data, with few culture data to support the theory, in part because of frequent contamination of viral respiratory culture, especially in patients infected with *P aeruginosa*.[15] With the advent of NAAT testing for viruses, a clearer picture of the role of viruses will likely emerge. It is now well recognized that acute exacerbations are associated with both influenza and respiratory syncytial virus.[104–106] One of the most interesting observations was the high rate of detection of rhinovirus, approximately 30% when a multiplex polymerase chain reaction was used.[107,108] Rhinovirus tends to persist in the CF airway, and viral loads increase during pulmonary exacerbations.[108] Using a CF airway epithelial model, it has been shown that rhinovirus superinfection of *P aeruginosa*–infected cells causes a release of planktonic bacteria. This release of planktonic bacteria during viral infection may be an important trigger of CF pulmonary exacerbations.[6]

Diagnosis of CF respiratory viral infections has been revolutionized by the use of NAAT. NAAT diagnostics approved by the US Food and Drug Administration are available for influenza A/B only, the combination of influenza A/B and respiratory syncytial virus (RSV), and multiplex NAAT for adenovirus, coronavirus, influenza viruses A/B, metapneumovirus, parainfluenza virus 1-4, RSV, and rhinovirus.[109–111] Because there have been no evaluations comparing these different methods specifically in patients with CF, the reliability of these methods is unknown in this patient population. Given the unusual nature of the matrix in which the virus may be found, specific evaluation of NAAT performance in these specimens would be useful. A meta-analysis[112] for influenza antigen detection assays suggested that these methods lack sensitivity in patients with low viral loads. Because they have not been evaluated in patients with CF, they cannot be recommended for that patient population.

MICROBIOME CONSIDERATIONS

Microbiome analysis is changing our way of thinking about chronic infections. In patients with CF, we have learned that other organisms beyond those that we have

already discussed may play an important role in CF lung disease. Organisms typically believed as belonging to the normal microflora of the oropharynx, *Prevotella*, *Veillonella*, and the *Streptococcus anginosus* group, are all found in respiratory tract of patients with CF.[5,13] It is speculated that these organisms might upregulate or downregulate virulence genes in pathogens such as *P aeruginosa* to obtain essential nutrients through the activity of these pathogens.[12] Another observation that has arisen as a result of microbiome studies is that microbial communities are remarkably stable, even in the face of repeated rounds of antimicrobial therapy.[11,13] In addition, as chronic *P aeruginosa* infection is established, the diversity of the microbial community declines.[10] Is this a result of *P aeruginosa* being the apex predator in the CF airway microbiome eliminating competitors and adapting itself by downregulating virulence genes to allow long-term survival of its human host? Over the next decade, we should get closer to this answer.

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