Metagenomic Characterization of the Respiratory Microbiome
A Pièce de Résistance

The existence of complex communities of commensal microorganisms ("microbiota") within the respiratory tract has been well established for a number of years now. The first important step in confirming the existence of a microbiome within the lower respiratory tract came through the application of culture-independent, high-throughput sequencing techniques using PCR-based amplification of the ubiquitous bacterial 16S ribosomal RNA (rRNA) gene (1). To date, the majority of respiratory microbiome studies have harnessed the 16S rRNA–sequencing technology to provide the descriptive information that the lower respiratory tract harbors distinct communities of commensals in health, with alterations ("dysbiosis") induced in chronic lung disease and/or by its therapies (2–5). However, this technology has the following recognized limitations: similarities in 16S rRNA sequences make separation between closely related commensals difficult at the species level, and 16S rRNA–based approaches also, importantly, do not provide any information about bacterial traits such as virulence, adherence, or antimicrobial resistance.

Shotgun metagenomic sequencing is a more sophisticated technique that is based on unrestricted DNA sequencing of all genetic material within a sample to allow for an unbiased and deeper taxonomical analysis of the microbiome. The assembly of short sequencing reads into larger fragments can increase the discriminatory power between microbes, enhancing taxonomic resolution up to the species or strain level. This technique provides more detailed insight into the functional capacity of commensals within a community and has been used to good effect to provide a unique understanding about the intestinal tract (6). However, microbial metagenomic sequencing in pulmonary samples is considerably more challenging because of the lower bacterial burden, meaning that the host DNA reads vastly outnumber bacterial reads, and a high sequencing depth is thus required to provide meaningful information.

In this issue of the *Journal*, Mac Aogáin and colleagues (pp. 433–447) report the largest metagenomic evaluation of respiratory specimens performed to date (7). The study provides an in-depth evaluation of the airway metagenome across a range of chronic respiratory disease states (chronic obstructive pulmonary disease, severe asthma, and bronchiectasis) and health. This strategy identified a core airway resistome, harbored by the lung microbiome, which is dominated by macrolide-resistance genes and occurs independently of health status or antibiotic exposure. The methodology used differs from previous studies in this field, in which steps have been taken to deplete host DNA from respiratory samples (8); the authors adopt a novel approach in which they increased sequencing depth to ensure sufficient bacterial reads were captured without the need for prior host depletion.

These findings are of great interest to the respiratory community given that long-term prophylactic antibiotics, including macrolides, are being increasingly used in the management of patients with frequently exacerbating chronic respiratory disease (9). The concept that increasing macrolide use in chronic lung disease might alter the "resistome" within microbiota has important implications within the landscape of escalating antimicrobial resistance rates globally. However, the detection of macrolide-resistant genes does not necessarily indicate clinical resistance but, rather, signals a resistance potential. The identification of the same core resistome genes in sputa from healthy subjects in the study by Mac Aogáin and colleagues suggests that the occurrence of these genes is highly ubiquitous. Accordingly, a similar presence of resistance genes has been reported in a study of the intestinal and skin microbiome of uncontacted Amerindian subjects with no previous exposure to antibiotics (10). Any theoretical risk of macrolide-induced resistance must be weighed up carefully against the recognized beneficial effects of these antibiotics in certain disease groups (9, 11). These findings provide direction for future studies to assess the occurrence of these genes in patients chronically colonized with drug-resistant bacteria.

As with any cross-sectional human microbiome study, disentangling cause from effect is challenging. However, a recent study by Taylor and colleagues (12) supports a causal role for macrolide therapy in increasing the carriage of antibiotic-resistance genes. In a before-and-after analysis of the AMAZES (Effect of Azithromycin on Asthma Exacerbations) study, 48 weeks of azithromycin therapy in subjects with asthma was shown to induce metagenomic shifts in the abundance of antibiotic-resistance genes in cultured clinical sputum isolates (12), with some overlap of the same genes reported by Mac Aogáin and colleagues (7). Further validation of these data will require confirmation in human macrolide intervention studies with more detailed longitudinal sampling coupled with functional manipulations in disease-relevant animal models.

The authors correctly acknowledge that the use of sputum may not exclusively capture the lower respiratory tract microbiota (7). Multiple studies indicate an overlap between oropharyngeal and respiratory microbiome profiles in sputa (13, 14), suggesting that this sample type may reflect a composite of the gastrointestinal and respiratory tract microbiome. However, sputum analysis represents a pragmatic approach that yields more easily accessible samples than invasive sampling techniques, and the oral microbiome is well recognized to be a direct source of commensals within the lower respiratory tract.

A further interesting aspect of the study by Mac Aogáin and colleagues relates to their evaluation of inhaler swab metagenomes paired with sputum analyses from the same subjects. These data indicate significant overlap between the inhaler and sputum

resistome, raising speculation that resistance transfer may occur between the host and their environment. Furthermore, this simpler sampling method could perhaps represent an easy-to-access surrogate measure of the host microbiome. This has potential utility given that spontaneous sputum samples are typically obtained <50% of the time, even in a dedicated clinical trial environment (15). Further studies are now needed to validate these findings independently.

Overall, the authors should be commended for taking a novel methodological approach to address a clinically important question. This intriguing study may herald the beginning of a new era in which we evolve from descriptive studies using 16S rRNA sequencing–based characterization of the microbiome toward more sophisticated techniques that offer greater insight into the functional roles and metabolic capacities ascribed to respiratory commensals. Ultimately, the hope is that this increased understanding will have huge implications for the development of personalized medicine-based approaches to patient management and also stimulate exciting new microbiota-focused therapies for respiratory disease. ∎

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