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Editorial

Challenges of landscaping and modulation strategies in human intestinal microbiota☆

Desafíos para la caracterización y estrategias de la modulación en la microbiota intestinal humana

The incorporation of molecular techniques based on massive sequencing and the bioinformatics tools to interpret the results is still a pending challenge in Clinical Microbiology Laboratories. Being able to identify microorganisms without cultivation has allowed us to expand our knowledge of complex ecosystems, such as the intestinal microbiome. In the article by Ventero et al., 2020, these techniques (DNA extraction of microorganisms present in stool, PCR amplification of the 16S rDNA gene and massive sequencing by Illumina technology) were applied to monitor the bacterial composition of the microbiota of a patient who received a faecal microbiota transfer (FMT) from a healthy donor by colonoscopy.[1] The main objective of this work was to compare the usefulness of different bioinformatics tools in the analysis of metagenomic results.

For the microbiome characterization, ecological parameters are used to define the total number of species and their distribution (alpha diversity: Shannon and Chao1 indexes) and to compare ecosystems of different subjects or times (beta diversity: Bray-Curtis and Unifrac indexes). Cut-off points to classify a microbiome as healthy or pathological have not been yet established, although the general rule is that the higher the alpha diversity, the healthier and more resilient the ecosystem. Similarly, there is no consensus on the "normal" microbiota composition, either for the presence/absence of particular taxa or for their abundance, although we can compare different samples from the same patient over time, as in the case of Ventero et al., or between groups of subjects. As cited in the article that originated this editorial, LEfSe (Linear discriminant analysis Effect Size) is one of the most demanding tools for identifying taxa with statistically significant differential abundance and is also one of the most widely used in research work.[2] This tool, as many others, is usually available in freely accessible web repositories for global analysis (https://www.microbiomeanalyst.ca/, https://huttenhower.sph.harvard.edu/galaxy/, https://microbiome-tools.embl.de/, etc.) or as downloadable and executable software in a Linux environment (https://github.com/SegataLab/lefse/).

It is highly recommended that a bioinformatician with biological knowledge integrating integrate the available clinical information (metadata) in the analysis, since without both facets it is difficult to reach solid conclusions. This whole process must always be carried out in a clinical context by qualified personnel, since, as mentioned above, there is currently no parameter that can differentiate a healthy microbiota from a pathological one, and the particularities of each patient must always considered. In recent years these molecular technologies have contributed to revealing the importance of the gut ecosystem in human health. Most of the studies have focused on understanding the bacterial composition, being the characterization of the virome or mycobiome still pending, and most importantly, the global microbial metabolism and the interaction of their metabolites with our human cells.

Increasing knowledge about the microbiota composition and functionality will be essential to define and identify specific pathological situations, but we will also need to integrate the physical and biological rules governing microorganisms in order to be able to carry out specific therapeutic actions. Definitively modifying the composition of a microbiome is beyond our reach today. All studies point to diet as the main factor modulating the composition of the intestinal microbiota, while available probiotics, most of them based on food-derived species, have shown limited action and almost always no engraftment ability. Second-generation probiotics based on bacteria that are a common part of the human microbiota from healthy subjects, such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii* and *Clostridium leptum*, are expected to appear in the near future.[3]

Currently the most drastic strategy available for microbiota modulation is FMT, being the treatment of recurrent *Clostridioides difficile* infection the exclusive indication with sufficient scientific evidence of their usefulness, as described by Ventero et al. This pathology has become increasingly important in recent years due to its rising incidence, high morbidity and mortality, high consumption of healthcare resources and prolonged hospitalisation of patients suffering from it.[4] The prevalence of this infection is

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increasing in all developed countries, with an estimated 124,000 cases per year in Europe, representing the sixth most frequent nosocomial pathogen during 2016–2017.[5] In Spain, a notable increase in this pathology has also been documented due to a better diagnosis based on clinical suspicion, a higher prevalence of predisposing factors such as advanced age, pluripathological status, and mainly antibiotic use, and finally the irruption of hyper-virulent hyperepidemic strains as 027 and 078 ribotypes.[6]

The natural history of infection begins with exposure to antibiotics that lead to the disruption of the gut microbiota with decrease of their alpha diversity. Subsequently, *C. difficile* increases its population and begins to synthesise toxins responsible for diarrhoea and tissue damage. Interestingly, the study of the microbiome shows that during infection *C. difficile* coexists with other microorganisms, contradicting the theory that the gut microbiota must be totally devastated for infection to occur.[7] Current evidence supports that it is the loss of functionality of the ecosystem that triggers this disease, rather than the alteration of its taxonomic composition,[8] as different species may have common metabolic pathways and, conversely, some pathways are only present in certain bacterial species.

However, the active fraction of faecal material that limits *C. difficile* toxin production is currently unknown, and the therapeutic effect of FMT is attributed to the restoration of ecosystem biodiversity, but this theory is increasingly questioned, as Ventero et al.[1] show that *C. difficile* coexists with other species during the infection. In fact, some patients during the diarrhoea period have been shown to maintain their microbiota.[7] Another fact that has challenged the central theory of the FMT effect is the therapeutic success achieved using pre-filtered (bacteria-free) faeces, although so far this has only been reported once.[9] In recent years, some alternatives to FMT have been proposed that involve greater control of the intervention, such as the use of probiotics and bacterial consortia, especially those strains producing bacteriocins with activity against *C. difficile*.[10] *Lactobacillus casei* has a protective role,[11] while a lower density of *Bacteroidetes* has been significantly associated with a worse prognosis.[7] Recent metabolomic studies[12] have identified alterations in the functionality of the microbiota during *C. difficile* infection, especially related to bile acids and leucine fermentation, which would give more weight to the functional role of the ecosystem than to its specific taxonomic composition. In this regard, it has been shown how FMT affects the metabolism of the patients' ecosystem, especially the production of short-chain fatty acids and endogenous amines.[13]

The future expectations of FMT for therapeutic purposes are numerous and affect numerous pathologies. There is growing evidence of its usefulness in the intestinal decolonisation of antibiotic multirresistant bacteria,[14,15] or in the maintenance of immunity during oncological treatments.[16,17] The preservation of healthy microbiota prior to therapeutic procedures that affect their functionality for a subsequent reintroduction (autologous transfer) is also increasing acceptance. All these aspects will increase the quality of life of our patients and must be centralised in Clinical Microbiology Laboratories, so it is a priority to incorporate the necessary methodology to determine the composition and functionality of the microbiome, as well as the tools to modulate it.

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Conflict of interest

The authors certify that there are no potential conflicts of interest regarding this study.

References

1. Ventero MP, Espinosa N, Jover R, Guillen Y, Merino E, Rodríguez JC. Evolution of intestinal microbiome in a process of faecal microbiota transfer (FMT) in a patient with *Clostridioides difficile* infection: NGS analysis with different software programs. Enferm Infecc Microbiol Clin. 2021;39:184–7.
2. Segata N, Izard J, Walron L, Gevers D, Miropolsky L, Garrett W, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12:R60, http://dx.doi.org/10.1186/gb-2011-12-6-r60.
3. O'Toole PW, Marchesi JR, Hill C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. Nat Microbiol. 2017;2:17057, http://dx.doi.org/10.1038/nmicrobiol.2017.57.
4. Marra AR, Perencevich EN, Nelson RE, Samore M, Khader K, Chiang HY, et al. Incidence and outcomes associated with *Clostridium difficile* infections: a systematic review and meta-analysis. JAMA Netw Open. 2020;3:e1917597, http://dx.doi.org/10.1001/jamanetworkopen.2019.17597.
5. Suetens C, Latour K, Kärki T, Ricchizzi E, Kinross P, Moro ML, et al. Prevalence of healthcareassociated infections, estimated incidence and composite antimicrobial resistance index in acute care hospitals and longterm care facilities: results from two European point prevalence surveys, 2016 to 2017. Euro Surveill. 2018;23:e1002150, http://dx.doi.org/10.2807/1560-7917.ES.2018.23.46.1800516.
6. Esteban-Vasallo MD, Naval Pellicer S, Domínguez-Berjón F, Cantero Caballero M, Asensio Á, Saravia G, et al. *Clostridium difficile*-related hospitalizations in Madrid (Spain) between 2003 and 2014, a rising trend. J Infect. 2016;72:401–3, http://dx.doi.org/10.1016/j.jinf.2015.12.003.
7. Hernández M, de Frutos M, Rodríguez-Lázaro D, López-Urrutia L, Quijada NM, Eiros JM. Fecal microbiota of toxigenic *Clostridioides difficile*-associated diarrhea. Front Microbiol. 2019;9:3331, http://dx.doi.org/10.3389/fmicb.2018.03331.
8. Pérez-Cobas AE, Artacho A, Ott SJ, Moya A, Gosalbes MJ, Latorre A. Structural and functional changes in the gut microbiota associated to *Clostridium difficile* infection. Front Microbiol. 2014;5:335, http://dx.doi.org/10.3389/fmicb.2014.00335.
9. Ott SJ, Waetzig GH, Rehman A, Moltzau-Anderson J, Bharti R, Grasis JA, et al. Efficacy of sterile fecal filtrate transfer for treating patients with *Clostridium difficile* infection. Gastroenterology. 2017;152:799–811, http://dx.doi.org/10.1053/j.gastro.2016.11.010, e7.
10. Valdés-Varela L, Alonso-Guervos M, García-Suárez O, Gueimonde M, Ruas-Madiedo P. Screening of *Bifidobacteria* and *Lactobacilli* able to antagonize the cytotoxic effect of *Clostridium difficile* upon intestinal epithelial ht29 monolayer. Front Microbiol. 2016;7:577, http://dx.doi.org/10.3389/fmicb.2016.00577.
11. Ma Y, Yang JY, Peng X, Xiao KY, Xu Q, Wang C. Which probiotic has the best effect on the prevention of *Clostridium difficile*-associated diarrhea? A systematic review and network meta-analysis. J Dig Dis. 2020;21:69–80, http://dx.doi.org/10.1111/1751-2980.12839.
12. Robinson JI, Weir WH, Crowley JR, Hink T, Reske KA, Kwon JH, et al. Metabolomic networks connect host-microbiome processes to human *Clostridioides difficile* infections. J Clin Invest. 2019;130:3792–806, http://dx.doi.org/10.1172/JCI126905.
13. Bruno G, Gagliardi A, Oliva A, Trancassini M, Macone A, Cicerone C, et al. Fecal microbial transplantation impact on gut microbiota composition and metabolome, microbial translocation and T-lymphocyte immune activation in recurrent *Clostridium difficile* infection patients. New Microbiol. 2019;42:221–4.
14. Seong H, Lee SK, Cheon JH, Yong DE, Koh H, Kang YK, et al. Fecal microbiota transplantation for multidrug-resistant organism: efficacy and response prediction. J Infect. 2020;81:719–25, http://dx.doi.org/10.1016/j.jinf.2020.09.003.
15. Bar-Yoseph H, Carasso S, Shklar S, Korytny A, Even Dar R, Daoud H, et al. Oral capsulized fecal microbiota transplantation for eradication of carbapenemase-producing Enterobacteriaceae colonization with a metagenomic perspective. Clin Infect Dis. 2020;8, http://dx.doi.org/10.1093/cid/ciaa737.
16. Stower H. Microbiome transplant-induced response to immunotherapy. Nat Med. 2021;27:21, http://dx.doi.org/10.1038/s41591-020-0-60122.
17. Lau HCH, Sung JJ, Yu J. Gut microbiota: impacts on gastrointestinal cancer immunotherapy. Gut Microbes. 2021;13:1–21, http://dx.doi.org/10.1080/19490976.2020.1869504.

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