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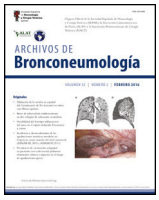

Editorial

The Respiratory Microbiome: Beyond the Culture☆

El microbioma respiratorio: más alla del cultivo

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In healthy individuals, if cultures of samples obtained distal to the glottis were negative, the bronchial tree and the lung were assumed to be sterile. Culture-independent microbiological techniques have confirmed that the bronchopulmonary tree hosts a wide range of microorganisms, known as the microbiome, composed of bacteria, viruses, and fungi. Only 1% of this microbiota grows on culture.

Until now, culture-independent analysis of bacterial communities been based on the gene coding for ribosomal RNA (16S rRNA). The ribosome is essential for the transcription of messenger RNA and, in bacteria, the 16S rRNA gene has remained unaltered over centuries, since any mutations would limit its viability. The taxonomic classification of all bacterial strains can be determined by analyzing the 16S rRNA gene, and the sequencing of this gene is used to describe the bacterial composition of an ecosystem.[1] Reference databases for the 16S rRNA gene allow the sequences of a sample to be classified from the highest (phylum) to the lowest (genus) taxonomic levels, although the data may sometimes be inadequate for achieving the species level. Bacteria are identified in terms of "operational taxonomic units" (OTU), and each taxonomic unit similar to the reference is considered an OTU. Thus, an OTU will be considered as equivalent to a genus if similarity with the reference is 95%, or equivalent to a species if it is 97%. The microbiome composition is usually expressed in terms of relative abundance, understood as the proportion of 16S rRNA gene copies that correspond to each OTU identified. The greatest limitation of 16S rRNA gene analysis is that it cannot be used to obtain information on viruses and fungi.

The normal individual hosts a phylogenetically diverse microbial flora in the bronchopulmonary tree,[2,3] the most common phyla being Firmicutes, Bacteroidetes, and Proteobacteria, while rates of OTUs corresponding to potentially pathogenic microorganisms, such as those of the *Haemophilus* genus, are low.[4] Recent same-day testing of samples taken using different oropharyngeal and bronchoalveolar lavage techniques aimed at preventing crossover contamination have confirmed the similarity of the oropharyngeal and the bronchopulmonary microbiome in healthy individuals, a parallelism that has been attributed to the aspiration of secretions during sleep.[5,6]

Very little information is available on the effect of tobacco smoke on the respiratory microbiome in healthy individuals. Early studies on oropharyngeal samples have shown dysbiosis, understood as a clearly increased relative abundance of one or more genera.[7] Studies of respiratory flora, however, showed no differences between smokers and never-smokers,[8] indicating that the response to tobacco smoke components is greater in the oropharyngeal flora than in the bronchopulmonary microbiota.

Bronchial colonization by potentially pathogenic microorganisms in COPD has been well established by culture techniques, and the appearance of respiratory symptoms during exacerbations has been related mainly with new strains appearing in the flora. Studies of the bronchopulmonary microbiome in patients with stable COPD have found clear differences compared to the typical flora of a healthy individual.[2,9] Proteobacteria, Bacteroidetes, Acinetobacteria, and Firmicutes occur more frequently in COPD, and *Pseudomonas, Streptococcus, Prevotella* and *Haemophilus* are common genera in these patients. This predominance has been observed both in samples of bronchial mucosa and in bronchoalveolar lavage or protected specimen brushing samples, which have been shown to be equivalent.[5,10] Similar results have been obtained in sputum, although this specimen comes from a different region of the respiratory tract, and hosts a different microbial community.[10]

The study of the microbiome in respiratory secretions during exacerbations reveals an increase in the relative abundance of specific genera, while colonizing flora remain unchanged.[11] In some of these episodes, this increase in abundance of the causative bacteria is not identified on culture, which only recovers microorganisms that have not changed in relative abundance compared to samples obtained during periods of stability, even though these may be potentially pathogenic, such as *Pseudomonas aeruginosa*. Thus, examination of the respiratory microbiome confirms that in some exacerbations, the increase in a bacterial pathogen is not detected by conventional microbiology, due to its limited sensitivity. Instead, the culture identifies microorganisms that in reality are only colonizers, giving misleading information to the clinician. Viral infections help promote these changes in the bacterial composition of the respiratory microbiome during exacerbation, since in the weeks immediately subsequent to a common cold, the relative abundance of Proteobacteria increases.[12] Treating a

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COPD exacerbation with antibiotics reduces the abundance of Proteobacteria, while treatment with corticosteroids alone promotes the overrepresentation of specific taxons, including genera of the Proteobacteria phylum.[13]

The recent characterization of the respiratory microbiome in idiopathic pulmonary fibrosis revealed an overrepresentation of microorganisms of the *Streptococcus, Staphylococcus* and *Prevotella* genera.[14] The presence of dysbiosis with a predominance of microorganisms of the Proteobacteria or Firmicutes phyla in bronchoalveolar lavage samples is associated with an inflammatory response, while overrepresentation of Bacteroidetes is associated with remodeling,[15] which may be of particular importance in pulmonary parenchymal diseases.

Although we are beginning to understand the microbial composition of the respiratory flora in chronic respiratory disease, the involvement of the microbiome in the pathogenesis of these diseases, and particularly how it acts on components thought of as non-pathogenic according to culture-based microbiology, is practically unknown. The loss of bacterial diversity, often related with an increase in the relative abundance of Proteobacteria, is associated with increased severity in most chronic respiratory pathologies, and it is very likely that this change in the composition of the microbiome is one of the factors that influences the course of the disease, as has already been demonstrated in idiopathic pulmonary fibrosis.[14] Recently introduced techniques, such as analysis of bacterial RNA and metagenomics, will help provide functional information about the respiratory microbiome, clarify interactions between viruses, fungi and bacteria, and potentially, facilitate the design of interventional studies aimed at conserving the mutualism of the microbial flora, thus maintaining the functionality of the respiratory system against the respiratory pathogens that progressively substitute the microbiota in the case of COPD or idiopathic pulmonary fibrosis.

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