

# Gastrointestinal microbiome, what is behind faecal microbiota transplantation?

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## Abstract

The intestinal microbiota is made up of billions of microorganisms that coexist in an organised ecosystem, where strict and facultative anaerobic bacteria predominate. The alteration or imbalance of these microorganisms, known as dysbiosis, can be associated with both gastrointestinal and extraintestinal diseases.

Based on a review of the literature, the intestinal microbiota is described in its state of health, the changes associated with some gastrointestinal diseases and the potential role that faecal microbiota transplantation has in the reestablishment of an altered ecosystem. Undoubtedly, the information revealed makes us reflect on the indication of faecal microbiota transplantation in various pathologies of intestinal origin. However, to ensure the efficacy and safety of this therapy, more studies are needed to obtain more evidence.

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## Introduction

The gut microbiota is made up of more than 100 trillion different microorganisms, including bacteria, fungi, viruses and protozoa [1]. It is a complex ecosystem that varies in number, depending on the identification tools used; therefore, both metagenomics and culturomics approaches are required to better understand the diversity and richness of bacteria present in the human gut microbiota [2]. In healthy individuals, it is dominated by strict anaerobes, 87% corresponding to the bacterial phyla *Bacteroidetes* and *Firmicutes*. To a lesser extent, bacteria belonging to other

phyla such as *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* can be found [3,4].

The proportion of bacteria varies throughout the gastrointestinal tract. The colon contains the highest number and diversity of species, compared with the stomach and small intestine [3].

One of the main roles of the gut microbiota is to contribute to the fermentation of various types of indigestible fibres by the small intestine. This activity results in the release of short-chain fatty acids (SCFAs), acetate, propionate and butyrate, which are used as an energy source by the colonic epithelium [5,6]. These molecules are essential for intestinal integrity, regulating luminal pH and mucus production [7,8]. In addition, they directly impact the development and activity of the immune system. They regulate various inflammatory processes, considering that the intestinal epithelium represents a stable barrier between lymphoid tissue and the microbiome itself [9,10].

It has been observed that losses of microbiota homeostasis or dysbiosis are associated with some diseases, which are explained by the imbalance and loss some species, such as *Faecalobacterium prausnitzii*, considered one intestinal health

biomarker [11,12] (Fig. 1). Studies have shown that this dysbiosis is involved in the development of a wide range of diseases, both intestinal and extraintestinal [13].

However, it is not fully understood whether these changes in the microbiota the cause of the disease or of it a consequence are, except for *Clostridioides difficile* infection where there is a clear causality [14]. Faecal microbiota transplantation (FMT) represents a unique procedure, aimed at restoring the natural diversity of the gastrointestinal microbiota and preventing the recurrence of a nosocomial disease, by correcting these imbalances with a healthy external microbiota [15].

The objective of this review was to describe the normal intestinal microbiota and the changes that occur in different gastrointestinal pathologies, to understand the basis of FMT as a therapy (going beyond *Clostridioides difficile*). A systematised search was carried out in Embase, Web of Science and PubMed.

## Gut microbiota in gastrointestinal diseases

### Inflammatory bowel disease (IBD)

IBD mainly encompasses Crohn's disease (CD) and ulcerative colitis, both of chronic course characterised by alternating periods of relapse and remission. Their clinical symptoms that include abdominal pain, diarrhoea, fatigue and extraintestinal manifestations that significantly affect patients' quality of life [16,17].

The aetiology of this disease is known as multifactorial, where there is interaction between the microbiome, genetic, environmental and immunological factors [18,19]. To respect,

studies have shown that an alteration in the composition and function of the microbiome [20].

Characteristic findings in IBD include a significant decrease in bacterias of the phyla *Firmicutes* and *Bacteroidetes*, including species such as *Clostridium symbiosum* and *Bacteroides dorei*. These bacteria are SCFA producers, such as butyrate, propionate and acetate [14,21]. The latter deliver energy to cells of the colonic epithelium but can also induce the differentiation of regulatory T cells of the immune response [22]. Furthermore, Brown et al. add that the sphingolipids produced by *Bacteroides* also play an important role in maintaining homeostasis, reducing inflammation of the intestine [23]. Other studies point out that both the transfer of proinflammatory bacteria and the transplantation of microbiota from sick to healthy mice can induce inflammation [24]. Based on these findings, FMT has been evaluated as a possible therapy for IBD through the restoration of the microbiome. It has demonstrated a clinical response in 53.8%, with a clinical and endoscopic remission in 37% [25]. Recently a meta-analysis that included 28 articles containing 976 IBD patients showed that responses and remission rated were more favourable for patients receiving repeated FMT regimens and antibiotic pretreatment strategies. However, the heterogeneity for all pooled analyses was high. Fifteen of the 18 studies (83%) that evaluated for shifts in specific gut microbial taxa reported increases in the abundance of anaerobes purported to produce health promoting anti-inflammatory SCFAs, such as *Bifidobacterium*, *Roseburia*, *Lachnospiraceae*, *Prevotella*, *Ruminococcus* and *Clostridioides* related species. Overall, FMT in patients with IBD was shown to be safe and well tolerated [26]. Others have also

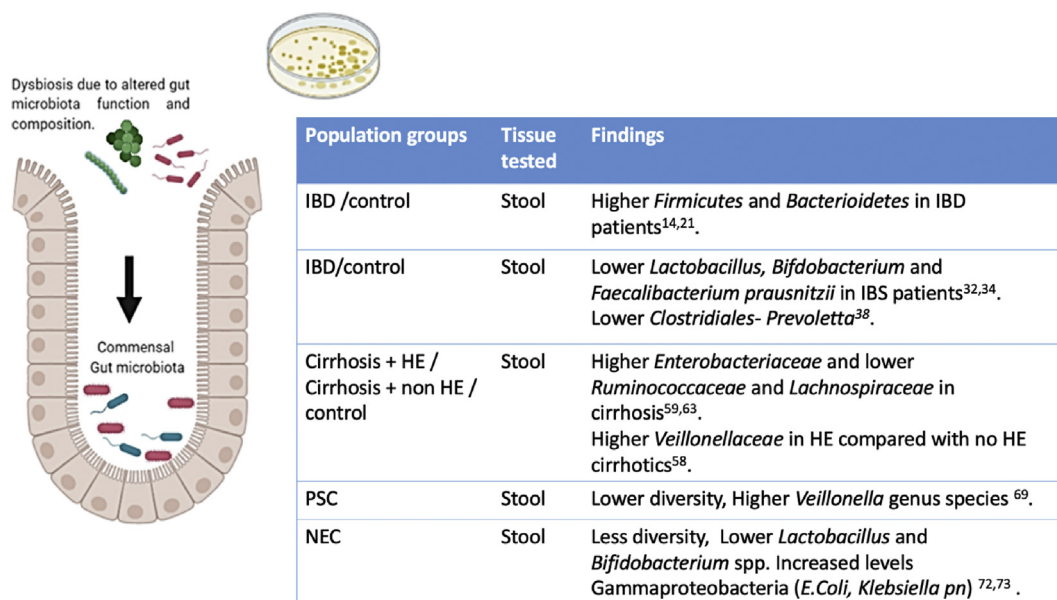


FIG. 1. Human microbiomes in gastrointestinal diseases.

confirmed that FMT could be an effective and safe therapy for CD. Interestingly, subanalyses suggested the rate of clinical remission with fresh stool FMT was higher than with frozen stool FMT (73% vs 43%;  $p < 0.05$ ) [27]. A systematic review that included nine studies containing 69 patients with chronic pouchitis showed that FMT has promising results and could be a useful and secure strategy in this scenario [28]. However, more studies are still needed to provide categorical evidence. Thus, the Third European consensus on Ulcerative Colitis of the European Crohn's and Colitis Organization highlights the need for additional studies to identify the best strategy, considering the route of administration, type of donor and security profile [29].

### Irritable bowel syndrome

Irritable bowel syndrome is a functional gastrointestinal disorder with symptoms including abdominal pain associated with a change in the shape or frequency of stool and colonic distention. The pathophysiology is not fully understood, but an altered connection between the gut and the brain is well established, leading to motility disturbances, visceral hypersensitivity [30].

The intestinal bacterial profile in patients with irritable bowel syndrome (IBS) differs from that in healthy subjects. The intestine of IBS patients has a lower abundance of butyrate-producing bacteria (*Erysipelotrichaceae* and *Ruminococcaceae* spp.) and a higher abundance of methane-producing bacteria (*Methanobacteriales* spp.) [31]. Also, the production of bile acids (BAs) has been investigated due to its ability to modulate intestinal function and its close connection with the intestinal microbiome and its relationship with irritable bowel syndrome. The study establishes an association between the presence of diarrhoea and visceral hypersensitivity and alteration of the metabolism of BAs in the intestine of patients with IBS, attributing this to an intestinal dysbiosis with a special reduction in genera of the *Ruminococcaceae* family [32], known for its high production of SCFAs [33].

A study carried out from the faeces of individuals with this syndrome identified lower levels of *Lactobacillus*, *Bifidobacterium*, *Eubacterium rectale* and *Faecalibacterium prausnitzii* [34], all of which are SCFA producers. Particularly, *F. prausnitzii* is one of the major producers of butyrate, which is why it is considered an indicator or biomarker of intestinal health [12,13]. On the contrary, low levels of these microorganisms could be predictive of CD [35].

Clearly, there are differences between the microbiota of IBS patients and the one of healthy patients. Despite this background, a single bacterial species has not been identified as a causative agent. Several authors agree that patients with IBS have an increase in microorganisms with proinflammatory properties, like some belonging to the *Veillonellaceae*,

*Pasteurellaceae* and *Fusobacteriaceae* families. Species such as enteroinvasive and adherent *Escherichia coli* and *Ruminococcus gnavus* have also been identified [36,37].

Other authors have also reported a decrease in the *Clostridiales-Prevotella* enterotype in individuals with IBS [38]. Undoubtedly, one of the strongest links between IBS and the intestinal microbiota is pointed out by a report in which FMT was performed. Faeces from IBS patients was transferred to healthy mice, which was associated with IBS phenotype characteristics, such as intestinal dysmotility, increased intestinal permeability and visceral hypersensitivity [39].

El-Salhy et al. analysed the potential role of FMT in patients with IBS and their results showed that this therapeutic strategy would be an effective treatment in this scenario. The response to FMT increased with the dose (76.9% and 89.1% of the patients who received 30 g FMT and 60 g FMT, respectively). The intestinal bacterial profiles changed also significantly in the groups received FMT (higher signals for *Eubacterium bifforme*, *Lactobacillus* spp. and *Alistipes* spp. after transplantation, and lower signals for *Bacteroides* spp.). The FMT adverse events were mild self-limiting gastrointestinal symptoms [40]. FMT increases the faecal SCFA and butyric levels in IBS patients. The increase in the butyric acid level was inversely correlated with abdominal symptoms and fatigue in IBS patients following FMT. These results suggest that SCFAs and the butyric acid play a role in the pathophysiology of IBS [41]. However, a systematic review and metaanalysis that included 4 studies showed not an overall clinical benefit from FMT for IBS patients. A discrepancy in efficacy of FMT for IBS may be related to the differences in route of administration, placebo treatment, FMT frequency and IBS subtype included among the studies [42].

### Steatosis/non-alcoholic fatty liver disease

In recent years, a direct relationship between obesity and metabolic disorders related to changes in the intestinal microbiota has been described [43,44]. In general, obese people have a higher concentration of bacteria of the phylum *Firmicutes* and less *Bacteroidetes*. Other studies have reported a decrease in *Bifidobacterium* concentrations in overweight subjects [45]. Metabolic syndrome, which includes insulin resistance, dyslipidemia, high blood pressure and increased abdominal circumference, is strongly associated with long-term development of type 2 diabetes and cardiovascular disease. The hepatic manifestation of this syndrome, although not part of its criteria, is non-alcoholic fatty liver disease. In this regard, metagenomic studies in humans have identified specific changes in the gut microbiota [46], including an increase in bacteria that contribute to insulin resistance, such as *Prevotella copri* and *Bacteroides vulgatus* [47]. Another study in obese mice, to which metformin was administered, described an increase in

*Akkermansia muciphila*, which has been associated to glucose homeostasis [48,49].

Likewise, it has been described that signalling molecules produced by intestinal bacteria can affect the intestinal integrity, the sensation of satiety and the metabolic phenotype of the host. SCFAs are the best-studied example; in this regard, butyrate exerts a positive regulation of tight-junction proteins such as claudin-1, induces apoptosis of T cells to eliminate the source of inflammation [50], while suppressing inflammation mediated by IFN- $\gamma$  in colon epithelial cells [51] and induces intestinal gluconeogenesis by different pathways [52]. For its part, acetate generates an increase in plasma incretin hormones, reduces TNF $\alpha$  and indirectly generates changes in the metabolism of lipids and glucose [53]. Bile acids are another class of molecules that play a role in microbiome-host communication, mediated by the nuclear farnesoid X receptor, which has been related to the regulation of glucose in obese mice [54].

### Liver cirrhosis and its complications

Changes in the gut microbiome play a role in the progression of cirrhosis and its complications. On the one hand, inflammatory mediators, endotoxemia and haemodynamic alterations lead to complications such as spontaneous bacterial peritonitis and hepatic encephalopathy (HE) [55]. The latter is the most studied in relation to the gut-brain axis [56]. Hepatic encephalopathy begins with cognitive changes or psychomotor deficits that can only be detected with psychometric or neurophysiological tests. This entity called minimal HE already presents alterations in the intestinal microbiota. Thus, Zhang et al. observed an increase in urease-producing bacteria such as *Streptococcus salivarius* in cirrhotic patients. This finding was significantly higher in patients with minimal HE than in those without HE ( $p = 0.03$ ) and was associated with a greater accumulation of ammonia in those with HE ( $R = 0.58$ ,  $p = 0.003$ ) than in the other group [57].

In turn, a change in the microbiota has been observed depending on the stage of progression of cirrhosis. There are two scoring systems, the Child-Turcotte-Pugh (includes albumin, bilirubin, prothrombin time, degree of encephalopathy and ascites) and MELD Model for end-stage liver disease (logarithmic score that includes bilirubin, creatinine and INR of prothrombin time). The higher the score, the worse the prognosis. Thus, a higher Child score has been associated with an increase of *Streptococcaceae*, *Veillonellaceae* and *Enterobacteriaceae* bacterial families, and a decrease in *Lachnospiraceae* [58]. Another study reported a higher MELD was associated with a microbiota with a higher number of *Enterobacteriaceae* and a lower proportion of *Lachnospiraceae* and *Ruminococcaceae* [59].

Current evidence, despite being scarce, points towards FMT could be an effective, safe and tolerable strategy in some liver

diseases. However, some unanswered questions remain about the optimal dose, the administration route, the long-term effects and the selection of the optimal donor. The gut microbiota appears to play a critical role in age-related immune clearance of hepatitis B virus [60]. An open-label pilot trial of FMT for chronic hepatitis B patients showed that this therapeutic strategy could induce HBeAg clearance in patients who have persistent positive HBeAg even after long-term antiviral treatment [61]. Given the current evidence of the paramount role of intestinal microbiota in HE and the limited therapeutic options, some studies have evaluated the efficacy and safety of FMT in this scenario [62]. An open-label randomised clinical trial included 20 cirrhotic patients with recurrent HE who were randomised 1:1 into FMT following 5 days of antibiotic pretreatment or standard of care. The study showed that FMT was safe, tolerated and effective with lower hospitalisations and improved cognitive tests. A relative increase in *Lachnospiraceae* and *Ruminococcaceae* was observed in the FMT arm compared with the SOC arm [63]. The same group, in a randomised and placebo-controlled trial (phase I), showed that encapsulated FMT did not reduce the rate of HE episodes compared with standard of care, despite improved cognitive functioning and increased duodenal microbial diversity [64]. Nevertheless, this study has some limitations that limited the conclusions about the efficacy of the oral route for FMT: the small sample size included in the study (20 patients), rifaximin and was not proton-pump inhibitors discontinued, scenarios that can modify intestinal microbiota.

### Primary sclerosing cholangitis

BAs, which participate in the emulsification and absorption of fats from the diet, are actively reabsorbed in the terminal ileum in a percentage close to 95%. The rest is unconjugated and transformed by the colonic microbiota, then passively passing to the portal circulation. BAs play a role in the control of bacterial overgrowth by binding to FXR (Farnesoid X receptor), which allows the production of antimicrobial peptides [24]. In primary sclerosing cholangitis (PSC), secondary to cholestasis there is an alteration of these BAs, leading to an alteration of the microbiota [65,66]. There is a decrease in the abundance and diversity of bacterial species [67,68]. Some subtypes such as *Veillonella* genus has been described up to 4.8 times more frequent in individuals with PSC versus healthy individuals and 7.8 times compared to ulcerative colitis, so this change is thought to be related to a liver disease rather than IBD [69].

### Necrotising enterocolitis

Necrotising enterocolitis (NEC) is a life-catastrophic disease almost exclusively affecting in preterm infants. NEC has a mortality rate as high as 20-30% [70]. The aetiology of NEC is

multifactorial with prematurity, low birth weight, administration of enteral feeds and antibiotic exposure associated with development of the disease. The inflammation of the intestine leading to bacterial invasion causing cellular damage and death which causes necrosis of the colon and intestine [62,71]. Compared with term infants, the intestinal microbiota of preterm infants has fewer bacterial species, less diversity, smaller proportions of beneficial bacteria including *Lactobacillus* and *Bifidobacterium* species and increased levels of bacteria that can become pathogenic Gammaproteobacteria (i.e. *E. coli*, *Klebsiella pneumoniae*) [72,73]. Since then, numerous trials evaluating the efficacy of probiotics in preventing NEC have been conducted, with some demonstrating efficacy [66,74]. Oral administration of *Lactobacillus* and *Bifidobacterium* was shown to prevent NEC in preterm infants [75], and when administered in combination with breast milk, there was significant reduction in the incidence of NEC compared with infants receiving breast milk alone [76].

## Conclusion

At present, FMT is recommended in patients with recurrent disease caused by *Clostridioides difficile*. However, owing to the association that exists between the alteration of some species of the intestinal microbiota and the presentation of pathologies such as IBD, IBS, steatosis/non-alcoholic fatty liver disease, PSC and others, a few studies carried out in animal models and others in humans, have shown that this procedure could be used as a therapeutic alternative in other intestinal diseases.

Some bacterial species present in the gut microbiota have been identified as healthy biomarkers, being associated with a high production of SCFAs. Butyrate is an energy source of the colonic epithelium and can modulate various inflammatory processes that directly impact the development and activity of the immune system.

These findings give continuity to the idea of using faecal transplantation in other pathologies. Further research in this area is needed to consider this therapy as effective and safe in human patients.

## Author contributions

P Thomson made substantial contributions in conception and design of the work, interpretation of data, revising the work critically and approved the final version of the review. R Quera made substantial contributions in the review conception, revising it critically and approved the final version. P Nuñez made the conception of the work, with the analysis and

interpretation of data works, drafted and revised it critically and approved the final version and finally C Bay drafting the work and final approval of the version to be published.

## Transparency declaration

No conflict of interest.

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