ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

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

James F. Chmiel[1], Timothy R. Aksamit[2], Sanjay H. Chotirmall[3], Elliott C. Dasenbrook[4], J. Stuart Elborn[5], John J. LiPuma[6], Sarath C. Ranganathan[7], Valerie J. Waters[8], Felix A. Ratjen[9]

[1]Department of Pediatrics, Case Western Reserve University School of Medicine and Rainbow Babies and Children's Hospital, Cleveland, OH, USA; [2]Department of Medicine, Division of Pulmonary Disease and Critical Care Medicine, Mayo Clinic, Rochester, MN, USA; [3]Department of Respiratory Medicine, Beaumont Hospital, Dublin, Republic of Ireland and Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore; [4]Departments of Medicine and Pediatrics, Case Western Reserve University School of Medicine and University Hospitals Case Medical Center, Rainbow Babies and Children's Hospital, Cleveland, Ohio, USA; [5]Medicine and Surgery, Queens University Belfast and Belfast City Hospital, Belfast, Northern Ireland, UK; [6]Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, MI, USA; [7]Department of Respiratory Medicine, Royal Children's Hospital, Infection and Immunity Theme, Murdoch Children's Research Institute, Department of Pediatrics, University of Melbourne, Melbourne, Australia; [8]Division of Infectious Diseases, Department of Pediatrics, University of Toronto and The Hospital for Sick Children, Toronto, ON, CAN; [9]Division of Respiratory Medicine, Department of Pediatrics, University of Toronto and The Hospital for Sick Children, Toronto, ON, CAN

Corresponding Author

James F. Chmiel, M.D., M.P.H.

Division of Pediatric Pulmonology and Allergy/Immunology, Room 3001

Rainbow Babies and Children's Hospital

11100 Euclid Avenue

Cleveland, OH 44106

Telephone: (216) 844-3267

Facsimile: (216) 844-5916

E-Mail: james.chmiel@uhhospitals.org

Running Title: Difficult Bacterial Lung Infections in CF

Sources of Support: Grant support from the National Institutes of Health Grant P30-DK27651 and the U.S. Cystic Fibrosis Foundation is gratefully acknowledged.

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

Revised Submission

25 June 2014

Word Count: 3471

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

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

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

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

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

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

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

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

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

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 multi-drug resistant *P. aeruginosa*, *S. maltophilia* and *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).

P. aeruginosa, *B. cepacia* complex and *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 ß-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, ß-lactams and fluoroquinolones by *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 (**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_{max}/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 ß-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 ß-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).

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

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

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

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

The authors wish to acknowledge Jay Hilliard for his help with editing and refining the figures and tables.

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

References

1. Chmiel JF, Berger M, Konstan MW. The role of inflammation in the pathophysiology of cf lung disease. *Clin Rev Allergy Immunol* 2002;23(1):5-27.

2. Szaff M, Hoiby N, Flensborg EW. Frequent antibiotic therapy improves survival of cystic fibrosis patients with chronic *Pseudomonas aeruginosa* infection. *Acta Paediatr Scand* 1983;72(5):651-657.

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* 2011;66(7):579-584.

6. LiPuma J. The new microbiology of cystic fibrosis: It takes a community. *Thorax* 2012;67(10):851-852.

7. Waters V, Ratjen F. Standard versus biofilm antimicrobial susceptibility testing to guide antibiotic therapy in cystic fibrosis. *Cochrane Database Syst Rev* 2012;11:CD009528.

8. 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(5):627-632.

9. Doring G, Conway SP, Heijerman HG, Hodson ME, Hoiby N, Smyth A, Touw DJ. Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: A European consensus. *Eur Respir J* 2000;16(4):749-767.

10. Doring G, Flume P, Heijerman H, Elborn JS, Consensus Study Group. Treatment of lung infection in patients with cystic fibrosis: Current and future strategies. *J Cyst Fibros* 2012;11(6):461-479.

11. Flume PA, Mogayzel PJ, Jr., Robinson KA, Goss CH, Rosenblatt RL, Kuhn RJ, Marshall BC, Clinical Practice Guidelines for Pulmonary Therapies Committee. Cystic fibrosis pulmonary guidelines: Treatment of pulmonary exacerbations. *Am J Respir Crit Care Med* 2009;180(9):802-808.

12. Flume PA, O'Sullivan BP, Robinson KA, Goss CH, Mogayzel PJ, Jr., Willey-Courand DB, Bujan J, Finder J, Lester M, Quittell L, Rosenblatt R, Vender RL, Hazle L, Sabadosa K, Marshall B; 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.

13. Mogayzel PJ, Jr., Naureckas ET, Robinson KA, Mueller G, Hadjiliadis D, Hoag JB, Lubsch L, Hazle L, Sabadosa K, Marshall B, Pulmonary Clinical Practice Guidelines Committee. Cystic fibrosis pulmonary guidelines. Chronic medications for maintenance of lung health. *Am J Respir Crit Care Med* 2013;187(7):680-689.

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

14. Cox MJ, Allgaier M, Taylor B, Baek MS, Huang YJ, Daly RA, Karaoz U, Andersen GL, Brown R, Fujimura KE, Wu B, Tran D, Koff J, Kleinhenz ME, Nielson D, Brodie EL, Lynch SV. Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. *PLoS One* 2010;5(6):e11044.

15. Fodor AA, Klem ER, Gilpin DF, Elborn JS, Boucher RC, Tunney MM, Wolfgang MC. The adult cystic fibrosis airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations. *PLoS One* 2012;7(9):e45001.

16. Guss AM, Roeselers G, Newton IL, Young CR, Klepac-Ceraj V, Lory S, Cavanaugh CM. Phylogenetic and metabolic diversity of bacteria associated with cystic fibrosis. *ISME J* 2011;5(1):20-29.

17. Harris JK, De Groote MA, Sagel SD, Zemanick ET, Kapsner R, Penvari C, Kaess H, Deterding RR, Accurso FJ, Pace NR. 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.

18. Klepac-Ceraj V, Lemon KP, Martin TR, Allgaier M, Kembel SW, Knapp AA, Lory S, Brodie EL, Lynch SV, Bohannan BJ, Green JL, Maurer BA, Kolter R. Relationship between cystic fibrosis respiratory tract bacterial communities and age, genotype, antibiotics and *Pseudomonas aeruginosa*. *Environ Microbiol* 2010;12(5):1293-1303.

19. 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.

365 20. Sibley CD, Parkins MD, Rabin HR, Duan K, Norgaard JC, Surette MG. A polymicrobial
366 perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients.
367 *Proc Natl Acad Sci U S A* 2008;105(39):15070-15075.
368 21. Stressmann FA, Rogers GB, van der Gast CJ, Marsh P, Vermeer LS, Carroll MP,
369 Hoffman L, Daniels TW, Patel N, Forbes B, Bruce KD. Long-term cultivation-independent
370 microbial diversity analysis demonstrates that bacterial communities infecting the adult cystic
371 fibrosis lung show stability and resilience. *Thorax* 2012;67(10):867-873.
372 22. van der Gast CJ, Walker AW, Stressmann FA, Rogers GB, Scott P, Daniels TW, Carroll
373 MP, Parkhill J, Bruce KD. Partitioning core and satellite taxa from within cystic fibrosis lung
374 bacterial communities. *ISME J* 2011;5(5):780-791.
375 23. Zhao J, Schloss PD, Kalikin LM, Carmody LA, Foster BK, Petrosino JF, Cavalcoli JD,
376 VanDevanter DR, Murray S, Li JZ, Young VB, LiPuma JJ. Decade-long bacterial community
377 dynamics in cystic fibrosis airways. *Proc Natl Acad Sci U S A* 2012;109(15):5809-5814.
378 24. Filkins LM, Hampton TH, Gifford AH, Gross MJ, Hogan DA, Sogin ML, Morrison HG,
379 Paster BJ, O'Toole GA. Prevalence of streptococci and increased polymicrobial diversity
380 associated with cystic fibrosis patient stability. *J Bacteriol* 2012;194(17):4709-4717.
381 25. Rogers GB, Carroll MP, Serisier DJ, Hockey PM, Jones G, Kehagia V, Connett GJ,
382 Bruce KD. Use of 16s rrna gene profiling by terminal restriction fragment length polymorphism
383 analysis to compare bacterial communities in sputum and mouthwash samples from patients with
384 cystic fibrosis. *J Clin Microbiol* 2006;44(7):2601-2604.
385 26. Madan JC, Koestler DC, Stanton BA, Davidson L, Moulton LA, Housman ML, Moore
386 JH, Guill MF, Morrison HG, Sogin ML, Hampton TH, Karagas MR, Palumbo PE, Foster JA,
387 Hibberd PL, O'Toole GA. Serial analysis of the gut and respiratory microbiome in cystic fibrosis

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

388 in infancy: Interaction between intestinal and respiratory tracts and impact of nutritional
389 exposures. *MBio* 2012; 3(4):e00251-12.
390 27. Goddard AF, Staudinger BJ, Dowd SE, Joshi-Datar A, Wolcott RD, Aitken ML, Fligner
391 CL, Singh PK. Direct sampling of cystic fibrosis lungs indicates that DNA-based analyses of
392 upper-airway specimens can misrepresent lung microbiota. *Proc Natl Acad Sci U S A*
393 2012;109(34):13769-13774.
394 28. Carmody LA, Zhao J, Schloss PD, Petrosino JF, Murray S, Young VB, Li JZ, LiPuma JJ.
395 Changes in cystic fibrosis airway microbiota at pulmonary exacerbation. *Ann Am Thorac Soc*
396 2013;10(3):179-187.
397 29. Cystic fibrosis foundation, patient registry 2012 annual data report. Bethesda, MD; 2013.
398 30. Goss CH, Muhlebach MS. Review: Staphylococcus aureus and MRSA in cystic fibrosis.
399 *J Cyst Fibros* 2011;10(5):298-306.
400 31. Dasenbrook EC, Checkley W, Merlo CA, Konstan MW, Lechtzin N, Boyle MP.
401 Association between respiratory tract methicillin-resistant *Staphylococcus aureus* and survival in
402 cystic fibrosis. *JAMA* 2010;303(23):2386-2392.
403 32. Dasenbrook EC, Merlo CA, Diener-West M, Lechtzin N, Boyle MP. Persistent
404 methicillin-resistant *Staphylococcus aureus* and rate of FEV_1 decline in cystic fibrosis. *Am J*
405 *Respir Crit Care Med* 2008;178(8):814-821.
406 33. Lo DK, Hurley MN, Muhlebach MS, Smyth AR. Interventions for the eradication of
407 methicillin-resistant *Staphylococcus aureus* (MRSA) in people with cystic fibrosis. *Cochrane*
408 *Database Syst Rev* 2013;2:CD009650.
409 34. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer
410 AW, Levine DP, Murray BE, J Rybak M, Talan DA, Chambers HS. Clinical practice guidelines

411 by the infectious diseases society of america for the treatment of methicillin-resistant
412 staphylococcus aureus infections in adults and children: Executive summary. *Clin Infect Dis*
413 2011;52(3):285-292.
414 35. Walkey AJ, O'Donnell MR, Wiener RS. Linezolid vs glycopeptide antibiotics for the
415 treatment of suspected methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia: A
416 meta-analysis of randomized controlled trials. *Chest* 2011;139(5):1148-1155.
417 36. Zobell JT, Epps K, Young DC, Hatch M, Olson J, Ampofo K, Chin M, Marshall B,
418 Dasenbrook EC. Utilization of antibiotics for methicillin-resistant *Staphylococcus aureus*
419 infection in cystic fibrosis patients. *Pediatr Pulmonol* 2013;48(S36):311.
420 37. Santos RP, Prestidge CB, Brown ME, Urbancyzk B, Murphey DK, Salvatore CM, Jafri
421 HS, McCracken GH, Jr., Ahmad N, Sanchez PJ, Siegel JD. Pharmacokinetics and
422 pharmacodynamics of linezolid in children with cystic fibrosis. *Pediatr Pulmonol*
423 2009;44(2):148-154.
424 38. Keel RA, Schaeftlein A, Kloft C, Pope JS, Knauft RF, Muhlebach M, Nicolau DP, Kuti
425 JL. Pharmacokinetics of intravenous and oral linezolid in adults with cystic fibrosis. *Antimicrob*
426 *Agents Chemother* 2011;55(7):3393-3398.
427 39. FDA drug safety communication: Serious CNS reactions possible when linezolid (zyvox)
428 is given to patients taking certain psychiatric medications. Retrieved November 15, 2013, from
429 http://www.fda.gov/Drugs/DrugSafety/ucm265305.htm.
430 40. Endimiani A, Blackford M, Dasenbrook EC, Reed MD, Bajaksouszian S, Hujer AM,
431 Rudin SD, Hujer KM, Perreten V, Rice LB, Jacobs MR, Konstan MW, Bonomo RA. Emergence
432 of linezolid-resistant *Staphylococcus aureus* after prolonged treatment of cystic fibrosis patients
433 in cleveland, ohio. *Antimicrob Agents Chemother* 2011;55(4):1684-1692.

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

434 41. Doe SJ, McSorley A, Isalska B, Kearns AM, Bright-Thomas R, Brennan AL, Webb AK,
435 Jones AM. Patient segregation and aggressive antibiotic eradication therapy can control
436 methicillin-resistant *Staphylococcus aureus* at large cystic fibrosis centres. *J Cyst Fibros*
437 2010;9(2):104-109.
438 42. Rifampin [package insert]. Pantheon pharmaceuticals inc. Cincinnati, oh; 2004.
439 43. di Sant'Agnese PA, Andersen DH. Celiac syndrome: Iv. Chemotherapy in infections of
440 the respiratory tract associated with cystic fibrosis of the pancreas; observations with penicillin
441 and drugs of the sulphonamide group, with special reference to penicillin aerosol. *Am J Dis*
442 *Child* 1946;72(1):17-61.
443 44. Trapnell BC, McColley SA, Kissner DG, Rolfe MW, Rosen JM, McKevitt M,
444 Moorehead L, Montgomery AB, Geller DE, Phase II FTI Study Group. Fosfomycin/tobramycin
445 for inhalation in patients with cystic fibrosis with pseudomonas airway infection. *Am J Respir*
446 *Crit Care Med* 2012;185(2):171-178.
447 45. Generali J, Cada D. Off-label drug uses: Vancomycin: Aerolsolizaton. *Hospital*
448 *Pharmacy* 2004;39(7):638-647.
449 46. Jennings M.J., Boyle MP, Bucur C., Konstan MW, Dasenbrook EC. Pharmacokinetics
450 and safety of inhaled vancomycin in patients with cystic fibrosis. *Pediatr Pulmonol*
451 2012;47(S35):320.
452 47. Savara, Inc. Efficacy and safety study of AeroVanc for the treatment of persistent MRSA
453 lung infection in cystic fibrosis patients. In: Clinicaltrials.Gov [internet]. Bethesda (MD):
454 National Library of Medicine (US). 2012- [cited 2014 jan 20]. Available from:
455 Http://clinicaltrials.Gov/ct2/show/nct01746095 nlm identifier: Nct01746095.

456 48. Boyle MP. Persistent methicillin resistant *Staphylococcus aureus* eradication protocol
457 (pmep). In: Clinicaltrials.Gov [internet]. Bethesda (MD): National Library of Medicine (US).
458 2012- [cited 2014 jan 20]. Available from: Http://clinicaltrials.Gov/ct2/show/nct01594827 nlm
459 identifier: Nct01594827.
460 49. Muhlebach M. Early methicillin-resistant *Staphylococcus aureus* (MRSA) therapy in
461 cystic fibrosis (CF) (star-too). In: Clinicaltrials.Gov [internet]. Bethesda (MD): National Library
462 of Medicine (US). 2011- [cited 2014 jan 20]. Available from:
463 http://clinicaltrials.Gov/ct2/show/nct01349192 nlm identifier: Nct01349192. 2013.
464 50. Al-Aloul M, Crawley J, Winstanley C, Hart CA, Ledson MJ, Walshaw MJ. Increased
465 morbidity associated with chronic infection by an epidemic *Pseudomonas aeruginosa* strain in
466 CF patients. *Thorax* 2004;59(4):334-336.
467 51. Alexander BD, Petzold EW, Reller LB, Palmer SM, Davis RD, Woods CW, LiPuma JJ.
468 Survival after lung transplantation of cystic fibrosis patients infected with *Burkholderia cepac*ia
469 complex. *Am J Transplant* 2008;8(5):1025-1030.
470 52. Corey M, Farewell V. Determinants of mortality from cystic fibrosis in canada, 1970-
471 1989. *Am J Epidemiol* 1996;143(10):1007-1017.
472 53. Tablan OC, Chorba TL, Schidlow DV, White JW, Hardy KA, Gilligan PH, Morgan WM,
473 Carson LA, Martone WJ, Jason JM, Jarvis WR. *Pseudomonas cepacia* colonization in patients
474 with cystic fibrosis: Risk factors and clinical outcome. *J Pediatr* 1985;107(3):382-387.
475 54. Waters V, Atenafu EG, Lu A, Yau Y, Tullis E, Ratjen F. Chronic *Stenotrophomonas*
476 *maltophilia* infection and mortality or lung transplantation in cystic fibrosis patients. *J Cyst*
477 *Fibros* 2013;12(5):482-486.

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

478 55. Waters V, Yau Y, Prasad S, Lu A, Atenafu E, Crandall I, Tom S, Tullis E, Ratjen F.
479 *Stenotrophomonas maltophilia* in cystic fibrosis: Serologic response and effect on lung disease.
480 *Am J Respir Crit Care Med* 2011;183(5):635-640.
481 56. Waters V. New treatments for emerging cystic fibrosis pathogens other than
482 pseudomonas. *Curr Pharm Des* 2012;18(5):696-725.
483 57. Clinical laboratory standards institute. Performance standards for antimicrobial
484 susceptibility testing. Wayne, PA: CLSI;2012.
485 58. Geller DE, Flume PA, Griffith DC, Morgan E, White D, Loutit JS, Dudley MN.
486 Pharmacokinetics and safety of MP-376 (levofloxacin inhalation solution) in cystic fibrosis
487 subjects. *Antimicrob Agents Chemother* 2011;55(6):2636-2640.
488 59. Geller DE, Konstan MW, Smith J, Noonberg SB, Conrad C. Novel tobramycin inhalation
489 powder in cystic fibrosis subjects: Pharmacokinetics and safety. *Pediatr Pulmonol*
490 2007;42(4):307-313.
491 60. McCoy KS, Quittner AL, Oermann CM, Gibson RL, Retsch-Bogart GZ, Montgomery
492 AB. Inhaled aztreonam lysine for chronic airway *Pseudomonas aeruginosa* in cystic fibrosis. *Am*
493 *J Respir Crit Care Med* 2008;178(9):921-928.
494 61. Moriarty TF, McElnay JC, Elborn JS, Tunney MM. Sputum antibiotic concentrations:
495 implications for treatment of cystic fibrosis lung infection. Pediatr Pulmonol 2007;42:1008-
496 1017.62. Ratjen F, Rietschel E, Kasel D, Schwiertz R, Starke K, Beier H, van
497 Koningsbruggen S, Grasemann H. Pharmacokinetics of inhaled colistin in patients with cystic
498 fibrosis. *J Antimicrob Chemother* 2006;57(2):306-311.
499 63. Reed MD, Stern RC, O'Riordan MA, Blumer JL. The pharmacokinetics of colistin in
500 patients with cystic fibrosis. *J Clin Pharmacol* 2001;41(6):645-654.

64. Clancy JP, Dupont L, Konstan MW, Billings J, Fustik S, Goss CH, Lymp J, Minic P, Quittner AL, Rubenstein RC, Young KR, Saiman L, Burns JL, Govan JR, Ramsey B, Gupta R; Arikace Study Group. Phase II studies of nebulised arikace in cf patients with *Pseudomonas aeruginosa* infection. *Thorax* 2013;68(9):818-825.

65. Canis F, Husson MO, Turck D, Vic P, Launay V, Ategbo S, Vincent A, Courcol RJ. Pharmacokinetics and bronchial diffusion of single daily dose amikacin in cystic fibrosis patients. *J Antimicrob Chemother* 1997;39(3):431-433.

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

67. Konstan MW, Flume PA, Kappler M, Chiron R, Higgins M, Brockhaus F, Zhang J, Angyalosi G, He E, Geller DE. Safety, efficacy and convenience of tobramycin inhalation powder in cystic fibrosis patients: The EAGER trial. *J Cyst Fibros* 2011;10(1):54-61.

68. Ratjen A, Yau Y, Wettlaufer J, Matukas L, Ratjen F, Zlosnik J, Speert DP, Tullis E, Waters V. Tobramycin susceptibilities of *Burkholderia cepacia* complex isolates from pediatric and adult cystic fibrosis patients. *Pediatr Pulmol* 2013;Suppl 36:31837

69. Assael BM, Pressler T, Bilton D, Fayon M, Fischer R, Chiron R, Larosa M, Knoop C, McElvaney N, Lewis SA, Bresnik M, Montgomery AB, Oermann CM, For the AZLI Active Comparator Study Group. Inhaled aztreonam lysine vs. Inhaled tobramycin in cystic fibrosis: A comparative efficacy trial. *J Cyst Fibros* 2012.

70. Tullis DE, Burns JL, Retsch-Bogart GZ, Bresnik M, Henig NR, Lewis SA, LiPuma JJ. Inhaled aztreonam for chronic Burkholderia infection in cystic fibrosis: A placebo-controlled trial. *J Cyst Fibros* 2014;13(3):296-305.

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

523 71. Hassett DJ, Cuppoletti J, Trapnell B, Lymar SV, Rowe JJ, Yoon SS, Hilliard GM,
524 Parvatiyar K, Kamani MC, Wozniak DJ, Hwang SH, McDermott TR, Ochsner UA. Anaerobic
525 metabolism and quorum sensing by *Pseudomonas aeruginosa* biofilms in chronically infected
526 cystic fibrosis airways: Rethinking antibiotic treatment strategies and drug targets. *Adv Drug*
527 *Deliv Rev* 2002;54(11):1425-1443.
528 72. Yu Q, Griffin EF, Moreau-Marquis S, Schwartzman JD, Stanton BA, O'Toole GA. *In*
529 *vitro* evaluation of tobramycin and aztreonam versus *Pseudomonas aeruginos*a biofilms on
530 cystic fibrosis-derived human airway epithelial cells. *J Antimicrob Chemother*
531 2012;67(11):2673-2681.
532 73. Waters V, Ratjen F, Tullis E, Corey M, Matukas L, Leahy R, Yau Y. Randomized double
533 blind controlled trial of the use of a biofilm antimicrobial susceptibility assay to guide antibiotic
534 therapy in chronic *Pseudomonas aeruginosa* infected cystic fibrosis patients. *Pediatr Pulmonol*
535 2010;Suppl 33:330.
536 74. Geller DE, Flume PA, Staab D, Fischer R, Loutit JS, Conrad DJ, Mpex 204 Study Group.
537 Levofloxacin inhalation solution (MP-376) in patients with cystic fibrosis with *Pseudomonas*
538 *aeruginosa*. *Am J Respir Crit Care Med* 2011;183(11):1510-1516.
539 75. Wu K, Yau YC, Matukas L, Waters V. Biofilm compared to conventional antimicrobial
540 susceptibility of *Stenotrophomonas maltophilia* isolates from cystic fibrosis patients. *Antimicrob*
541 *Agents Chemother* 2013;57(3):1546-1548.
542 76. Tsivkovskii R, Sabet M, Tarazi Z, Griffith DC, Lomovskaya O, Dudley MN.
543 Levofloxacin reduces inflammatory cytokine levels in human bronchial epithelia cells:
544 Implications for aerosol MP-376 (levofloxacin solution for inhalation) treatment of chronic
545 pulmonary infections. *FEMS Immunol Med Microbiol* 2011;61(2):141-146.

546 77. McCaughey G, Gilpin D, Elborn J, Tunney MM. The future of antimicrobial therapy in
547 the era of antibiotic resistance in cystic fibrosis pulmonary infection. *Expert Rev Respir Med*
548 2013;7(4):385-396.
549 78. Pattison SH, Rogers GB, Crockard M, Elborn JS, Tunney MM. Molecular detection of cf
550 lung pathogens: Current status and future potential. *J Cyst Fibros* 2013;12(3):194-205.
551 79. Foweraker JE, Govan JR. Antibiotic susceptibility testing in early and chronic respiratory
552 infections with pseudomonas aeruginosa. *J Cyst Fibros* 2013;12(3):302.
553 80. Gillham MI, Sundaram S, Laughton CR, Haworth CS, Bilton D, Foweraker JE. Variable
554 antibiotic susceptibility in populations of *Pseudomonas aeruginosa* infecting patients with
555 bronchiectasis. *J Antimicrob Chemother* 2009;63(4):728-732.
556 81. Hurley MN, Ariff AH, Bertenshaw C, Bhatt J, Smyth AR. Results of antibiotic
557 susceptibility testing do not influence clinical outcome in children with cystic fibrosis. *J Cyst*
558 *Fibros* 2012;11(4):288-292.
559 82. Parkins MD, Rendall JC, Elborn JS. Incidence and risk factors for pulmonary
560 exacerbation treatment failures in patients with cystic fibrosis chronically infected with
561 *Pseudomonas aeruginosa*. *Chest* 2012;141(2):485-493.
562
563
564

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

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

Drug	Mean peak serum concentrations (µg/ml)	Mean peak sputum concentrations (µg/g)
Tobramycin		
• Intravenous (8 mg/kg/day) (range)	29.4 (23.1-35.5)	3.88 (1.8-5.7)
• Aerosolized		
Solution (300mg) (±SD)	1.04±0.58	737±1,028
Powder (112 mg) (±SD)	1.02±0.53	1,048±1,080
Amikacin		
• Intravenous (35 mg/kg) (±SD)	121.4±37.3	10.95+7.55
• Aerosolized (560 mg) (±SD)	1.29±0.77	2,286 (11.6-11,220)
Levofloxacin		
• Oral (500 mg)	6.5	5.1
• Aerosolized (240 mg) (±SD)	1.71±0.62	4,691±4,516
Aztreonam		
• Intravenous (2 g)	80.1	5.2
• Aerosolized (75 mg) (range)	0.622 (0.31-1.7)	537 (0.2-3,010)
Colistimethate (Colistin)		
• Intravenous (7mg/kg/day) (±SD)	23±6	N/A
• Aerosolized (2 million units) (±SEM)	0.178±0.018	40±5

SD: standard deviation; SEM: standard error of the mean

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

Table 2.§ Empiric antibiotic therapy for the treatment of difficult pulmonary bacterial infections in CF

Organism	Antibiotic	Pediatric Dose	Adult Dose	Side Effects
Gram-positive organisms				
Methicillin-resistant *Staphylococcus aureus*	Vancomycin	15 mg/kg intravenously every 6h	1 g intravenously every 12h	-Oto/nephro toxicity, red man syndrome
	OR Linezolid	if <11yrs: 10 mg/kg intravenously or orally every 8h if >11yrs: 10 mg/kg intravenously or orally every 12h	600 mg intravenously or orally every 12h	-Optic/peripheral neuropathy, myelosuppression
Gram-negative organisms				
Pseudomonas aeruginosa	Tobramycin*	10 mg/kg intravenously every 24h	10 mg/kg intravenously every 24h	-Ototoxicity, nephrotoxocity
	OR Amikacin**	30 mg/kg intravenously every 24h	30 mg/kg intravenously every 24h	
	OR Colistin (colistimethate sodium)	8 mg/kg/day intravenously divided every 8h	8 mg/kg/day intravenously divided every 8h (max 480 mg/day)	-Nephrotoxicity, neurotoxicity
	PLUS (choose one): Ticarcillin/ clavulanate	100 mg/kg of ticarcillin component intravenously every 6h	3 g of ticarcillin component intravenously every 6h	-GI, rash, hepatitis, neutropenia
	Ceftazidime	50 mg/kg intravenously every 6h††	2 g intravenously every 8h††	-GI, rash
	Meropenem	40 mg/kg intravenously every 8h	2 g intravenously every 8h	GI, rash, hepatitis, neutropenia
	Ciprofloxacin		400 mg intravenously	-GI, rare seizure,

		15 mg/kg intravenously or 20 mg/kg orally every 12h	or 750 mg orally every 12h	tendinopathy
***Burkholderia cepacia* complex**	Meropenem	40 mg/kg intravenously every 8h	2 g intravenously every 8h	-GI, rash, hepatitis, neutropenia
	PLUS (choose 1):			
	Ceftazidime	50 mg/kg intravenously every 6h	2 g intravenously every 8h	-GI, rash
	Chloramphenicol°	15-20 mg/kg intravenously every 6h	1 g intravenously every 6h	-Bone marrow suppression /failure
	Trimethoprim/ sulfamethoxazole	4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h	4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h	-GI, hypersensitivity neutropenia, serum sickness
	Aztreonam	50 mg/kg intravenously every 8h	2 g intravenously every 8h	-GI, rash
Stenotrophomonas maltophilia	Trimethoprim/ sulfamethoxazole	4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h	4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h	-GI, hypersensitivity neutropenia, serum sickness
	PLUS (choose 1):			
	Ticarcillin/ clavulanate	100 mg/kg of ticarcillin component intravenously every 6h	3 g of ticarcillin component intravenously every 6h	-GI, rash, hepatitis, neutropenia
	Levofloxacin	if <5yrs: 10 mg/kg intravenously or orally every 12h if >5 yrs: 10 mg/kg intravenously or orally once daily	500-750 mg intravenously or orally once daily	-GI, rarely seizure, tendinopathy
	Doxycycline†	2 mg/kg intravenously or orally every 12h	100 mg intravenously or orally every 12h	-GI, photosensitivity

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

	Tigecycline	1.2 mg/kg intravenously every 12h	50mg intravenously every 12h	-GI, cholestasis
***Achromobacter* species**	Meropenem	40 mg/kg intravenously every 8h	2 g intravenously every 8h	-GI, rash, hepatitis
	OR			
	Imipenem	15-25 mg/kg intravenously every 6h	500 mg-1 g intravenously every 6h	-GI, rarely seizures
	PLUS (choose 1):			
	Trimethoprim/ sulfamethoxazole	4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h	4-5 mg/kg (max 240 mg) of trimethoprim component intravenously or orally every 12h	-GI, hypersensitivity neutropenia, serum sickness
	Ciprofloxacin	15 mg/kg intravenously or 20 mg/kg orally every 12h	400 mg intravenously or 750 mg orally every 12h	-GI, rarely seizure, tendinopathy
	Minocycline†	2 mg/kg intravenously or orally every 12h	100 mg orally every 12h	-GI, photosensitivity

GI: gastrointestinal

§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.

* 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 <1 mg/L.

** 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 <1 mg/L.

° Serum concentrations should be monitored; the peak concentration ranges from 15-25 μg/ml and the trough from 5-15 μg/ml.

† Should not be given to children less than 8 years of age.

†† Continuous infusion of ceftazidime may be considered in cases of clinical failure or for the treatment of multi-drug resistant *P. aeruginosa* in order to maximize the time above the minimum inhibitory concentration (MIC)

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

Bacteria	Antibiotic					
	CAZ	PIP/TAZ	MER	AZT	TOB	COL
S. maltophilia	✓	+/-	----	----	----	✓
Achromobacter spp.	✓	✓	----	----	----	+/-
BCC	+/-	+/-	+/-	----	----	+/-
MSSA	+/-	✓	✓	----	✓	----
MRSA	----	✓	----	----	✓	----
Streptococci	+/-	✓	✓	----	----	----
H. influenzae	✓	✓	✓	+/-	✓	----
P. aeruginosa	✓	✓	✓	✓	✓	✓
Anaerobes	----	✓	✓	----	----	----

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

Bacteria	Antibiotic				
	CO-T	DOX	CHL	FOS	TIG
S. maltophilia	✓	✓	+/-	----	✓
Achromobacter spp.	✓	✓	+/-	----	✓
BCC	+/-	+/-	+/-	+/-	+/-
MSSA	✓	✓	✓	✓	✓
MRSA	+/-	✓	----	✓	✓
Streptococci	✓	✓	✓	+/-	✓
H. influenzae	✓	✓	✓	?	✓
P. aeruginosa	----	----	+/-	✓	----
Anaerobes	----	----	✓	----	✓

✓: 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*

ANNALSATS Articles in Press. Published on 07-August-2014 as 10.1513/AnnalsATS.201402-050AS

Figures

Fig. 1