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Review article

Microbiota and Human Health: Characterization techniques and transference[☆]

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ABSTRACT

The human microbiota comprises all the microorganisms of our body, which can also be categorised as commensals, mutualists and pathogens according to their behaviour. Our knowledge of the human microbiota has considerably increased since the introduction of 16S rRNA next generation sequencing (16S rDNA gene). This technological breakthrough has seen a revolution in the knowledge of the microbiota composition and its implications in human health. This article details the different human bacterial ecosystems and the scientific evidence of their involvement in different diseases. The faecal microbiota transplant procedure, particularly used to treat recurrent diarrhoea caused by *Clostridium difficile*, and the methodological bases of the new molecular techniques used to characterise microbiota are also described.

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Microbiota en la Salud Humana: técnicas de caracterización y transferencia

RESUMEN

La microbiota es el conjunto de microorganismos que reside en nuestro cuerpo, que a su vez pueden diferenciarse según su comportamiento en comensales, mutualistas y patógenos. El conocimiento de este ecosistema se ha visto considerablemente incrementado tras la introducción de las técnicas de secuenciación masiva del gen 16S ARNr (gen ADNr 16S). Este avance ha supuesto una verdadera revolución en el conocimiento de la composición de la microbiota y de su implicación en los estados de salud y enfermedad del ser humano. En este documento se detallan los diferentes ecosistemas bacterianos que podemos encontrar en el cuerpo humano y las evidencias científicas que existen en relación con diferentes enfermedades. También se describe el procedimiento de transferencia de materia fecal, particularmente utilizado para el tratamiento de las recidivas de la diarrea por *Clostridium difficile*, y las bases metodológicas de las nuevas técnicas moleculares utilizadas en la caracterización de la microbiota.

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Introduction

The microbiota is the set of microorganisms (bacteria, fungi, Archaea, viruses and parasites) residing in our bodies. These microorganisms may be classified as commensal, mutualistic or pathogenic. The term microbiome refers to the entire habitat, including the microorganisms, their genes and the environmental conditions. However, in practice, the two terms are used interchangeably as the suffix *-biome* (community) and the suffix *-oma* (set) are conflated.¹ In each of the different locations of our body we can find complex microbial ecosystems. The most complex, diverse and numerous is the one associated with the digestive tract, particularly in the caecum, where the density of micro-organisms is the highest in our body. These communities have a symbiotic and mutualistic behaviour with human eukaryotic cells, are essential for the proper functioning of our body, maintain an important dialogue with the immune system and have homeostatic functions that condition our health.² Numerous pieces of scientific evidence have implicated the intestinal microbiome and its metabolic potential in diverse pathological states over the last years, creating new therapeutic strategies to control and regulate this ecosystem.³ Among these new approaches is the transfer of faecal microbiota, with increasing popularity given its success in the treatment of recurrent diarrhoea caused by *Clostridium difficile*.⁴

The knowledge of our microbiome has been considerably expanded after the use of molecular massive sequencing techniques, especially next-generation sequencing techniques. Microbiological cultures have always been used to determine the composition of the microbiota, but nowadays it is known that most of the micro-organisms of this ecosystem cannot be cultivated using traditional means, and their detection is only possible after DNA sequencing as a genetic fingerprint. The use of molecular techniques has enabled identifying and taxonomically assigning most microorganisms without the need to culture them.⁵ This advance, as well as other microbiological techniques that will be detailed in this article, have led to a true revolution in the knowledge of the microbiota and its implication in the health and illness of human beings.

Clinical considerations

From birth, there is a symbiotic relationship between the microbiota and our cells that evolves over time, adapting to changes.⁶ Due to its enormous metabolic capacity, the microbiota has been considered as an essential "organ" for life, with an influence on health and illness.⁷ It is composed of unique features and characteristics of each individual, and may vary depending on genetics, diet and interaction with the environment.

The study of this ecosystem is a field of rapid scientific progress, with it being universally accepted that to reach an adequate state of health it is also necessary to have a "healthy" microbiota. Our microbiota undergoes changes due to the influence of multiple factors, in a way similar to those experienced by any organ of our body from ontogeny to death. We are continually exposed to factors that may influence, although one of its characteristics is its great capacity for resilience⁸ (ability to adapt to a disturbing agent or an adverse situation, with subsequent recovery of the initial state when the alteration ceases), immediately recovering its natural state, which is termed "eubiosis". The level of these changes is defined not only by the nature, strength and duration of the alteration, but also by the composition and stability of each microbiota, assuming that each is unique to each person. On some occasions, the nature of the alteration is so strong that it conditions alterations in its composition or functioning, reaching a state of dysbiosis. Dysbiosis can occur in a matter of days, particularly after the ingestion of

antibiotics, but it can also be a consequence of other longer-term actions, fundamentally related to diet.

In an adult, the gastrointestinal tract can be home to between 500 and 1000 species of micro-organisms, with bacteria of the *Bacteroidetes* (~25%) and *Firmicutes* (~60%) phyla being the majority. In a lesser proportion, *Proteobacteria*, *Verrucomicrobia*, *Fusobacteria*, *Cyanobacteria*, *Actinobacteria* and *Spirochaetes*, Archaea, fungi, protozoa, viruses and other micro-organisms are detected. It is also important to keep the proportions balanced, and therefore the *Firmicutes/Bacteroides* ratio has been established as a parameter to evaluate the balance of the intestinal microbiota and its functionality. In obese people, this ratio is greatly altered by the increase in *Firmicutes*. The increase of *Firmicutes* has also been described physiologically in elderly people as a consequence of age.

The main functions of the intestinal microbiota are to prevent colonisation by other pathogenic microorganisms, help digest food, produce vitamins B and K that the human body is not able to synthesise and, finally, and by no means less important, to stimulate the immune system. After birth, the cells of the immune system lack stimuli, recognising all the antigens around them as part of the body and blocking the inflammatory response against them. That is why the first contacts of the microbiota with the undifferentiated immunological cell lines are very important, and they will help define what belongs to "the body" from what is "foreign". This system and the intestinal microbiota maintain a continuous mutualistic dialogue, but if this situation becomes unbalanced, a pathological process can begin. This seems to be the basis of certain autoimmune diseases where the antigens of the intestinal microbiota represent a stimulus large enough to trigger an inflammatory response. In other diseases, such as metabolic syndrome and obesity, the origin of the stimulus that causes a continuous basal inflammatory response is also attributed to the intestinal microbiota.

The existence of the gut-brain axis (GBA) has recently been described, which connects the central nervous system with the intestinal microbiota through the vagus nerve, the parasympathetic nervous system and bacterial metabolites, which can act as neurotransmitters, and the endocrine system associated with the digestive tract.^{9,10} Thus, in addition to the diseases that have traditionally been related to alterations in the microbiota, such as obesity, type 2 diabetes, inflammatory bowel diseases and allergies, other diseases of the central nervous system have recently been associated with it, such as autism, anxiety, depression and alcohol dependence.

It is currently accepted that in order to reach a complete state of health it is necessary for our microbiota, particularly that associated with the gastrointestinal tract, to also be healthy. The main health indicators of the microbiota are its richness (number of microorganisms) and its biodiversity (number of species). Both parameters are evaluated with alpha-type biodiversity indices, such as the Shannon diversity index (reflects the heterogeneity of a community based on the number of species present and their relative abundance), and the Chao index (abundance and representation of each species in all samples).

Numerous associations between pathological states and alterations of the microbiota have been published, either due to the presence or increase of certain genera, or the complete opposite, i.e., the absence or reduction of their concentration. Computational methods are used to evaluate and compare the microbiota of a patient with that of a healthy subject, such as the clustering method, or reduction of the dimensions of the distance matrices that define the set of samples of each study. It is also possible to perform a principal component analysis, which allows the addition of other clinical variables.

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