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Antibiotic Management of Lung Infections in Cystic Fibrosis: Part I. The Microbiome, MRSA, Gram-Negative Bacteria, and Multiple Infections

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Key Words: Achromobacter species, Burkholderia cepacia, Methicillin-resistant Staphylococcus aureus, Microbiome, Pseudomonas aeruginosa, Stenotrophomonas maltophilia,

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Abstract

Despite significant advances in treatment strategies targeting the underlying defect in cystic fibrosis (CF), airway infection remains an important cause of lung disease. In this two part series, we review recent evidence related to the complexity of CF airway infection, explore data suggesting the relevance of individual microbial species, and discuss current and future treatment options. In Part I, the evidence with respect to the spectrum of bacteria present in the CF airway, known as the lung microbiome is discussed. Subsequently, the current approach to treat methicillin-resistant Staphylococcus aureus (MRSA), Gram-negative bacteria, as well as multiple co-infections is reviewed.

Newer molecular techniques have demonstrated that the airway microbiome consists of a large number of microbes, and the balance between microbes, rather than the mere presence of a single species, may be relevant for disease pathophysiology. A better understanding of this complex environment could help define optimal treatment regimens that target pathogens without affecting others. While relevance of these organisms is unclear, the pathologic consequences of MRSA infection in CF patients have been recently determined. New strategies for eradication and treatment of both acute and chronic infections are discussed. P. aeruginosa plays a prominent role in CF lung disease, but many other non-fermenting Gram-negative bacteria are also found in the CF airway. Many new inhaled antibiotics specifically targeting P. aeruginosa have become available with the hope that they will improve the quality of life for patients. Part I concludes with a discussion of how best to treat patients with multiple co-infections.

Abstract word count: 250
Introduction

Cystic fibrosis (CF) lung disease is characterized by airway obstruction, chronic bacterial infection, and a vigorous host inflammatory response (1). Antibiotic therapy of bacterial lung infections has tremendously contributed to the increased survival in CF (2). However, many bacteria form biofilms in the CF lung that make their eradication difficult (3). In addition, it has also become clear that only a small fraction of the microbes present in the CF airway are being identified with routine laboratory techniques (4, 5), and both extended culture methods and molecular techniques have identified organisms that previously were not routinely cultured (6). Traditional antibiotic susceptibility testing performed on planktonic bacteria has been found to be of limited clinical use in chronic airway infection as most bacteria in the CF lung exist in biofilms (7). While it has long been recognized that patients clinically respond even when their infecting organisms are pan-resistant, 25% of patients do not reach pre-exacerbation values in lung function measures despite aggressive treatment for their bacterial lung infections (8), demonstrating that current treatment is inadequate when addressing the complexity of airway infection. In addition to bacteria identified by routine sputum culture methods, clinicians are often faced with an array of multi-drug resistant organisms that are difficult to treat. In this manuscript and its companion manuscript, we provide a summary of current aspects of airway infection in CF. These manuscripts are derived from a symposium organized by the Scientific Assemblies on Pediatrics and Clinical Problems and presented at the 2013 American Thoracic Society (ATS) International Conference in Philadelphia, PA. In Part I, we discuss the lung microbiome in CF, methicillin-resistant Staphylococcus aureus (MRSA), Gram-negative bacteria, and approaches to treating multiple infections. In Part II, we discuss nontuberculous mycobacteria, anaerobic bacteria, and fungi. The current evidence for treatment of these lung
infections in CF, which we summarized, is limited. Within these documents, we also provide a pragmatic approach as to how one might treat these infections. However, it is important to note that these manuscripts are not meant to represent definitive treatment guidelines or consensus recommendations. For available guideline recommendations, the reader is referred elsewhere in the published literature (9-13).

**The Lung Microbiome in Cystic Fibrosis**

The conventional view of CF airway microbiology has been based on the recovery in culture of a suite of bacterial pathogens, including *S. aureus* and opportunists such as *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, *Achromobacter* species, and *Stenotrophomonas maltophilia*. The Human Microbiome Project, an NIH sponsored initiative launched in 2007, applying *culture-independent* methods to assess bacterial ecology, has significantly broadened this view. Numerous studies now provide compelling evidence that the airways of persons with CF may be inhabited by diverse bacterial communities comprised of dozens of species (14-23). In addition to ‘typical’ CF pathogens, ‘non-pathogenic oral bacteria’, including many obligate and facultative anaerobic species, are often present in densities that well exceed those of the traditional opportunists associated with CF (4). Although most of these studies analyzed expectorated sputum samples that would be expected to be ‘contaminated’ with bacteria residing in the non-sterile oropharynx during expectoration, several lines of evidence indicate that this has only a marginal impact on measures of airway microbiota (17, 24, 25).

Limited studies of children with CF indicate that bacterial community diversity (a measure of both the number and relative abundances of the species present) increases with age (14, 26). In contrast, several cross-sectional and longitudinal studies of adults have shown that
diversity decreases with age and declining lung function (14, 18, 22, 23). Analyses of respiratory specimens from persons with end-stage lung disease or from lung explants show very constrained communities, often limited to a single dominant species (23, 27). These observations suggest that after an initial increase during childhood, airway bacterial diversity peaks in young adulthood, and then declines with advancing age and disease progression (Fig. 1). Antibiotic use may be the primary driver of decreasing diversity with advancing disease (23).

The change in the airway microbiota around the time of exacerbations of pulmonary symptoms is an area of intense interest. Fodor and colleagues (15) showed that bacterial community richness decreased transiently with antibiotic therapy, but rebounded quickly thereafter. Zhao and colleagues (23) similarly showed a significant decrease in diversity with antibiotic therapy of exacerbation, but did not find significant changes in diversity when comparing samples from periods of clinical stability to those taken at the onset of exacerbation symptoms; a finding that was subsequently confirmed in a larger study (28). Interestingly, this latter study showed a decrease in the relative and absolute abundance of *P. aeruginosa* in communities dominated by this species at the onset of symptoms leading to exacerbation.

Thus, our traditional view of CF airway microbiology is changing. Whereas bacterial communities likely become more diverse during childhood, they become increasing confined with advancing lung disease and antibiotic treatment in adulthood. Eventually, a single species representing one of the traditional CF pathogens (*S. aureus*, *P. aeruginosa*, *B. cepacia* complex or *Achromobacter* spp.) dominates the community. Despite this dramatic decrease in diversity, total bacterial density appears to remain rather constant. The dynamics of bacterial communities around the time of exacerbation suggest that the dominant pathogen in the community may decrease with onset of symptoms. As our understanding of the dynamics of airway bacterial
ecology continues to expand, so too will the opportunities to develop novel strategies to better
manage airway infection in CF.

**Methicillin-Resistant S. aureus**

The prevalence of MRSA has increased dramatically over the last decade and now is
detected in the respiratory tract of greater than 25% of patients with CF in the United States (29).
The prevalence of MRSA in Canada and Europe is lower, ranging from 3 to 11% of patients with
CF (30). Chronic MRSA infection in patients with CF is associated with increased rate of lung
function decline, failure to recover lung function after a pulmonary exacerbation, and decreased
survival (8, 31, 32). Currently, there are no conclusive studies demonstrating an effective and
safe treatment protocol for MRSA respiratory infection in CF (33). Here, we provide a practical
framework on how to treat MRSA infection in different clinical scenarios by first describing
antibiotic choices and then subsequently provide guidance for defining patients that require
treatment.

In CF patients infected with MRSA who are experiencing an acute pulmonary
exacerbation, vancomycin and linezolid are the first-line antimicrobial choices (34, 35). Dosing
of vancomycin is based on the patient’s weight and creatinine clearance. A trough concentration
of 15 to 20 mcg/mL, which is the practice of most CF physicians based on a recent survey (36),
should be the considered target. Linezolid dosing may need adjustment in children with CF (37),
but not adults (38). Because approximately 80% of CF patients with chronic MRSA may also be
infected with *P. aeruginosa*, linezolid, rather than vancomycin with its associated renal effects,
may be a good option in these patients who require anti-*Pseudomonal* treatment with other
nephrotoxic drugs such as aminoglycosides. As depression is detected in greater than 20% of
adults with CF (29), it is important to note that linezolid can be associated with the serotonin syndrome in CF patients taking serotonergic psychiatric drugs (39). Chronic linezolid use may also be associated with the development of potentially irreversible peripheral and optic neuropathies and linezolid resistant MRSA (40). In patients with allergies or contraindications to the above medications, alternative antibiotics include rifampin, fucidin (not available in the United States), ceftaroline, tigecycline, chloramphenicol, and/or clindamycin.

The approach to CF patients with MRSA infection seen in the outpatient clinic has recently been reviewed (30). Doe and colleagues (41) reported that patient segregation and aggressive antibiotic eradication therapy can achieve eradication in the majority of patients with CF. Numerous antibiotic regimens were used; however, the most successful were those regimens that included two oral antibiotics (one of which was rifampin) and nebulized vancomycin. Rifampin has been a component of successful MRSA eradication protocols due to its high mucosal concentrations and activity against biofilms, but it should be used in combination with another antibiotic as resistance develops quickly with monotherapy (30). Rifampin can also be associated with worsening gastroesophageal reflux and decreased efficacy of oral contraceptives (42).

Inhaled antibiotics have been used as a treatment for CF respiratory tract infections since penicillin first became available (43). Fosfomycin tobramycin (FTI) for inhalation has activity against anaerobic, Gram-negative, and Gram-positive bacteria including MRSA. In a clinical trial of FTI in CF patients with *P. aeruginosa* infection, 29 patients with MRSA at baseline had significant decreases in the concentration of MRSA after 28 days of FTI (n=19) compared to placebo (n=10) (44). Current published experience with aerosolized vancomycin suggests that it is safe and well tolerated (45, 46). There are two ongoing studies investigating the use of inhaled
vancomycin, including one assessing a novel dry powder formulation (47, 48). The results of these trials will help to delineate the risks and benefits of treating chronic MRSA infection. Because there are no definitive studies to guide the decision to treat (or not to treat) MRSA infections in CF, the following approach is based on uncontrolled studies and anecdote.

The MRSA population can be split into four groups: 1) new MRSA infection in an asymptomatic patient, 2) new MRSA infection in a symptomatic patient, 3) chronic MRSA infection in an asymptomatic patient, and 4) chronic MRSA infection in a symptomatic patient. There is no standard definition for chronic MRSA infection, but previous and ongoing studies typically have defined chronic infection as having at least 3 MRSA positive cultures within the previous 6-12 months (32, 47, 48). The most straightforward decision for treatment occurs in those patients in whom MRSA is cultured from the respiratory tract and who are also experiencing an acute pulmonary exacerbation. Ninety-eight percent of CF providers in the US who responded to a survey regarding MRSA treatment stated they would give oral or IV antibiotics in this situation (36). The advantages and disadvantages of various treatment regimens are detailed in the above paragraphs.

Many CF providers have wondered if there is a role for eradication of respiratory-tract MRSA infection. Arguments for recommending and withholding systemic therapy can be made for eradication of a new MRSA infection. Previous studies have suggested that 1/3 of new MRSA infections may subsequently clear; suggesting the risks of treatment may not outweigh the benefits (32). However, the easiest time to eradicate MRSA is most likely when it is first cultured before it becomes entrenched in the lung. Unfortunately, at the time of first culture, it is not possible to determine which patients will clear spontaneously and which will progress to chronic MRSA infection. For these reasons, one approach to a new MRSA infection is to
perform an eradication attempt with oral antibiotics. Because MRSA is often found outside of the respiratory tract, an eradication attempt may also include treatment with nasal mupirocin and chlorhexidine or bleach baths. Interestingly, in contrast to *P. aeruginosa*, there is some evidence that chronic MRSA may be eradicated from the respiratory tract (41). Given that oral antibiotics alone may not be enough to eradicate chronic MRSA, the addition of inhaled antibiotics targeting MRSA also may be considered.

The most difficult to treat patients with MRSA are those who are chronically infected and who do not have enough symptoms to trigger the administration of IV antibiotics, but who have persistent respiratory symptoms. These patients may respond temporarily to repeated courses of oral antibiotics, but eventually this treatment may become associated with decreased efficacy, resistance, and/or side effects. One suggested approach is to administer 250mg of the IV formulation of vancomycin reconstituted in 5cc of sterile water via nebulization mist treatment (48). The patient inhales the medication twice daily for 28 days. Albuterol is often inhaled prior to the administration of the antibiotic, although a pilot study did not demonstrate that bronchospasm was a significant issue (46). At the conclusion of the 28-day treatment period, a repeat culture is obtained to determine if MRSA can still be detected in the respiratory tract. If the patient becomes symptomatic when not taking inhaled vancomycin, and has not eradicated MRSA, then suppressive treatment with either every other month or continuous inhaled vancomycin may be given. Again, these are potential options for patients with new or chronic MRSA infection while we are awaiting the results of ongoing clinical trials that will further inform treatment decisions (47-49).

**Gram-Negative Bacteria**
As individuals with CF age, their airways become more frequently infected with Gram-negative bacteria. In the United States, the overall prevalence of pulmonary infection with multidrug resistant \textit{P. aeruginosa}, \textit{S. maltophilia} and \textit{B. cepacia} complex in CF patients is 9%, 14% and 3%, respectively (29). Infection with these Gram-negative organisms is associated with poorer clinical outcomes, such as rapid lung function decline, increased risk of pulmonary exacerbation and greater rates of mortality or need for lung transplantation (50-55).

\textit{P. aeruginosa}, \textit{B. cepacia} complex and \textit{S. maltophilia} are found in the environment and have consequently developed ways of surviving in harsh milieus with exposure to naturally occurring antimicrobials. Treatment is thus difficult due to their impressive array of antimicrobial resistance mechanisms, which include efflux pumps, chromosomally encoded \(\beta\)-lactamases, decreased outer membrane permeability and biofilm formation(56). Given these numerous mechanisms of antimicrobial resistance, these bacteria are deemed resistant to drugs such as aminoglycosides, \(\beta\)-lactams and fluoroquinolones by \textit{in vitro} testing according to Clinical Laboratory Standards Institute (CLSI) guidelines(57). However, aerosolized antibiotics can yield higher sputum concentrations, in areas of the lung that remain well ventilated, through direct delivery to the site of infection (\textbf{Table 1}) (58-65). There is a relationship between the maximal drug concentration achieved and the minimum inhibitory concentration (MIC) required to inhibit bacterial growth, with higher ratios associated with greater reduction in bacterial density (66).

Therefore, newer inhaled antibiotics, herein discussed, have the potential to be used as chronic suppressive treatment for pathogens traditionally considered resistant to these agents.

One of the new inhalational antibiotics available is tobramycin inhalation powder (TIP) delivered by the podhaler device. TIP has been shown to result in comparable increases in forced expiratory volume in 1 second (FEV\(_1\)) and decreases in hospitalization as tobramycin inhalation...
solution (TIS) in the treatment of chronic *P. aeruginosa* in CF patients (67). However, TIP can achieve up to 1.5-2 fold higher sputum tobramycin concentrations (up to 2,000 µg/g) than TIS. *In vitro* studies of 180 *B. cepacia* complex and 103 *S. maltophilia* isolates demonstrated an MIC$_{50}$ of 100 µg/ml, tested by planktonic and biofilm growth (68). This suggests that a \( C_{\text{max}}/\text{MIC} \) ratio of up to 20 may be achievable with TIP treatment of these pathogens. Clinical trials of TIP in CF patients with *B. cepacia* complex and *S. maltophilia* infection to decrease sputum bacterial density are planned.

Inhaled aztreonam solution is another aerosolized antimicrobial for the treatment of chronic *P. aeruginosa* in CF. Non-inferiority studies have shown that it is comparable, if not superior, to TIS in non-treatment naive individuals with respect to increases in lung function (69). When used in trials for CF patients with chronic *B. cepacia* complex infection, however, inhaled aztreonam did not result in any statistically significant improvement in FEV$_1$ or decreases in sputum bacterial density compared to placebo (70). The ability of \( \beta \)-lactam antibiotics to function in the CF lung could be limited by the slow, anaerobic, biofilm growth of organisms (71). *In vitro* studies of biofilm growth of *P. aeruginosa* on CF airway cells have demonstrated little additional benefit of aztreonam in combination with tobramycin, likely due to bacterial exopolysaccharide production causing tolerance to aztreonam (72). In addition, in an ongoing clinical trial of biofilm susceptibility testing of over 1,000 clinical *P. aeruginosa* CF isolates, the percentage of \( \beta \)-lactam susceptible isolates was reduced when grown as a biofilm compared to planktonically. These data suggest that, despite the known limitations of antimicrobial susceptibility testing in CF, this class of antimicrobials may be less effective in this context (73).
Finally, studies of aerosolized levofloxacin have demonstrated improvements in lung function (8.7% increase in FEV$_1$ vs. placebo) and decreases in bacterial pulmonary burden (0.96 log difference in density vs. placebo) in *P. aeruginosa* infected patients with CF (74).

Levofloxacin is a second generation fluoroquinolone that in addition to having anti-*P. aeruginosa* effects has activity against *S. maltophilia* (56). In an *in vitro* study of a large number of clinical *S. maltophilia* CF isolates, levofloxacin, at levels achievable by inhalation, was the most active antibiotic alone and in combination, against *S. maltophilia* grown as a biofilm or planktonically (75). In addition to achieving high levels of drug in the lung (4,000 µg/g), levofloxacin also has anti-inflammatory effects (58, 76). Inhaled levofloxacin may thus be an effective chronic suppressive antimicrobial therapy in CF patients with chronic *S. maltophilia* infection and warrants further investigation as it may have utility beyond the treatment of *P. aeruginosa* infection.

The treatment of multidrug resistant Gram-negative bacteria in CF patients with advanced lung disease is challenging given the intrinsic resistance of these organisms to antimicrobials of several different classes. Engaging a microbiologist and/or infectious disease expert in a discussion about potential therapeutic options for these patients may thus be fruitful.

**Treating Multiple Infections**

The recognition that there is a diverse microbiota in sputum samples from people with CF raises questions about how we approach antibiotic therapy. Conventional bacterial culture in aerobic conditions allows isolation of a limited number of organisms. Extended culture methods identify a much wider range of bacteria, which include more difficult to culture bacteria such as anaerobic bacteria (4, 15, 23). At present, there is no readily available methodology to identify
all of these organisms in a way that makes this information valuable for clinical treatment (77).

Studies are under way to develop technologies to allow molecular identification without prior
culture (78). The choice of antibiotics for pulmonary exacerbations associated with multiple
bacteria is an area that has not been extensively studied. A number of different oral and
intravenous antibiotics may be combined to tailor antibiotic therapy as best possible to particular
combinations of positive bacterial culture results. Antimicrobial susceptibility testing with single
agents or synergy protocols for *P. aeruginosa* and *B. cepacia* complex organisms are not helpful
as they do not predict response to treatment (79-81). However, treatment with antibiotics for a
pulmonary exacerbation to which the main bacterial species is resistant is associated with
treatment failure (82). These studies have been observational, and there are few randomized
controlled trials to help in choosing antibiotics for pulmonary exacerbations. The choice is
largely empirical and based upon the experience of the physician, patient and previous
occurrence of drug allergy. In addition, there are no data to suggest that this also applies to other
bacteria cultured in CF sputum. The dosages and side effects of common antibiotics used to treat
MRSA and Gram-negative bacteria are provided in Table 2.

Combinations of organisms that are commonly encountered with *P. aeruginosa* are *S.
aureus, H. influenzae, S. maltophilia, B. cepacia* complex and *Achromobacter* spp. Other
combinations can occur, and studies describing the airway microbiome indicate that co-infection
is common and often complex. Two or more organisms may be cultured in approximately 25%
of sputum samples. Table 3 indicates the susceptibility of these bacteria to antibiotics used to
treat *P. aeruginosa*. These susceptibilities are a guide to consider combinations of IV and oral
antibiotics for pulmonary exacerbations where multiple bacteria are present to maximize the
appropriateness of antibiotic choice against the organisms isolated. As molecular diagnostics for
a wider range of bacteria in the CF airway microbiome become available, clinical trials will be
needed to better inform the choice of antibiotics for long-term bacterial suppression and
treatment (6).

Summary

MRSA and *P. aeruginosa* are two of the most prevalent bacteria isolated from CF sputum
from patients in the United States (29). In addition, several Gram-negative bacteria, which
rapidly become resistant to multiple antibiotics, have been described over the last several years.
These bacteria are often difficult to treat, and have garnered much attention from clinicians,
investigators, and pharmaceutical companies with respect to the development of drugs for the
treatment of acute exacerbations and chronic suppression. Antibiotics have been the cornerstone
of CF care for decades (11). However, the frequent use of antibiotics likely alters the host’s
microbiota with yet poorly defined consequences. Because of this selective pressure and with
the advent of new laboratory isolation techniques, many previously unrecognized
microorganisms are being identified from CF lung secretions. The pathogenic significance of
many of these microorganisms is still unknown. This information is important to the CF
clinician and patient, as we need to understand which organisms to treat whereas treatment of
other organisms may actually be detrimental by enabling pathogenic bacteria to expand. As
antimicrobials will likely remain a cornerstone of CF therapy far into the future, research into CF
lung microbiology must continue until that time when the disease is cured and its associated
airway infection is eradicated.
Acknowledgements

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References

1. Chmiel JF, Berger M, Konstan MW. The role of inflammation in the pathophysiology of

2. Szaff M, Hoiby N, Flensborg EW. Frequent antibiotic therapy improves survival of cystic
   fibrosis patients with chronic Pseudomonas aeruginosa infection. Acta Paediatr Scand

3. Chmiel JF, Davis PB. State of the art: Why do the lungs of patients with cystic fibrosis
   become infected and why can't they clear the infection? Respir Res 2003;4:8-21.

4. Tunney MM, Field TR, Moriarty TF, Patrick S, Doering G, Muhlebach MS, Wolfgang
   MC, Boucher R, Gilpin DF, McDowell A, Elborn JS Detection of anaerobic bacteria in high
   numbers in sputum from patients with cystic fibrosis. Am J Respir Crit Care Med
   2008;177(9):995-1001.

5. Tunney MM, Klem ER, Fodor AA, Gilpin DF, Moriarty TF, McGrath SJ, Muhlebach
   MS, Boucher RC, Cardwell C, Doering G, Elborn JS, Wolfgang MC. Use of culture and
   molecular analysis to determine the effect of antibiotic treatment on microbial community
   diversity and abundance during exacerbation in patients with cystic fibrosis. Thorax

6. LiPuma J. The new microbiology of cystic fibrosis: It takes a community. Thorax

7. Waters V, Ratjen F. Standard versus biofilm antimicrobial susceptibility testing to guide


in infancy: Interaction between intestinal and respiratory tracts and impact of nutritional

27. Goddard AF, Staudinger BJ, Dowd SE, Joshi-Datar A, Wolcott RD, Aitken ML, Fligner
CL, Singh PK. Direct sampling of cystic fibrosis lungs indicates that DNA-based analyses of
upper-airway specimens can misrepresent lung microbiota. *Proc Natl Acad Sci U S A*

28. Carmody LA, Zhao J, Schloss PD, Petrosino JF, Murray S, Young VB, Li JZ, LiPuma JJ.
Changes in cystic fibrosis airway microbiota at pulmonary exacerbation. *Ann Am Thorac Soc*


31. Dasenbrook EC, Checkley W, Merlo CA, Konstan MW, Lechtzin N, Boyle MP.
Association between respiratory tract methicillin-resistant *Staphylococcus aureus* and survival in
cystic fibrosis. *JAMA* 2010;303(23):2386-2392.

32. Dasenbrook EC, Merlo CA, Diener-West M, Lechtzin N, Boyle MP. Persistent
methicillin-resistant *Staphylococcus aureus* and rate of FEV$_1$ decline in cystic fibrosis. *Am J

33. Lo DK, Hurley MN, Muhlebach MS, Smyth AR. Interventions for the eradication of
methicillin-resistant *Staphylococcus aureus* (MRSA) in people with cystic fibrosis. *Cochrane

34. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer
AW, Levine DP, Murray BE, J Rybak M, Talan DA, Chambers HS. Clinical practice guidelines


66. LiPuma JJ. Microbiological and immunologic considerations with aerosolized drug delivery. *Chest* 2001;120(3 Suppl):118S-123S.


Figure Legends

**Figure 1.** Schematic representation of airway bacterial community diversity versus patient age or lung disease severity. Available data suggest that after an initial increase during childhood, airway bacterial diversity peaks in young adulthood, and then declines with advancing age and lung disease progression. At end-stage disease, bacterial communities may be dominated by a single species, most often a “typical” CF opportunistic pathogen.
## Tables

### Table 1. Serum and sputum antibiotic concentrations

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<th>Drug</th>
<th>Mean peak serum concentrations (µg/ml)</th>
<th>Mean peak sputum concentrations (µg/g)</th>
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<tr>
<td><strong>Tobramycin</strong></td>
<td></td>
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<tr>
<td>• Intravenous (8 mg/kg/day) (range)</td>
<td>29.4 (23.1-35.5)</td>
<td>3.88 (1.8-5.7)</td>
</tr>
<tr>
<td>• Aerosolized</td>
<td></td>
<td></td>
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<tr>
<td>Solution (300mg) (+SD)</td>
<td>1.04±0.58</td>
<td>737±1,028</td>
</tr>
<tr>
<td>Powder (112 mg) (+SD)</td>
<td>1.02±0.53</td>
<td>1,048±1,080</td>
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<tr>
<td><strong>Amikacin</strong></td>
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<td>• Intravenous (35 mg/kg) (+SD)</td>
<td>121.4±37.3</td>
<td>10.95±7.55</td>
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<tr>
<td>• Aerosolized (560 mg) (+SD)</td>
<td>1.29±0.77</td>
<td>2,286 (11.6-11,220)</td>
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<td><strong>Levofloxacin</strong></td>
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<tr>
<td>• Oral (500 mg)</td>
<td>6.5</td>
<td>5.1</td>
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<td>• Aerosolized (240 mg) (+SD)</td>
<td>1.71±0.62</td>
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<td><strong>Aztreonam</strong></td>
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<td>• Intravenous (2 g)</td>
<td>80.1</td>
<td>5.2</td>
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<td>• Aerosolized (75 mg) (range)</td>
<td>0.622 (0.31-1.7)</td>
<td>537 (0.2-3,010)</td>
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<td><strong>Colistimethate (Colistin)</strong></td>
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<tr>
<td>• Intravenous (7mg/kg/day) (+SD)</td>
<td>23±6</td>
<td>N/A</td>
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<td>• Aerosolized (2 million units) (+SEM)</td>
<td>0.178±0.018</td>
<td>40±5</td>
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SD: standard deviation; SEM: standard error of the mean
Table 2.§ Empiric antibiotic therapy for the treatment of difficult pulmonary bacterial infections in CF

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<tr>
<th>Organism</th>
<th>Antibiotic</th>
<th>Pediatric Dose</th>
<th>Adult Dose</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-positive organisms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
<td>Vancomycin</td>
<td>15 mg/kg intravenously every 6h</td>
<td>1 g intravenously every 12h</td>
<td>-Oto/nephro toxicity, red man syndrome</td>
</tr>
<tr>
<td></td>
<td>OR Linezolid</td>
<td>if &lt;11 yrs: 10 mg/kg intravenously or orally every 8h</td>
<td>600 mg intravenously or orally every 12h</td>
<td>-Optic/peripheral neuropathy, myelosuppression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>if &gt;11 yrs: 10 mg/kg intravenously or orally every 12h</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gram-negative organisms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Tobramycin*</td>
<td>10 mg/kg intravenously every 24h</td>
<td>10 mg/kg intravenously every 24h</td>
<td>-Ototoxicity, nephrotoxicity</td>
</tr>
<tr>
<td></td>
<td>OR Amikacin**</td>
<td>30 mg/kg intravenously every 24h</td>
<td>30 mg/kg intravenously every 24h</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OR Colistin (colistimethate sodium)</td>
<td>8 mg/kg/day intravenously divided every 8h</td>
<td>8 mg/kg/day intravenously divided every 8h (max 480 mg/day)</td>
<td>-Nephrotoxicity, neurotoxicity</td>
</tr>
<tr>
<td></td>
<td>PLUS (choose one):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ticarcillin/ clavulanate</td>
<td>100 mg/kg of ticarcillin component intravenously every 6h</td>
<td>3 g of ticarcillin component intravenously every 6h</td>
<td>-GI, rash, hepatitis, neutropenia</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime</td>
<td>50 mg/kg intravenously every 6h</td>
<td>2 g intravenously every 8h††</td>
<td>-GI, rash</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
<td>40 mg/kg intravenously every 8h</td>
<td>2 g intravenously every 8h††</td>
<td>GI, rash, hepatitis, neutropenia</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>400 mg intravenously</td>
<td></td>
<td>-GI, rare seizure,</td>
</tr>
<tr>
<td><strong>Burkholderia cepacia complex</strong></td>
<td><strong>Meropenem</strong></td>
<td>15 mg/kg intravenously or 20 mg/kg orally every 12h</td>
<td>or 750 mg orally every 12h</td>
<td>tendinopathy</td>
</tr>
<tr>
<td>---------------------------------</td>
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<td>--------------------------------------------------</td>
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<td>--------------</td>
</tr>
<tr>
<td>PLUS (choose 1):</td>
<td><strong>40 mg/kg intravenously every 8h</strong></td>
<td><strong>2 g intravenously every 8h</strong></td>
<td>-GI, rash, hepatitis, neutropenia</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>50 mg/kg intravenously every 6h</strong></td>
<td><strong>2 g intravenously every 8h</strong></td>
<td>-GI, rash</td>
<td></td>
</tr>
<tr>
<td><strong>Ceftazidime</strong></td>
<td><strong>15-20 mg/kg intravenously every 6h</strong></td>
<td><strong>1 g intravenously every 6h</strong></td>
<td>-Bone marrow suppression/failure</td>
<td></td>
</tr>
<tr>
<td><strong>Chloramphenicol°</strong></td>
<td><strong>4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h</strong></td>
<td><strong>4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h</strong></td>
<td>-GI, hypersensitivity, neutropenia, serum sickness</td>
<td></td>
</tr>
<tr>
<td><strong>Trimethoprim/ sulfamethoxazole</strong></td>
<td><strong>50 mg/kg intravenously every 8h</strong></td>
<td><strong>2 g intravenously every 8h</strong></td>
<td>-GI, rash</td>
<td></td>
</tr>
<tr>
<td><strong>Aztreonam</strong></td>
<td><strong>50 mg/kg intravenously every 8h</strong></td>
<td><strong>2 g intravenously every 8h</strong></td>
<td>-GI, rash</td>
<td></td>
</tr>
<tr>
<td><strong>Stenotrophomonas maltophilia</strong></td>
<td><strong>Trimeo</strong></td>
<td><strong>4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h</strong></td>
<td><strong>4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h</strong></td>
<td>-GI, hypersensitivity, neutropenia, serum sickness</td>
</tr>
<tr>
<td>PLUS (choose 1):</td>
<td><strong>4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h</strong></td>
<td><strong>4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h</strong></td>
<td>-GI, rash, hepatitis, neutropenia</td>
<td></td>
</tr>
<tr>
<td><strong>Ticarcillin/ clavulanate</strong></td>
<td><strong>100 mg/kg of ticarcillin component intravenously every 6h</strong></td>
<td><strong>3 g of ticarcillin component intravenously every 6h</strong></td>
<td>-GI, rarely seizure, tendinopathy</td>
<td></td>
</tr>
<tr>
<td><strong>Levofloxacin</strong></td>
<td>if &lt;5yrs: <strong>10 mg/kg intravenously or orally every 12h</strong></td>
<td><strong>500-750 mg intravenously or orally once daily</strong></td>
<td>-GI, photosensitivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>if &gt;5 yrs: <strong>10 mg/kg intravenously or orally once daily</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Doxycycline†</strong></td>
<td><strong>2 mg/kg intravenously or orally every 12h</strong></td>
<td><strong>100 mg intravenously or orally every 12h</strong></td>
<td>-GI, photosensitivity</td>
<td></td>
</tr>
<tr>
<td><strong>GI:</strong> gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>§ The antibiotic doses given in this table come from a compilation of sources and practice patterns including commonly prescribed off-label doses and uses. Sources include the pharmacy formulary of The Hospital for Sick Children in Toronto, ON, which is based upon product inserts and the published literature. The doses given are general guidelines, and may vary somewhat between institutions. It is recommended that the clinician consult his/her institution's pharmacy, product inserts, and published literature before prescribing these drugs.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Serum concentrations should be monitored and aim for a maximum serum concentration (Cmax) in the range of 20–40 mg/L with a minimum serum concentration (Cmin) of &lt;1 mg/L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>** Serum concentrations should be monitored and aim for a maximum serum concentration (Cmax) in the range of 80–120 mg/L with a minimum serum concentration (Cmin) of &lt;1 mg/L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>° Serum concentrations should be monitored; the peak concentration ranges from 15-25 µg/ml and the trough from 5-15 µg/ml.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>† Should not be given to children less than 8 years of age.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>†† Continuous infusion of ceftazidime may be considered in cases of clinical failure or for the treatment of multi-drug resistant <em>P. aeruginosa</em> in order to maximize the time above the minimum inhibitory concentration (MIC).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Tigecycline</th>
<th>1.2 mg/kg intravenously every 12h</th>
<th>50mg intravenously every 12h</th>
<th>-Gi, cholestasis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Achromobacter species</strong></td>
<td>Meropenem</td>
<td>40 mg/kg intravenously every 8h</td>
<td>2 g intravenously every 8h</td>
<td>-Gi, rash, hepatitis</td>
</tr>
<tr>
<td>OR</td>
<td>Imipenem</td>
<td>15-25 mg/kg intravenously every 6h</td>
<td>500 mg-1 g intravenously every 6h</td>
<td>-Gi, rarely seizures</td>
</tr>
<tr>
<td>PLUS (choose 1):</td>
<td>Trimethoprim/sulfamethoxazole</td>
<td>4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h</td>
<td>4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h</td>
<td>-Gi, hypersensitivity, neutropenia, serum sickness</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>15 mg/kg intravenously or 20 mg/kg orally every 12h</td>
<td>400 mg intravenously or 750 mg orally every 12h</td>
<td>-Gi, rarely seizure, tendinopathy</td>
</tr>
<tr>
<td>Minocycline†</td>
<td>2 mg/kg intravenously or orally every 12h</td>
<td>100 mg orally every 12h</td>
<td>-Gi, photosensitivity</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Typical susceptibilities to anti-pseudomonal antibiotics of bacteria commonly cultured from the CF airway

A. Commonly used antibiotics to treat *P. aeruginosa* infection in the CF airway

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Antibiotic</th>
<th>CAZ</th>
<th>PIP/TAZ</th>
<th>MER</th>
<th>AZT</th>
<th>TOB</th>
<th>COL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. maltophilia</em></td>
<td></td>
<td>✓</td>
<td>+/-</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>✓</td>
</tr>
<tr>
<td><em>Achromobacter</em> spp.</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>+/-</td>
</tr>
<tr>
<td>BCC</td>
<td></td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>----</td>
<td>----</td>
<td>+/-</td>
</tr>
<tr>
<td>MSSA</td>
<td></td>
<td>+/-</td>
<td>✓</td>
<td>✓</td>
<td>----</td>
<td>✓</td>
<td>----</td>
</tr>
<tr>
<td>MRSA</td>
<td></td>
<td>----</td>
<td>✓</td>
<td>----</td>
<td>----</td>
<td>✓</td>
<td>----</td>
</tr>
<tr>
<td><em>Streptococci</em></td>
<td></td>
<td>+/-</td>
<td>✓</td>
<td>✓</td>
<td>----</td>
<td>✓</td>
<td>----</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>+/-</td>
<td>✓</td>
<td>----</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Anaerobes</td>
<td></td>
<td>----</td>
<td>✓</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

B. Less Commonly used antibiotics to treat *P. aeruginosa* infection in the CF airway

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Antibiotic</th>
<th>CO-T</th>
<th>DOX</th>
<th>CHL</th>
<th>FOS</th>
<th>TIG</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. maltophilia</em></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>+/-</td>
<td>----</td>
<td>✓</td>
</tr>
<tr>
<td><em>Achromobacter</em> spp.</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>+/-</td>
<td>----</td>
<td>✓</td>
</tr>
<tr>
<td>BCC</td>
<td></td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>MSSA</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>MRSA</td>
<td></td>
<td>+/-</td>
<td>✓</td>
<td>----</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><em>Streptococci</em></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>+/-</td>
<td>✓</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td>----</td>
<td>----</td>
<td>+/-</td>
<td>✓</td>
<td>----</td>
</tr>
<tr>
<td>Anaerobes</td>
<td></td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>✓</td>
</tr>
</tbody>
</table>

✓: in vitro susceptibility; +/-: borderline susceptibility; ----: resistance not known; CAZ: ceftazidime; PIP/TAZ: piperacillin-tazobactam; MER: meropenem; AZT: aztreonam; TOB: tobramycin; COL: Colistimethate; CO-T: co-trimoxazole; DOX: doxycycline; CHL: chloramphenicol; FOS: fosfomycin; TIG: tigicycline; BCC: *Burkholderia cepacia* complex; MSSA: methicillin-sensitive *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*
Figures

Fig. 1

Dynamic Polymicrobial Communities

Increasing

Community Diversity

S. aureus,
H. influenzae
....others

P. aeruginosa,
Burkholderia,
Achromobacter

Increasing

Patient Age or
Lung Disease Severity