

Assessing Airway Microbiota in Cystic Fibrosis: What More Should Be Done?

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The use of culture-independent methods has deepened our appreciation of the complexity of the bacterial communities that typically reside in the airways of persons with cystic fibrosis (CF). New insights into how changes in the structure of these communities relate to lung disease progression will likely raise expectations for more in-depth microbiologic analysis of CF respiratory specimens. An article in this issue of the *Journal of Clinical Microbiology* (W. G. Flight, A. Smith, C. Paisey, J. R. Marchesi, M. J. Bull, P. J. Norville, K. J. Mutton, A. K. Webb, R. J. Bright-Thomas, A. M. Jones, and E. Mahenthiralingam, *J Clin Microbiol* 53: 2022–2029, 2015, <http://dx.doi.org/10.1128/JCM.00432-15>) describes the application of a culture-independent approach to the assessment of CF airway microbiota.

The role that chronic infection of the airways plays in contributing to mortality in people with cystic fibrosis (CF) has been recognized since the 1940s. During the 1950s, when most children with CF did not survive beyond the first few years of life, *Staphylococcus aureus* and, to a lesser degree, *Haemophilus influenzae* were considered the primary respiratory pathogens. As the life expectancy of CF patients increased during the 1960s and 1970s, *Pseudomonas aeruginosa* emerged as the dominant opportunistic pathogen in the growing number of patients living into adolescence (1). A small number of other bacterial species, including *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*, and species within the *Burkholderia cepacia* complex, were subsequently recognized in the 1980s and 1990s as less commonly occurring opportunistic respiratory pathogens in CF. Although other bacterial species (e.g., *Ralstonia* spp., *Pandoraea* spp., and *Inquilinus limosus*) have been since identified as occasionally causing infection in CF, the suite of “CF species of interest” has remained relatively limited (2). Antimicrobial management of CF, as well as practices pertaining to the processing of CF respiratory specimens by clinical microbiology laboratories, has focused on this set of opportunistic bacterial species.

Within the past decade, the use of culture-independent methods to detect the presence of microbial species in biological specimens has been applied to CF respiratory samples (3). These methods, employed primarily by research laboratories to this point, have provided important new insights into CF microbiology. First, it is now clear that CF airways typically harbor a greater number of bacterial species than the relatively small number of species reported by standard culture. Many of these species, including anaerobic species such as *Prevotella* spp., *Veillonella* spp., and *Fusobacterium* spp., are not specifically sought in routine CF sputum culture protocols. Other species, including *Rothia* spp., *Gemella* spp., *Granulicatella* spp., and facultative anaerobic *Streptococcus* spp., may be recovered in culture but not reported or reported collectively as “oral flora.” Although upper-airway inhabitants such as these have been attributed to oral contamination of expectorated sputum, culture-independent studies now provide compelling evidence supporting their presence in the lower airways (4).

Another insight derived from culture-independent analyses in several studies is that in adolescents and adults with CF, airway

bacterial community diversity—a function of both the number of species present (community richness) and their relative abundances (community evenness)—decreases with age and lung disease progression (4). While the causal relationship(s) between these observations is not clear, evidence suggests that antibiotic therapy, which often accelerates as patients age, is the primary driver of decreasing airway microbial diversity (5). Regardless of the pathophysiologic underpinnings, the association between low bacterial diversity and decreased lung function is clear (6).

Culture-independent detection of bacterial species in CF respiratory specimens has focused on analysis of the 16S ribosomal subunit gene (16S rRNA). Initial studies employing 16S rRNA terminal restriction fragment length polymorphism analysis (7) have been largely supplanted by deep sequencing of PCR-amplified hypervariable regions of the 16S rRNA gene. More recent studies have employed a metagenomic approach, sequencing all the DNA in a biological specimen to identify the bacterial species present therein. Although DNA sequence analyses are increasingly finding their way into the clinical microbiology laboratory, methods requiring detailed analysis of the large data sets resulting from deep sequencing remain primarily in the domain of research laboratories.

In this issue of the *Journal of Clinical Microbiology*, Flight and colleagues (8) describe the use of ribosomal intergenic spacer analysis (RISA) to assess CF sputum samples. This method employs PCR amplification of the intergenic transcribed spacer (ITS) region between the bacterial 16S and 23S rRNA subunit genes, which varies in length among bacterial species. The various ITS fragments amplified in a sputum sample are separated using a

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microfluidic platform and analyzed using commercially available software that takes ITS fragment density (an approximation of species relative abundance) into account. This analysis thus provides, in theory, a profile of the membership and relative abundance (i.e., diversity) of the bacterial species present in the specimen.

Among a set of 200 sputum samples analyzed by RISA, culture results were available for 179 samples, and 59 samples were analyzed by deep sequencing of hypervariable regions V4 to V6 of the 16S rRNA gene, allowing comparison between these methods. The authors interpreted the cluster analysis of the 200 RISA profiles as showing two broad groups, which were designated the “*Pseudomonas* group” and the “emerging nonfermenting Gram-negative (eNFGN) group.” As an aside, the use of the term “emerging” in this context seems misplaced considering that the main species in this group (*Burkholderia* spp., *Achromobacter* spp., *Ralstonia* spp., and *S. maltophilia*) have been causing infection in CF with steady annual incidences for the past couple of decades. There was considerable overlap in the bacterial species detected by culture between these two groups, with 74% and 20% of the sputum samples being culture positive for *P. aeruginosa* in the *Pseudomonas* and eNFGN groups, respectively. In the subset of 59 sputum samples for which RISA, culture, and 16S rRNA sequencing were performed, many more species were detected by RISA and sequence analysis than were reported from routine CF culture. While this finding is not unexpected, it is noteworthy that 16S rRNA sequence analysis detected the presence of “known CF pathogens,” including *Pseudomonas*, *Burkholderia*, *Achromobacter*, and *Stenotrophomonas*, in several sputum samples that were culture negative for these species. Similarly, 10 (42%) of the 24 samples that RISA cluster analysis placed in the eNFGN group were culture negative for either *Burkholderia*, *Stenotrophomonas*, or *Achromobacter*, all of which were detected by 16S rRNA sequence analysis.

The authors also noted that the presence of a dominant ITS band in the RISA profile is likely indicative of a community with lower bacterial diversity than a community for which the RISA profile shows no dominant band. This is reflected in the Shannon diversity index, which measures both community richness and community evenness; decreased evenness, due to the dominance of one species, results in a decreased measure of diversity.

As the authors acknowledge, RISA lacks the specificity to provide definitive species identification. Further, precisely what constitutes a dominant ITS band—and, by extension, a bacterial community with relatively low diversity—is left undefined. The utility of RISA then is that it provides a means to screen CF sputum to differentiate bacterial communities that are dominated by *Pseu-*

domonas or by another species. Samples that fall into the latter group should yield a culture that is positive for a species other than *Pseudomonas*. The utility of differentiating airway communities with and without a dominant species (i.e., communities that are more or less diverse) will require a better understanding of how this information could impact clinical management of CF.

Despite the limitations of RISA, this study demonstrates that analysis of CF sputum beyond routine culture, employing techniques that are currently available in clinical microbiology laboratories, can provide a more informative assessment of CF respiratory samples. As we continue to expand our understanding of the microbial ecology of the CF airways, it is likely that CF caregivers will expect analyses that go beyond the detection of traditional “CF species of interest.” In the not-too-distant future, detection of nontraditional species, assessment of species’ relative abundance, and measures of overall microbial community diversity may become important components of “routine” analysis of CF respiratory specimens.

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