



Editorial

 Look at the wood and not at the tree: The microbiome in chronic obstructive lung disease and cystic fibrosis[☆]


Mirar el bosque y no el árbol: el microbioma en la enfermedad pulmonar obstructiva crónica y la fibrosis quística

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Sequencing of the human microbiome

Currently available culture-based microbiological techniques are inadequate for the identification of up to 80% of the microorganisms that are hosted on mucosal surfaces. This is because many of the bacteria that form part of the human microbiome are difficult to culture using conventional methods, and culture of obligate anaerobic flora or samples with low bacterial loads often produces false negatives. The sequencing of the gene that encodes the 16S component of ribosomal RNA (16S rRNA) after amplification has permitted a highly-detailed study of microbial diversity, genotyping the bacterial presence as a whole through to the level of genera, and in many cases even of species, irrespective of the results obtained on culture.

Respiratory microbiome

The implementation of culture-independent techniques in the respiratory tract proved that the bronchial tree of normal subjects is not sterile, and in the absence of respiratory disease, it hosts a microbial flora that is very similar to that of the oropharyngeal environment, due to migration to the bronchial tree. The study of the microbiome hosted by the various compartments of the respiratory system has allowed us to rule out the possibility that bronchial flora retrieved from sputum and identified

by sequencing is attributable to contamination by oropharyngeal flora. Similarities between the bronchopulmonary and oropharyngeal microbiome are determined by migration of the supraglottic flora to the tracheobronchial tree. Indeed, the similarity between the two bacterial compositions is higher when the bronchial area examined is closer to the oropharynx, as in the case of the right upper lobe.¹ The migration of oropharyngeal bacteria to the tracheobronchial tree is facilitated by microaspiration, a phenomenon that occurs periodically and intermittently at night.¹ The flora originating in the oropharynx is subsequently modulated by the microenvironment of the bronchopulmonary space, which favors the growth of some of its components.² This modification is magnified in the presence of chronic lung disease (COPD), a situation in which the tracheobronchial flora is clearly different from that of the oropharynx.³

Stable disease

In COPD, analysis of the respiratory microbiome has shown that bacterial diversity in the tracheobronchial tree becomes reduced as the disease becomes more severe. This can be identified by a decline in the Shannon Diversity Index, a marker that recognizes both reduced diversity and reduced abundance in the presence of bacterial types. This anomaly is mainly attributable to a decrease in anaerobic flora, especially the genera *Prevotella* and *Veillonella*, which are partially replaced by proteobacteria such as *Haemophilus* and *Pseudomonas*, genera that include common respiratory pathogens that become a key part of the flora hosted in the respiratory tree of these patients.^{4,5} The same pattern has been observed in cystic fibrosis, which has also been associated with repeated use of antibiotics.⁶

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Exacerbation

Although the respiratory flora as a whole only changes in about half of COPD exacerbations,⁴ the relative abundance of genera that include specific respiratory pathogens increases significantly compared to previous samples obtained from the same patients when they are stable.^{4,7} The sensitivity of conventional bacterial culture for identifying the causative agent of an exacerbation in COPD is limited. In more than 70 % of these episodes, a pathogen identified in the sequencing of the patient's sample increases in abundance from stable state levels to a level of dominance of over 50 %, but this microorganism is not identified in culture in about half of these events.^{8,9} This limitation has also been identified in cystic fibrosis.^{10,11} Analysis of the respiratory microbiome in COPD found that potentially pathogenic microorganisms, such as *Moraxella catarrhalis*, are responsible for exacerbations in more than one-third of cases, after it was determined that the genus *Moraxella* occurs at relative abundances that are clearly higher than those observed in the same patients in a previous period of stability.^{4,7}

Culture and the microbiome

Positive cultures for *Pseudomonas aeruginosa* in COPD and cystic fibrosis raise specific problems when examined in the light of the results obtained from analysis of the microbiome of the respiratory samples. Several studies have shown a good correlation between the relative abundance of the genus *Pseudomonas* in sequencing and the quantification of *Pseudomonas aeruginosa* in culture,^{7,8,12} and both sequencing and culture have been useful for identifying chronic colonization by this microorganism when it continued to be isolated over time.¹³ When an exacerbation occurs in a chronically colonized patient, however, the finding of positive cultures for *Pseudomonas aeruginosa* can be ambiguous, since the colonizing organism is sometimes identified, and not the organism actually causing the exacerbation. This occurs in about 20 % of exacerbations in patients colonized with *Pseudomonas*, in whom sequencing reveals that the organism causing the exacerbation is not *Pseudomonas aeruginosa*, despite this pathogen appearing in the culture.^{8,9}

Sputum and bronchoalveolar lavage

It is often difficult in respiratory diseases to select the most suitable sample for analysis of the respiratory microbiome. Clearly sputum, whether spontaneous or induced, is the most easily obtained sample, although is only representative of the bronchial airway.¹⁴ Results from bronchoalveolar lavage are similar to those from distal bronchial brushing obtained in sterile conditions.² The relative advantage of lavage (i.e., collection of a large volume of material that extends to the alveolar region) is counteracted by the dilution effect of the technique, which limits the validity of the results obtained. Sampling situations in which the material obtained contains a lower amount of biomass due to dilution, as is the case with bronchoalveolar lavage, reduce the number of copies of the *16S*rRNA gene in the sample.¹ As a result, the low concentrations of bacterial contaminants that are ubiquitous in laboratory reagents are magnified when determining relative abundance, overshadowing the low proportion of material of bacterial origin and complicating the interpretation of the results obtained.¹⁵ Thus, when analyzing the microbiome in respiratory samples, particularly bronchoalveolar lavage, it is important to use controls that

have undergone the same analytical process and that, therefore, contain the same contaminants inherent in that process. This will allow technicians to exclude the types of bacteria present in the control samples from the final readings obtained in the study sample, if needed.

By analyzing the respiratory microbiome, we have been able to confirm the abundance and diversity of the microbial flora hosted in the tracheobronchial tree, and the changes associated with the development of a chronic disease and its exacerbations. It is now clear that a change in the bacterial load of a microorganism is not the only fundamental element in the microbiological progress of a certain disease, and that these specific alterations must be interpreted only as a component of the changes that occur in the overall microbial flora and its all-important interaction. The tree is only part of the forest.

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