Reconciling Antimicrobial Susceptibility Testing and Clinical Response in Antimicrobial Treatment of Chronic Cystic Fibrosis Lung Infections

Valerie J. Waters,1 Timothy J. Kidd,2 Rafael Canton,3 Miquel B. Ekkelenkamp,4 Helle Krog Johansen,5 John J. LiPuma,6 Scott C. Bell,7 J. Stuart Elborn,8 Patrick A. Flume,9 Donald R. VanDevanter,10 and Peter Gilligan11; for the Antimicrobial Resistance International Working Group in Cystic Fibrosis

1Division of Infectious Diseases, Department of Pediatrics, Hospital for Sick Children, University of Toronto, Canada; 2School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia; 3Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain; 4Department of Medical Microbiology, University Medical Center Utrecht, The Netherlands; 5Department of Clinical Microbiology, Rigshospitalet, Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark; 6Department of Pediatrics, University of Michigan Medical School, Ann Arbor; 7Department of Thoracic Medicine, Prince Charles Hospital and QIMR Berghofer Medical Research Institute, Brisbane, Australia; 8Imperial College Hospital, Queen’s University Belfast, Northern Ireland; 9Departments of Medicine and Pediatrics, Medical University of South Carolina, Charleston; 10Department of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, Ohio; and 11Department of Pathology-Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill

Median cystic fibrosis (CF) survival has increased dramatically over time due to several factors, including greater availability and use of antimicrobial therapies. During the progression of CF lung disease, however, the emergence of multidrug antimicrobial resistance can limit treatment effectiveness, threatening patient longevity. Current planktonic-based antimicrobial susceptibility testing lacks the ability to predict clinical response to antimicrobial treatment of chronic CF lung infections. There are numerous reasons for these limitations including bacterial phenotypic and genotypic diversity, polymicrobial interactions, and impaired antibiotic efficacy within the CF lung environment. The parallels to other chronic diseases such as non-CF bronchiectasis are discussed as well as research priorities for moving forward.

Keywords. cystic fibrosis; antimicrobial susceptibility.

Cystic fibrosis (CF) is a life-shortening genetic disease due to mutations in the gene encoding for the anion channel, cystic fibrosis transmembrane conductance regulator (CFTR) [1, 2]. The survival of individuals with CF has increased dramatically over time, with a current predicted median age of survival of 43 years, but median age of death of 29 years [3]. This is due to a number of factors including improved nutrition, physical airway clearance techniques, and mucoactive and antibiotic treatments [4]. With the development of modulator compounds that can increase the quantity and function of CFTR, the life span on CF patients is expected to continue to improve [5]. During the progression of the disease, however, the emergence of multidrug antimicrobial-resistant microorganisms can limit the effectiveness of antibiotic treatment of pulmonary infections, threatening patient longevity [6].

CHALLENGES OF TREATING CHRONIC INFECTIONS

Defective CFTR activity in airway epithelial cells leads to abnormal sodium chloride and water transport, ultimately resulting in the accumulation of viscous mucus in the airways [1, 2]. This creates a niche that allows for colonization and subsequent chronic infection due to opportunistic pathogens such as Pseudomonas aeruginosa, which cannot be effectively cleared due to impaired innate immunity, particularly ciliary dysfunction. The subsequent inflammatory response leads to cytokine release and tissue damage in the form of bronchiectasis and lung parenchymal loss [7], leading to respiratory failure, which is the primary cause of death in individuals with CF [3].

Chronic infections can persist lifelong in patients with CF, and antibiotic therapy is key in their management. Unlike the treatment of acute infections such as bacteremia, in the setting of chronic infection, clearance of the microorganism is not the goal. Rather, the objective of antibiotic treatment is to suppress the resident bacterial population and ameliorate symptoms [8]. Chronic P. aeruginosa infection in persons with CF is treated with long-term inhaled antibiotics and regular intravenous antibiotic therapy to decrease the pulmonary bacterial burden [9]. This type of chronic suppressive antibiotic therapy maintains lung function, reduces risk and rates of flares of respiratory symptoms (pulmonary exacerbations), and prolongs survival of
people with CF and chronic *P. aeruginosa* infection [10]. Finally, antimicrobial treatment of pulmonary exacerbations themselves contributes to maintenance of lung function [11].

As lung disease advances in an individual with CF, however, it becomes more difficult to achieve the same degree of clinical response with antibiotic therapy, and lung infections become more refractory to treatment. With persistence of infection, *P. aeruginosa* adapts itself, both phenotypically and genotypically, to the CF lung environment. Such adaptations include switching from a planktonic to a biofilm mode of growth; bacterial cells within the biofilm matrix have a reduced rate of growth and exist within an anaerobic environment, impeding the efficacy of many antibiotics [12, 13]. Furthermore, *P. aeruginosa* can also exist as persister cells, metabolically dormant cells of a clonal bacterial population that can survive exposure to high concentrations of an antibiotic [14]. Repeated courses of antibiotics may select for mutations associated with antimicrobial resistance [15, 16]. Hypermutator strains may also arise and are recognized by their increased spontaneous mutation rate arising from defects in DNA repair or error avoidance systems [17]. In a CF patient treated for chronic *P. aeruginosa* infection for many years, results of conventional, planktonic-based antimicrobial susceptibility testing (AST) will demonstrate an increasing number of antibiotics to which *P. aeruginosa* is deemed resistant, reflecting antibiotic exposure over time. However, AST results lack the ability to predict clinical response during the treatment of chronic CF lung infections [18].

**DISCONNECT BETWEEN ANTIMICROBIAL SUSCEPTIBILITY TESTING RESULTS AND CLINICAL OUTCOMES**

In the treatment of monomicrobial acute infections where the microorganism is growing planktonically, or “free-floating,” such as in bloodstream or uncomplicated urinary tract infections, the ability of current AST methods to predict clinical outcomes is quite good; there is little evidence of this association in CF [8, 18]. It is not uncommon for a CF patient to improve on antibiotic therapy targeting a microorganism that is deemed resistant by AST and, conversely, for a patient to fail to respond to antibiotics to which a microorganism is categorized as susceptible in vitro.

There are many reasons why AST may fail in the setting of CF. The aforementioned phenotypic adaptations of *P. aeruginosa* in the establishment of chronic infection are not reproduced in traditional AST, such as standardized dilution, concentration gradient, and disk diffusion tests, which are all based on planktonic growth of bacteria [19]. With persistence of infection, *P. aeruginosa* undergoes phenotypic but also genotypic diversification within different regions, or microenvironments, of the CF lung [20, 21]. *Pseudomonas aeruginosa* isolates are commonly recovered from spontaneously expectorated CF sputum (or throat swabs), which may not be representative of the entire CF lung. This limited depth of sampling of the phenotypic and genotypic diversity of *P. aeruginosa* may contribute to the failure of AST as a predictive test of outcome. Finally, there is an inherent assumption in current AST testing that all bacterial isolates that can be tested contribute to clinical disease and, by extension, must all be effectively targeted to achieve antimicrobial efficacy. There are, however, few data supporting this assumption and it may be that some bacterial subpopulations within a given bacterial species may be more important to test than others. For instance, it has been suggested that because antipseudomonal antibiotics commonly used to treat CF pulmonary exacerbations, such as β-lactams, have relatively little in vitro activity against organisms grown as biofilms compared to their activity against the same strains growing planktonically, clinical response to these therapies is probably realized through activity against planktonic organisms [22]. Unfortunately, current AST test methods do not include separation of bacterial colonies derived from planktonic phenotypes from those derived from “nonplanktonic” phenotypes.

Additionally, CF pulmonary infections are not monomicrobial but rather polymicrobial [23, 24]. When sputum and throat swab samples are processed in the clinical microbiology laboratory, they are cultured in selective conditions that promote the recovery and identification of specific organisms that have traditionally been defined as CF pathogens, often due to their predominant nature within the CF airway microbiome [25]. Many other organisms may be ignored because they are difficult to recover, grow slowly, are present in small amounts, are considered to be sample contamination with “normal oral flora,” or are of unknown significance. When AST does not predict outcomes, we must ask ourselves: “Did we choose the right pathogen?” [26]. Furthermore, interactions between microorganisms can affect their behavior, including antimicrobial susceptibility. For instance, certain *P. aeruginosa* strains when grown as biofilms in the presence of *Staphylococcus aureus* will aggregate and become tolerant to high concentrations of tobramycin, resisting killing [27]. Such phenotypes are not observed when AST is performed for a single bacterial isolate on an agar plate. Replicating these complex polymicrobial interactions in the laboratory is not feasible and our inability to accurately do so further explains the limitations of AST in CF.

Last, AST aims to test the antibiotic efficacy in vitro. Current AST methodologies measure the minimum inhibitory concentration (MIC) of an antibiotic and interpret these results according to “clinical breakpoints,” classifying an organism as either susceptible, intermediate, or resistant to a particular drug [19]. Breakpoints are mainly determined based on the known achievable serum or tissue concentration of a drug administered systemically, in addition to other factors such as pharmacokinetic/pharmacodynamic parameters and outcome data from clinical studies [28–30]. However, in CF, antibiotics are often administered via inhalation, achieving much higher drug concentrations to the airways while minimizing systemic toxicities [31, 32]. The results
generated from AST of CF pathogens such as \textit{P. aeruginosa} are thus not relevant in this setting. Antibiotics may also have effects against bacteria that go beyond growth inhibition or direct killing. Azithromycin, for example, has been shown to be of benefit in CF patients with chronic \textit{P. aeruginosa} infection by increasing forced expiratory volume in 1 second (FEV\textsubscript{1}) and decreasing the frequency of exacerbations \cite{33}. Its main effect does not seem to be due to \textit{P. aeruginosa} killing, but may be attributed to anti-inflammatory effects by decreasing cytokine production, modulation of bacterial virulence factors, inhibiting quorum sensing responsible for biofilm formation, or effects against bacterial species other than \textit{P. aeruginosa} \cite{34, 35}. These effects are not captured in conventional AST methods. Furthermore, as previously discussed, the goals of antibiotic treatment of chronic CF lung infections are different than acute infections. It would be expected that the efficacy of an antibiotic in inhibiting bacterial growth would predict eradication of an organism, but inhibiting bacterial growth may not predict improvement in lung function, as decreases in sputum bacterial density do not always correlate with increases in FEV\textsubscript{1} \cite{36}. Tests of antimicrobial efficacy should thus use an appropriate measure of response.

**PARALLELS IN OTHER CHRONIC DISEASES**

Other conditions share similarities with the chronic infections observed in CF. Patients with bronchiectasis due to diseases other than CF, such as primary ciliary dyskinesia, chronic obstructive pulmonary disease, and common variable immune deficiency, similarly have impaired mucociliary clearance leading to the retention of airway secretions, permitting the establishment of persistent infection \cite{37}. These infections can last for years, are polymicrobial, and are often dominated by organisms such as \textit{Haemophilus influenzae}, \textit{Moraxella} species, \textit{S. aureus}, \textit{P. aeruginosa}, and anaerobic bacteria such as \textit{Prevotella} and \textit{Veillonella} species \cite{38, 39}. Nontuberculous mycobacteria (NTM), pathogens of concern in the CF population \cite{40}, have also been reported as a cause of a significant proportion of bronchiectatic pulmonary infections \cite{41}. In chronic NTM infections of the airways, there is considerable bacterial diversity, with multistain and even multispecies infections often noted within a single patient \cite{40, 42}. As with CF, individuals with non-CF bronchiectasis often experience pulmonary exacerbations, and treatment with intravenous antibiotics has been associated with decreases in airway bacterial load and inflammatory markers \cite{43}. Additionally, long-term use of antibiotics, in the form of inhaled antibiotics or oral macrolides, have also been shown to be associated with a decreased risk of exacerbation in bronchiectasis \cite{44, 45}. Given the shared characteristics of chronic infections between conditions, clinicians caring for patients with bronchiectasis thus face similar challenges, as in CF, in reconciling measures of antimicrobial resistance and clinical outcomes.

**FUTURE DIRECTIONS**

The data generated by current AST methodologies are limited in terms of guiding antibiotic management of chronic CF lung infections. AST is a time-consuming and labor-intensive process, and CF centers that have restricted the frequency of AST of CF pathogens to once yearly (rather than on every sputum sample culture) have not noted worse clinical status in their patient population \cite{46}. Most importantly, clinicians must recognize the inability of AST to predict outcomes in CF and not change antibiotic therapy based on AST results in patients who are clinically responding to treatment.

Developing a better antimicrobial susceptibility test has been a longstanding goal in CF care. Much effort has been expended in this area of research such as designing biofilm antimicrobial susceptibility testing assays \cite{47–49} and combination antimicrobial testing panels \cite{50–52}; to date, all have failed to improve a patient’s response to antibiotic treatment \cite{53–55}. For an in vitro test to be successful, it will need to capture the phenotypic and genotypic diversity, polymicrobial interactions, and antibiotic efficacy within the CF lung environment. Attempts to obtain more representative specimens of the lower airways using routine bronchoalveolar lavage sampling, for example, have not resulted in improved clinical outcomes such as a lower prevalence of \textit{P. aeruginosa} infection or less structural lung disease \cite{56}. Using whole genome sequencing to define the genetic determinants of antimicrobial resistance, sometimes referred to as the “resistome” \cite{57}, has been suggested as a way of overcoming the limitations of culture-based AST methods. However, the genotypic to phenotypic correlation of antimicrobial resistance has been quite poor for \textit{P. aeruginosa} \cite{58}. Measuring the expression of bacterial genes in the form of RNA sequencing or proteomics may be more successful in defining antimicrobial resistance within the CF lung, but this is as of yet undetermined. The success of an in vitro test will most likely also depend on defining a more clinically relevant measure rather than antibiotic inhibitory concentrations for a single presumed pathogen. Further research is needed to define the CF microbiome changes associated with clinical response such as lung function improvement. Only then will we know what we should be measuring in the laboratory to predict outcome.

Even in the absence of a better antimicrobial susceptibility test, can we use antibiotics more rationally in CF? We may be able to by carefully and systematically observing responses to antibiotic treatment at a population level. Standardized protocols for the management of CF pulmonary exacerbations, for example, may shed light on the optimal antibiotic choices for certain pulmonary infections. The STOP trial (Standardized Treatment of Pulmonary Exacerbations in Patients With CF; ClinicalTrials.gov identifier NCT02109822) studied current treatment practices of pulmonary exacerbations using the Cystic Fibrosis Foundation Registry, and the follow-up STOP
II trial aims to compare durations of intravenous antibiotic therapy for pulmonary exacerbations (ClinicalTrials.gov identifier NCT02781610). These types of study designs could be used to address other practical antibiotic management issues in CF, such as whether previous response to an antibiotic regimen for the treatment of pulmonary exacerbation predicts future response to the same antibiotic choices.

In summary, until such time that we have a more robust way of predicting response to antibiotic therapy in CF, clinicians should prioritize a patient’s clinical response over in vitro AST results and not change an antibiotic regimen based only on these findings if the patient is clinically improving. The search for a strategy to rationalize antibiotic use in the treatment of CF lung infections needs to be a research priority.

**Notes**

**Acknowledgments.** The authors acknowledge the work of the other members of the Antimicrobial Resistance International Working Group in Cystic Fibrosis: Wendy Bullington (Medical University of South Carolina, Charleston); Pierre-Regis Burgel (Paris Descartes University, France); Catherine Byrnes (Auckland Hospital, New Zealand); Pavel Drevinek (University Hospital Motol, Prague, Czech Republic); Alison Holmes (Imperial College London, United Kingdom); Barbara Kahl (University Hospital Münster, Germany); Holly Maples (University of Arkansas for Medical Sciences, Little Rock); Stacey Martinniano (Children's Hospital Colorado, Aurora); Susanna McColley (Children's Hospital of Chicago, Illinois); Andrew Morris (University Health Network, Sinai Health System, Toronto, Canada); Marianne Muhlebach (University of North Carolina, Chapel Hill); Michael Parkins (University of Calgary, Canada); Felix Ratjen (Hospital for Sick Children, Toronto, Canada); Jason Roberts (Royal Brisbane and Women's Hospital, Australia); Lisa Saiman (Columbia University Medical Center, New York); Anand Shah (Royal Brompton Hospital, London, United Kingdom); Alan Smyth (Nottingham Children's Hospital, United Kingdom); Ranjani Somayaji (University of Calgary, Canada); Giovanni Taccetti (Children's Hospital Colorado, Aurora); Michael Tunney (Queen's University Belfast, United Kingdom); Kevin Winthrop (Oregon Health and Science University, Portland); and Edith Zemanick (Children's Hospital Colorado, Aurora).

**Financial support.** This work was supported by the European Cystic Fibrosis Society, Cystic Fibrosis Foundation, Cystic Fibrosis Trust, Cystic Fibrosis Canada, and Cystic Fibrosis Australia.

**Potential conflicts of interest.** V. J. W. has received honoraria from Novartis Canada and Teva Pharmaceutical Industries and grants from Novartis Canada, Innovotech Inc, and Gilead outside the submitted work. S. C. B. has received grants from Vertex Pharma and has served on advisory boards for Vertex Pharma, Galapagos, and AbbVie outside the submitted work. J. E. E. has received grants from Vertex Pharma and Novartis, and has served on advisory boards for Vertex Pharma, AbbVie, and Cellaxis. P. A. F. received grants from the European Cystic Fibrosis Society, Cystic Fibrosis Foundation, Cystic Fibrosis Trust, Cystic Fibrosis Canada, and Cystic Fibrosis Australia during the conduct of the study, and has also received grants from Bayer Healthcare, Insmed, and Novartis and personal fees from Bayer Healthcare AG, Insmed, Eloxox Pharmaceuticals, and Horizon Pharma outside the submitted work. J. J. L. has received personal fees from Aradigm, CURx, Horizon, Raptor, and VAST Therapeutics outside the submitted work. R. C. has received personal fees from Chiesi Spain, Pfizer, and Merck Sharp & Dohme outside the submitted work. D. R. V. has received personal fees from AbbVie, Albumedix, AN2 Therapeutics Inc, Aradigm, Calithera, Chiesi USA, Concert, CURx, Eloxox, Enbiotix, Genentech, Horizon, IBF Biotechnics, ICON Clinical Sciences, Ionis, Kala, Life Science Strategies, OrbiMed, Protalix, PTC Therapeutics, Pulmocide, Raptor, Recida, Respiration, Savara, VAST, and Vertex outside the submitted work. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


32. LiPuma JJ. Microbiological and immunologic considerations with aerosolized drug delivery. Chest 2001; 120:118–23S.


37. Flume PA, Chalmers JD, Olivier KN. Advances in bronchiectasis: endotyping, genetics, microbiome, and disease heterogeneity. Lancet 2018; 392:880–90.


