frontiers | Research Topics

Improving the gut microbiome: Applications of fecal transplantation in disease

Edited by
Angel Lanas

Published in
Frontiers in Medicine

ISSN 1664-8714
ISBN 978-2-8325-2617-0
DOI 10.3389/978-2-8325-2617-0

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Improving the gut microbiome: Applications of fecal transplantation in disease

Topic editor
Angel Lanas — University of Zaragoza, Spain

Citation
Lanas, A., ed. (2023). *Improving the gut microbiome: Applications of fecal transplantation in disease*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-2617-0

Table of contents

frontiers | Frontiers in Medicine

TYPE Editorial
PUBLISHED 19 May 2023
DOI 10.3389/fmed.2023.1203448

OPEN ACCESS

EDITED AND REVIEWED BY
Ana Isabel Alvarez-Mercado,
Granada Biosanitary Research Institute, Spain

*CORRESPONDENCE
Angel Lanas
✉ alanas@unizar.es

RECEIVED 10 April 2023
ACCEPTED 08 May 2023
PUBLISHED 19 May 2023

CITATION
García-Mateo S and Lanas A (2023) Editorial: Improving the gut microbiome: applications of fecal transplantation in disease.
Front. Med. 10:1203448.
doi: 10.3389/fmed.2023.1203448

Editorial: Improving the gut microbiome: applications of fecal transplantation in disease

Sandra García-Mateo[1,2,3,4] and Angel Lanas[1,2,3,4]*

[1]Service of Digestive Diseases, University Clinic Hospital Lozano Blesa, Zaragoza, Spain, [2]Instituto de Investigación Sanitaria de Aragón (IIS Aragón), Zaragoza, Spain, [3]University of Zaragoza, Zaragoza, Spain, [4]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain

KEYWORDS

fecal transplant, bacteria, microbioma, gastrointestinal, inflammatory bowel conditions

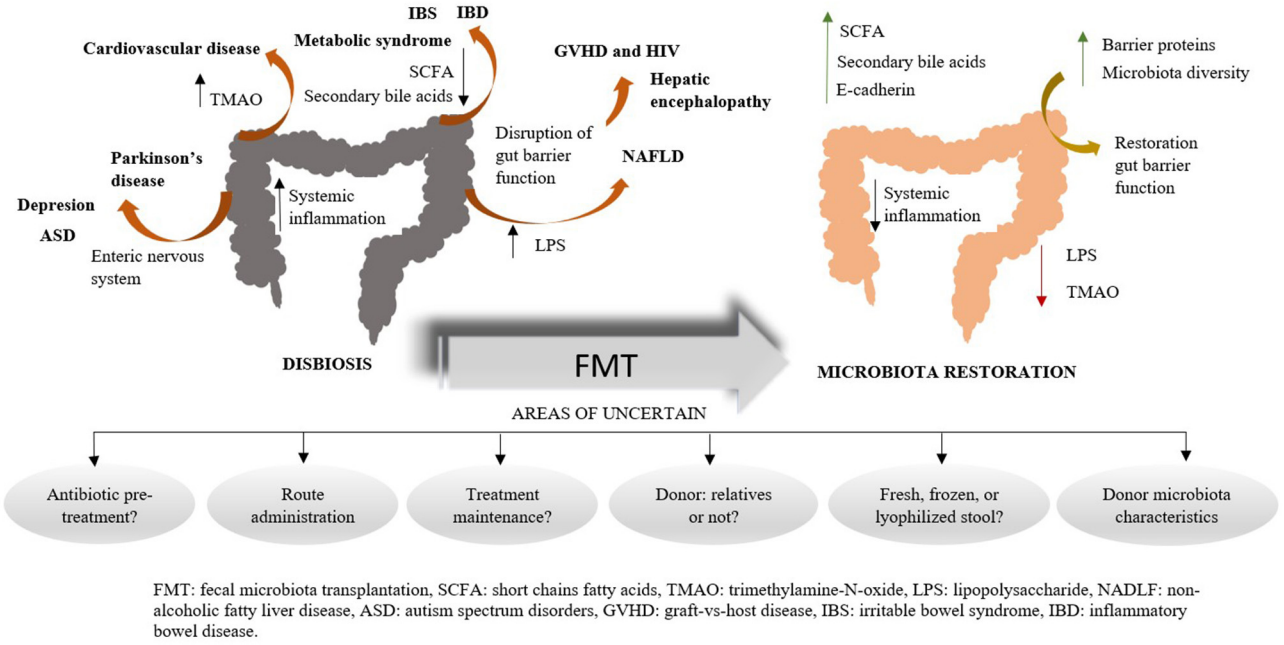
Editorial on the Research Topic
Improving the gut microbiome: applications of fecal transplantation in disease

"*Improving the gut microbiome: applications of fecal transplantation in disease*" is a Research Topic with the aim of point out the advances in gut microbiome as a therapeutic tool, not only for gastrointestinal disorders, but also for non-communicable diseases. As such, this editorial focuses on recent advances in fecal microbiota transplantation (FMT) in a wide spectrum of diseases highlighting both the windfalls and challenges of this promising therapeutic weapon as reflect the manuscripts submitted and published as part of this novel topic.

The progressive worsening of global obesity pandemic which predispose individuals to metabolic syndrome or cardiovascular disease in parallel with a severe downturn of lifestyle and diet despite the development of new, more personalized, and powerful drugs, has driven researchers to focus on new therapeutic targets. In line with this, in recent decades has emerged a more holistic paradigm of diseases. It assigns not only to microbiota, which englobe bacteria, virus, or protozoa, but also to microbioma, which encompasses also other molecules resulting of the interaction of microbiota and the immune system of the host, a principal role in the physiopathology of illness, being dysbiosis the new goal of the modern therapeutic approaches.

Microbiota-based therapies have been investigated since the 1950s as a treatment of gut dysbiosis to return metabolite levels and profiles to a healthy state as a result of a normal host enzymatic activity. Over the last decades, fecal microbiota transplantation (FMT) has become a potential treatment strategy by restoring a balanced microbiome to the host. However, recurrent *Clostridioides difficile* infection is currently the only indication of FMT as Chopra et al. have widely reported, despite their promising results in other non-communicable diseases.

Several clinical studies have been conducted using FMT in the setting of metabolic syndrome, obesity, and non-alcoholic fatty liver disease, not only in rats but also in humans as Liptak et al. describe in their review. In those studies, patients showed decreased gut microbial diversity, and after FMT from lean and healthy donors, an increase in microbial diversity, butyrate producing microorganisms, insulin sensitivity or improvement in liver necrosis were observed. Hopeful results were also noticed in hepatic encephalopathy, autism disorder, depression or Parkinson's disease, where improvements in behavioral and depressive symptoms, as well as constipation and other gastrointestinal symptoms were



FMT: fecal microbiota transplantation, SCFA: short chains fatty acids, TMAO: trimethylamine-N-oxide, LPS: lipopolysaccharide, NADLF: non-alcoholic fatty liver disease, ASD: autism spectrum disorders, GVHD: graft-vs-host disease, IBS: irritable bowel syndrome, IBD: inflammatory bowel disease.

FIGURE 1
Fecal microbiota transplantation as a promising and developing tool for gastrointestinal and non-gastrointestinal diseases by microbiota restoration. FMT, fecal microbiota transplantation; SCFA, short chains fatty acids; TMAO, trimethylamine-N-oxide; LPS, lipopolysaccharide; NADLF, non-alcoholic fatty liver disease; ASD, autism spectrum disorders; GVHD, graft-vs-host disease; IBS, irritable bowel syndrome; IBD, inflammatory bowel disease.

pointed out. Moreover, FMT not only plays a significant role among non-communicable or neurological diseases, but also surprisingly in others such as graft-vs-host disease or HIV infection. Ouyang et al. thoroughly reviewed the evidence and highlighted the importance of clarifying some areas of uncertainty in these specific diseases claiming more clinical trials, a statement that we had not overlook either.

However, even when the results reported were positive, most of these studies had some common weaknesses: FMT impact was only evaluated after a short time after the procedure, studies were scarce in some fields and had substantial methodological differences between them. All these shortcomings make hard to assume their conclusions (Figure 1).

To date, more robust evidence has been reported through several randomized controlled trials to demonstrate the efficacy of FMT in the setting of inflammatory bowel disease (IBD) (Zhang X. et al.). A systematic review of 30 studies carried out by Zhang J. et al. showed that the microbiome of FMT responders were similar to their donors, with an increase of short-chain fatty acids (SCFA) producing taxa. However, these results should be taken with care once again, because there were important differences among the results of the studies included in the systematic review, probably due to their methodological differences, especially in the ways of FMT delivery and the number of infusions.

Although colonoscopy seems to be the most effective mechanism of FMT administration, other delivery methods such as nasal tube, capsules, sigmoidoscopy, or retention enema are available as Hamman et al. reported. Tkach et al. performed a randomized clinical trial to demonstrate the efficacy and safety of FMT via colonoscopy in patients with mild-to-moderate ulcerative colitis (UC), with great outcomes for tolerability and safety, but with no differences in stool frequency ($p = 0.583$), fecal calprotectin and microbiota composition between FMT and the standard care group. It is important to note that due to severity of some diseases, standard therapy should be continued during the trials, so the role of FMT in the improvement of patient conditions is usually difficult to ascertain.

In Crohn's disease (CD), clinical trials with FMT are scarce, with clearly worse steroid-free clinical remission rates after FMT compared with the results observed in UC patients. These differences may be explained by the presence of extensive lesions in the small intestine in CD. In line with this, other complementary options apart from FMT, which mainly treats the colon microbioma, should be considered. Given that microbioma of small intestine participates in the pathogenesis of some diseases such as CD, intestinal fluid transplantation (HIFT) (Chen et al.) may have a crucial role. In fact, treating the whole microbiota and not only the one limited to the colon could be more effective than FMT alone, as has been reported by Chen et al.

One of the weaknesses in that field is the absence of standard protocols. Researchers determine different follow-up periods and commonly tend to perform short-term follow-up studies with small sample sizes. Although it makes studies more ease to compare, they loss strength because the lack of long-term data.

In this way, it is important to outline the retrospective study reported by Cui et al. in 227 patients with irritable bowel syndrome (IBS) who underwent FMT and were evaluated at different follow-up time points with a maximum of 60 months. The conclusion was that the treatment effect declined over time and that repeated and periodic FMT treatment can significantly guarantee the long-term efficacy of this therapy. It is clear, however, that more studies are needed to determine the frequency and number of FMT to obtain a successful and long-lasting therapeutic effect.

On another note, Cui et al. also compare the efficacy among different ways of delivery. They found significant differences in the efficacy rates after the administration of FMT capsules when compared to nasointestinal tube and colonoscopy administration. Although colonoscopy seems to be preferable (Hamamah et al.), oral capsule features an easy route to implement, with less side effects being a non-invasive method which leads to better medical compliance.

It is important to highlight the potential role of FMT as a promising therapeutic tool. However, it is necessary to draw attention to the fact that it may involve certain risks. One of the most side effects reported among different studies is the transference of unknown pathogenic microorganism to the host (Orr). Thereby it has been reported not only an increased risk for sepsis or death, but also the risks of developing diseases in the future such a colorectal cancer or metabolic syndrome. Without a doubt whatsoever, the whole microbiota is a complex entity with microbes that have never been fully characterized, and need much more research in the different fields being explored so far.

Orr emphasized the importance of a good donor selection, because seemingly healthy donors may not necessarily be appropriate donors for FMT. He highlights the importance of developing tools to identify and prioritize factors that best support a healthy gut biota among recipients, being crucial not only donor biota diversity but also patient's preparation with antibiotics, and an adequate diet without industrial or processed food. However, much more remains to be learned in that field.

In that line, a new list of endogenous microorganisms with potential benefits but still without a long history of clinical use called next-generation probiotics (NGP), are currently being investigated as the next step of traditional probiotics in order to mitigate those limitations and risk of FMT (Wortelboer et al.). NGP have shown promising results restoring the gut microbiota in *Clostridioides difficile infection* (Chopra et al.). Wortelboer et al. published the case of *Anaerobutyricum soehngenii*, a NGP which has demonstrated to improve insulin-resistance with hopeful perspectives in the field of metabolic syndrome and obesity in both *in vivo* and *in vitro* studies as well as in humans.

By and large, although the promising results that have been currently publishing, more controlled and personalized procedures are needed to improve the long term success after FMT an mitigate the potential side effects of the procedures.

Author contributions

Both authors listed have conducted the research and investigation process as well as they have prepared and presented the work approved for publication.

Acknowledgments

SG-M is a recipient of a Rio Hortega by the National Institute Carlos III, Madrid.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers in Medicine

REVIEW
published: 06 August 2020
doi: 10.3389/fmed.2020.00421

Treating From the Inside Out: Relevance of Fecal Microbiota Transplantation to Counteract Gut Damage in GVHD and HIV Infection

Jing Ouyang[1,2,3†], *Stéphane Isnard*[1,2†], *John Lin*[1,2], *Brandon Fombuena*[1,2,4], *Xiaorong Peng*[1,2,5], *Seema Nair Parvathy*[6], *Yaokai Chen*[3], *Michael S. Silverman*[6] *and Jean-Pierre Routy*[1,2,7*]

[1] *Infectious Diseases and Immunity in Global Health Program, McGill University Health Centre, Research Institute, Montreal, QC, Canada,* [2] *Chronic Viral Illness Service, McGill University Health Centre, Montreal, QC, Canada,* [3] *Chongqing Public Health Medical Center, Chongqing, China,* [4] *Department of Microbiology and Immunology, McGill University, Montreal, QC, Canada,* [5] *State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, College of Medicine, The First Affiliated Hospital, Zhejiang University, Hangzhou, China,* [6] *Infectious Diseases Division, St. Joseph's Health Care, London, ON, Canada,* [7] *Division of Hematology, McGill University Health Centre, Montreal, QC, Canada*

OPEN ACCESS

Edited by:
Levinus Albert Dieleman, University of Alberta, Canada

Reviewed by:
Rosica S. Valcheva, University of Alberta, Canada
Angel Lanas, University of Zaragoza, Spain

***Correspondence:**
Jean-Pierre Routy
jean-pierre.routy@mcgill.ca

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *15 April 2020*
Accepted: *30 June 2020*
Published: *06 August 2020*

Citation:
Ouyang J, Isnard S, Lin J, Fombuena B, Peng X, Nair Parvathy S, Chen Y, Silverman MS and Routy J-P (2020) Treating From the Inside Out: Relevance of Fecal Microbiota Transplantation to Counteract Gut Damage in GVHD and HIV Infection. Front. Med. 7:421. doi: 10.3389/fmed.2020.00421

The gastrointestinal (GI) tract is a complex and well-balanced milieu of anatomic and immunological barriers. The epithelial surface of the GI tract is colonized by trillions of microorganisms, known as the gut microbiota, which is considered an "organ" with distinctive endocrine and immunoregulatory functions. Dysregulation of the gut microbiota composition, termed dysbiosis, has been associated with epithelial damage and translocation of microbial products into the circulating blood. Dysbiosis, increased gut permeability and chronic inflammation play a major role on the clinical outcome of inflammatory bowel diseases, graft-vs.-host disease (GVHD) and HIV infection. In this review, we focus on GVHD and HIV infection, conditions sharing gut immune damage leading to dysbiosis. The degree of dysbiosis and level of epithelial gut damage predict poor clinical outcome in both conditions. Emerging interventions are therefore warranted to promote gut microbiota homeostasis and improve intestinal barrier function. Interventions such as anti-inflammatory medications, and probiotics have toxicity and/or limited transitory effects, justifying innovative approaches. Fecal microbiota transplantation (FMT) is one such approach where fecal microorganisms are transferred from healthy donors into the GI tract of the recipient to restore microbiota composition in patients with *Clostridium difficile*-induced colitis or inflammatory bowel diseases. Preliminary findings point toward a beneficial effect of FMT to improve GVHD and HIV-related outcomes through the engraftment of beneficial donor bacteria, notably

those producing anti-inflammatory metabolites. Herein, we critically review the potential for FMT in alleviating dysbiosis and gut damage in patients with GVHD or HIV-infection. Understanding the underlying mechanism by which FMT restores gut function will pave the way toward novel scalable and targeted interventions.

Keywords: fecal microbiota transplantation, graft-vs.-host disease, HIV infection, gut epithelial damage, dysbiosis

INTRODUCTION

Trillions of microorganisms reside in the human gut, encompassing not only bacteria but also fungi, archaea, viruses, and eukaryotic microbes, collectively termed microbiota. The gut microbiota was recently considered as an essential organ, playing a critical role in various host functions such as maintenance of the gut barrier and modulation of systemic immune response (1). Furthermore, the endocrine function of the gut microbiota was demonstrated through the production of vitamins and immunoregulatory short chain fatty acids (SCFA) (2). Dysregulation of gut microbiota composition, also known as dysbiosis, can lead to barrier dysfunction and translocation of microbial products leading to systemic inflammation (3). Recent evidence has shown that patients with diabetes, inflammatory bowel diseases (IBD), cancer, graft-vs.-host disease (GVHD) or HIV infection present with gut dysbiosis, gut damage, and microbial translocation (4–7).

Allo-hematopoietic stem cell transplantation (HSCT) is used in the treatment of hematological cancers where donor derived T-cells and natural killer cells target cancer cells in the recipient (4). Occurring after chemotherapy conditioning and HSCT, GVHD may develop as a serious complication when donor immune cells recognize the recipient as foreign and attack healthy cells in host's tissues. GVHD mostly occurs in the gut through the disruption of epithelial tight junctions, destruction of epithelial cells and inflammation in association with dysbiosis (5–8). A large multicenter study showed that gut microbiota composition independently predicted mortality in 1,362 HSCT patients with GVHD (9–11). Similarly, immune damage observed in the gut of people living with HIV (PLWH) was associated with gut dysbiosis, inflammation and clinical outcome (12–15). Despite long-term antiretroviral therapy (ART), damage to the gut mucosa and dysbiosis persist in PLWH, leading to systemic inflammation (8, 10, 11, 15). Like for people with GVHD, PLWH present with a disrupted gut epithelial barrier, immune-mediated intestinal damage, and increased gut permeability (15–22).

Given the association between microbiota composition and clinical outcome in both GVHD and HIV infection (5–11), strategies to modify the gut microbiota have come to light through dietary interventions, the antidiabetic drug metformin, selective antibiotics, probiotics, prebiotics, and fecal microbiota transplantation (FMT) (5, 23, 24). FMT refers to the transfer of fecal microorganisms from healthy donors into the GI tract of patients. It has shown to be effective in *Clostridium difficile* colitis (CDC), IBDs or obesity (25–28). As FMT has been recently shown to improve intestinal barrier function through promotion of gut microbiota homeostasis in GVHD and HIV infection, we discuss its relevance in both conditions in this review (29).

DYSBIOSIS AND INCREASED GUT PERMEABILITY ARE COMMON FEATURES IN PATIENTS WITH GVHD OR HIV-INFECTION

In GVHD or HIV infection, a decrease of gut microbiota diversity is observed and associated with poor clinical outcome (30–33). Compared to patients undergoing allogeneic HSCT without GVHD, patients experiencing GVHD had decreased stool microbial diversity (32). Taur et al. reported that lower bacterial diversity was associated with increased transplant-related mortality in HSCT recipients (33). Nowak et al. also reported that the bacterial diversity of the gut microbiota was correlated with CD4 T-cell counts and inversely correlated with markers of microbial translocation and monocyte activation in PLWH (30).

The gut barrier is organized as a multi-layered and complex system which allows nutrient absorption while preventing the translocation of microbes and their products. Epithelial gut damage occurs in patients with GVHD and PLWH, with damaged enterocytes (basal barrier), non-functional Paneth cells (antimicrobial peptide production) and less mucosal-associated invariant T (MAIT) cells (5, 34–37). Several proteins have been used as gut damage markers. Plasma concentrations of regenerating islet-derived 3-alpha (REG3α), secreted by Paneth cells, were 3-fold higher in patients with gut GVHD at the onset of the disease compared to other HSCT patients (36, 37). Lower levels of REG3α at GVHD onset are correlated with higher 1 year survival (37). In PLWH, we observed that REG3α but not intestinal fatty acid binding protein (I-FABP) plasma levels were correlated with HIV disease progression, microbial translocation and immune activation (36). Similarly, soluble suppression of tumorigenicity (sST2) was also used to predict gut damage and clinical outcomes in patients with GVHD and PLWH (38–42).

Epithelial gut damage allows microbial translocation of microbial products from the lumen to the bloodstream, inducing local and systemic inflammation (43). Circulating levels of lipopolysaccharide (LPS), a pro-inflammatory bacterial cell wall component, is a clinically significant marker to assess the level of microbial translocation (44). LPS leakage in the circulation could induce innate immune activation, in association with mortality in GVHD (45–47). In PLWH, we and others have shown that LPS translocation is correlated

with immune dysfunction and increased risk of non-AIDS comorbidities (48–51). Additionally, cytomegalovirus (CMV) primarily replicates in mucosal epithelial cells, decreasing gut barrier integrity. In patients with GVHD and PLWH, CMV latent infection or reactivation is associated with poor clinical outcomes (52–57). These findings suggest that patients with GVHD and HIV infection share similar features in gut damage and related microbial translocation.

Moreover, the gut microbiota can influence host cell physiology via production of metabolites such as SCFAs and bile acids. SCFAs, especially butyrate, constitute the primary energy source for colon epithelial cells. SCFAs play an important role in protecting intestinal barrier function, preventing microbial translocation and reducing inflammation through regulation of host epigenetics (58–60). GVHD patients or PLWH present with a lower abundance of SCFA-producing bacteria and a lower level of SCFAs, compared to non-GVHD HSCT patients or HIV-negative individuals, respectively (61–64). In both conditions, lower levels of SCFAs have been associated with gut damage and inflammation (62, 64–66). Conflicting results exist on the role of butyrate in GVHD as one report shows that patients developing GVHD had higher butyrate production (67). Furthermore, microbiota modulation leading to poor bile acids reabsorption could also be associated with gut damage in both patients with GVHD or PLWH (68–72).

Globally, gut dysbiosis, increased gut permeability, inflammation and systemic immune activation are common features of patients with GVHD or PLWH.

FMT IN PATIENTS WITH GUT GVHD

Given the dysbiosis and gut permeability in patients with GVHD, and regarding the vital role of gut microbiota in intestinal barrier and homeostasis, strategies targeting the microbiota offer one promising avenue for preventing or treating this condition. In the 1990s, investigators attempted to prevent the development of acute GVHD by drastically reducing the gut microbiota mass with antibiotics, removing the triggers of inflammation (73–75). However, newer studies have proven that gut microbiota-depleted patients had a higher risk of developing acute GVHD following HSCT than non-depleted patients (76, 77). Therefore, strategies promoting a "healthy" microbiota including FMT have attracted recent attention. Kakihana et al. (78) conducted a pilot study on four patients with steroid-resistant or steroid-dependent gut GVHD to observe the effects of FMT from spouses or relatives via nasoduodenal tube. All patients responded to FMT with three complete responses, one partial response, all in absence of severe adverse events. Spindelboeck et al. (79) reported successful FMT in three patients with severe acute GVHD. After one to six FMTs delivered via colonoscopy, all three patients showed increased diversity of the gut microbiota, with two complete remissions of GVHD and one partial remission. Qi et al. (80) reported eight patients with steroid-refractory gut GVHD receiving FMT through a nasoduodenal tube, from a stool bank. After FMT, all patients' clinical symptoms were relieved, bacteria diversity was enriched, and the gut microbiota diversity was restored. Compared to those who did not receive FMT, these eight patients achieved a longer progression-free survival. These case studies suggest that FMT can serve as a promising therapeutic option for gut GVHD, however larger controlled studies are required to confirm these effects.

FMT IN PLWH

In PLWH, the mucosal immune system is disturbed by HIV infection. Th17 and Th22 cells, important components of mucosal immunity, are rapidly depleted following HIV or simian immunodeficiency virus (SIV) infection, contributing to a reduced barrier integrity, microbial translocation, and systemic immune activation (81–83). In a pilot study, Hensley-McBain et al. (84) reported that FMT significantly increases the number of peripheral Th17 and Th22 cells and reduced CD4 T-cell activation in the gut in SIV-infected macaques receiving ART. Moreover, the transplant was well-tolerated and no side effects were observed (84).

A pilot study in ART-suppressed individuals who received one-time FMT from stool bank via colonoscopy reported no serious adverse effects during the 24 weeks of follow-up. Microbial engraftment occurred but was partial, and limited to specific bacterial taxa including an increase of Faecalibacterium (85), which has been shown to exert anti-inflammatory effects in murine experimental colitis (86, 87). The authors considered that the limited effects of FMT might be related to the single dose of FMT given and the absence of antibiotic pre-treatment to "provide space" before FMT (85). Serrano-Villar et al. reported that repeated oral capsular FMT was one way to safely introduce incremental compositional changes into the gut microbiota in ART-treated PLWH (88). Compared to placebo, FMT significantly decreased the gut damage marker I-FABP 4 weeks after initiating FMT. Furthermore, mild engraftment of the donor's microbiota persisted until week 36 after initiating FMT and greater engraftment was observed among the four subjects who had received antibiotics in the 12 week period before FMT (88) (**Figure 1**).

Safety should be the primary focus of any intervention. Concern persists on the safety of FMT administration, even more so in immunosuppressed recipients. However, PLWH with low CD4 T-cell count were shown to have the most profound modification of their gut microbiota and therefore would benefit greatly from FMT (89). As reviewed by Shogbesan et al. (27), FMT is successful in the treatment of recurrent CDC in immunocompromised patients including organ transplant recipients and PLWH. Encouragingly, FMT showed similar rates of adverse events in immunocompromised participants compared to immunocompetent ones including PLWH with CD4 counts lower than 200 cells/mm^3 (90–93). Additionally, Schunemann et al. showed that FMT increased CD4 counts in an individual with HIV (94). To better assess the efficacy and safety of FMT, well-designed RCT clinical trials are ongoing and presented in **Table 1**. However, large studies assessing the influence of FMT in PLWH are still needed.

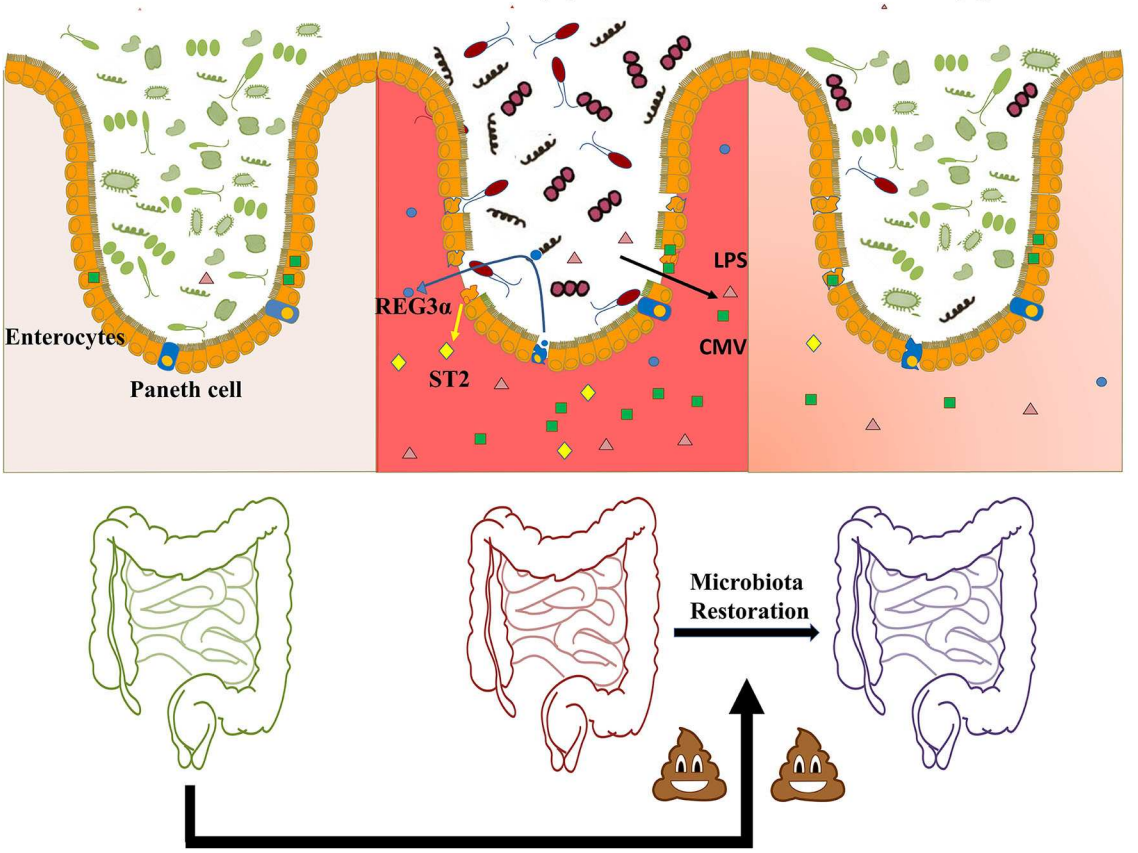


FIGURE 1 | Potential of FMT in GVHD or HIV infection-related gut damage.

CHALLENGES OF FMT FOR PATIENTS WITH GVHD AND PLWH

FMT needs further confirmation of its efficacy in decreasing gut damage in patient with GVHD or PLWH since studies assessing FMT with GVHD or PLWH involved a small number of participants. Moreover, safety needs to be validated as rare side effects may not have been observed in small studies. Therefore, challenges in designing formulations, preventing potential risks and implementing application in clinic for patients with GVHD and PLWH still remain.

Firstly, both healthy donors and patients have a microbiota composition with a high inter-person variability, and the key factors causing microbiota composition variation over time are not fully characterized. The precise influence of different microbiota composition and metabolites on epithelial barrier and clinical outcomes remain poorly understood and need further studies to define their distinctive role on the development of GVHD and HIV infection. Therefore, it remains difficult to select donors and special products for FMT formulation. Moreover, FMT treatment may carry pathogens for digestive and bloodstream infection, as DeFilipp et al. recently reported two cases of drug-resistant *Escherichia coli* bacteremia transmitted by FMT (95). Therefore, despite the absence of a uniform standard for "qualified" microbial communities, donors have to be thoroughly screened for transmissible diseases (e.g., HIV and hepatitis) and other non-infectious conditions (e.g., obesity and diabetes) that may be influenced by changes in the microbiome. In the light of Coronavirus Disease 2019, efforts to screen for novel infectious diseases should be implemented in the future. SARS-CoV-2, the virus that causes this disease, was found in stools even after diminution of respiratory symptoms and could be transmitted through a fecal-oral route (96, 97). Donors who may transfer undesirable agents (e.g., antibiotics, anti-acid proton pump inhibitors, systemic immunosuppressive agents,

TABLE 1 | Ongoing clinical trials using FMT as a treatment for GVHD and HIV.

Condition and Aim	Design	Intervention	Number of participants	Country	Clinical trial number
Patients with GVHD					
GVHD prevention	RCT	ARM I: total gut decontamination + FMT via enema ARM II: FMT via enema Arm III: standard therapy	120	US	NCT03862079
GVHD prevention	RCT	ARM I: Oral FMT Capsule ARM II: Oral placebo Capsule	120	US	NCT03678493
GVHD prevention	RCT	ARM I: FMT capsules ARM II: placebo capsules	48	US	NCT03720392
Acute GVHD treatment	Single arm	Autologous FMT via nasogastric tube	70	Israel	NCT03492502
Steroid refractory acute GI GVHD treatment	Single arm	FMT	32	France	NCT03359980
Acute GI GVHD treatment	Single arm	FMT under colonoscopy or gastroscopy	30	China	NCT03812705
Refractory GVHD treatment	Single arm	FMT via nasojejunal tube	15	China	NCT03549676
Acute GVHD treatment	Single arm	FMT instilled into caecum or terminal ileum	15	Austria	NCT03819803
Gut acute GVHD treatment	Single arm	Oral FMT capsules	4	Israel	NCT03214289
Severe acute gut GVHD treatment	Single arm	Oral FMT capsules	20	US	NCT04280471
Severe acute intestinal GVHD treatment	Single arm	FMT capsules +ruxolitinib+steroids	20	Russia	NCT04269850
GI acute GVHD treatment	Single arm	Oral FMT capsules	17	US	NCT04059757
High-risk acute GVHD treatment	Single arm	Oral FMT capsules	11	US	NCT04139577
Steroid resistant gut acute GVHD treatment	Single arm	FMT via colonoscopy or duodenal tube	30	China	NCT04285424
PLWH					
HIV infections treatment	RCT	ARM I: FMT capsules and ART ARM II: placebo capsules and ART	22	Mexico	NCT04165200
Safety of FMT in PLWH	Single arm	FMT capsules	6	US	NCT03329560
Microbiota restoration in PLWH	RCT	ARM I: FMT capsules ARM II: Placebo capsules	30	Spain	NCT03008941

FMT, Fecal microbiota transplantation; GVHD, Graft-vs.-host disease; PLWH, People living with HIV; ART, Antiretroviral therapy; RCT, Randomized Controlled Trial.

antineoplastic agents, and glucocorticoids) which can affect the safety or efficacy of FMT should also be excluded (98). Hence, screening for potential donors is costly and time consuming (99). Fortunately, new techniques allow freezing and storage of donor stools for extended periods of time, possibly facilitating FMT implementation (100).

As donor selection is a difficult process, and in order to favor clinical improvement, engraftment of the donor's microbiota should be optimal. Antibiotic conditioning given to the recipient just before FMT seems to improve microbiota engraftment (88). This procedure may destabilize the existing microbial community and promote engraftment of another community. By preventing niche competition in the mucosa between the xenomicrobiota and indigenous microbiota, preparing the gut with antibiotics was shown to facilitate xenomicrobiota colonization, thus enhancing the overall gut microbiota modification efficiency (101). Preliminary results by Serrano-villar et al. showed greater engraftment in four PLWH who had received antibiotics before FMT (88). Pre-therapy with antibiotics before FMT to alleviate GVHD is currently under study (NCT03862079, **Table 1**).

Encouragingly, multiple clinical trials studying the potential of FMT as a treatment for GVHD or HIV-related gut damage are ongoing (**Table 1**). In these trials, several routes of administration for FMT are under investigations, including oral capsules, nasal tube, colonoscopy, or enema. The optimal administration route may depend on the characteristics of the disease, and general condition of the patient. Compared with enema, colonoscopy could deliver the FMT to deep cecum, and increase engraftment while the donor stools are expelled less rapidly. However, colonoscopy remains a relatively invasive procedure (102): Kelly et al. reported one case of death from lung-aspiration injury during sedation for FMT administered via colonoscopy (103). Furthermore, nasal administration is considered inconvenient as some cases of intestinal bleeding and rare peritonitis have been reported (104). However, oral capsules have been developed to pass through the acidic environment of the stomach and ensure a delayed delivery of live microbial communities into the intestine (105). By using questionnaires, this route is considered to be most convenient for patients. Kao et al. compared oral capsule and colonoscopy delivered FMT on recurrent CDC showed similar efficacy, with less adverse events (106). Further studies should

analyze the preferential route of FMT to alleviate gut damage patients in GVHD and PLWH.

CONCLUSION

Both gut GVHD and HIV infection have been associated with dysbiosis and increased gut permeability, contributing to microbial translocation, inflammation, and poor clinical outcomes. Progress has been made in discerning the role of the microbiota in GVHD patients and PLWH. Manipulating the gut microbiota with FMT has been successfully used to treat CDC through microbiota restoration and has paved the way as a novel strategy to improve the outcomes of GVHD patients and PLWH. Several clinical trials are ongoing to assess the efficacy and safety of treating GVHD and HIV-induced gut damage with FMT. However, most trials and published studies are pilot or case series, thus making it difficult to confirm its efficacy and safety. Only large multicentre RCT studies will address the merit of such intervention. Moreover, a standard FMT procedure needs to be implemented and described, including pre-treatment with antibiotics and delivery with oral capsules to favor engraftment. Overall, collaborative efforts encompassing microbiology, clinical care, and pharmacy will define the optimal procedure and number of FMT to obtain a significant and lasting benefit from FMT for individuals with GVHD and HIV. In the future, FMT will pave avenues toward the characterization of important species and their metabolites in modulation of gut damage in patient with GVHD or PLWH, leading to more effective interventions.

AUTHOR CONTRIBUTIONS

JO and SI wrote the first draft of the manuscript. JL, BF, XP, SN, YC, and MS provided critical revision of the manuscript. J-PR conceived and designed the manuscript. All authors approved it for publication.

FUNDING

Our research is funded by the Fonds de la Recherche Québec-Santé (FRQ-S): Réseau SIDA/Maladies infectieuses and Thérapie cellulaire; the Canadian Institutes of Health Research (CIHR; grants MOP 103230 and PTJ 166049); the Vaccines & Immunotherapies Core of the CIHR Canadian HIV Trials Network (CTN; CTN PT038); the Canadian Foundation for AIDS Research (CANFAR; grant 02-512); CIHR- funded Canadian HIV Cure Enterprise (CanCURE) Team Grant HB2-164064; and Chongqing Basic and Frontier Research project (No. cstc2018jcyjAX0652).

ACKNOWLEDGMENTS

We are highly grateful to Angie Massicotte, Josée Girouard, and Cezar Iovi for coordination and assistance.

REFERENCES

1. Sircana A, Framarin L, Leone N, Berrutti M, Castellino F, Parente R, et al. Altered gut microbiota in type 2 diabetes: just a coincidence? *Curr Diab Rep.* (2018) 18:98. doi: 10.1007/s11892-018-1057-6
2. Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. *N Engl J Med.* (2016) 375:2369–79. doi: 10.1056/NEJMra1600266
3. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. *Nutrients.* (2011) 3:858–76. doi: 10.3390/nu3100858
4. Ingham AC, Kielsen K, Cilieborg MS, Lund O, Holmes S, Aarestrup FM, et al. Specific gut microbiome members are associated with distinct immune markers in pediatric allogeneic hematopoietic stem cell transplantation. *Microbiome.* (2019) 7:131. doi: 10.1186/s40168-019-0745-z
5. Fredricks DN. The gut microbiota and graft-versus-host disease. *J Clini Invest.* (2019) 129:1808–17. doi: 10.1172/JCI125797
6. Ghimire S, Weber D, Mavin E, Wang XN, Dickinson AM, Holler E. Pathophysiology of GvHD and other HSCT-related major complications. *Front Immunol.* (2017) 8:79. doi: 10.3389/fimmu.2017.00079
7. Nalle SC, Zuo L, Ong M, Singh G, Worthylake AM, Choi W, et al. Graft-versus-host disease propagation depends on increased intestinal epithelial tight junction permeability. *J Clin Invest.* (2019) 129:902–14. doi: 10.1172/JCI98554
8. Gilliam AC, Whitaker-Menezes D, Korngold R, Murphy GF. Apoptosis is the predominant form of epithelial target cell injury in acute experimental graft-versus-host disease. *J Invest Dermatol.* (1996) 107:377–83. doi: 10.1111/1523-1747.ep12363361
9. Peled JU, Gomes ALC, Devlin SM, Littmann ER, Taur Y, Sung AD, et al. Microbiota as predictor of mortality in allogeneic hematopoietic-cell transplantation. *N Engl J Med.* (2020) 382:822–34. doi: 10.1056/NEJMoa1900623
10. Zitvogel L, Kroemer G. Immunostimulatory gut bacteria. *Science.* (2019) 366:1077–8. doi: 10.1126/science.aaz7595
11. Stein-Thoeringer CK, Nichols KB, Lazrak A, Docampo MD, Slingerland AE, Slingerland JB, et al. Lactose drives *Enterococcus* expansion to promote graft-versus-host disease. *Science.* (2019) 366:1143–9. doi: 10.1126/science.aax3760
12. Gianella S, Letendre SL, Iudicello J, Franklin D, Gaufin T, Zhang Y, et al. Plasma (1 –> 3)-beta-D-glucan and suPAR levels correlate with neurocognitive performance in people living with HIV on antiretroviral therapy: a CHARTER analysis. *J Neurovirol.* (2019) 25:837–43. doi: 10.1007/s13365-019-00775-6
13. Hoenigl M, de Oliveira MF, Perez-Santiago J, Zhang Y, Morris S, McCutchan AJ, et al. (1–>3)-beta-D-Glucan levels correlate with neurocognitive functioning in HIV-infected persons on suppressive antiretroviral therapy: a cohort study. *Medicine.* (2016) 95:e3162. doi: 10.1097/MD.0000000000003162
14. Gelpi M, Vestad B, Hansen SH, Holm K, Drivsholm N, Goetz A, et al. Impact of HIV-related gut microbiota alterations on metabolic comorbidities. *Clin Infect Dis.* (2020). doi: 10.1093/cid/ciz1235. [Epub ahead of print].
15. Vujkovic-Cvijin I, Sortino O, Verheij E, Sklar J, Wit FW, Kootstra NA, et al. HIV-associated gut dysbiosis is independent of sexual practice and correlates with noncommunicable diseases. *Nat Commun.* (2020) 11:2448. doi: 10.1038/s41467-020-16222-8
16. Mehandru S, Tenner-Racz K, Racz P, Markowitz M. The gastrointestinal tract is critical to the pathogenesis of acute HIV-1 infection. *J Allergy Clin Immunol.* (2005) 116:419–22. doi: 10.1016/j.jaci.2005.05.040
17. Brenchley JM, Douek DC. HIV infection and the gastrointestinal immune system. *Mucosal Immunol.* (2008) 1:23–30. doi: 10.1038/mi.2007.1
18. Estes JD, Kityo C, Ssali F, Swainson L, Makamdop KN, Del Prete GQ, et al. Defining total-body AIDS-virus burden with implications for curative strategies. *Nat Med.* (2017) 23:1271–6. doi: 10.1038/nm.4411

19. Hensley-McBain T, Berard AR, Manuzak JA, Miller CJ, Zevin AS, Polacino P, et al. Intestinal damage precedes mucosal immune dysfunction in SIV infection. *Mucosal Immunol.* (2018) 11:1429–40. doi: 10.1038/s41385-018-0032-5
20. Batman PA, Miller AR, Forster SM, Harris JR, Pinching AJ, Griffin GE. Jejunal enteropathy associated with human immunodeficiency virus infection: quantitative histology. *J Clin Pathol.* (1989) 42:275–81. doi: 10.1136/jcp.42.3.275
21. Batman PA, Kotler DP, Kapembwa MS, Booth D, Potten CS, Orenstein JM, et al. HIV enteropathy: crypt stem and transit cell hyperproliferation induces villous atrophy in HIV/Microsporidia-infected jejunal mucosa. *AIDS.* (2007) 21:433–9. doi: 10.1097/QAD.0b013e3280142ee8
22. Heise C, Dandekar S, Kumar P, Duplantier R, Donovan RM, Halsted CH. Human immunodeficiency virus infection of enterocytes and mononuclear cells in human jejunal mucosa. *Gastroenterology.* (1991) 100:1521–7. doi: 10.1016/0016-5085(91)90648-5
23. Li J, Riaz Rajoka MS, Shao D, Jiang C, Jin M, Huang Q, et al. Strategies to increase the efficacy of using gut microbiota for the modulation of obesity. *Obes Rev.* (2017) 18:1260–71. doi: 10.1111/obr.12590
24. Vujkovic-Cvijin I, Somsouk M. HIV and the gut microbiota: composition, consequences, and avenues for amelioration. *Curr HIV/AIDS Rep.* (2019) 16:204–13. doi: 10.1007/s11904-019-00441-w
25. Cammarota G, Ianiro G, Tilg H, Rajilic-Stojanovic M, Kump P, Satokari R, et al. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut.* (2017) 66:569–80. doi: 10.1136/gutjnl-2016-313017
26. Mullish BH, Quraishi MN, Segal JP, McCune VL, Baxter M, Marsden GL, et al. The use of faecal microbiota transplant as treatment for recurrent or refractory *Clostridium difficile* infection and other potential indications: joint British society of gastroenterology (BSG) and healthcare infection society (HIS) guidelines. *Gut.* (2018) 67:1920–41. doi: 10.1136/gutjnl-2018-316818
27. Shogbesan O, Poudel DR, Victor S, Jehangir A, Fadahunsi O, Shogbesan G, et al. a systematic review of the efficacy and safety of fecal microbiota transplant for *Clostridium difficile* infection in immunocompromised patients. *Can J Gastroenterol Hepatol.* (2018) 2018:1394379. doi: 10.1155/2018/1394379
28. Konturek PC, Koziel J, Dieterich W, Haziri D, Wirtz S, Glowczyk I, et al. Successful therapy of *Clostridium difficile* infection with fecal microbiota transplantation. *J Physiol Pharmacol.* (2016) 67:859–66. doi: 10.1016/S0016-5085(17)31403-8
29. Kang Y, Cai Y. Altered gut microbiota in hiv infection: future perspective of fecal microbiota transplantation therapy. *AIDS Res Hum Retroviruses.* (2019) 35:229–35. doi: 10.1089/aid.2017.0268
30. Nowak P, Troseid M, Avershina E, Barqasho B, Neogi U, Holm K, et al. Gut microbiota diversity predicts immune status in HIV-1 infection. *AIDS.* (2015) 29:2409–18. doi: 10.1097/QAD.0000000000000869
31. Tuddenham SA, Koay WLA, Zhao N, White JR, Ghanem KG, Sears CL. The impact of human immunodeficiency virus infection on gut microbiota alpha-diversity: an individual-level meta-analysis. *Clin Infect Dis.* (2020) 70:615–27. doi: 10.1093/cid/ciz258
32. Jenq RR, Ubeda C, Taur Y, Menezes CC, Khanin R, Dudakov JA, et al. Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *J Exp Med.* (2012) 209:903–11. doi: 10.1084/jem.20112408
33. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood.* (2014) 124:1174–82. doi: 10.1182/blood-2014-02-554725
34. Troeger H, Hering NA, Bojarski C, Fromm A, Barmeyer C, Uharek L, et al. Epithelial barrier dysfunction as permissive pathomechanism in human intestinal graft-versus-host disease. *Bone Marrow Transplant.* (2018) 53:1083–6. doi: 10.1038/s41409-018-0144-2
35. Nalle SC, Turner JR. Endothelial and epithelial barriers in graft-versus-host disease. *Adv Exp Med Biol.* (2012) 763:105–31. doi: 10.1007/978-1-4614-4711-5_5
36. Isnard S, Ramendra R, Dupuy FP, Lin J, Fombuena B, Kokinov N, et al. Plasma levels of C-type lectin REG3alpha and gut damage in people with human immunodeficiency virus. *J Infect Dis.* (2020) 221:110–21. doi: 10.1093/infdis/jiz423
37. Ferrara JL, Harris AC, Greenson JK, Braun TM, Holler E, Teshima T, et al. Regenerating islet-derived 3-alpha is a biomarker of gastrointestinal graft-versus-host disease. *Blood.* (2011) 118:6702–8. doi: 10.1182/blood-2011-08-375006
38. Vander Lugt MT, Braun TM, Hanash S, Ritz J, Ho VT, Antin JH, et al. ST2 as a marker for risk of therapy-resistant graft-versus-host disease and death. *N Engl J Med.* (2013) 369:529–39. doi: 10.1056/NEJMoa1213299
39. Rowan C, Pike F, Cooke KR, Krance R, Carpenter PA, Duncan C, et al. Assessment of ST2 for risk of death following graft-versus-host disease in the pediatric and adult age groups. *Blood.* (2020) 135:1428–37. doi: 10.2139/ssrn.3409311
40. Younas M, Psomas C, Mehraj V, Cezar R, Portales P, Tuaillon E, et al. Plasma level of soluble ST2 in chronically infected HIV-1 patients with suppressed viremia. *Open AIDS J.* (2017) 11:32–5. doi: 10.2174/1874613601711010032
41. Mehraj V, Jenabian MA, Ponte R, Lebouche B, Costiniuk C, Thomas R, et al. The plasma levels of soluble ST2 as a marker of gut mucosal damage in early HIV infection. *AIDS.* (2016) 30:1617–27. doi: 10.1097/QAD.0000000000001105
42. Mehraj V, Ponte R, Routy JP. The dynamic role of the IL-33/ST2 axis in chronic viral-infections: alarming and adjuvanting the immune response. *EBioMedicine.* (2016) 9:37–44. doi: 10.1016/j.ebiom.2016.06.047
43. Mu J, Chen Q, Zhu L, Wu Y, Liu S, Zhao Y, et al. Influence of gut microbiota and intestinal barrier on enterogenic infection after liver transplantation. *Curr Med Res Opin.* (2019) 35:241–8. doi: 10.1080/03007995.2018.1470085
44. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med.* (2006) 12:1365–71. doi: 10.1038/nm1511
45. Price KS, Nestel FP, Lapp WS. Progressive accumulation of bacterial lipopolysaccharide *in vivo* during murine acute graft-versus-host disease. *Scand J Immunol.* (1997) 45:294–300. doi: 10.1046/j.1365-3083.1997.d01-404.x
46. Daguindau E, Gautier T, Chague C, Pais de Barros JP, Deckert V, Lagrost L, et al. Is it time to reconsider the lipopolysaccharide paradigm in acute graft-versus-host disease? *Front Immunol.* (2017) 8:952. doi: 10.3389/fimmu.2017.00952
47. Hill GR, Crawford JM, Cooke KR, Brinson YS, Pan L, Ferrara JL. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. *Blood.* (1997) 90:3204–13. doi: 10.1182/blood.V90.8.3204
48. Jenabian MA, El-Far M, Vyboh K, Kema I, Costiniuk CT, Thomas R, et al. Immunosuppressive tryptophan catabolism and gut mucosal dysfunction following early HIV infection. *J Infect Dis.* (2015) 212:355–66. doi: 10.1093/infdis/jiv037
49. Ramendra R, Isnard S, Mehraj V, Chen J, Zhang Y, Finkelman M, et al. Circulating LPS and (1–>3)-beta-D-Glucan: a folie a deux contributing to HIV-associated immune activation. *Front Immunol.* (2019) 10:465. doi: 10.3389/fimmu.2019.00465
50. Morris A, Hillenbrand M, Finkelman M, George MP, Singh V, Kessinger C, et al. Serum (1–>3)-beta-D-glucan levels in HIV-infected individuals are associated with immunosuppression, inflammation, and cardiopulmonary function. *J Acquir Immune Defic Syndr.* (2012) 61:462–8. doi: 10.1097/QAI.0b013e318271799b
51. Xu W, Luo Z, Alekseyenko AV, Martin L, Wan Z, Ling B, et al. Distinct systemic microbiome and microbial translocation are associated with plasma level of anti-CD4 autoantibody in HIV infection. *Sci Rep.* (2018) 8:12863. doi: 10.1038/s41598-018-31116-y
52. Maidji E, Somsouk M, Rivera JM, Hunt PW, Stoddart CA. Replication of CMV in the gut of HIV-infected individuals and epithelial barrier dysfunction. *PLoS Pathog.* (2017) 13:e1006202. doi: 10.1371/journal.ppat.1006202
53. Ramendra R, Isnard S, Lin J, Fombuena B, Ouyang J, Mehraj V, et al. CMV seropositivity is associated with increased microbial translocation in people living with HIV and uninfected controls. *Clin Infect Dis.* (2019). doi: 10.1093/cid/ciz1001. [Epub ahead of print].
54. Collins-McMillen D, Buehler J, Peppenelli M, Goodrum F. Molecular determinants and the regulation of human cytomegalovirus latency and reactivation. *Viruses.* (2018) 10:444. doi: 10.3390/v10080444

55. Camargo JF, Wieder ED, Kimble E, Benjamin CL, Kolonias DS, Kwon D, et al. Deep functional immunophenotyping predicts risk of cytomegalovirus reactivation after hematopoietic cell transplantation. *Blood.* (2019) 133:867–77. doi: 10.1182/blood-2018-10-878918
56. Cantoni N, Hirsch HH, Khanna N, Gerull S, Buser A, Bucher C, et al. Evidence for a bidirectional relationship between cytomegalovirus replication and acute graft-versus-host disease. *Biol Blood Marrow Transplant.* (2010) 16:1309–14. doi: 10.1016/j.bbmt.2010.03.020
57. Broers AE, van Der Holt R, van Esser JW, Gratama JW, Henzen-Logmans S, Kuenen-Boumeester V, et al. Increased transplant-related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after allogeneic T-cell-depleted stem cell transplantation. *Blood.* (2000) 95:2240–5. doi: 10.1182/blood.V95.7.2240.007k08_2240_2245
58. Woo V, Alenghat T. Host-microbiota interactions: epigenomic regulation. *Curr Opin Immunol.* (2017) 44:52–60. doi: 10.1016/j.coi.2016.12.001
59. Wang HB, Wang PY, Wang X, Wan YL, Liu YC. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. *Dig Dis Sci.* (2012) 57:3126–35. doi: 10.1007/s10620-012-2259-4
60. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol.* (2014) 121:91–119. doi: 10.1016/B978-0-12-800100-4.00003-9
61. Gonzalez-Hernandez LA, Ruiz-Briseno MDR, Sanchez-Reyes K, Alvarez-Zavala M, Vega-Magana N, Lopez-Iniguez A, et al. Alterations in bacterial communities, SCFA and biomarkers in an elderly HIV-positive and HIV-negative population in western Mexico. *BMC Infect Dis.* (2019) 19:234. doi: 10.1186/s12879-019-3867-9
62. Dillon SM, Kibbie J, Lee EJ, Guo K, Santiago ML, Austin GL, et al. Low abundance of colonic butyrate-producing bacteria in HIV infection is associated with microbial translocation and immune activation. *AIDS.* (2017) 31:511–21. doi: 10.1097/QAD.0000000000001366
63. Biagi E, Zama D, Nastasi C, Consolandi C, Fiori J, Rampelli S, et al. Gut microbiota trajectory in pediatric patients undergoing hematopoietic SCT. *Bone Marrow Transplant.* (2015) 50:992–8. doi: 10.1038/bmt.2015.16
64. Mathewson ND, Jenq R, Mathew AV, Koenigsknecht M, Hanash A, Toubai T, et al. Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nat Immunol.* (2016) 17:505–13. doi: 10.1038/ni.3400
65. Kohler N, Zeiser R. Intestinal microbiota influence immune tolerance post allogeneic hematopoietic cell transplantation and intestinal GVHD. *Front Immunol.* (2018) 9:3179. doi: 10.3389/fimmu.2018.03179
66. Serrano-Villar S, Vazquez-Castellanos JF, Vallejo A, Latorre A, Sainz T, Ferrando-Martinez S, et al. The effects of prebiotics on microbial dysbiosis, butyrate production and immunity in HIV-infected subjects. *Mucosal Immunol.* (2017) 10:1279–93. doi: 10.1038/mi.2016.122
67. Golob JL, DeMeules MM, Loeffelholz T, Quinn ZZ, Dame MK, Silvestri SS, et al. Butyrogenic bacteria after acute graft-versus-host disease (GVHD) are associated with the development of steroid-refractory GVHD. *Blood Adv.* (2019) 3:2866–9. doi: 10.1182/bloodadvances.2019000362
68. Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab.* (2013) 17:225–35. doi: 10.1016/j.cmet.2013.01.003
69. Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci USA.* (2006) 103:3920–5. doi: 10.1073/pnas.0509592103
70. Cramp ME, Hing MC, Marriott DJ, Freund J, Cooper DA. Bile acid malabsorption in HIV infected patients with chronic diarrhoea. *Aust N Z J Med.* (1996) 26:368–71. doi: 10.1111/j.1445-5994.1996.tb01924.x
71. Steuerwald M, Bucher HC, Muller-Brand J, Gotze M, Roser HW, Gyr K. HIV-enteropathy and bile acid malabsorption: response to cholestyramine. *Am J Gastroenterol.* (1995) 90:2051–3.
72. Joshi NM, Hassan S, Jasani P, Dixon S, Cavenagh JD, Oakervee HE, et al. Bile acid malabsorption in patients with graft-versus-host disease of the gastrointestinal tract. *Br J Haematol.* (2012) 157:403–7. doi: 10.1111/j.1365-2141.2011.09014.x
73. Vossen JM, Heidt PJ, van den Berg H, Gerritsen EJ, Hermans J, Dooren LJ. Prevention of infection and graft-versus-host disease by suppression of intestinal microflora in children treated with allogeneic bone marrow transplantation. *Eur J Clin Microbiol Infect Dis.* (1990) 9:14–23. doi: 10.1007/BF01969527
74. Beelen DW, Haralambie E, Brandt H, Linzenmeier G, Muller KD, Quabeck K, et al. Evidence that sustained growth suppression of intestinal anaerobic bacteria reduces the risk of acute graft-versus-host disease after sibling marrow transplantation. *Blood.* (1992) 80:2668–76. doi: 10.1182/blood.V80.10.2668.bloodjournal80102668
75. Beelen DW, Elmaagacli A, Muller KD, Hirche H, Schaefer UW. Influence of intestinal bacterial decontamination using metronidazole and ciprofloxacin or ciprofloxacin alone on the development of acute graft-versus-host disease after marrow transplantation in patients with hematologic malignancies: final results and long-term follow-up of an open-label prospective randomized trial. *Blood.* (1999) 93:3267–75. doi: 10.1182/blood.V93.10.3267.410k22_3267_3275
76. Routy B, Letendre C, Enot D, Chenard-Poirier M, Mehraj V, Seguin NC, et al. The influence of gut-decontamination prophylactic antibiotics on acute graft-versus-host disease and survival following allogeneic hematopoietic stem cell transplantation. *Oncoimmunology.* (2017) 6:e1258506. doi: 10.1080/2162402X.2016.1258506
77. Gjaerde LK, Schmidt M, Sengelov H. Gut decontamination during allogeneic hematopoietic stem cell transplantation and the risk of acute graft-versus-host disease. *Bone Marrow Transplant.* (2018) 53:1061–4. doi: 10.1038/s41409-018-0131-7
78. Kakihana K, Fujioka Y, Suda W, Najima Y, Kuwata G, Sasajima S, et al. Fecal microbiota transplantation for patients with steroid-resistant acute graft-versus-host disease of the gut. *Blood.* (2016) 128:2083–8. doi: 10.1182/blood-2016-05-717652
79. Spindelboeck W, Schulz E, Uhl B, Kashofer K, Aigelsreiter A, Zinke-Cerwenka W, et al. Repeated fecal microbiota transplantations attenuate diarrhea and lead to sustained changes in the fecal microbiota in acute, refractory gastrointestinal graft-versus-host-disease. *Haematologica.* (2017) 102:e210–3. doi: 10.3324/haematol.2016.154351
80. Qi X, Li X, Zhao Y, Wu X, Chen F, Ma X, et al. Treating steroid refractory intestinal acute graft-vs.-host disease with fecal microbiota transplantation: a pilot study. *Front Immunol.* (2018) 9:2195. doi: 10.3389/fimmu.2018.02195
81. Klatt NR, Estes JD, Sun X, Ortiz AM, Barber JS, Harris LD, et al. Loss of mucosal CD103+ DCs and IL-17+ and IL-22+ lymphocytes is associated with mucosal damage in SIV infection. *Mucosal Immunol.* (2012) 5:646–57. doi: 10.1038/mi.2012.38
82. Klatt NR, Brenchley JM. Th17 cell dynamics in HIV infection. *Curr Opin HIV AIDS.* (2010) 5:135–40. doi: 10.1097/COH.0b013e3283364846
83. Brenchley JM, Paiardini M, Knox KS, Asher AI, Cervasi B, Asher TE, et al. Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. *Blood.* (2008) 112:2826–35. doi: 10.1182/blood-2008-05-159301
84. Hensley-McBain T, Zevin AS, Manuzak J, Smith E, Gile J, Miller C, et al. Effects of fecal microbial transplantation on microbiome and immunity in simian immunodeficiency virus-infected macaques. *J Virol.* (2016) 90:4981–9. doi: 10.1128/JVI.00099-16
85. Paquin-Proulx D, Ching C, Vujkovic-Cvijin I, Fadrosh D, Loh L, Huang Y, et al. Bacteroides are associated with GALT iNKT cell function and reduction of microbial translocation in HIV-1 infection. *Mucosal Immunol.* (2017) 10:69–78. doi: 10.1038/mi.2016.34
86. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA.* (2008) 105:16731–6. doi: 10.1073/pnas.0804812105
87. Frank DN, St. Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA.* (2007) 104:13780–5. doi: 10.1073/pnas.0706625104
88. Serrano-Villar S, Talavera A, Madrid-Elena N, Pérez-Molina JA, Gosalbes MJ, Budree S, et al. Rational donor fecal microbiota transplantation in HIV (refresh study). In: *Conference on Retroviruses and Opportunistic Infections (CROI).* Boston, MA (2020).

89. Lu W, Feng Y, Jing F, Han Y, Lyu N, Liu F, et al. Association between gut microbiota and CD4 recovery in HIV-1 infected patients. *Front Microbiol.* (2018) 9:1451. doi: 10.3389/fmicb.2018.01451
90. Mandalia A, Ward A, Tauxe W, Kraft CS, Dhere T. Fecal transplant is as effective and safe in immunocompromised as non-immunocompromised patients for *Clostridium difficile*. *Int J Colorectal Dis.* (2016) 31:1059–60. doi: 10.1007/s00384-015-2396-2
91. 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.e7. doi: 10.1053/j.gastro.2016.11.010
92. Elopre L, Rodriguez M. Fecal microbiota therapy for recurrent *Clostridium difficile* infection in HIV-infected persons. *Ann Intern Med.* (2013) 158:779–80. doi: 10.7326/0003-4819-158-10-201305210-00021
93. Gathe JC Jr, Diejomaoh EM, Mayberry CC, Clemmons JB. Fecal transplantation for *Clostridium difficile*-"all stool may not be created equal". *J Int Assoc Provid AIDS Care.* (2016) 15:107–8. doi: 10.1177/2325957415627695
94. Schunemann M, Oette M. Fecal microbiota transplantation for *Clostridium difficile*-associated colitis in a severely immunocompromized critically ill AIDS patient: a case report. *AIDS.* (2014) 28:798–9. doi: 10.1097/QAD.0000000000000148
95. DeFilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N Engl J Med.* (2019) 381:2043–50. doi: 10.1056/NEJMoa1910437
96. Song Y, Liu P, Shi XL, Chu YL, Zhang J, Xia J, et al. SARS-CoV-2 induced diarrhoea as onset symptom in patient with COVID-19. *Gut*. (2020) 69:1143–4. doi: 10.1136/gutjnl-2020-320891
97. Wu Y, Guo C, Tang L, Hong Z, Zhou J, Dong X, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol*. (2020) 5:434–35. doi: 10.1016/S2468-1253(20)30083-2
98. Canada H. *Guidance Document: Fecal Microbiota Therapy Used in the Treatment of Clostridium difficile Infection Not Responsive to Conventional Therapies: Health Canada*. (2019). Available online at: https://www.canada.ca/en/health-canada/services/drugs-health-products/biologics-radiopharmaceuticals-genetic-therapies/applications-submissions/guidance-documents/regulation-fecal-microbiota-therapy-treatment-difficile-infections.html (accessed February 21, 2020).
99. Craven LJ, Nair Parvathy S, Tat-Ko J, Burton JP, Silverman MS. Extended screening costs associated with selecting donors for fecal microbiota transplantation for treatment of metabolic syndrome-associated diseases. *Open Forum Infect Dis.* (2017) 4:ofx243. doi: 10.1093/ofid/ofx243
100. Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent *Clostridium difficile* infection. *Am J Gastroenterol.* (2012) 107:761–7. doi: 10.1038/ajg.2011.482
101. Ji SK, Yan H, Jiang T, Guo CY, Liu JJ, Dong SZ, et al. Preparing the gut with antibiotics enhances gut microbiota reprogramming efficiency by promoting xenomicrobiota colonization. *Front Microbiol.* (2017) 8:1208. doi: 10.3389/fmicb.2017.01208
102. Konig J, Siebenhaar A, Hogenauer C, Arkkila P, Nieuwdorp M, Noren T, et al. Consensus report: faecal microbiota transfer - clinical applications and procedures. *Aliment Pharmacol Ther.* (2017) 45:222–39. doi: 10.1111/apt.13868
103. Kelly CR, Ihunnah C, Fischer M, Khoruts A, Surawicz C, Afzali A, et al. Fecal microbiota transplant for treatment of *Clostridium difficile* infection in immunocompromised patients. *Am J Gastroenterol.* (2014) 109:1065–71. doi: 10.1038/ajg.2014.133
104. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for *Clostridium difficile* infection: systematic review and meta-analysis. *Am J Gastroenterol.* (2013) 108:500–8. doi: 10.1038/ajg.2013.59
105. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing *Clostridium difficile* infection. *JAMA*. (2014) 312:1772–8. doi: 10.1001/jama.2014.13875
106. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, et al. Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent *Clostridium difficile* infection: a randomized clinical trial. *JAMA*. (2017) 318:1985–93. doi: 10.1001/jama.2017.17077

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 30 July 2021
doi: 10.3389/fmed.2021.710452

Long-Term Follow-Up Results of Fecal Microbiota Transplantation for Irritable Bowel Syndrome: A Single-Center, Retrospective Study

*Jiaqu Cui†, Zhiliang Lin†, Hongliang Tian†, Bo Yang, Di Zhao, Chen Ye, Ning Li, Huanlong Qin and Qiyi Chen**

Intestinal Microenvironment Treatment Center of General Surgery, Shanghai Tenth People's Hospital, Tenth People's Hospital of Tongji University, Shanghai, China

OPEN ACCESS

Edited by:
Ruggiero Francavilla, University of Bari Aldo Moro, Italy

Reviewed by:
Felix Broecker, Idorsia Pharmaceuticals Ltd, Switzerland
Xiao Jing Wang, Mayo Clinic, United States

***Correspondence:**
Qiyi Chen
qiyichen2011@163.com

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *17 May 2021*
Accepted: *29 June 2021*
Published: *30 July 2021*

Citation:
Cui J, Lin Z, Tian H, Yang B, Zhao D, Ye C, Li N, Qin H and Chen Q (2021) Long-Term Follow-Up Results of Fecal Microbiota Transplantation for Irritable Bowel Syndrome: A Single-Center, Retrospective Study. Front. Med. 8:710452. doi: 10.3389/fmed.2021.710452

Objective: This study aimed to investigate the long-term efficacy of fecal microbiota transplantation (FMT) in patients with irritable bowel syndrome (IBS).

Study Methods: In this single-center long-term follow-up study, FMT treatment was administered to patients with moderate to severe IBS (IBS severity scoring system (IBS-SSS) > 175). After 1 year of treatment, it was decided whether to repeat FMT based on IBS-SSS score (IBS-SSS > 175). Baseline characteristics before and after FMT and questionnaires were completed at 1, 3, 6, 12, 24, 36, 48, and 60 months after FMT. The study outcomes included treatment efficacy rates, change of IBS-SSS, IBS-specific quality of life and fatigue, effect on stool frequency, Bristol Stool Scale for IBS-C and IBS-D, and side effects.

Results: A total of 227 patients (47.58% IBS-C, 39.21% IBS-D, and 13.22% IBS-M) were recruited (142 females and 85 males with a mean age of 41.89 ± 13.57 years). The efficacy rates were 108 (51.92%), 147 (74.62%), 125 (74.41 %), 88 (71.54%), 78 (75.00%), 65 (73.03%), 45 (61.64%), and 37 (62.71%) at different follow-up time points. The total IBS-SSS score was 321.37 ± 73.89 before FMT, which significantly decreased after 1 month. The IBS-specific quality of life (IBS-QoL) score was 40.24 ± 11.34 before FMT, increased gradually, and was significantly higher at 3 months compared to before FMT. The total Fatigue Assessment Scale (FAS) score was 47 ± 8.64 before FMT and was significantly lower at 3 months. During follow-up, 89 (39.21%) side effects occurred that were alleviated by symptomatic treatment, and no serious adverse events were detected.

Conclusion: Based on 60 months of long-term follow-up, the safety and efficacy of FMT for IBS was established. However, as the treatment effect declines over time, periodic and repetitive FMT is required for a sustained effect.

Keywords: irritable bowel syndrome, fecal microbiota transplantation, efficacy, safety, retrospective study

INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most commonly diagnosed gastrointestinal (GI) conditions. It is a symptom-based condition defined by the presence of abdominal pain or discomfort, with altered bowel habits, in the absence of any other disease to cause these sorts of symptoms (1). The prevalence of IBS in the global population ranges from 5.7 to 34% (2, 3), and in Southeast Asia, it is relatively infrequent (7.0%) (2). With the rapid economic growth and current environmental changes, the incidence of IBS in China has been on the rise year by year, showing a 5–10% prevalence in adults (4).

While medical treatment for IBS is still limited, the overall illness burden is high, patients report a low quality of life, low work efficiency and absenteeism in the workplace, and significant direct and indirect healthcare costs (5). The etiology of IBS is not fully understood, and there is no effective treatment for the condition. Current evidence suggests that the microbiota of the GI tract could be a significant factor in the etiology of IBS (6). The gut microbiota of patients with IBS differs from that of healthy subjects, with the former having a lower bacterial diversity (dysbiosis), for example (7, 8). It is speculated that changes in the intestinal environment will lead to an imbalance in the composition of gut microbiota, termed "dysbiosis," which has been associated with the occurrence of IBS (9). Consequently, probiotics and antibiotics have been studied as a potential treatment option for IBS (10, 11); however, the reported magnitude of improvement in associated symptoms was limited.

Fecal microbiota transplantation (FMT), also known as fecal bacteriotherapy or fecal infusion, consists of administration of a liquid filtrate of feces from a healthy donor into the GI tract of a recipient individual (12). In recurrent *Clostridioides difficile* infections, FMT has shown excellent effects. The cure rate of FMT is higher than conventional treatment with antibiotics (13, 14), and studies have shown that FMT can restore intestinal microbial balance in treated patients (13, 15). Using the FMT method, our team has treated 2,010 cases of various GI dysfunction diseases, including IBS. The long-term (36 months) effective rate has exceeded 60% (16). A number of short-term follow-up studies with small sample sizes showed that FMT can improve symptoms and restore the intestinal microbiota diversity in IBS patients (17–19). The current study retrospectively analyzed the long-term efficacy of FMT in IBS by applying a large sample size and conducting a 5-year follow-up period. Furthermore, the differences in efficacy between various transplantation approaches were compared.

MATERIALS AND METHODS

Participants and Study Design

In this single-center, retrospective study, consecutive patients treated at the Intestinal Microenvironment Diagnosis and Treatment Center, Tenth People's Hospital of Tongji University (Shanghai, China), between January 2014 and January 2019 were included if they met the following criteria: (1) aged 18–65 years and complied with the diagnostic criteria of Rome III or Rome IV; (2) had moderate to severe disease activity (IBS severity scoring system (IBS-SSS) $\geq$ 175); (3) had normal colonoscopy (performed within 1 year) if the patient was $\geq$40 years or had blood in the stool; and (4) had no response shown to conventional treatment for IBS. The exclusion criteria were as follows: (1) other chronic GI diseases; (2) fecal sample positive for enteropathogenic microorganisms; (3) positive screening for HIV, HBV, or HCV antibodies; (4) a history of surgical interventions in the GI region (except for appendectomy, hernia repair, cholecystectomy, and gynecological or urological procedures); (5) severe psychiatric disorders; (6) fecal calprotectin $\geq$50 mg/kg; (7) severe allergies or asthma; (8) abnormal biochemistry screening result; (9) abnormal colonoscopy findings; (10) pregnancy, planned pregnancy, or breastfeeding females; (11) ingestion of probiotics or antibiotics <4 weeks prior to inclusion; (12) immunocompromised patients or those using immunosuppressive drugs; and (13) GI or systemic malignancies.

The data used in this study were obtained from the follow-up system of the Intestinal Microenvironment Diagnosis and Treatment Center, Tenth People's Hospital of Tongji University, Shanghai, China. All patients were checked during study visits for baseline (before FMT) and 1, 3, 6, 12, 24, 36, 48, and 60 months. At the end of the follow-up period, they completed the IBS-SSS and IBS-specific quality of life (IBS-QoL) questionnaire. Additional questionnaires included the following: Bristol Stool Form Scale, stool frequency, and Fatigue Assessment Scale (FAS). Any complications within 7 days after the first transplantation were recorded. Adverse events were evaluated by the use of the modified Common Terminology Criteria for Adverse Events version 3.0 (20). All enrolled patients signed the FMT treatment informed consent.

The Donor Screening

A total of 19 fecal donors were recruited for this study. Once enrolled, full-time donor managers were employed to manage the diet, lifestyle, and physical condition of the donors during the collecting period. All donors were screened according to guidelines (21, 22) and were recruited based on the following inclusion criteria: (1) 18–30 years of age; (2) good previous and current health status; (3) normal body weight (body mass index (BMI) between 18 and 22 kg/m^2); (4) normal bowel movements (defined as one to two times per day and type 3–4 on the Bristol Stool Form Scale); and (5) no medications taken. The exclusion criteria were as follows: (1) history of antibiotic treatment within 3 months preceding donation; (2) history of intrinsic GI illnesses; and (3) metabolic syndromes, obesity, or any ongoing diseases. A single universal donor was recruited for our trial, who was a 24-year-old healthy University student. For the purposes of informed consent, the donor was required to be over 18 years of age. Current guidelines recommend using a donor questionnaire that is similar to current protocols for screening blood donors. Blood collection was performed before FMT donation, which included a complete blood count, chemistry, and iron profile. The donor blood sample was negative for common viruses (hepatitis A, B, and C; HIV-1 and HIV-2; cytomegalovirus; Epstein–Barr virus; herpes simplex; and varicella zoster) and

Treponema pallidum. The donor feces were negative for common enteric pathogens (*Yersinia* spp., *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, *Clostridioides difficile* toxin, helminths, ova, parasites, and *Helicobacter pylori*). Multidrug-resistant bacteria were determined using standard screening methods.

Preparation of FMT

Preparation of Fresh FMT Solution

According to the fresh FMT solution preparation method previously established by our team (23), fresh stool (200 g) was immediately mixed in a blender with 500 ml 0.9% sterile saline for several seconds until it developed a smooth consistency. The obtained stool suspension was filtered several times through gauze screens with decreasing apertures (2.0–0.7 ± 0.2 mm) to remove large and small particles that could clog the nasointestinal tube. The resulting concentrated fecal bacterial suspension was either administered to the patient without delay or amended with glycerol to a final concentration of 10%. The latter suspension was stored frozen at −20°C for 1–4 weeks until further use. The stool suspension was poured into a sterile bottle for administration within 2 h. The study used standardized, processed stool from the same universal donor and the same amount of stool for FMT for each patient.

Preparation of Freeze-Dried FMT Capsules

The FMT capsules were prepared according to the method previously established by our team (24). After the preparation of the above fresh FMT solution, centrifugation was carried out at 4°C, the supernatant was removed, and freeze-drying protectant was added. The bacterial suspension was mixed well with an oscillator, prepared for pre-freezing, and the frozen sample was quickly transferred to the freeze dryer for freeze-drying. Finally, the freeze-dried powder was put into acid-resistant hydroxypropyl methylcellulose. The capsules were sealed and stored at −20°C (48 capsules/200 g feces).

FMT Procedure

An initial dose of oral antibiotic (500 mg vancomycin orally twice per day) was administered for 3 consecutive days. The day before FMT, polyethylene glycol was administered orally or through a nasointestinal tube to prepare the bowel. Patients received fresh FMT for 6 consecutive days through a nasointestinal tube or colonoscopy. Altogether, 100 g of stool suspension was administered through the nasointestinal tube or colonoscopy within 6 min daily for 6 consecutive days. Meanwhile, patients who could not tolerate the nasointestinal tube or endoscopic approach received four capsules twice daily on an empty stomach for 6 consecutive days. The nasointestinal tube was flushed with 50 ml of saline solution before and after each procedure to ensure that the entire volume of stool suspensions was transplanted into the intestine. For the capsule group, the 48 capsules contained sieved, concentrated, and freeze-dried powders derived from 200 g of donor stool.

Twelve months after FMT treatment, the total IBS-SSS score of the annual follow-up results was used to decide whether the FMT treatment would be continued. If this score decreased by

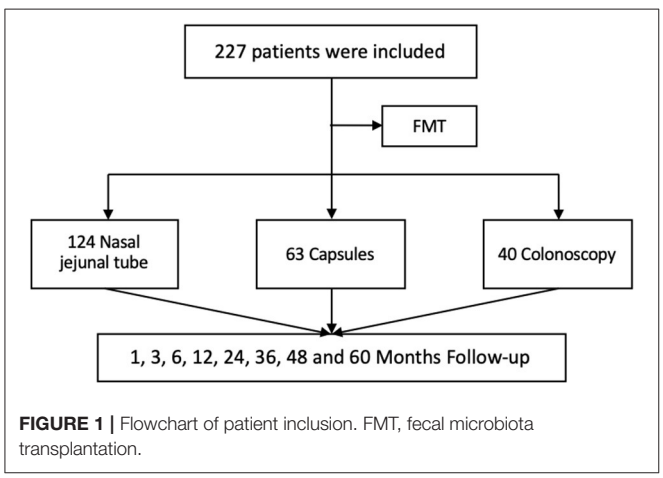


FIGURE 1 | Flowchart of patient inclusion. FMT, fecal microbiota transplantation.

TABLE 1 | Baseline characteristics of patients.

Characteristics	Overall
n	227
Age (mean ± SD)	41.89 ± 13.57
Sex, female/male	142/85
BMI (mean ± SD)	20.86 ± 1.63
Weight (mean ± SD)	61.33 ± 10.58
Type of IBS (%)	
IBS with constipation	108 (47.58)
IBS with diarrhea	89 (39.21)
IBS mixed	30 (13.22)
History of IBS-related medications (%)	
Laxatives	132 (58.15)
Prokinetic drugs	84 (37)
Antidiarrheal	106 (47)
Psychotropic drugs	97 (42.73)
Painkillers	65 (28.63)
PPI	183 (80.62)
Antibiotics	152 (66.96)
Probiotics	197 (86.78)
Traditional Chinese medicine	118 (51.98)
Spasmolytic	149 (65.64)
IBS-SSS score (mean ± SD)	321.37 ± 73.89
IBS-QoL score (mean ± SD)	40.24 ± 11.34
FAS score (mean ± SD)	47 ± 8.64
FMT pathway (%)	
Capsules	63 (27.75)
Nasointestinal tube	124 (54.63)
Colonoscopy	40 (17.62)
Average course of FMT (times)	3.93 ± 2.30
Nasointestinal tube (times)	3.83 ± 1.78
Capsules (times)	5.19 ± 2.94
Colonoscopy (times)	2.25 ± 1.24

BMI, body mass index; FMT, fecal microbiota transplantation; IBS-QoL, IBS-specific quality of life; IBS-SSS, IBS severity scoring system; PPI, proton pump inhibitor.

more than 50 but was still over 175 after FMT, it was suggested that FMT should be continued. On the contrary, if the total IBS-SSS score after FMT was <175, no further treatment was considered necessary. If the total IBS-SSS score after FMT had no obvious change or increase, the FMT treatment was set to be stopped, and conventional treatment would be adopted.

Questionnaires

This study used the questionnaires discussed below. All steps were completed under the direct supervision of the investigators to ensure that participants understood and completed all questions. All questionnaires were formally translated to Mandarin Chinese and validated. Abdominal symptoms were assessed using the IBS-SSS questionnaires, which included five dimensions: abdominal distension/bloating, abdominal pain frequency, abdominal pain severity, satisfaction with bowel habits, and quality of life. Fatigue was evaluated on the FAS. Quality of life was determined using the IBS-QoL questionnaires, where higher IBS-QoL scores indicated a better quality of life. Patients whose total IBS-SSS score decreased by ≥50 points after FMT were considered responders. A decrease of ≥175 points in the IBS-SSS total score, a decrease of ≥4 points in the FAS score, and an increase of ≥14 points in the IBS-QoL score were considered to indicate significant clinical improvements in abdominal symptoms, fatigue, and quality of life, respectively (25). The fulfillment of all these criteria at the same time was considered effective in the treatment of IBS by FMT.

Statistical Methods

Statistical analysis was performed by descriptive methods and SPSS 20.0 software. The count data were expressed by the number of cases (%), and the measurement data that conform to the normal distribution were expressed by $x \pm s$. A chi-square test or Fisher's exact probability method was used to compare the treatment efficacy rate between groups. The comparison of time points before and after treatment was performed by univariate analysis of variance. The IBS-QoL score was transformed into a 0–100 scale using the following formula: total score = (sum of the items – 34/170) × 100.

RESULTS

Patient Characteristics

A total of 227 patients were enrolled in this study (**Figure 1**), including 142 females and 85 males with a median age of 41.89 ± 13.57 years, BMI of 20.86 ± 1.63, and weight of 61.33 ± 10.58 kg. According to the classification of IBS, there were 108 (47.58%) constipation-predominant IBS (IBS-C), 89 (39.21%) diarrhea-predominant IBS (IBS-D), and 30 (13.22%) mixed-type IBS (IBS-M) cases. The history of IBS-related drug use included laxatives (132, 58.15%), prokinetic drugs (84, 37%), antidiarrheal drugs (106, 47%), psychotropic drugs (97, 42.73%), painkillers (65, 28.63%), PPI (183, 80.62%), antibiotics (152, 66.96%), probiotics (197, 86.78%), traditional Chinese medicine (118, 51.98%), and spasmolytic agents (149, 65.64%). The total scores of IBS-SSS, IBS-QoL, and FAS were 321.37 ± 73.89, 40.24 ± 11.34, and 47 ± 8.64, respectively, before FMT. According to the transplantation method, 124 (54.63%) patients received the transplant through a nasointestinal tube, 63 (27.75%) in the form of oral capsules, and 40 (17.62%) through colonoscopy. The average course of FMT was 3.93 ± 2.30, including 3.83 ± 1.78 for nasointestinal tube, 5.19 ± 2.94 for capsule, and 2.25 ± 1.24 for colonoscopy (**Table 1**).

Rate of Effective Follow-Up

In this study, a total of 227 patients were enrolled. Based on 60 months of long-term follow-up data, the effective follow-up rates at 1, 3, 6, 12, 24, 36, 48, and 60 months after FMT were 51.92% (108/208), 74.62% (147/197), 74.41% (125/168), 71.54% (88/123), 75.00% (78/104), 73.03% (65/89), 61.64% (45/73), and 62.71% (37/59), respectively.

Effect of Different Transplantation Routes on the Treatment Efficacy

Three transplantation groups were included in this study: the nasointestinal tube group ($n = 124$), capsule group ($n = 63$), and colonoscopy group ($n = 40$).

The effective follow-up rates at 1, 3, 12, and 60 months, respectively, were 60 (53.10%), 80 (74.07%), 48 (70.59%), and 23 (60.53%) for the nasointestinal tube group; 31 (54.39%), 43 (78.18%), 30 (83.33%), and 12 (75.00%) for the capsule group;

TABLE 2 | The effect of different transplantation routes on efficacy.

Follow-up time	Nasointestinal tube group ($n = 124$)		Capsules group ($n = 63$)		Colonoscopy group ($n = 40$)		χ^2	p
	No.	Effective number (%)	No.	Effective number (%)	No.	Effective number (%)		
1 month	113	60 (53.10)	57	31 (54.39)	38	17 (44.74)	0.987	0.61
3 months	108	80 (74.07)	55	43 (78.18)	34	24 (70.59)	0.677	0.713
6 months	92	68 (73.91)	47	39 (82.98)	29	18 (62.07)	4.143	0.126
12 months	68	48 (70.59)	36	30 (83.33)	19	10 (52.63)	5.826	0.054
24 months	61	44 (72.13)	31	27 (87.10)	12	7 (58.33)	4.465	0.107
36 months	53	37 (69.81)	26	23 (88.46)	10	5 (50.00)	6.116	0.047*
48 months	47	28 (59.57)	20	14 (70.00)	6	3 (50.00)	1.02	0.601
60 months	38	23 (60.53)	16	12 (75.00)	5	2 (40.00)	2.214	0.331

*$p < 0.05$.

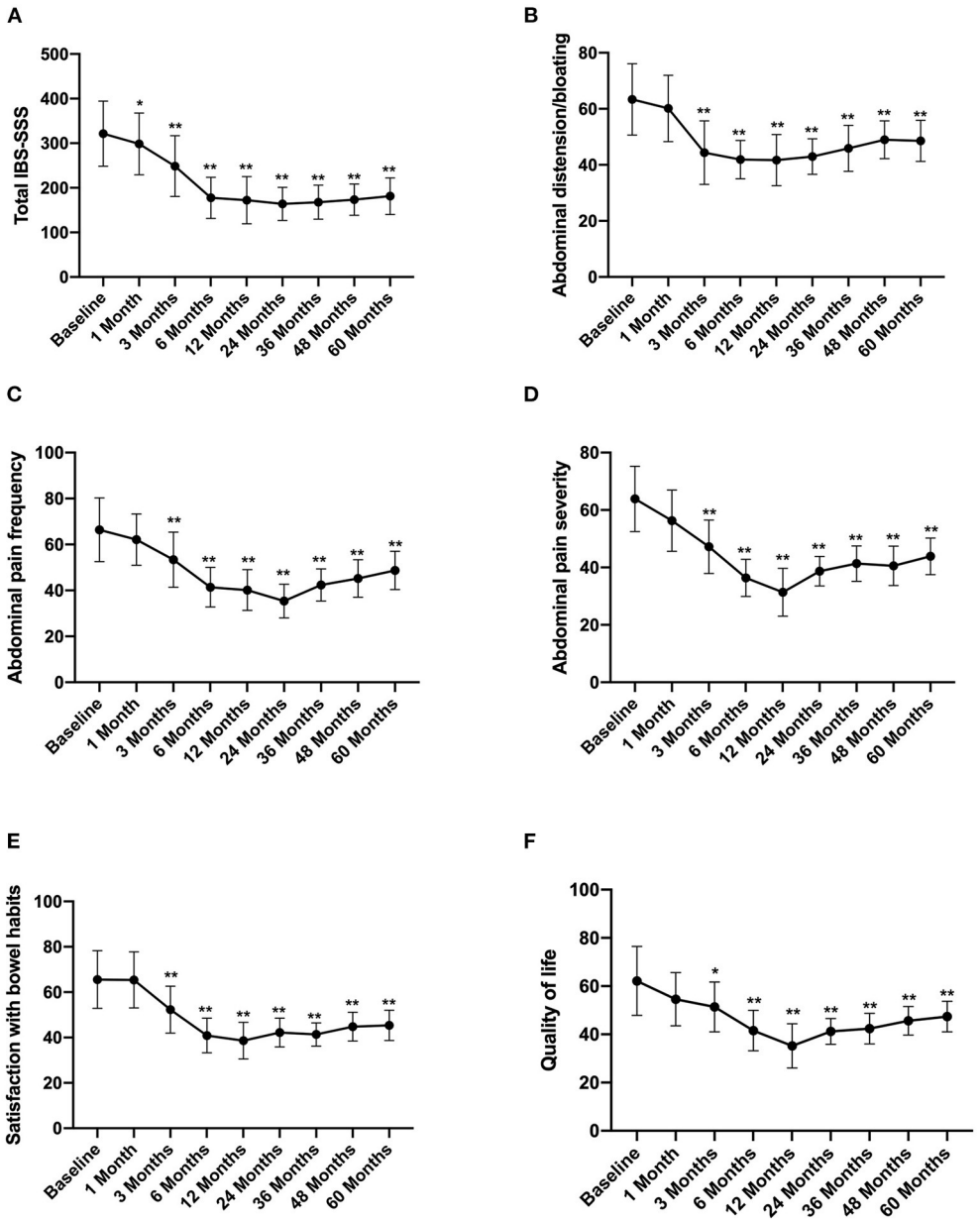


FIGURE 2 | IBS-SSS score between groups and the change over time. Difference was compared between each time point of follow-up and before FMT (baseline). **(A)** Total IBS-SSS score; **(B)** abdominal distension/bloating score; **(C)** abdominal pain frequency score; **(D)** abdominal pain severity score; **(E)** satisfaction with bowel habits score; **(F)** quality of life score. IBS-SSS, IBS severity scoring system. Data are presented as $x \pm s$, statistical analyses: univariate analysis of variance, $^{*}p < 0.05$, $^{**}p < 0.01$.

and 17 (44.74%), 24 (70.59%), 10 (52.63%), and 2 (40.00%) for the colonoscopy group (**Table 2**). A significant difference in the efficacy rates among the three groups was observed only at 36 months after FMT.

Long-Term Follow-Up of IBS-SSS

According to the long-term follow-up research data, after FMT, the abdominal symptoms assessed by the IBS-SSS questionnaires were significantly reduced. The total IBS-SSS score was 321.37 ± 73.89 before FMT, which significantly decreased after 1 month of FMT to 298.57 ± 69. Moreover, abdominal distension bloating, abdominal pain, and abdominal pain severity also decreased, whereas satisfaction with bowel habits and quality of life improved after 1 month of FMT (**Figure 2**).

Long-Term Follow-Up of IBS-QoL

The IBS-QoL score gradually increased after FMT, rising from 40.24 ± 11.34 before FMT to 50.13 ± 9.34 at 3 months after treatment ($p < 0.05$) (**Figure 3**).

Long-Term Follow-Up of FAS

The total FAS score was 47 ± 8.64 before FMT, which decreased gradually after FMT and was significantly lower at 3 months after FMT (32.58 ± 4.86) than that before FMT (**Figure 4A**). At the same time, the physical fatigue and mental health scale scores also reduced significantly at 3 months after FMT, with scores of 15.89 ± 3.86 and 16.78 ± 4.1, respectively (**Figures 4B,C**) and then remained at a stable level. At the 5th year of follow-up, the total FAS, physical fatigue scale, and mental health scale scores were significantly lower than those before FMT, with values of 31.89 ± 5.74, 18.12 ± 4.28, 17.77 ± 3.55, respectively ($p < 0.01$) (**Figure 4**).

Change of Stool Frequency and Bristol Stool Scale for IBS-C and IBS-D

In this study, patients with IBS-C and IBS-D were followed up to evaluate the stool frequency and the Bristol Stool Scale score. The results showed that the stool frequency of IBS-C patients increased from 1.5 ± 1.38 times per week before treatment to 2.68 ± 1.15 times per week at 1 month after FMT treatment (compared with that before FMT, $p < 0.05$) and increased to 4.33 ± 1.56 times per week in the 5th year after FMT (compared with that before FMT, $p < 0.01$) (**Figure 5A**). In contrast, the stool frequency of IBS-D patients decreased from 4.67 ± 1.87 times per day before treatment to 3.26 ± 1.42 times per day at 1 month after FMT treatment. By the 5th year, this reduced to 2.25 ± 1.87 times per day (compared with that before FMT, $p < 0.01$) (**Figure 5B**).

The Bristol Stool Scale score of IBS-C patients increased from 2.13 ± 0.88 before treatment to 2.94 ± 1.3 at 1 month after FMT treatment (compared with that before FMT, $p < 0.05$) and further increased to 3.71 ± 1.21 by the 5th year after FMT (compared with that before FMT, $p < 0.01$) (**Figure 6A**). In contrast, the Bristol Stool Scale score of IBS-D patients reduced from 5.88 ± 1.15 before FMT to 3.38 ± 0.85 at 3 months after FMT treatment. By the 5th year, this declined to 3.71 ± 0.88 (compared with that before FMT, $p < 0.01$) (**Figure 6B**).

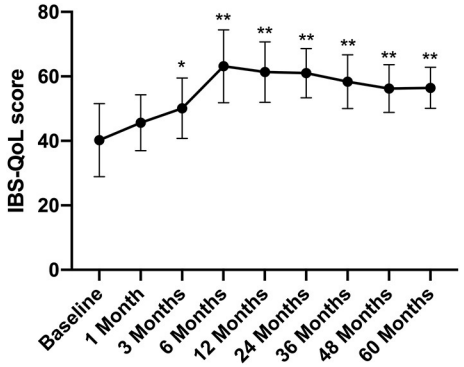


FIGURE 3 | IBS-QoL score between groups and the change over time. Difference was compared between each time point of follow-up and before FMT (baseline). IBS-QoL, IBS-specific quality of life. Data are presented as $x \pm s$, statistical analyses: univariate analysis of variance, $^{*}p < 0.05$, $^{**}p < 0.01$.

Side Effects of FMT

Any side effects directly related to and during FMT treatment and within 1 week after FMT were considered to be adverse effects of FMT. At the same time, different side effects were observed for different FMT pathways (colonoscopy, nasointestinal, and capsule). A total of 89 (39.21%) adverse reactions occurred during follow-up. Of these, 83 were mild, and no interventions or medications were indicated (grade 1). The other six adverse events were classified as grade 2 effects. No serious adverse reactions (grade 3 or above) were observed. The main adverse events were abdominal pain in 15 (6.61%) patients, of which six (15%) with the highest incidence were in the colonoscopy group; thus, this event may be related to the colonoscopy procedure. Furthermore, seven (5.65%) and two (3.18%) cases were in the nasointestinal tube and capsule group, respectively. Of the 17 cases of abdominal distension/bloating, eight (6.45%) occurred in the nasointestinal tube pathway, three (4.76%) in the capsule pathway, and six (15%) in the colonoscopy pathway. Diarrhea presented in 13 cases, including five (12.50%) in

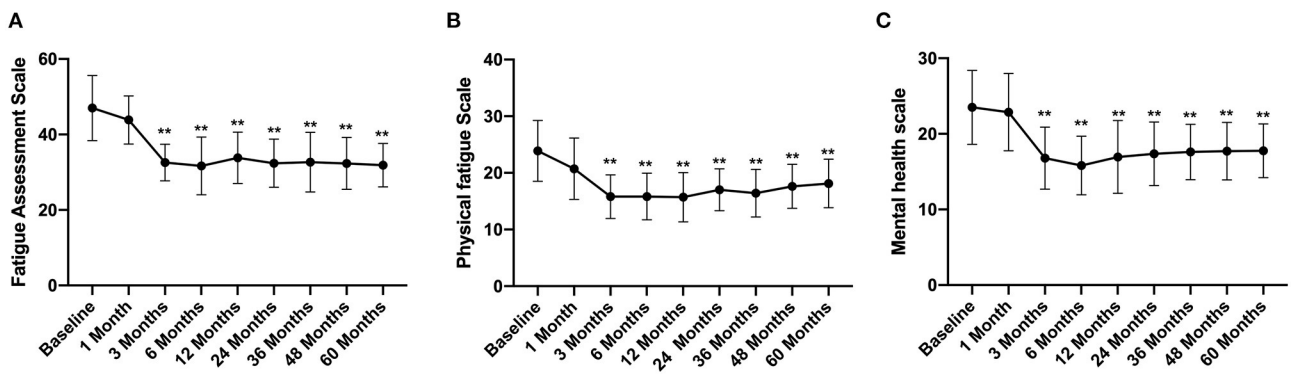


FIGURE 4 | FAS score between groups and their change over time. Difference was compared between each time point of follow-up and before FMT (baseline). **(A)** total score; **(B)** physical fatigue; **(C)** mental health. FAS, Fatigue Assessment Scale. Data are presented as $x \pm s$, statistical analyses: univariate analysis of variance, $^{**}p < 0.01$.

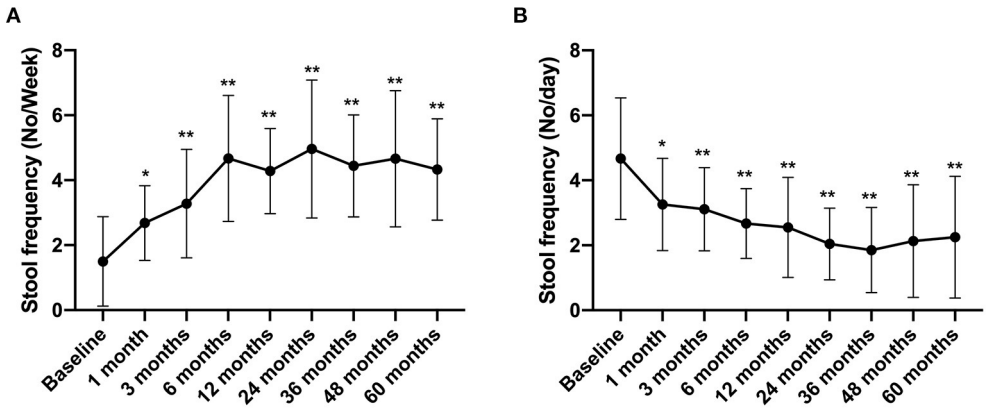


FIGURE 5 | Stool frequency between groups and their change over time. Difference was compared between each time point of follow-up and before FMT (baseline). **(A)** IBS-C; **(B)** IBS-D. Data are presented as $x \pm s$, statistical analyses: univariate analysis of variance, $^{*}p < 0.05$, $^{**}p < 0.01$.

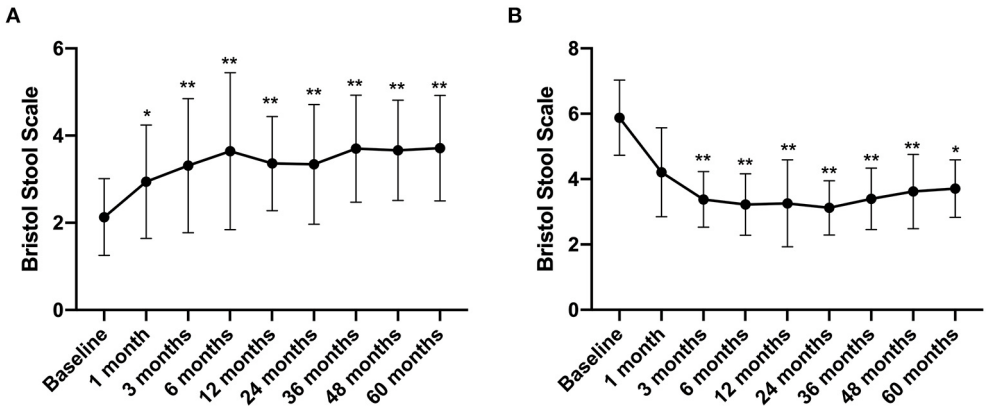


FIGURE 6 | Bristol Stool Scale between groups and their change over time. Difference was compared between each time point of follow-up and before FMT (baseline). **(A)** IBS-C; **(B)** IBS-D. Data are presented as $x \pm$ s, statistical analyses: univariate analysis of variance, $^{*}p < 0.05$, $^{**}p < 0.01$.

the colonoscopy group, six (4.84%) in the nasointestinal tube group, and two (3.18%) in the capsule group. The highest incidence rate of diarrhea in the colonoscopy pathway may be related to the stimulation effect of colonoscopy. There were 16 cases of nausea, of which 11 (8.87%) occurred in the nasointestinal tube pathway, 1 (1.59%) in the capsule pathway, and 4 (10%) in the colonoscopy pathway. Of the 10 cases of vomiting, six (4.84%) occurred in the nasointestinal tube group, two (3.18%) in the capsule group, and two (5%) in the colonoscopy group. Headache occurred in seven (3.08%) cases, a single case of GI bleeding occurred after colonoscopy, and allergic reactions were detected in two (0.88%) cases, namely, one (0.81%) in the nasointestinal tube group and one (2.5%) in the colonoscopy group. Fever occurred in eight (3.52%) cases, of which five (4.03%) were in the nasointestinal tube group, two (5%) were in the colonoscopy group, and one (1.59%) was in the capsule group. No significant differences were observed concerning the adverse events among the three groups. All symptoms were cured by symptomatic treatment, and no serious adverse events were reported during treatment or follow-up (**Table 3**).

DISCUSSION

In this study, 227 patients presenting IBS were enrolled. Previously, FMT was reported to reduce IBS symptoms in small-scale samples and short-term follow-up (17–19). Whether FMT can produce long-term effects on IBS has not yet been proven. Herein, the effect of FMT on IBS was studied through a long-term (5-year) follow-up and a large sample size (227 cases). The study endpoints included effective follow-up rates, change of IBS-SSS score, IBS-related quality of life and fatigue, effect on stool frequency, Bristol Stool Scale for IBS-C and IBS-D, and side effects of FMT.

Current evidence suggests that the microbiota of the GI tract could be a significant factor in the etiology of IBS (6). The gut microbiota of IBS patients differs from that of healthy subjects,

TABLE 3 | Side effects of FMT.

	Nasointestinal tube (124)	Capsules (63)	Colonoscopy (40)	Total complications (%)	*p*
Abdominal pain (%)	7 (5.65)	2 (3.18)	6 (15)	15 (6.61)	0.06
Abdominal distension/bloating (%)	8 (6.45)	3 (4.76)	6 (15)	17 (7.49)	0.133
Diarrhea (%)	6 (4.84)	2 (3.18)	5 (12.50)	13 (5.73)	0.125
Nausea (%)	11 (8.87)	1 (1.59)	4 (10)	16 (7.05)	0.094
Vomiting (%)	6 (4.84)	2 (3.18)	2 (5)	10 (4.41)	0.828
GI bleeding (%)	0 (0)	0 (0)	1 (2.5)	1 (0.44)	0.176
Headache (%)	5 (4.03)	0 (0)	2 (5)	7 (3.08)	0.146
Allergic reactions (%)	1 (0.81)	0 (0)	1 (2.5)	2 (0.88)	0.398
Fever (%)	5 (4.03)	1 (1.59)	2 (5)	8 (3.52)	0.624
Total complications (%)				89 (39.21)	

with the former having low bacterial diversity (dysbiosis), for example (7, 8). Changes in the intestinal environment were hypothesized to induce a compositional imbalance of the gut microbiota, termed "dysbiosis," which was associated with IBS (9). Consequently, probiotics and antibiotics were studied as potential treatment for IBS (10, 11); however, the scale of improvement in symptoms was limited. FMT provides a creative approach to restore the abnormal gut microbiome in patients with IBS. Our team has treated 2,010 cases of various GI dysfunction diseases including IBS through FMT, and the resulting long-term (36 months) efficacy rates were >60% (16). Although the current clinical studies have confirmed the efficacy of FMT in the treatment of IBS, these were short-term studies with small sample sizes; therefore, large-scale long-term studies are still lacking in this field (26).

In 2017, the first randomized controlled trial (RCT) on FMT treatment for IBS was conducted in Norway. Patients were assigned to a group ($n = 60$) comprising subjects who received 50–80 g of fresh FMT (used on the same day) or frozen FMT and a group ($n = 30$) consisting of subjects who received his or her own feces as placebo. Transplantation was performed with colonoscopy. After 3 months of FMT treatment, the IBS-SSS scores decreased by more than 75 points for 36 out of 55 subjects who were actively treated (65%) and 12 out of 28 subjects who received placebo (43%) ($p = 0.049$), indicating that the therapeutic efficacy was significantly better in the treatment group than in the placebo group (27). Since then, in several other randomized controlled studies that have been established, FMT has appeared to be effective at improving the symptoms (IBS-SSS) and the quality of life of patients with IBS, as well as reducing their fatigue (25, 28).

At present, FMT can be administered through a variety of methods, such as oral fecal capsules, nasointestinal injection, or endoscopy. Due to the bacterial overgrowth in the small intestine of IBS patients (29), the upper GI route is more recommended. In this study, we compared the following three methods to treat IBS: nasointestinal tube, capsule, and colonoscopy. The results showed that the capsule approach had the most obvious advantages. The main reason might be that the implementation of this approach is simpler and more convenient and has better medical compliance. Nonetheless, no significant differences were observed concerning the adverse events among the three groups.

Microbiota transplantation has been reported to have significant effects within the 1st day after administration (30), while the engraftment of transferred microbiota may take at least 7 months after FMT (31). The decline of donor strain populations has been detected within 1.5–3 months after FMT (32), and 39 ± 23% of the species showed resistance to introduced strains. Along with the decline of donor strains, the theoretical effect of FMT will also decrease significantly (32). The study by Johnsen et al. showed that, after 3 months of treatment, the efficacy rate of FMT was 65% (36 cases), while treatment response was observed in 12 cases (43%) of the placebo group. There was a significant statistical difference between the FMT and the placebo groups ($p = 0.049$). However, after 12 months of FMT, its effect decreased, and it had a similar effect on participants as the placebo (FMT vs. placebo groups, $p = 0.075$) (28). Therefore, repeated FMT treatments might be required. Previous research showed that a high-dose transplant and/or repeated FMT for IBS may increase the response rate and the intensity of the effects of FMT (25). In our previous clinical studies, we had observed that, following the FMT treatment period, the response decreased over time. Therefore, a repetitive and periodic FMT treatment strategy was subsequently established (33). Herein, it was confirmed that repeated and periodic FMT treatment can significantly ensure the long-term efficacy of FMT.

The present study indicated that the average course of FMT was 3.93 ± 2.30, including 3.83 ± 1.78 for nasointestinal tube patients, 5.19 ± 2.94 for capsule patients, and 2.25 ± 1.24 for colonoscopy patients. The reason behind the larger number of capsule transplants is that it is a simple, non-invasive, and easy route to implement, which leads to better medical compliance. Due to the trauma and discomfort of the nasointestinal tube and colonoscopy, their medical compliance is poor, and the frequency of repeated treatments is limited. Consequently, the good patient compliance and high repetition rate of capsule transplantation may be the main reasons for its high efficacy.

Adverse reactions to FMT treatment should also be addressed. It has been shown that most of these events are GI symptoms, as most patients experience transient diarrhea after FMT treatment, and a few may manifest symptoms such as bloating and belching that usually disappear after 2–3 days (34). In this study, 89 (39.21%) adverse reactions occurred during follow-up. The most common of these were abdominal pain, abdominal distension/bloating, diarrhea, nausea, vomiting, headache, allergic reactions, and fever. The capsules had the least side effects when compared to the nasointestinal tube and colonoscopy. All side effects were reduced by symptomatic treatment, and no serious adverse events occurred during the follow-up period. In 2019, the U.S. Food and Drug Administration (FDA) issued a warning that two donors who had not been tested for multidrug-resistant bacteria caused severe infections after FMT, and one patient died as a result (35). Our team has administered FMT therapy in 5,757 cases of various diseases, and no deaths have occurred. Donors have been tested for multidrug-resistant bacteria and resistant genes since the beginning of the study. At the same time, recipients are being selected according to rigorous standards. In addition to routine tests, we also evaluate the immune function of the recipient, such as lymphocyte count and T lymphocyte subgroup, since patients with immunodeficiency are extremely prone to enteric infections.

Certain limitations of this study need to be highlighted. First, it is a retrospective analysis rather than a prospective randomized controlled study. Second, we mainly focused on the clinical symptoms of IBS patients after FMT but did not follow up the changes of intestinal flora after the treatment period. Extensive research has shown that FMT improves symptoms in patients with IBS by improving the intestinal flora. However, one study indicated that the intestinal flora of FMT significantly enhanced, while the symptoms of IBS did not show any improvement (17).

CONCLUSION

In this retrospective study, the effects of three approaches of FMT therapy to treat IBS were evaluated during 5 years of long-term follow-up. The results demonstrated the safety and efficacy of FMT for IBS patients; however, as the treatment effect declines over time, periodic and repetitive treatment is necessary.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of Shanghai Tenth Hospital affiliated to Tongji University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QC, NL, and HQ conceived and designed the study. JC and CY conducted the data collections. Analysis and interpretation of data were done by ZL, HT, BY, and DZ. Statistical analysis was done by QC and JC. Writing and revision of the manuscript were done by JC, ZL, and QC. All authors read and approved the final manuscript.

FUNDING

This work was supported by Shanghai Shenkang Hospital Development Center: SHDC2020CR1030B and SHDC2020CR4026.

REFERENCES

1. Chey WD, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. *JAMA*. (2015) 313:949–58. doi: 10.1001/jama.2015.0954
2. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol*. (2012) 10:712–21. doi: 10.1016/j.cgh.2012.02.029
3. Soares RL. Irritable bowel syndrome: a clinical review. *World J Gastroenterol*. (2014) 20:12144–60. doi: 10.3748/wjg.v20.i34.12144
4. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology*. (2006) 130:1480–91. doi: 10.1053/j.gastro.2005.11.061
5. Zhang F, Xiang W, Li CY, Li SC. Economic burden of irritable bowel syndrome in China. *World J Gastroenterol*. (2016) 22:10450–60. doi: 10.3748/wjg.v22.i47.10450
6. Lee KN, Lee OY. Intestinal microbiota in pathophysiology and management of irritable bowel syndrome. *World J Gastroenterol*. (2014) 20:8886–97. doi: 10.3748/wjg.v20.i10.2456
7. Casen C, Vebo HC, Sekelja M, Hegge FT, Karlsson MK, Ciemniejewska E, et al. Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. *Aliment Pharmacol Ther*. (2015) 42:71–83. doi: 10.1111/apt.13236
8. El-Salhy M, Mazzawi T. Fecal microbiota transplantation for managing irritable bowel syndrome. *Expert Rev Gastroenterol Hepatol*. (2018) 12:439–45. doi: 10.1080/17474124.2018.1447380
9. Barbara G, Feinle-Bisset C, Ghoshal UC, Quigley EM, Santos J, Vanner S, et al. The intestinal microenvironment and functional gastrointestinal disorders. *Gastroenterology*. (2016) 150:1305–1318. doi: 10.1053/j.gastro.2016.02.028
10. Lee KJ, Tack J. Altered intestinal microbiota in irritable bowel syndrome. *Neurogastroenterol Motil*. (2010) 22:493–8. doi: 10.1111/j.1365-2982.2010.01482.x
11. Pimentel M, Lembo A, Chey WD, Zakko S, Ringel Y, Yu J, et al. Rifaximin therapy for patients with irritable bowel syndrome without constipation. *N Engl J Med*. (2011) 364:22–32. doi: 10.1056/NEJMoa1004409
12. Mugie SM, Di Lorenzo C, Benninga MA. Constipation in childhood. *Nat Rev Gastroenterol Hepatol*. (2011) 8:502–11. doi: 10.1038/nrgastro.2011.130
13. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med*. (2013) 368:407–15. doi: 10.1056/NEJMoa1205037
14. Lee CH, Steiner T, Petrof EO, Smieja M, Roscoe D, Nematallah A, et al. Frozen vs fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent clostridium difficile infection: a randomized clinical trial. *JAMA*. (2016) 315:142–9. doi: 10.1001/jama.2015.18098
15. Khoruts A, Dicksved J, Jansson JK, Sadowsky MJ. Changes in the composition of the human fecal microbiome after bacteriotherapy for

recurrent Clostridium difficile-associated diarrhea. *J Clin Gastroenterol.* (2010) 44:354–60. doi: 10.1097/MCG.0b013e3181c87e02
16. Li N, Tian HL, Chen QY, Yang B, Ma CL, Lin ZL, et al. [Efficacy analysis of fecal microbiota transplantation in the treatment of 2010 patients with intestinal disorders]. *Zhonghua Wei Chang Wai Ke Za Zhi.* (2019) 22:861–8. doi: 10.3760/cma.j.issn.1671-0274.2019.09.011
17. Halkjaer SI, Christensen AH, Lo BZS, Browne PD, Gunther S, Hansen LH, et al. Faecal microbiota transplantation alters gut microbiota in patients with irritable bowel syndrome: results from a randomised, double-blind placebo-controlled study. *Gut.* (2018) 67:2107–15. doi: 10.1136/gutjnl-2018-316434
18. Aroniadis OC, Brandt LJ, Oneto C, Feuerstadt P, Sherman A, Wolkoff AW, et al. Faecal microbiota transplantation for diarrhoea-predominant irritable bowel syndrome: a double-blind, randomised, placebo-controlled trial. *Lancet Gastroenterol Hepatol.* (2019) 4:675–85. doi: 10.1016/S2468-1253(19)30198-0
19. El-Salhy M, Hatlebakk JG, Gilja OH, Brathen Kristoffersen A, Hausken T. Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. *Gut.* (2020) 69:859–67. doi: 10.1136/gutjnl-2019-319630
20. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol.* (2003) 13:176–81. doi: 10.1016/S1053-4296(03)00031-6
21. Cammarota G, Ianiro G, Tilg H, Rajilic-Stojanovic M, Kump P, Satokari R, et al. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut.* (2017) 66:569–80. doi: 10.1136/gutjnl-2016-313017
22. Mullish BH, Quraishi MN, Segal JP, McCune VL, Baxter M, Marsden GL, et al. The use of faecal microbiota transplant as treatment for recurrent or refractory Clostridium difficile infection and other potential indications: joint British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. *Gut.* (2018) 67:1920–41. doi: 10.1136/gutjnl-2018-316818
23. Tian H, Ge X, Nie Y, Yang L, Ding C, McFarland LV, et al. Fecal microbiota transplantation in patients with slow-transit constipation: a randomized, clinical trial. *PLoS ONE.* (2017) 12:e0171308. doi: 10.1371/journal.pone.0171308
24. Tian H, Ding C, Gong J, Wei Y, McFarland LV, Li N. Freeze-dried, Capsulized Fecal Microbiota Transplantation for Relapsing Clostridium difficile Infection. *J Clin Gastroenterol.* (2015) 49:537–8. doi: 10.1097/MCG.0000000000000330
25. El-Salhy M, Hausken T, Hatlebakk JG. Increasing the dose and/or repeating faecal microbiota transplantation (FMT) increases the response in patients with irritable bowel syndrome (IBS). *Nutrients.* (2019) 11:1415. doi: 10.3390/nu11061415
26. Wang Y, Zheng F, Liu S, Luo H. Research progress in fecal microbiota transplantation as treatment for irritable bowel syndrome. *Gastroenterol Res Pract.* (2019) 2019:9759138. doi: 10.1155/2019/9759138
27. Ford AC. Stool as a treatment for IBS: more questions than answers? *Lancet Gastroenterol Hepatol.* (2018) 3:2–3. doi: 10.1016/S2468-1253(17)30337-0
28. Johnsen PH, Hilpusch F, Cavanagh JP, Leikanger IS, Kolstad C, Valle PC, et al. Faecal microbiota transplantation versus placebo for moderate-to-severe irritable bowel syndrome: a double-blind, randomised, placebo-controlled, parallel-group, single-centre trial. *Lancet Gastroenterol Hepatol.* (2018) 3:17–24. doi: 10.1016/S2468-1253(17)30338-2
29. Wu KQ, Sun WJ, Li N, Chen YQ, Wei YL, Chen DF. Small intestinal bacterial overgrowth is associated with Diarrhea-predominant irritable bowel syndrome by increasing mainly Prevotella abundance. *Scand J Gastroenterol.* (2019) 54:1419–25. doi: 10.1080/00365521.2019.1694067
30. Jalanka J, Mattila E, Jouhten H, Hartman J, de Vos WM, Arkkila P, et al. Long-term effects on luminal and mucosal microbiota and commonly acquired taxa in faecal microbiota transplantation for recurrent Clostridium difficile infection. *BMC Med.* (2016) 14:155. doi: 10.1186/s12916-016-0698-z
31. Broecker F, Klumpp J, Schuppler M, Russo G, Biedermann L, Hombach M, et al. Long-term changes of bacterial and viral compositions in the intestine of a recovered Clostridium difficile patient after fecal microbiota transplantation. *Cold Spring Harb Mol Case Stud.* (2016) 2:a000448. doi: 10.1101/mcs.a000448
32. Li SS, Zhu A, Benes V, Costea PI, Hercog R, Hildebrand F, et al. Durable coexistence of donor and recipient strains after fecal microbiota transplantation. *Science.* (2016) 352:586–9. doi: 10.1126/science.aad8852
33. Zhang X, Tian H, Ma C, Yang B, Hua Y, Zhu Y, et al. [Efficacy observation of periodic fecal microbiota transplantation in the treatment of refractory constipation]. *Zhonghua Wei Chang Wai Ke Za Zhi.* (2017) 20:1355–9. doi: 10.3760/cma.j.issn.1671-0274.2017.12.008
34. Jiang ZD, Ajami NJ, Petrosino JF, Jun G, Hanis CL, Shah M, et al. Randomised clinical trial: faecal microbiota transplantation for recurrent Clostridum difficile infection - fresh, or frozen, or lyophilised microbiota from a small pool of healthy donors delivered by colonoscopy. *Aliment Pharmacol Ther.* (2017) 45:899–908. doi: 10.1111/apt.13969
35. DeFilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N Engl J Med.* (2019) 381:2043–50. doi: 10.1056/NEJMoa1910437

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

REVIEW
published: 07 September 2021
doi: 10.3389/fmed.2021.665520

Fecal Microbiota Transplantation as a Tool for Therapeutic Modulation of Non-gastrointestinal Disorders

Robert Liptak[1,2], *Barbora Gromova*[3] *and Roman Gardlik*[3]*

[1] Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [2] Emergency Department, University Hospital in Bratislava, Bratislava, Slovakia, [3] Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Fecal microbiota transplantation has been primarily investigated as a therapeutic tool for a number of gut disorders. Optimistic results from clinical studies on Clostridium difficile infection, inflammatory bowel disease and irritable bowel syndrome have stimulated the expansion of possible indications in which FMT might represent a game changing approach. Microbial dysbiosis was shown in a number of non-gastrointestinal disorders. Moreover, FMT was proven to be effective in therapy of numerous animal models of disease. However, only a proportion of these disorders have been addressed in clinical studies using FMT. These include obesity, non-alcoholic fatty liver disease, cardiovascular inflammation and neurological disorders such as autism, depression and Parkinson's disease. Results from preclinical and clinical studies also outlined possible molecular mechanisms that contribute to alleviation of the disease. These range from increasing the circulating levels of microbial metabolites (trimethylamine N-oxide, lipopolysaccharide, short chain fatty acids) to stimulation of the enteric nervous system. Several methodological shortcomings are still to be addressed; however, positive results of the clinical studies indicate that further investigation of FMT as a therapeutic tool for non-gastrointestinal disorders can be expected in upcoming years.

Keywords: intestinal microbiota, metabolic syndrome, liver disease, cardiovascular health, autism spectrum disorder, depression, Parkinson's disease, enteric nervous system

OPEN ACCESS

Edited by:
Angel Lanas,
University of Zaragoza, Spain

Reviewed by:
Christopher Velez,
Massachusetts General Hospital and Harvard Medical School, United States
Levinus Albert Dieleman,
University of Alberta, Canada

***Correspondence:**
Roman Gardlik
romangardlik@gmail.com

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *28 April 2021*
Accepted: *13 August 2021*
Published: *07 September 2021*

Citation:
Liptak R, Gromova B and Gardlik R (2021) Fecal Microbiota Transplantation as a Tool for Therapeutic Modulation of Non-gastrointestinal Disorders. Front. Med. 8:665520. doi: 10.3389/fmed.2021.665520

GUT MICROBIOTA AND FECAL MICROBIOTA TRANSPLANTATION

Gut microbiota have gained tremendous scientific attention over the last 15 years. With the advances in biotechnology we have been able to, at least partially, describe the microbial environment and its effects on the host. Virtually all parts of the human body have been studied from the microbial point of view. However, the most studied site of the human body remains the gut.

The most abundant members of gut microbiota are bacteria, followed by viruses, archaea, and microbial eukaryotes. Predominant bacterial phyla in healthy individuals are *Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria* (1). Interaction between the gut microbiota and the host is closely associated with maturation of the immune system (2), immune homeostasis (3), modulation of xenobiotics (4), and protection against pathogens (5). Gut microbiota dysbiosis either compositional or functional has been linked to autistic spectrum disorder (6), depression and anxiety (7), cardiovascular health (8), metabolic syndrome (9), development of non-alcoholic fatty liver disease (10), chemotherapy effectiveness modulation (11), and even in sepsis (12).

Fecal microbiota transplantation (FMT) is a method for modulating host microbiome in order to restore gut microbiota dysbiosis toward eubiosis. This method is fairly simple, during the procedure healthy stool from a donor is placed into the gastrointestinal system of the recipient via nasogastric tube, colonoscope, capsule or combination of these methods. The first report of FMT in medical literature comes from 1958 and was used to treat pseudomembranous colitis (13). First randomized trial using FMT was conducted in 2013 and since then it has gained more and more attention as an effective tool for alleviating certain maladies (14).

In this review, we summarize the therapeutic applications of FMT for disorders that primarily affect tissues and organs outside the gastrointestinal tract. We focus only on disorders with at least one clinical study using FMT as a therapeutic tool. Studies on metabolic syndrome/obesity, non-alcoholic fatty liver disease/non-alcoholic steatohepatitis, cardiovascular disease, autism spectrum disorders, depression and Parkinson's disease represent a knowledge base for further clinical investigation. **Figure 1** depicts the suggested mechanisms by which FMT can modulate the pathogenesis of these disorders. **Table 1** summarizes the details of relevant clinical studies employing FMT for the treatment of the reviewed disorders.

METABOLIC SYNDROME

Metabolic syndrome as a set of central obesity, dyslipidemia, decreased insulin sensitivity and arterial hypertension has been established in 1988 and has been intensively studied ever since (30). First evidence of gut being at least partly responsible for metabolic syndrome came in 2007 with studies where rodents were fed a high fat diet. After 4 weeks of the diet the rodents showed signs of metabolic syndrome with increased lipopolysaccharide (LPS) concentration in blood. LPS caused a proinflammatory state which decreased insulin sensitivity (31). Landmark studies were performed by Jeffrey Gordon's group, in which they proved that increased adiposity might be a transmissible trait, as was first shown by FMT from *ob/ob* mice into germ free-recipients (32). Association of gut microbiota with obesity was nicely shown in a more recent study where FMT was performed from twins discordant for obesity to germ free mice. Mice that received bacteria from obese twin had increased adiposity and decreased diversity of the gut microbiome (33).

Healthy gut microbiota positively affects host energy metabolism. Bacteria within the gut using their respective metabolic pathways produce molecules that pose a signal for the host cells. Bacteria ferment the indigestible polysaccharides into short chain fatty acids (SCFAs) which act as energy sources for colonocytes but more importantly as signal molecules. SCFAs enhance insulin sensitivity and stimulate fatty acid oxidation and lipolysis (34). Gut bacteria convert primary bile acids into secondary bile acids which affects the farnesoid X receptor, a regulator of host glucose and fat homeostasis (35).

High fructose diet-induced metabolic syndrome in rats was associated with higher abundances of *Coprococcus* and *Ruminococcus* genera. FMT from non-obese healthy rat donors were able to colonize rats fed high fructose diet. Colonization led to reduction of markers of metabolic syndrome and decreased abundance of *Coprococcus* and *Ruminococcus* genera (36). In a similar study diet-induced obese mice received FMT from lean mice. The recipient obese mice were treated with antibiotics prior to FMT to enhance engraftment of donor microbiota. After FMT gut microbiota of obese mice showed greater diversity and regained some functionality showed by metaproteomic approach (37). Similarly, stool from lean mice that exercised transferred into obese mice improved obesity and inflammatory status in obese mice (38). Recent study showed that when autologous stool obtained before induction of obesity is transferred into obese hosts, it results in increased lipolysis and caloric restriction. However, the FMT with caloric restriction group compared to the caloric restriction group without FMT did not show significant difference in gut microbiota composition with only differences *Bifidobacterium* and *Blautia* genera were observed. Authors proposed different mechanisms apart from microbiota engraftment that induced this effect which might include bacteriophages or bacterial metabolites in the stool. However, mice were not observed for long-term effects after the FMT, so the metabolic improvement could have been only temporary (39).

There are several human studies available today. A study by Vrieze et al. showed that FMT from lean donors transferred by single administration via duodenal tube into obese participants increased insulin sensitivity. Obese patients showed decreased gut microbial diversity compared to lean patients. After FMT from lean donors, the gut microbiota diversity was increased significantly. Moreover, sixteen bacterial groups increased in abundance after FMT including potent butyrate producers *Roseburia intestinalis* and *Eubacterium hallii*. Increased butyrate reduces the translocation of endotoxins into the bloodstream, which drives insulin resistance. Whether this is the sole mechanism or there are others at play is currently unknown (15). Subsequently, a similar effect was observed following FMT from lean donors to obese patients via duodenal tube. At 6 weeks after FMT increased insulin sensitivity accompanied by decreased glycated hemoglobin was observed. The gut microbiome changes in patients who responded to FMT showed increased abundance of *Akkermansia muciniphila* and *Eubacterium ventriosum*. There was no difference in gut microbiota diversity among responders and non-responders (16). Another human study had a different design than the previous ones. Stool donors were patients after gastric bypass surgery or obese individuals without intervention. Recipients were obese individuals with metabolic syndrome. The main outcome, insulin sensitivity, showed significant difference, however, this was mainly due to decreased insulin sensitivity of the control group (obese individuals receiving FMT from obese donors). However, a slight increase in insulin sensitivity was observed in the intervention group. The intervention group showed decreased subcutaneous fat inflammation post FMT with decreased expression of chemokine CCL2. The intervention group had increased abundance of *Bacteroides* sp. compared to the control group. In analysis of the intervention group responders and non-responders to FMT were identified. Higher baseline abundances of *Alistipes shahii* and *Anaerostipes hadrus* were associated with better glycemic control after FMT (17).

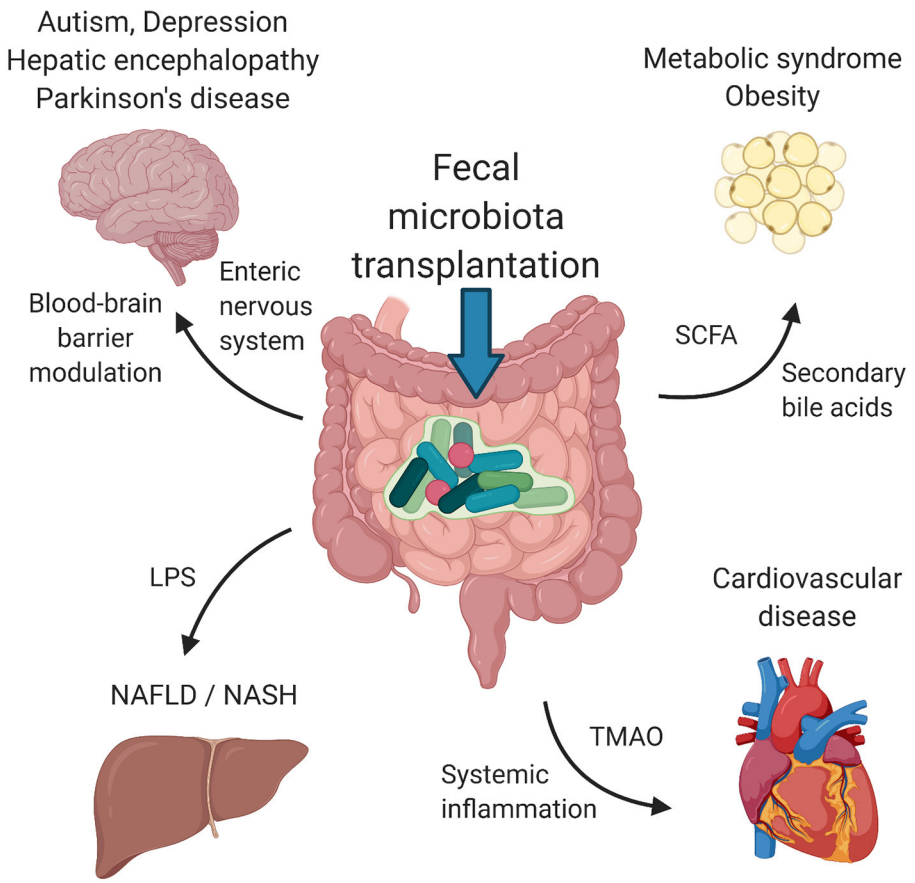


FIGURE 1 | Suggested mechanisms of FMT. Signaling via enteric nervous system and blood-brain barrier modulation by microbial metabolites influence psychiatric/neurological disorders, including autism spectrum disorders, depression, hepatic encephalopathy and Parkinson's disease. Metabolic syndrome and obesity seem to be modulated by the presence of SCFA and secondary bile acids produced by bacteria. LPS and other structural molecules from bacteria entering the portal circulation affect the liver health. Cardiovascular health was found to be regulated by bacterially produced TMAO as well as systemic inflammation induced by the presence of circulating bacteria and their metabolites. FMT, fecal microbiota transplantation; SCFA, short chains fatty acids; LPS, lipopolysaccharide; TMAO, trimethylamine-N-oxide. Created with BioRender.com.

These three studies, however, are from the same study group. Yu et al. performed double blind placebo controlled pilot trial administering oral capsules of FMT from lean donors to obese individuals. Participants were administered 15 capsules during two consecutive days, followed by a capsule once per week for 5 weeks. The primary outcome was insulin sensitivity measured at week 0 and week 6 and several other secondary outcomes such as HbA1c, body composition, and resting energy expenditure. There were no differences between the intervention and placebo group despite engraftment of donor bacteria as assessed by 16S V4 amplicon sequencing (18). There are several differences between the previous three studies and this one. Most importantly, the route of administration (endoscopy vs. capsule), FMT material (fresh vs. frozen), and colon preparation with laxatives (yes vs. no bowel preparation) was different. Another fact to consider is the geographical region in which these studies were performed (Netherlands vs. USA) which can affect both the donor and recipient microbiota. These questions need to be addressed in future studies.

CARDIOVASCULAR HEALTH

Growing body of evidence has linked gut microbiota to cardiovascular diseases such as atherosclerosis or arterial hypertension (8, 40, 41). Interaction between gut microbiota metabolites and their proinflammatory activity has been suggested. Microbiota metabolism of phosphatidylcholine through the production of proatherogenic metabolite trimethylamine-N-oxide (TMAO). Increased levels of TMAO are associated with increased incidence of major cardiovascular events, as was shown in healthy participants and during a 3 years of follow-up in patients undergoing elective coronary angiography (42). Besides metabolites, gut microbiota dysbiosis with decrease of SCFA producing bacteria may induce systemic inflammation with increased neutrophil infiltration of aortic root, thus exhibiting proatherogenic effect (43). Gut microbiota obtained from donors with hypertension transferred into germ-free mice resulted in increased blood pressure in an animal model (41). Similarly, in high-salt induced hypertension

TABLE 1 | Clinical studies using FMT for non-gastrointestinal disorders.

Disease	Donors	Recipients	Placebo arm	Administration route	Dose of feces	Frequency	Follow up time	Primary outcome	References
Metabolic syndrome	Lean male donors *n = 9*	Obese participants *n = 18*	Yes	Duodenal infusion	500 ml in 0.9% NaCl	One time?	6 weeks	Insulin sensitivity	(15)
Metabolic syndrome	Lean male donors *n = 11*	Obese participants *n = 38*	Yes	Nasoduodenal infusion	500 ml in 0.9% NaCl	One time	6 and 18 weeks	Insulin sensitivity	(16)
Metabolic syndrome	(a) post-Roux-en-Y gastric bypass, *n = 5* (b) metabolic syndrome, *n = 6*	Obese participants (a) *n = 11* (b) *n = 12*	Yes	Duodenal infusion	500 ml	One time	2 weeks	Insulin sensitivity	(17)
Metabolic syndrome	Lean donors *n* = 24	Obese participants *n = 24*	Yes	Oral capsules	15 capsules + 1 capsule weekly	2 days + weekly for 5 weeks	6 weeks	Insulin sensitivity	(18)
Cardiovascular health	Lean vegan donors *n = 10* recipients themselves *n = 10*	Obese participants *n = 20*	Yes	Nasoduodenal infusion	500 ml in 0.9% NaCl	One time	2 weeks	TMAO and PET/CT scan of abdominal aorta	(19)
NAFLD/NASH	Lean donors *n = 21*	Obese participants with hepatic steatosis *n = ?*	Yes	Duodenal infusion	–	Three times at 8-weeks intervals	24 weeks	Liver necrosis score and hepatic gene expression	(20)
Hepatic encephalopathy	Healthy volunteer from OpenBiome	Outpatient cirrhotic men with recurrent HE *n = 20*	No	FMT enema	Frozen-then-thawed FMT units (90 ml total) 2.7×10^{12} CFU	One time	5 months	FMT-related serious adverse events (SAEs) and endpoint of death	(21)
Hepatic encephalopathy	Healthy volunteer from OpenBiome	Outpatient cirrhotic men with recurrent HE *n = 20*	No	FMT enema	Frozen-then-thawed FMT units (90 ml total) 2.7×10^{12} CFU	One time	15 months from	FMT-related serious adverse events (SAEs) and endpoint of death	(22)
Hepatic encephalopathy	Healthy volunteer from OpenBiome	Outpatient cirrhotic men with recurrent HE *n = 20*	Yes	Oral capsules	15 capsules	–	5 months	Tolerability, FMT-related serious adverse events (SAEs)	(23)
Autism spectrum disorder	Healthy adults	Children with ASD *n = 18*	No	Oral infusion ectal infusion	2.5×10^{12} cells/day	2 days – three times per day, 1 h	10–18 weeks	GI and ASD-related symptoms	(24)
Autism spectrum disorder	–	Children with ASD *n = 18*	No	–	–	–	2 years	GI and ASD-related symptoms	(25)
Autism spectrum disorder	Healthy adults	Children with ASD *n = 24*	No	Oral infusion b) rectal infusion	–	–	2 months	GI and ASD-related symptoms	(26)
Depression	Healthy adults	58 years old male 66 years old female 48 years old male	No	Rectal infusion	–	10× over 2 weeks 6× over 1 week 5× over 1 week	6 months 4 years 6 months	GI and depression symptoms	(27)
Parkinson's disease	Healthy adults	PD patients *n = 10* PD patients *n = 5*	No	Rectal infusion nasoduodenal infusion	–	<1 h	1 and 3 months	Motor and non-motor symptoms	(28)
Parkinson's disease	Frozen fecal microbiota was obtained from the China fmtBank	PD patients with constipation, *n = 11*	No	Nasoduodenal infusion	40–50 ml of frozen fecal microbiota in 200 ml of warm normal saline, fresh every time	–	6–12 weeks	16S ribosomal DNA, motor and non-motor symptoms	(29)

in rats this phenotype was transferable by gut microbiota. Moreover, hypertension was alleviated by transferring healthy gut microbiota. This beneficial effect was accompanied by decreased intestinal derived corticosterone and increased levels of *Bacteriodes fragilis* and arachidonic acid levels in the intestine (44).

Despite this evidence there is relatively small amount of studies exploring the potential effect of FMT to improve cardiovascular health. In a murine model of myocarditis FMT from a healthy donor alleviated myocardial damage by reducing inflammatory infiltration and restoring gut microbiota eubiosis (45). Other authors showed that transplantation of healthy stool to spontaneously hypertensive rats alleviated hypertension via modulation of sympathetic nervous activity (46).

The only human study conducted so far explored the effect of single FMT from vegan donors on TMAO levels and vascular inflammation in a double blind randomized fashion. Recipients received one time only FMT via nasoduodenal tube from lean vegan donors or autologous gut microbiota. After FMT there was no difference in gut microbiota diversity; however, some compositional differences were observed. In the lean donor group, the *Lachnospiraceae* showed increased abundance whereas the autologous group showed increased *Clostridiales* which are known producers of trimethylamine - a TMAO precursor. Vegan donor FMT did not alter fasting or urinary 24 h excretion of TMAO, nor there were changes in vascular inflammation assessed by ^{18}F-FDG PET/CT (19).

NON-ALCOHOLIC FATTY LIVER DISEASE/NON-ALCOHOLIC STEATOHEPATITIS

Non-alcoholic fatty liver disease (NAFLD) is characterized by steatosis affecting at least 5% of the liver volume or weight in non-alcoholic patients. About 30% of people with NAFLD progress into non-alcoholic steatohepatitis (NASH) which is characterized by progressive inflammation. About 20% of patients with NASH will progress into liver fibrosis with decline in liver function (47). The cause of this accumulation is unknown, however, it is often associated with signs of metabolic syndrome (48). The pathophysiology of NASH is poorly understood, however, interaction between genetics, environment, and possibly also gut microbiota is suggested (49). Germ free mice that were fed a high fat diet had a lower rate of liver steatosis than conventional mice, suggesting that gut microbiome might play a role (50). Liver receives the majority of blood supply from the portal vein which drains nutrients along with bacterial compounds from intestines (51). During dysbiosis gut barrier function is disrupted and more bacterial derived compounds enter the circulation, thus the first site these compounds hit is the liver. Afterwards, these molecules, such as LPS, are able to initiate and maintain chronic inflammation. This may potentiate NAFLD and subsequently its progression to NASH (52). Moreover, after transferring gut microbiota from mice with NASH into germ free mice, these mice had more adipose tissue than their counterparts receiving FMT from healthy mice (53).

Gut microbiome changes in NAFLD have been observed, however with conflicting results. Authors found that people with NAFLD and NASH have increased abundances of *Proteobacteria* including increased *Enterobacteriaceae* and decreased *Rikenellaceae* and *Ruminococcaceae* (54). Moreover, some of the bacterial signatures were common with metabolic syndrome and obesity.

In a murine model of diet induced steatohepatitis FMT was successful in restoring gut microbiota dysbiosis. This was accompanied by increased SCFA production and decrease in proinflammatory cytokines production (55).

Recent human double blinded, randomized study investigated the effect of allogenic FMT using stool obtained from individuals eating plant based diet compared with autologous FMT administered three times at 8-weeks intervals via duodenal tube. After the FMT there was no difference in gut microbiota diversity after 24 weeks. However, there were some compositional differences. Individuals receiving allogeneic FMT had increased *Ruminococcus, Eubacterium hallii, Faecalibacterium, and Prevotella copri;* however, the difference did not reach statistical significance. Recipients of allogeneic FMT showed improvement in liver necrosis score which was in line with expression of several hepatic genes including genes responsible for liver endothelial integrity. Changes in gut microbiome might result in decreased levels of microbial aromatic amino acid production, especially phenyllactic acid which is linked to NAFLD. Thus, reducing the production of toxic metabolites by dysbiotic gut microbiota might alleviate NAFLD (20).

HEPATIC ENCEPHALOPATHY

Under normal physiologic circumstances the gut provides a barrier for various metabolites (e.g., pro-inflammatory molecules, adipokines, TMA etc.) arising in the gut. Metabolites that penetrate this barrier pass through the liver where they are metabolized and thus, the brain is protected from toxic substances. However, in advanced liver disease, such as cirrhosis, these barrier mechanisms are compromised.

Patients with advanced liver disease show gut microbial dysbiosis, increased gut permeability and decreased liver capacity to detoxify toxins. All of which perpetuates one another and ultimately leads to neuronal dysfunction and damage resulting in hepatic encephalopathy (HE) (56).

Patients with HE have reduced abundances of *Lachnospiraceae, Ruminococcaceae* and Clostridiales XIV and increased abundances of *Staphylococcaeae, Enterobacteriaceae,* and *Enterococcaceae* (57). The latter taxa are associated with disease progression and endotoxemia (58). Traditional treatment of HE consisted of lactulose and rifaximin, both of which change bacterial composition without reducing the absolute amount of bacteria in GI tract (59–61).

In a rat model of carbon tetrachloride induced acute liver failure, the rats received FMT with three different concentrations of bacteria or probiotic solution for 3 weeks after acute liver failure induction. All of the rats receiving FMT or probiotics showed increased memory function, improved liver function,

decreased intestinal permeability, and reduced ammonia levels and systemic proinflammatory cytokines concentration. However, no analysis of the microbiome was performed (62).

Participants of the first open label clinical trial received a single FMT via enema from a healthy donor. The donor was selected based on relative abundance of *Lachnospiraceae* and *Ruminococcaceae* since these taxa are indicative of gut microbiome health (63). Patients were divided into standard care (SC) group and SC + FMT group. Both groups had 10 participants. SC consisted of lactulose, rifaximin and proton pump inhibitor. FMT patients received antibiotic treatment before FMT. The FMT group had significantly fewer HE episodes and had significant improvement in cognitive function. MELD score was similar in both groups. FMT patients had increased relative abundance of *Lachnospiraceae* and *Ruminococcaceae*. Patients were followed up to 5 months (21). Afterwards, authors decided to expand the follow up period up to 15 months. There were significantly less hospitalizations in the FMT group than SC group and cognitive function was better in the FMT group. Microbiome analysis revealed increased *Burkholderiaceae* and decreased *Acidaminococcus* in FMT patients, however *Lachnospiraceae* and *Ruminococcaceae* were similar between groups (22).

The same authors performed a single center, randomized, single blinded placebo controlled trial with similar design. In this subsequent study FMT was delivered via oral capsules and no pre-FMT antibiotics were administered. FMT patients had fewer serious adverse events, HE episodes, and improved cognitive functions. FMT patients underwent repeated endoscopies which showed decreased expression of IL6, and increased expression of barrier proteins (defensin A5), and E-cadherin in duodenum post FMT. Serum concentration of lipoprotein binding protein also decreased post FMT. Stool microbiota showed increased abundance of *Lachnospiraceae* in the FMT group. Duodenal mucosa in the FMT group showed increase in *Ruminococcaceae* and *Bifidobacteriaceae*, reduction in *Streptococaceae* and *Veillonellaceae* and increased Shannon diversity index post FMT (23).

AUTISM SPECTRUM DISORDER

Autism spectrum disorder (ASD) is a neurodevelopmental disorder which results in several behavioral abnormalities. Pathogenesis is unclear, but genes play a major role in developing ASD. However, gene-environment interactions have lately gained more attention in research. Some authors estimate that 50% of the neurobiology is caused by factors that are non-inherited (64). ASD is often accompanied by more or less severe gastrointestinal symptoms. Several studies have shown altered gut microbiota compositions (65, 66). Interestingly, ASD behavior can be transferred via FMT to germ-free mice (67).

In fragile X mental retardation 1 KO mice, a model in which mice elicit autistic like behavior, FMT can ameliorate abnormal behavior in mice (68). In human studies ASD children who received FMT for 8 weeks showed significant behavioral improvement for 8 weeks after the treatment ended (24). In subsequent study by the same author, bowel cleansing, antibiotics, and stomach acid suppressants followed by FMT. Participants were followed by up to 2 years after the treatment stopped. Gastrointestinal symptoms improvement was maintained and behavioral symptoms improved significantly after the treatment ended. Authors observed no adverse effects (25). Although this study was open-label with no placebo control the results are promising. The authors concluded that improvement of gastrointestinal and behavioral symptoms persisted for at least 2 months after FMT compared to the control group. In a conference abstract, a different group of authors showed that FMT in ASD individuals was well-tolerated, improved statistically ASD-related symptoms, and shifted the microbiome of ASD patients toward a healthy state. They reported adverse effects such as fever, allergy, and nausea, but these were mild and transient and could be associated with the mode of delivery of FMT - colonoscopy and gastroscopy. However, there is no information about the pretreatment of recipients and the amount of stool administered (26).

DEPRESSION

Depression has an increasing prevalence in Western world with substantial morbidity and mortality. More and more evidence is emerging associating gut microbiome with depression. The proposed mechanisms include neuroimmune, neuroendocrine and neural pathways (69). For example, mice suffering chronic social defeat stress show depression-like symptoms which are transferable via FMT. *Faecalibacterium rodentium* showed increased abundance in these mice and ingesting this bacterium alone can produce depression-like symptoms. Furthermore, these can be alleviated with subdiaphragmatic vagotomy suggesting that enteric nervous system plays a role (70). Altered gut microbiome composition has been found in patients with depression, a negative correlation between *Faecalibacterium* and depressive symptoms has been found (71). Transferring gut microbiota from depressed humans can induce depression like behavior in rats pretreated with antibiotics (72). Similar result is obtained when transferring gut microbiota from depressed humans into germ free mice (73). Only a small case series described the effect of FMT from a healthy donor into a depressed individual. These patients also suffered from irritable bowel syndrome. FMT was administered via colonoscopy with variable amounts of large bowel enemas based on attending clinician. FMT resulted in alleviating symptoms of both depression and irritable bowel syndrome (27). However, it is questionable whether decreased depression symptoms were the consequence of FMT on depression, or improved symptoms of irritable bowel syndrome.

PARKINSON'S DISEASE

Parkinson's disease (PD) is a neurodegenerative disorder which mainly affects the motor system of the central nervous system. Aggregation of α-synuclein (α-syn) is thought to be the cause of the disease. Dopaminergic neurons in substantia nigra are the

first neurons affected by this accumulation. Although multiple gene variants have been associated with the development of PD, the gut microbiome has gained more attention in the last years. The accumulation of α-syn in the enteric nervous system (ENS) has been reported years ago (74). Subsequent study has shown that α-syn is transported via the vagus nerve into the central nervous system after injection into the stomach and duodenal wall (75). *In vivo* studies showed that gut microbiome influences accumulation of α-syn in ENS (76). In mice that overexpress α-syn the presence of gut microbiota is required to promote pathological alterations similar to PD. Moreover, FMT from patients with PD induced PD phenotype in recipient mice (77).

In a murine model of PD FMT was sufficient to ameliorate PD symptoms and increased striatal dopamine and serotonin in recipient mice. FMT also reduced neuroinflammation. In PD mice the authors observed gut microbiota dysbiosis compared to healthy mice. FMT treatment was sufficient to remove these differences and tip the scale toward eubiosis. PD mice showed increased Proteobacteria at phylum level with decreased *Clostridiales* at the order level (78).

A human pilot study including 15 patients receiving FMT from healthy donors reported mixed effects of FMT in alleviating PD symptoms. Ten of the patients received FMT via colonoscopy and 5 of the patients received FMT via nasointestinal route. Colonic route appeared superior, some of the patients reported improved health status for up to 24 months after FMT, although gut microbiota changes were not examined. However, no control or placebo group was included (28).

More than 70% of PD patients suffer from constipation affecting their quality of life. Recent study included 11 PD patients with constipation that underwent single FMT from healthy donors via nasoduodenal tube in order to alleviate gastrointestinal symptoms. Patients were evaluated 6 and 12 weeks after the first FMT. Stool was collected pre-FMT at 4, 6, 8, and 12 weeks after FMT, afterwards 16S rDNA sequencing for microbiome analysis was performed. Overall the gut microbiome diversity was lower in pre-FMT samples and increased post-FMT. In pre-FMT samples increased abundance of *Bacteroides* and reduced abundance of *Faecalibacterium* was observed. At 12 weeks post-FMT abundance of these genera reversed. Abundance of *Blautia*, a butyrate producing bacteria, increased post-FMT. Increased levels of butyrate could explain decreased gastrointestinal symptoms, however, this hypothesis needs to be proven. Baseline gut microbiome showed high relative abundance of *Enterobacteriaceae* which was positively correlated with postural instability and gait difficulty (79). After FMT the abundance of *Enterobacteriaceae* decreased with improvement in postural instability and gait difficulty. Similarly as in previous study, no control group was included (29).

CONCLUSIONS

Advances in biotechnology and expansion of the knowledge on mechanisms of FMT have extended the spectrum of diseases treatable using FMT. Besides the well-known gastrointestinal indications such as *Clostridium difficile* infection, inflammatory bowel disease and irritable bowel syndrome that have been under massive clinical investigation for at least a decade, seemingly non-gastrointestinal disorders recently emerged as potential therapeutic targets for FMT. Dysbiosis was found in a number of metabolic, inflammatory, cardiovascular or neurological disorders, however, only a small number of clinical studies investigating the therapeutic effect of FMT have been published to date. Despite several methodological shortcomings, mostly positive results of these clinical studies indicate that further investigation of FMT as a therapeutic tool for non-gastrointestinal disorders can be expected in upcoming years.

AUTHOR CONTRIBUTIONS

RL and BG: conceptualization, writing, original draft preparation. RG: writing—review and editing, funding, and supervision. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was funded by the Slovak Research and Development Agency under the contract no. APVV-17-0505 and by the Ministry of Education, Science, Research and Sport of the Slovak Republic under the contract no. VEGA 1/0649/21.

REFERENCES

1. Das B, Ghosh TS, Kedia S, Rampal R, Saxena S, Bag S, et al. Analysis of the gut microbiome of rural and urban healthy indians living in sea level and high altitude areas. *Sci Rep.* (2018) 8:10104. doi: 10.1038/s41598-018-28550-3
2. Chung H, Pamp SJ, Hill JA, Surana NK, Edelman SM, Troy EB, et al. Gut immune maturation depends on colonization with a host-specific microbiota. *Cell.* (2012) 149:1578–93. doi: 10.1016/j.cell.2012.04.037
3. Daïen CI, Pinget GV, Tan JK, Macia L. Detrimental impact of microbiota-accessible carbohydrate-deprived diet on gut and immune homeostasis: an overview. *Front Immunol.* (2017) 8:548. doi: 10.3389/fimmu.2017.00548
4. Ursell LK, Knight R. Xenobiotics and the human gut microbiome: metatranscriptomics reveal the active players. *Cell Metab.* (2013) 17:317–8. doi: 10.1016/j.cmet.2013.02.013
5. Frost F, Weiss FU, Sendler M, Kacprowski T, Rühlemann M, Bang C, et al. The gut microbiome in patients with chronic pancreatitis is characterized by significant dysbiosis and overgrowth by opportunistic pathogens. *Clin Transl Gastroenterol.* (2020) 11:e00232. doi: 10.14309/ctg.0000000000000232
6. Vuong HE, Hsiao EY. Emerging roles for the gut microbiome in autism spectrum disorder. *Biol Psychiatry.* (2017) 81:411–23. doi: 10.1016/j.biopsych.2016.08.024
7. Foster JA, McVey Neufeld KA. Gut-brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci.* (2013) 36:305–12. doi: 10.1016/j.tins.2013.01.005
8. Jie Z, Xia H, Zhong SL, Feng Q, Li S, Liang S, et al. The gut microbiome in atherosclerotic cardiovascular disease. *Nat Commun.* (2017) 8:845. doi: 10.1038/s41467-017-00900-1

9. Mazidi M, Rezaie P, Kengne AP, Mobarhan MG, Ferns GA. Gut microbiome and metabolic syndrome. *Diabetes Metab Syndr.* (2016) 10:S150–7. doi: 10.1016/j.dsx.2016.01.024
10. Jadhav K, Cohen TS. Can you trust your gut? Implicating a disrupted intestinal microbiome in the progression of NAFLD/NASH. *Front Endocrinol.* (2020) 11:592157. doi: 10.3389/fendo.2020.592157
11. Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, Kinross JM. Gut microbiota modulation of chemotherapy efficacy and toxicity. *Nat Rev Gastroenterol Hepatol.* (2017) 14:356–65. doi: 10.1038/nrgastro.2017.20
12. Zhang D, Chen G, Manwani D, Mortha A, Xu C, Faith JJ, et al. Neutrophil ageing is regulated by the microbiome. *Nature.* (2015) 525:528–32. doi: 10.1038/nature15367
13. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery.* (1958) 44:854–9.
14. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med.* (2013) 368:407–15. doi: 10.1056/NEJMoa1205037
15. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology.* (2012) 143:913–6.e7. doi: 10.1053/j.gastro.2012.06.031
16. Kootte RS, Levin E, Salojärvi J, Smits LP, Hartstra AV, Udayappan SD, et al. Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metab.* (2017) 26:611–9.e6. doi: 10.1016/j.cmet.2017.09.008
17. de Groot P, Scheithauer T, Bakker GJ, Prodan A, Levin E, Khan MT, et al. Donor metabolic characteristics drive effects of faecal microbiota transplantation on recipient insulin sensitivity, energy expenditure and intestinal transit time. *Gut.* (2020) 69:502–12. doi: 10.1136/gutjnl-2019-318320
18. Yu EW, Gao L, Stastka P, Cheney MC, Mahabamunuge J, Torres Soto M, et al. Fecal microbiota transplantation for the improvement of metabolism in obesity: the FMT-TRIM double-blind placebo-controlled pilot trial. *PLoS Med.* (2020) 17:e1003051. doi: 10.1371/journal.pmed.1003051
19. Smits LP, Kootte RS, Levin E, Prodan A, Fuentes S, Zoetendal EG, et al. Effect of vegan fecal microbiota transplantation on carnitine- and choline-derived trimethylamine-N-oxide production and vascular inflammation in patients with metabolic syndrome. *J Am Heart Assoc.* (2018) 7:e008342. doi: 10.1161/JAHA.117.008342
20. Witjes JJ, Smits LP, Pekmez CT, Prodan A, Meijnikman AS, Troelstra MA, et al. Donor fecal microbiota transplantation alters gut microbiota and metabolites in obese individuals with steatohepatitis. *Hepatol Commun.* (2020) 4:1578–90. doi: 10.1002/hep4.1601
21. Bajaj JS, Kassam Z, Fagan A, Gavis EA, Liu E, Cox IJ, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. *Hepatology.* (2017) 66:1727–38. doi: 10.1002/hep.29306
22. Bajaj JS, Fagan A, Gavis EA, Kassam Z, Sikaroodi M, Gillevet PM. Long-term outcomes of fecal microbiota transplantation in patients with cirrhosis. *Gastroenterology.* (2019) 156:1921–3 e3. doi: 10.1053/j.gastro.2019.01.033
23. Bajaj JS, Salzman NH, Acharya C, Sterling RK, White MB, Gavis EA, et al. Fecal Microbial Transplant Capsules Are Safe in Hepatic Encephalopathy: A Phase 1, Randomized, Placebo-Controlled Trial. *Hepatology.* (2019) 70:1690–703. doi: 10.1002/hep.30690
24. Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome.* (2017) 5:10. doi: 10.1186/s40168-016-0225-7
25. Kang DW, Adams JB, Coleman DM, Pollard EL, Maldonado J, McDonough-Means S, et al. Long-term benefit of microbiota transfer therapy on autism symptoms and gut microbiota. *Sci Rep.* (2019) 9:5821. doi: 10.1038/s41598-019-42183-0
26. Zhao HJ, Gao XF, Xi L, Shi YC, Peng LH, Wang CY, et al. Fecal microbiota transplantation for children with autism spectrum disorder. *Gastrointestinal Endoscopy.* (2019) 89:AB512–3. doi: 10.1016/j.gie.2019.03.857
27. Collyer RC, A.; Borody, T. Faecal microbiota transplantation alleviates symptoms of depression in individuals with irritable bowel syndrome: a case series. *Med Microecol.* (2020) 6:100029. doi: 10.1016/j.medmic.2020.100029
28. Xue LJ, Yang XZ, Tong Q, Shen P, Ma SJ, Wu SN, et al. Fecal microbiota transplantation therapy for Parkinson's disease: a preliminary study. *Medicine.* (2020) 99:e22035. doi: 10.1097/MD.0000000000022035
29. Kuai XY, Yao XH, Xu LJ, Zhou YQ, Zhang LP, Liu Y, et al. Evaluation of fecal microbiota transplantation in Parkinson's disease patients with constipation. *Microb Cell Fact.* (2021) 20:98. doi: 10.1186/s12934-021-01589-0
30. Sarafidis PA, Nilsson PM. The metabolic syndrome: a glance at its history. *J Hypertens.* (2006) 24:621–6. doi: 10.1097/01.hjh.0000217840.26971.b6
31. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes.* (2007) 56:1761–72. doi: 10.2337/db06-1491
32. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* (2006) 444:1027–31. doi: 10.1038/nature05414
33. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science.* (2013) 341:1241214. doi: 10.1126/science.1241214
34. Utzschneider KM, Kratz M, Damman CJ, Hullar M. Mechanisms linking the gut microbiome and glucose metabolism. *J Clin Endocrinol Metab.* (2016) 101:1445–54. doi: 10.1210/jc.2015-4251
35. Calkin AC, Tontonoz P. Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. *Nat Rev Mol Cell Biol.* (2012) 13:213–24. doi: 10.1038/nrm3312
36. Di Luccia B, Crescenzo R, Mazzoli A, Cigliano L, Venditti P, Walser JC, et al. Rescue of fructose-induced metabolic syndrome by antibiotics or faecal transplantation in a rat model of obesity. *PLoS ONE.* (2015) 10:e0134893. doi: 10.1371/journal.pone.0134893
37. Guirro M, Costa A, Gual-Grau A, Herrero P, Torrell H, Canela N, et al. Effects from diet-induced gut microbiota dysbiosis and obesity can be ameliorated by fecal microbiota transplantation: a multiomics approach. *PLoS ONE.* (2019) 14:e0218143. doi: 10.1371/journal.pone.0218143
38. Lai ZL, Tseng CH, Ho HJ, Cheung CKY, Lin JY, Chen YJ, et al. Fecal microbiota transplantation confers beneficial metabolic effects of diet and exercise on diet-induced obese mice. *Sci Rep.* (2018) 8:15625. doi: 10.1038/s41598-018-33893-y
39. Pérez-Matute P, Íñiguez M, de Toro M, Recio-Fernández E, Oteo JA. Autologous fecal transplantation from a lean state potentiates caloric restriction effects on body weight and adiposity in obese mice. *Sci Rep.* (2020) 10:9388. doi: 10.1038/s41598-020-64961-x
40. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature.* (2011) 472:57–63. doi: 10.1038/nature09922
41. Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, et al. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome.* (2017) 5:14. doi: 10.1186/s40168-016-0222-x
42. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med.* (2013) 368:1575–84. doi: 10.1056/NEJMoa1109400
43. Brandsma E, Kloosterhuis NJ, Koster M, Dekker DC, Gijbels MJJ, van der Velden S, et al. A proinflammatory gut microbiota increases systemic inflammation and accelerates atherosclerosis. *Circ Res.* (2019) 124:94–100. doi: 10.1161/CIRCRESAHA.118.313234
44. Yan X, Jin J, Su X, Yin X, Gao J, Wang X, et al. Intestinal flora modulates blood pressure by regulating the synthesis of intestinal-derived corticosterone in high salt-induced hypertension. *Circ Res.* (2020) 126:839–53. doi: 10.1161/CIRCRESAHA.119.316394
45. Hu XF, Zhang WY, Wen Q, Chen WJ, Wang ZM, Chen J, et al. Fecal microbiota transplantation alleviates myocardial damage in myocarditis by restoring the microbiota composition. *Pharmacol Res.* (2019) 139:412–21. doi: 10.1016/j.phrs.2018.11.042
46. Toral M, Robles-Vera I, de la Visitación N, Romero M, Yang T, Sánchez M, et al. Critical role of the interaction gut microbiota - sympathetic nervous system in the regulation of blood pressure. *Front Physiol.* (2019) 10:231. doi: 10.3389/fphys.2019.00231
47. DeWeerdt S. Disease progression: divergent paths. *Nature.* (2017) 551:S92–s3. doi: 10.1038/d41586-017-06925-2

48. Kolodziejczyk AA, Zheng D, Shibolet O, Elinav E. The role of the microbiome in NAFLD and NASH. *EMBO Mol Med.* (2019) 11:e9302. doi: 10.15252/emmm.201809302
49. Abu-Shanab A, Quigley EM. The role of the gut microbiota in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol.* (2010) 7:691–701. doi: 10.1038/nrgastro.2010.172
50. Rabot S, Membrez M, Bruneau A, Gérard P, Harach T, Moser M, et al. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. *FASEB J.* (2010) 24:4948–59. doi: 10.1096/fj.10-164921
51. Macpherson AJ, Heikenwalder M, Ganal-Vonarburg SC. The liver at the nexus of host-microbial interactions. *Cell Host Microbe.* (2016) 20:561–71. doi: 10.1016/j.chom.2016.10.016
52. Luther J, Garber JJ, Khalili H, Dave M, Bale SS, Jindal R, et al. Hepatic injury in nonalcoholic steatohepatitis contributes to altered intestinal permeability. *Cell Mol Gastroenterol Hepatol.* (2015) 1:222–32. doi: 10.1016/j.jcmgh.2015.01.001
53. Mouries J, Brescia P, Silvestri A, Spadoni I, Sorribas M, Wiest R, et al. Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development. *J Hepatol.* (2019) 71:1216–28. doi: 10.1016/j.jhep.2019.08.005
54. Aron-Wisnewsky J, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, et al. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol.* (2020) 17:279–97. doi: 10.1038/s41575-020-0269-9
55. Zhou D, Pan Q, Shen F, Cao HX, Ding WJ, Chen YW, et al. Total fecal microbiota transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of gut microbiota. *Sci Rep.* (2017) 7:1529. doi: 10.1038/s41598-017-01751-y
56. Butterworth R. Neuronal cell death in hepatic encephalopathy. *Metab Brain Dis.* (2007) 22:309–20. doi: 10.1007/s11011-007-9072-3
57. Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol.* (2014) 60:940–7. doi: 10.1016/j.jhep.2013.12.019
58. Bajaj JS, Ridlon JM, Hylemon PB, Thacker LR, Heuman DM, Smith S, et al. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol.* (2012) 302:G168–75. doi: 10.1152/ajpgi.00190.2011
59. DuPont HL. Introduction: understanding mechanisms of the actions of rifaximin in selected gastrointestinal diseases. *Aliment Pharmacol Ther.* (2016) 43 (Suppl. 1):1–2. doi: 10.1111/apt.13406
60. Kawaguchi T, Suzuki F, Imamura M, Murashima N, Yanase M, Mine T, et al. Rifaximin-altered gut microbiota components associated with liver/neuropsychological functions in patients with hepatic encephalopathy: an exploratory data analysis of phase II/III clinical trials. *Hepatol Res.* (2019) 49:404–18. doi: 10.1111/hepr.13300
61. Wang JY, Bajaj JS, Wang JB, Shang J, Zhou XM, Guo XL, et al. Lactulose improves cognition, quality of life, and gut microbiota in minimal hepatic encephalopathy: a multicenter, randomized controlled trial. *J Dig Dis.* (2019) 20:547–56. doi: 10.1111/1751-2980.12816
62. Wang WW, Zhang Y, Huang XB, You N, Zheng L, Li J. Fecal microbiota transplantation prevents hepatic encephalopathy in rats with carbon tetrachloride-induced acute hepatic dysfunction. *World J Gastroenterol.* (2017) 23:6983–94. doi: 10.3748/wjg.v23.i38.6983
63. Antharam VC, Li EC, Ishmael A, Sharma A, Mai V, Rand KH, et al. Intestinal dysbiosis and depletion of butyrogenic bacteria in *Clostridium difficile* infection and nosocomial diarrhea. *J Clin Microbiol.* (2013) 51:2884–92. doi: 10.1128/JCM.00845-13
64. Mayer EA, Padua D, Tillisch K. Altered brain-gut axis in autism: comorbidity or causative mechanisms? *Bioessays.* (2014) 36:933–9. doi: 10.1002/bies.201400075
65. Liu S, Li E, Sun Z, Fu D, Duan G, Jiang M, et al. Altered gut microbiota and short chain fatty acids in Chinese children with autism spectrum disorder. *Sci Rep.* (2019) 9:287. doi: 10.1038/s41598-018-36430-z
66. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The central nervous system and the gut microbiome. *Cell.* (2016) 167:915–32. doi: 10.1016/j.cell.2016.10.027
67. Sharon G, Cruz NJ, Kang DW, Gandal MJ, Wang B, Kim YM, et al. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell.* (2019) 177:1600–18.e17. doi: 10.1016/j.cell.2019.05.004
68. Goo N, Bae HJ, Park K, Kim J, Jeong Y, Cai M, et al. The effect of fecal microbiota transplantation on autistic-like behaviors in Fmr1 KO mice. *Life Sci.* (2020) 262:118497. doi: 10.1016/j.lfs.2020.118497
69. Winter G, Hart RA, Charlesworth RPG, Sharpley CF. Gut microbiome and depression: what we know and what we need to know. *Rev Neurosci.* (2018) 29:629–43. doi: 10.1515/revneuro-2017-0072
70. Wang S, Ishima T, Qu Y, Shan J, Chang L, Wei Y, et al. Ingestion of *Faecalibaculum rodentium* causes depression-like phenotypes in resilient Ephx2 knock-out mice: a role of brain-gut-microbiota axis via the subdiaphragmatic vagus nerve. *J Affect Disord.* (2021) 292:565–73. doi: 10.1016/j.jad.2021.06.006
71. Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, Yin Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun.* (2015) 48:186–94. doi: 10.1016/j.bbi.2015.03.016
72. Kelly JR, Borre Y, C OB, Patterson E, El Aidy S, Deane J, et al. Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *J Psychiatr Res.* (2016) 82:109–18. doi: 10.1016/j.jpsychires.2016.07.019
73. Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol Psychiatry.* (2016) 21:786–96. doi: 10.1038/mp.2016.44
74. Braak H, Del Tredici K, Rüb U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging.* (2003) 24:197–211. doi: 10.1016/S0197-4580(02)00065-9
75. Holmqvist S, Chutna O, Bousset L, Aldrin-Kirk P, Li W, Björklund T, et al. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathol.* (2014) 128:805–20. doi: 10.1007/s00401-014-1343-6
76. Chen SG, Stribinskis V, Rane MJ, Demuth DR, Gozal E, Roberts AM, et al. Exposure to the functional bacterial amyloid protein curli enhances alpha-synuclein aggregation in aged fischer 344 rats and *Caenorhabditis elegans*. *Sci Rep.* (2016) 6:34477. doi: 10.1038/srep34477
77. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell.* (2016) 167:1469–80.e12. doi: 10.1016/j.cell.2016.11.018
78. Sun MF, Zhu YL, Zhou ZL, Jia XB, Xu YD, Yang Q, et al. Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: gut microbiota, glial reaction and TLR4/TNF-α signaling pathway. *Brain Behav Immun.* (2018) 70:48–60. doi: 10.1016/j.bbi.2018.02.005
79. Scheperjans F, Aho V, Pereira PA, Koskinen K, Paulin L, Pekkonen E, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord.* (2015) 30:350–8. doi: 10.1002/mds.26069

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Medicine

METHODS
published: 18 May 2022
doi: 10.3389/fmed.2022.830004

Fecal Microbiota and Human Intestinal Fluid Transplantation: Methodologies and Outlook

Ye Chen†, Lin Zhiliang†, Cui Jiaqu†, Lv Xiaoqiong, Zhang Shaoyi, Ma Chunlian, Yan Yinmei, Yang Bo, Zhao Di, Tian Hongliang, Li Ning, Chen Qiyi and Qin Huanlong**

Department of Colorectal Disease Specialty, Clinical Research Center for Digestive Diseases, The Tenth People's Hospital, Tongji University, Shanghai, China

OPEN ACCESS

Edited by:
Jozsef Soki, University of Szeged, Hungary

Reviewed by:
Felix Broecker, Idorsia Pharmaceuticals Ltd., Switzerland
Yongbo Kang, Shanxi Medical University, China

***Correspondence:**
Chen Qiyi
qiyichen2011@163.com
Qin Huanlong
huanlong_qin@live.cn

†*These authors share first authorship*

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *06 December 2021*
Accepted: *11 April 2022*
Published: *18 May 2022*

Citation:
Chen Y, Zhiliang L, Jiaqu C, Xiaoqiong L, Shaoyi Z, Chunlian M, Yinmei Y, Bo Y, Di Z, Hongliang T, Ning L, Qiyi C and Huanlong Q (2022) Fecal Microbiota and Human Intestinal Fluid Transplantation: Methodologies and Outlook. Front. Med. 9:830004. doi: 10.3389/fmed.2022.830004

Fecal microbiota transplantation (FMT) is a therapy that involves the transplantation of healthy human fecal microorganisms into the gut of patients to rebuild or consolidate the intestinal microecology. It has been utilized in many diseases. However, FMT had a limited effect on patients with small intestinal diseases because of the unique ecological characteristics of the microorganisms. Thus, we proposed a new microecology transplantation therapy called human intestinal fluid transplantation (HIFT). Human intestinal fluid can be collected through a nasojejunal tube and be made into capsules using the freeze-dried powder method. In addition, strict standards for donor screening and management have been established. We are currently developing a high-standard HIFT preparation system and conducting high-quality clinical studies to validate the safety and efficacy of HIFT combined with FMT.

Keywords: human intestinal fluid transplantation, fecal microbiota transplantation, gut microbial therapeutics, methodologies, donor

INTRODUCTION

Fecal microbiota transplantation (FMT) is a new therapy that involves the transplantation of healthy human fecal microorganisms into the gut of patients to rebuild or consolidate the intestinal microecology (1). Many diseases have been found to be associated with intestinal flora disturbance, including intestinal diseases, such as functional bowel disease (2), inflammatory bowel disease (3), and infectious diarrhea (4); and extra-intestinal diseases, such as Parkinson's disease (5), autism spectrum disorder (ASD) (6), and amyotrophic lateral sclerosis (7). Many studies have showed that the microbial community structure and functions could be normalized after FMT in human, instead of destroying the original structure (2–4, 6). However, FMT is not a panacea for all kinds of diseases, especially in diseases associated with the brain-gut axis and small intestinal bacterial overgrowth (8).

Given that FMT mainly treats the colon flora, the 4-meter-long small intestine is not given the priority it deserves. Small intestine is one of the most important organs in human beings, which has lots of digestive enzymes, microorganisms, immunoglobulins and other vital substances (9–11). It is involved in nutrient absorption, secretion, metabolism and immune functions. However,

few interventions are used in small intestinal diseases. Recent studies have also confirmed that bacteria from different segments of the gut colonize homologous segments after transplantation. These microorganisms have unique ecological characteristics (12), and the application of 16S rRNA technology has brought a new understanding to the study of microorganisms. It was reported that there were amounts of bacteria in the stomach and small intestine, where it was previously thought that there was only few (13, 14). In clinical practice, human intestinal fluids (HIF) reinfusion can significantly improve intestinal function in patients with severe intestinal dysfunction (15, 16). Homologous HIF may have better tolerance than enteral nutrient solution because of the living substance. Therefore, human intestinal fluid transplantation (HIFT) from the healthy population may be more effective for patients with intestinal function disturbances than FMT. But there is no effective method to collect human intestinal fluid in clinic. In this study, we summarize the first establishment of standardized HIFT preparation, which is used in the treatment of intestinal dysfunction diseases.

DONOR SELECTION AND MANAGEMENT

HIFT is a new therapy without a standardized methodology yet. To ensure the safety and efficacy of HIFT (17–19), the criteria for HIFT donor selection and management were based on the China expert consensus on the establishment of standardized methodology and clinical application for FMT (20). The strict donor screening criteria included objective criteria, psychological evaluation, and history of diseases, which fully evaluated the past and recent potentially harmful behaviors and the risks for infection transmission:

Donor screening criteria and management criteria:

- **Objective criteria**
  - Age 18–30, male or female, body mass index of 18.5–22.9 kg/m^2.
  - Normr negative hematology tests: blood routine test, hepatic and renal function, electrolytes and c-reactive protein, infectious hepatitis, HIV, syphilis, Epstein-Barr virus, cytomegalovirus, nematode, amoeba, and other pathogens.
  - Normal or negative stool tests: feces regular test, occult blood test, *Clostridium Difficile, Campylobacteria, Salmonella, Shigella, Shiga-toxigenic Escherichia coli*, worm eggs, vesicles, parasites, spores, norovirus, rotavirus, and multiple drug resistance genes (such as carbapenem-resistant enterobacteriaceae, extended-spectrum β-lactamase-producing bacteria, methicillin-resistant staphylococcus aureus and other drug-resistant bacteria).
- **Psychological evaluation**
  - Assessed as having a good psychological state by a cardiologist or psychological consultant.
  - Normal scores in Self-rating Depression Scale, Self-rating Anxiety Scale, and Pittsburgh Sleep Quality Index.
- **History of diseases**
  - Past History: without gastrointestinal symptoms in recent 2 weeks, no antibiotics, acid inhibitors, immunosuppressants, or chemotherapeutic drug use in the last 3 months, no chronic pain symptoms, no history of digestive surgery, no history of infection and infectious disease exposure, no allergic disease, no autoimmune disease, no metabolic disease, no cardiovascular and cerebrovascular diseases, no neuropathy, no psychosis, no malignancy, no growth hormone, insulin, coagulation factors, or other injection treatment.
  - Personal history: have a regular routine, healthy diet and harmonious family, and no bad sexual history; no smoking, drinking, and drug addiction history; no vaccination, drug trial, skin damage, and contact with tropical areas in the last 6 months.
  - Family history: no family history of gastrointestinal diseases, malignant tumors, or infectious diseases.
  - Others: not pregnant, not in menstrual period.
- **Archive and follow-up system establishment**
  - Standard donor file establishment, including recording each inspection result, HIF donation, and related treatment.
  - Follow-up system establishment, to ensure that the donors regularly complete and pass the physical examination and donation requirements.
- **Donor management group establishment**
  - The donor management should be in charge of the full-time donor managers of the HIFT center, including at least 1 principal and 2 assistants.
  - The donor managers should maintain regular communication with the donors, establish a good trust relationship, and carry out necessary management and intervention on the donor's lifestyle and diet structure. They should immediately correct the unhealthy lifestyle and diet structure, and eliminate unqualified donors according to the follow-up results.
- **Informed consent of the donors**
  - Donor candidates should be fully informed and signed the informed consent before screening.
  - The HIFT donor should be fully informed and signed the informed consent for nasointestinal tube catheterization before donation.
- **HIFT Donor donation requirements**
  - The donors shall ensure the continuity of the donation. Each donation can be made for 3–7 consecutive days, once every 1–2 months.
  - The amount of HIF donation should be no <350 mL per day after filtering, and the color of the HIF must be golden yellow.

The above items should be reviewed every 2 months. Only ∼2% of the population can be screened as ideal donors. After screening, HIFT donors provided informed consent for the donation and nasointestinal tube catheterization during the HIF collection period. Every donor should obey the criteria of donor management to ensure the stabilization and safety of HIF. Stool and HIF samples of each donor were saved for 16s rRNA sequencing and composition analysis to ensure the basic stability of the bacterial community and biological components, and to allow tracing when recipients have adverse effects. In addition, some donors should restrict certain types of food for 5 days prior to the donation of HIF when recipients have food allergy or food intolerance symptoms.

PREPARATION METHODS OF HIFT CAPSULES

Donors underwent nasojejunal tube catheterization by using a modified Flocare nasogastric feeding tube, with a depth of 175 cm away from the nose. The modified disposable sterile negative pressure collecting devices were connected to the catheter for continuous drainage, which was controlled below 6.7 kPa. The collecting devices were changed every 2 h because the HIF may be metamorphic outside. After collection, HIF was successively filtered through screen cloths of 2.0, 1.0, and 0.5 mm. Then, 10% glycerin was added to the filtrate as a cryoprotectant and HIF was turned into the lyophilized powder by lyophilizer (19, 21), keeping the water content below 5% and mucus content below 10%. Finally, the lyophilized powder was packaged in an enteric capsule shell of an acid-resistant acrylic resin, which would only disintegrate in small intestine. The packaged capsules were stored in the −80°C refrigerator, and these were valid for 6 months (**Figure 1**).

The entire preparation process required information registration, HIF identification, weighing, and testing. A 2 mL HIF sample from each donation must be set aside for at least 6 months to allow tracing in case of adverse events. Donor information code, donation date, production date, expiration date, dose, and storage temperature were recorded.

INDICATIONS AND CONTRAINDICATIONS

Although the efficiency of FMT for rCDI is 90% (22, 23), the response rates for other diseases do not have the same results. HIFT compensates for the loss of small intestinal microorganisms in the FMT. Thus, HIFT can be used in combination with FMT, in diseases that respond poorly to FMT treatment in preliminary, including digestive diseases (small intestinal bacterial overgrowth, inflammatory bowel disease, and obstructive functional constipation), neuropsychiatric disorders (ASD, anxious depression, and Parkinson's disease), metabolic disorders (diabetes, obesity, fatty liver, and hyperlipidemia), and immune systemic diseases (tumor immunity, allergic diseases, and chronic fatigue syndrome). The combination treatment, in other words, is a treatment of whole intestinal microbiota transplantation. However, the microorganism itself is an antigen. Bacterial translocation may be an important part of the exacerbation of the inflammatory response in the system (24). Abuse of FMT or HIFT can cause serious complications, including sepsis and death. Patients with congenital or acquired immune deficiency, recently received high-risk immunosuppressive or cytotoxic drugs, or with severely damaged intestinal mucosa, must not receive FMT or HIFT.

TREATMENT OF HIFT

Previous studies have suggested that the number of bacteria in the proximal small intestine is $<10^5$ cfu/ml. 1 cfu is almost several numbers of bacteria. However, with the application of 16S rRNA and Trypan Blue test, the number of bacteria recorded in HIF is $>3.0 \times 10^8$/ml. There is a significant difference between these two values, and many new microorganisms have been identified (25). According to the test results, the following requirements were drawn. The amounts of viable bacteria were used as the standard therapeutic dose, which in the HIFT liquid should be $\geq 5.0 \times 10^8$/ml, with the viable bacteria proportion ≥83%. The amounts of viable bacteria in the HIFT powder should be $\geq 2.0 \times 10^6$/g and the viable bacteria proportion should be ≥81%. For adults, the therapeutic dose of HIFT liquid was 50 ml at each time, and for children, each dose was 1 ml/kg. Based on current technologies, the route for HIFT is only upper gastrointestinal tract, including nasojejunal tube, endoscopy and oral administration capsule. And, the nasojejunal tube and oral administration capsule can be repeated dosed. The HIFT treatment courses were consistent with the FMT. A standard course of HIFT was administered once daily for 6 consecutive days. Treatment is one course per month for at least two consecutive treatments. To mitigate this possible interaction, FMT was first followed by HIFT therapy. The interval between the two should be more than half an hour.

MANAGEMENT OF ADVERSE EFFECTS

Although FMT has a low incidence of adverse effects (AEs) and its management varies from country to country, the risks of its clinical use must be carefully considered (24). The most common symptoms are nausea, emesis, abdominal distension, diarrhea, allergy, and fever. Most AEs are mild to moderate and are always self-limited (2). In fact, HIF contains fewer microorganisms and may be safer. Although HIF has not been thoroughly investigated, it can be considered as one of the most vital body fluids. Similarly, succus entericus reinfusion is an important therapy for treating severe patients with complex intestinal fistula, which can stabilize the intestinal mucosal barrier function and promote the recovery of intestinal function (15). It has been reported that autologous or allogeneic succus entericus reinfusion is safer and plays an extremely important role in the treatment of critically ill patients, and its effect was found to be even better than that of enteral nutrition alone (26, 27). The HIFT prevention and management of AEs were as follows: (1) Establishment of an AE reporting system; (2) Strict criteria on the indications and contraindications, and assessment of the risk of complications before HIFT; (3) Mild symptoms:

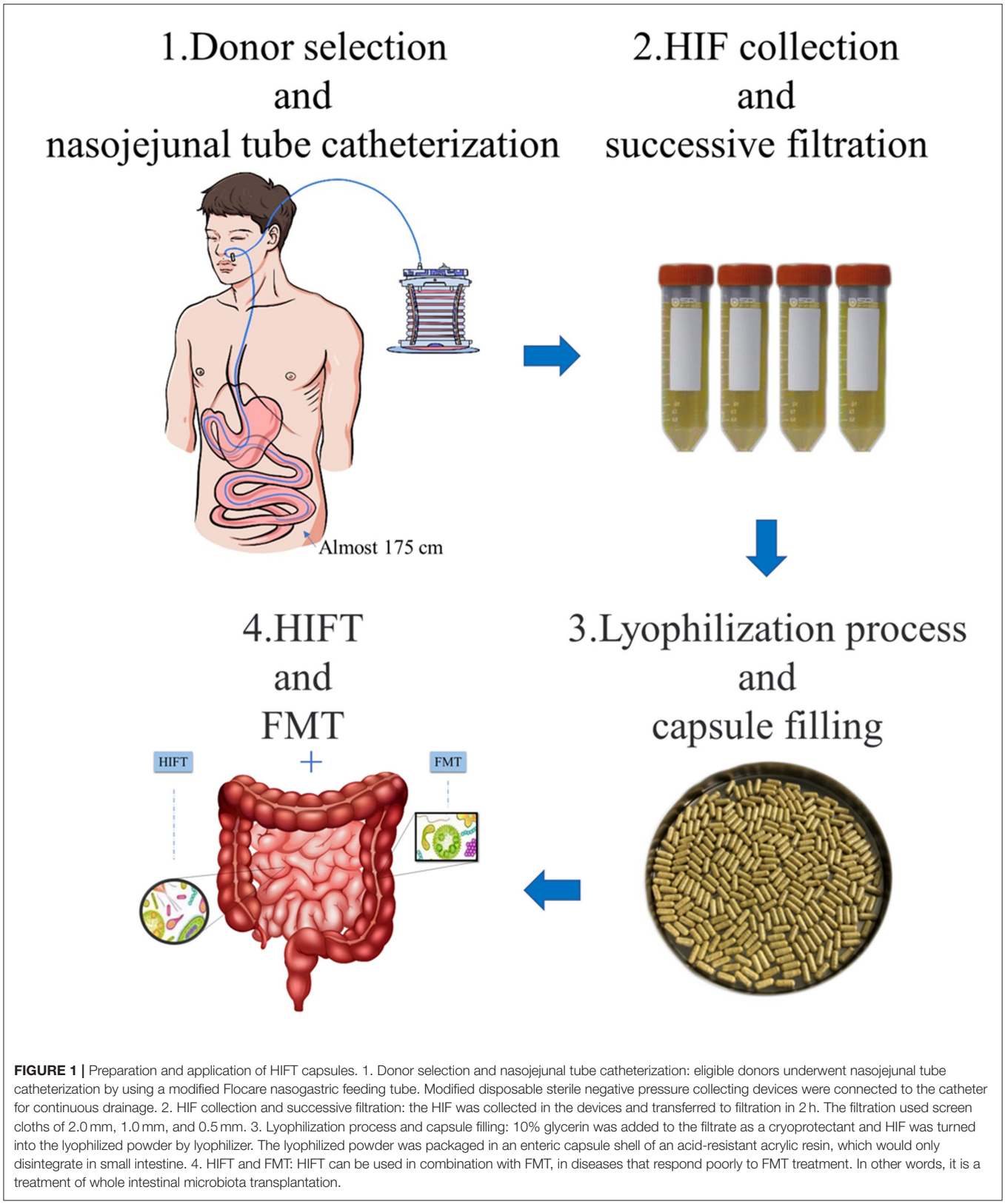


FIGURE 1 | Preparation and application of HIFT capsules. 1. Donor selection and nasojejunal tube catheterization: eligible donors underwent nasojejunal tube catheterization by using a modified Flocare nasogastric feeding tube. Modified disposable sterile negative pressure collecting devices were connected to the catheter for continuous drainage. 2. HIF collection and successive filtration: the HIF was collected in the devices and transferred to filtration in 2 h. The filtration used screen cloths of 2.0 mm, 1.0 mm, and 0.5 mm. 3. Lyophilization process and capsule filling: 10% glycerin was added to the filtrate as a cryoprotectant and HIF was turned into the lyophilized powder by lyophilizer. The lyophilized powder was packaged in an enteric capsule shell of an acid-resistant acrylic resin, which would only disintegrate in small intestine. 4. HIFT and FMT: HIFT can be used in combination with FMT, in diseases that respond poorly to FMT treatment. In other words, it is a treatment of whole intestinal microbiota transplantation.

TABLE 1 | Different methods in achieving HIF biopsy in recent 5 years.

References	Methods	Position	Recipients
Leite et al. (32)	Duodenoscopy	Duodenum	Human
Tziatzios et al. (35)	Gastroscopy	Duodenum	Human
Ding et al. (33)	Magnetically controlled sampling capsule endoscope	Jejunum and ileum	Pigs
Riethorst et al. (29)	Double-lumen catheter	Duodenum and jejunum	Human
de la Cruz-Moreno et al. (36)	Double-lumen catheter	Duodenum and jejunum	Human
Riethorst et al. (34)	Double-lumen catheter	Duodenum and jejunum	Human and simulated intestinal fluids

continuous observation of clinical symptoms, such as mild dizziness, nausea, and gastrointestinal discomfort; (4) Moderate symptoms: symptomatic treatment and suspension of HIFT if necessary, such as oral antidiarrheal for diarrhea, oral non-steroidal anti-inflammatory drugs for fever, and intramuscular injection of metoclopramide for nausea and vomiting; (5) Severe symptoms: emergency treatment and termination of HIFT. Blood tests and symptomatic treatments are urgently needed. If enterogenic infection is suspected, blood culture tests should be conducted, and intravenous anti-infection or selective digestive decontamination should be administered (28). In addition, the patient's fecal pathogens and the donor's fluid and/or powder should be evaluated. Last but not least, HIFT should be applied and managed through the ethic committee and the local government. Though the regulatory issues of FMT vary from country to country, the goal of treatment is the same that every step of HIFT should be recorded and tested, to ensure that the bacteria are eligible and effective.

CHALLENGES AND FUTURE CONSIDERATIONS

The whole intestinal microbiota is a complex consortium with many components that have never been totally characterized in FMT. Likewise, the microbiota construction in HIF is even less known. Previous studies have tested the pH, bile salts, phospholipids, cholesterol, free fatty acids, pancreatic lipase, and other life active substance in HIF (29). It is used to know that due to the presence of gastric acid, bile acid, immunoglobulin IgA, and other bactericidal and antibacterial substances in the upper digestive tract (10), the small intestinal microbiota is minimal, which is $<10^5$ cfu/ml (30, 31). This may be related to the innovation of microbiome detection technology and the discovery of many new microbiomes (13). Given that there are still numerous undiscovered bacteria, HIF may have more bacteria per unit volume. Nowadays, FMT has been proved as a therapy which has high security (1, 3, 8), though the knowledge has not been available enough either regarding the influence of transplanting the microbiota from person to person. Based on the standard treatment of FMT, HIFT is a brand-new treatment concept which may be safe. To ensure the safety, it is still important to carry out standard methodologies of HIFT, including donor/recipient screening, HIF preparation, route of transplantation, and informed consent.

The current preparation method of HIF is in a primary stage. It is the first time to achieve mass production. A variety of techniques have been used to achieve minimally invasive or non-invasive HIF biopsy in healthy people, including gastroscopy, capsule endoscopy, and nasointestinal tube insertion (32–34) (**Table 1**). Endoscopy is often limited by the limited depth of placement (32), and it is difficult to collect HIF in the fasting state (35). A new generation of capsule endoscope, called magnetically controlled sampling capsule endoscope, can collect 0.2–0.4 ml HIF through a negative pressure system (33). This yield cannot meet the treatment needs and the cost of this procedure is high (37). A double-lumen catheter, one kind of nasojejunal tubes, was mainly evaluated the physicochemical properties of HIF and the dissolution of drugs in the upper digestive tract (29, 36). The position of the catheter was proximal to the duodenum and distal to the jejunum, which needed the application of fluoroscopy (36). The tolerance of double-lumen catheter is poor, and in fact, the duodenum part is needless. The modified nasojejunal tube can be blindly placed into the distal jejunum in our center. It is non-invasive and simple, and the operating time of tube just need 2–5 min. The HIF should be continuously and repeatedly drained, and this preparation may be more optimized in the future.

It is certain that fecal therapy, no matter FMT or HIFT, will continue to be refined in methodologies and treatment concepts. According to the characteristics of microbiota distribution and functions of life active substances, HIFT may compensate the shortcomings of FMT. Thus, the whole intestinal microbiota transplantation can consist of FMT and HIFT, which may have greater impact on diseases. Although challenges exist, we will further analyze the components of HIF and conduct high-quality clinical studies to validate the safety and efficacy of HIFT combined with FMT.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Shanghai Tenth People's Hospital Ethics Committee. Written informed consent to participate in this study was provided by the participants or their legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

CQ, LN, and QH made an investigation plan. CQ and YC prepared the draft of the manuscript. YC, YY, LX, and MC carried on the research work and provided data. YB, ZD, LZ, TH, and CJ manage donors and recipients. ZS, LZ, and CJ proofread and revised the manuscript. All authors agree to be accountable for the content of the work. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Clinical Research Plan of SHDC (Nos. SHDC2020CR1030B and SHDC2020CR4026) and Shanghai Action Plan on Science, Technology and Innovation (21Y11908300).

ACKNOWLEDGMENTS

The authors are grateful to Dr. Zhou Mingfei for his help with the preparation in the recruit.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.830004/full#supplementary-material

REFERENCES

1. Kelly CR, Kahn S, Kashyap P, Laine L, Rubin D, Atreja A, et al. Update on fecal microbiota transplantation 2015: indications, methodologies, mechanisms, and outlook. *Gastroenterology*. (2015) 149:223–37. doi: 10.1053/j.gastro.2015.05.008
2. El-Salhy M, Hatlebakk JG, Gilja OH, Bråthen Kristoffersen A, Hausken T. Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. *Gut*. (2020) 69:859–67. doi: 10.1136/gutjnl-2019-319630
3. Costello SP, Hughes PA, Waters O, Bryant RV, Vincent AD, Blatchford P, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. *JAMA*. (2019) 321:156–64. doi: 10.1001/jama.2018.20046
4. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical practice guidelines for Clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis*. (2018) 66:e1–e48. doi: 10.1093/cid/cix1085
5. Nishiwaki H, Ito M, Ishida T, Hamaguchi T, Maeda T, Kashihara K, et al. Meta-analysis of gut dysbiosis in Parkinson's disease. *Mov Disord*. (2020) 35:1626–35. doi: 10.1002/mds.28119
6. Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome*. (2017) 5:10. doi: 10.1186/s40168-016-0225-7
7. Mandrioli J, Amedei A, Cammarota G, Niccolai E, Zucchi E, D'Amico R, et al. FETR-ALS study protocol: a randomized clinical trial of fecal microbiota transplantation in amyotrophic lateral sclerosis. *Front Neurol*. (2019) 10:1021. doi: 10.3389/fneur.2019.01021
8. Qiyi C, Bo Y, Hongliang T, Ma C, Ding C, Ge X, et al. A five-year follow-up analysis of efficacy and complications of 3 932 cases of fecal microbiota transplantation treatment. *Chin J Dig*. (2020) 2020:768–777. doi: 10.3760/cma.j.cn311367-20200706-00432
9. Kalantzi L, Goumas K, Kalioras V, Abrahamsson B, Dressman JB, Reppas C. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharm Res*. (2006) 23:165–76. doi: 10.1007/s11095-005-8476-1
10. Fuchs A, Dressman JB. Composition and physicochemical properties of fasted-state human duodenal and jejunal fluid: a critical evaluation of the available data. *J Pharm Sci*. (2014) 103:3398–411. doi: 10.1002/jps.24183
11. Kalantzi L, Persson E, Polentarutti B, Abrahamsson B, Goumas K, Dressman JB, et al. Canine intestinal contents vs. simulated media for the assessment of solubility of two weak bases in the human small intestinal contents. *Pharm Res*. (2006) 23:1373–81. doi: 10.1007/s11095-006-0207-8
12. Li N, Zuo B, Huang S, Zeng B, Han D, Li T, et al. Spatial heterogeneity of bacterial colonization across different gut segments following inter-species microbiota transplantation. *Microbiome*. (2020) 8:161. doi: 10.1186/s40168-020-00917-7
13. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, et al. Molecular analysis of the bacterial microbiota in the human stomach. *Proc Natl Acad Sci USA*. (2006) 103:732–7. doi: 10.1073/pnas.0506655103
14. Delgado S, Cabrera-Rubio R, Mira A, Suárez A, Mayo B. Microbiological survey of the human gastric ecosystem using culturing and pyrosequencing methods. *Microb Ecol*. (2013) 65:763–72. doi: 10.1007/s00248-013-0192-5
15. Ye X, Peng N, Jiang F, Ni Y, Tong Z, Li W, et al. Subsegmental bowel enteral nutrition infusion and succus entericus reinfusion in patients with severe acute pancreatitis complicated with multiple enteric fistulae: a successful experience. *Am Surg*. (2013) 79:E169–71. doi: 10.1177/000313481307900415
16. Davidovics ZH, Vance K, Etienne N, Hyams JS. Fecal transplantation successfully treats recurrent D-lactic acidosis in a child with short bowel syndrome. *JPEN J Parenter Enter Nutr*. (2017) 41:896–7. doi: 10.1177/0148607115619931
17. Choi HH, Cho YS. Fecal microbiota transplantation: current applications, effectiveness, and future perspectives. *Clin Endosc*. (2016) 49:257–65. doi: 10.5946/ce.2015.117
18. Wang JW, Kuo CH, Kuo FC, Wang YK, Hsu WH, Yu FJ, et al. Fecal microbiota transplantation: review and update. *J Formos Med Assoc*. (2019) 118(Suppl. 1):S23–31. doi: 10.1016/j.jfma.2018.08.011
19. Tang G, Yin W, Liu W. Is frozen fecal microbiota transplantation as effective as fresh fecal microbiota transplantation in patients with recurrent or refractory Clostridium difficile infection: A meta-analysis? Diagn. *Microbiol Infect Dis*. (2017) 88:322–9. doi: 10.1016/j.diagmicrobio.2017.05.007
20. Parenteral and Enteral Nutrition Branch of Chinese Medical Association, Enhanced Recovery After Surgery Branch of China International Health Care Promotion, Exchange Association, and China Microecological Treatment Innovation Alliance. Association MCoSPM. Chinese experts consensus on standardized methodology and clinical application of fecal microbiota transplantation. *Chin J Gastrointest Surg*. (2020) 23:5–13. doi: 10.3760/cma.j.cn.441530-20200420-00231
21. Wise J. Frozen faecal matter works as well as fresh for transplantation in C difficile patients. *BMJ*. (2016) 352:i138. doi: 10.1136/bmj.i138
22. Woodworth MH, Neish EM, Miller NS, Dhere T, Burd EM, Carpentieri C, et al. Laboratory testing of donors and stool samples for fecal microbiota transplantation for recurrent *Clostridium difficile* infection. *J Clin Microbiol*. (2017) 55:1002–10. doi: 10.1128/JCM.02327-16
23. Pomares Bascuñana RÁ, Veses V, Sheth CC. Effectiveness of fecal microbiota transplant for the treatment of Clostridioides difficile diarrhea: a systematic review and meta-analysis. *Lett Appl Microbiol*. (2021) 73:149–58. doi: 10.1111/lam.13486
24. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N Engl J Med*. (2019) 381:2043–50. doi: 10.1056/NEJMoa1910437
25. Alexandre A, Alex LM, Miguel B, et al. A new genomic blueprint of the human gut microbiota. *Nature*. (2019) 568:499–504. doi: 10.1038/s41586-019-0965-1

26. Picot D, Layec S, Dussaulx L, Trivin F, Thibault R. Chyme reinfusion in patients with intestinal failure due to temporary double enterostomy: a 15-year prospective cohort in a referral centre. *Clin Nutr.* (2017) 36:593–600. doi: 10.1016/j.clnu.2016.04.020
27. Niu DG, Yang F, Tian WL, Huang Q, Zhao YZ, Li C, et al. Method for establishing continuous reinfusion of succus entericus in complex high-output fistula. *Surg Laparosc Endosc Percutan Tech.* (2020) 30:227–32. doi: 10.1097/SLE.0000000000000757
28. Wittekamp BHJ, Oostdijk EAN, Cuthbertson BH, Brun-Buisson C, Bonten MJM. Selective decontamination of the digestive tract (SDD) in critically ill patients: a narrative review. *Intensive Care Med.* (2020) 46:343–9. doi: 10.1007/s00134-019-05883-9
29. Riethorst D, Mols R, Duchateau G, Tack J, Brouwers J, Augustijns P. Characterization of human duodenal fluids in fasted and fed state conditions. *J Pharm Sci.* (2016) 105:673–81. doi: 10.1002/jps.24603
30. Camp JG, Kanther M, Semova I, Rawls JF. Patterns and scales in gastrointestinal microbial ecology. *Gastroenterology.* (2009) 136:1989–2002. doi: 10.1053/j.gastro.2009.02.075
31. Gibson PR, Barrett JS. The concept of small intestinal bacterial overgrowth in relation to functional gastrointestinal disorders. *Nutrition.* (2010) 26:1038–43. doi: 10.1016/j.nut.2010.01.005
32. Leite G, Morales W, Weitsman S, Celly S, Parodi G, Mathur R, et al. The duodenal microbiome is altered in small intestinal bacterial overgrowth. *PLoS ONE.* (2020) 15:e0234906. doi: 10.1371/journal.pone.0234906
33. Ding Z, Wang W, Zhang K, Ming F, Yangdai T, Xu T, et al. Novel scheme for non-invasive gut bioinformation acquisition with a magnetically controlled sampling capsule endoscope. *Gut*. (2021) 70:2297–306. doi: 10.1136/gutjnl-2020-322465
34. Riethorst D, Mitra A, Kesisoglou F, Xu W, Tack J, Brouwers J, et al. Human intestinal fluid layer separation: the effect on colloidal structures and solubility of lipophilic compounds. *Eur J Pharm Biopharm.* (2018) 129:104–10. doi: 10.1016/j.ejpb.2018.05.026
35. Tziatzios G, Gkolfakis P, Papanikolaou IS, Mathur R, Pimentel M, Damoraki G, et al. High prevalence of small intestinal bacterial overgrowth among functional dyspepsia patients. *Dig Dis.* (2021) 39:382–90. doi: 10.1159/000511944
36. de la Cruz-Moreno MP, Montejo C, Aguilar-Ros A, Dewe W, Beck B, Stappaerts J, et al. Exploring drug solubility in fasted human intestinal fluid aspirates: impact of inter-individual variability, sampling site and dilution. *Int J Pharm.* (2017) 528:471–84. doi: 10.1016/j.ijpharm.2017.05.072
37. Amoako-Tuffour Y, Jones ML, Shalabi N, Labbe A, Vengallatore S, Prakash S. Ingestible gastrointestinal sampling devices: state-of-the-art and future directions. *Crit Rev Biomed Eng.* (2014) 42:1–15. doi: 10.1615/CritRevBiomedEng.2014010846

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

SYSTEMATIC REVIEW
published: 26 May 2022
doi: 10.3389/fmed.2022.773105

Features of Gut Microbiome Associated With Responses to Fecal Microbiota Transplantation for Inflammatory Bowel Disease: A Systematic Review

Jindong Zhang[1†], *Yangyang Guo*[2†] *and Liping Duan*[1*]

[1] *Department of Gastroenterology, Peking University Third Hospital, Beijing, China,* [2] *Department of Critical Care Medicine, Beijing Tiantan Hospital, Capital Medical University, Beijing, China*

OPEN ACCESS

Edited by:
Angel Lanas,
University of Zaragoza, Spain

Reviewed by:
Longxian Lv,
Zhejiang University, China
Faming Zhang,
Nanjing Medical University, China
Jingyuan Fu,
University Medical Center Groningen, Netherlands

***Correspondence:**
Liping Duan
duanlp@bjmu.edu.cn

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *29 September 2021*
Accepted: *19 April 2022*
Published: *26 May 2022*

Citation:
Zhang J, Guo Y and Duan L (2022) Features of Gut Microbiome Associated With Responses to Fecal Microbiota Transplantation for Inflammatory Bowel Disease: A Systematic Review. Front. Med. 9:773105. doi: 10.3389/fmed.2022.773105

Fecal microbiota transplantation (FMT) has been seen as a novel treatment for inflammatory bowel disease (IBD). The results on microbial alterations and their relationship to treatment efficacy are varied among studies. We performed a systematic review to explore the association between microbial features and therapy outcomes. We searched PubMed, Web of Science, Embase, and Cochrane Library databases from inception to November 2020. Studies that investigated the efficacy of FMT and baseline microbial features or dynamic alteration of the microbiome during FMT were included. The methodological quality of the included cohort studies and randomized controlled trials (RCTs) was assessed using the Newcastle–Ottawa Scale (NOS) and the Cochrane risk of bias tool, respectively. A total of 30 studies were included in the analysis. Compared to non-responders, the microbial structure of patients who responded to FMT had a higher similarity to that of their donors after FMT. Donors of responders (R-d) and non-responders (NR-d) had different microbial taxa, but the results were inconsistent. After FMT, several beneficial short-chain fatty acids- (SCFA-) producing taxa, such as *Faecalibacterium*, *Eubacterium*, *Roseburia*, and species belonging to them, were enriched in responders, while pathogenic bacteria (*Escherichia coli* and *Escherichia-Shigella*) belonging to the phylum Proteobacteria were decreased. Alterations of microbial functional genes and metabolites were also observed. In conclusion, the response to FMT was associated with the gut microbiota and their metabolites. The pre-FMT microbial features of recipients, the comparison of pre- and post-FMT microbiota, and the relationship between recipients and donors at baseline should be further investigated using uniform and standardized methods.

Keywords: gut microbiome, microbial metabolites, fecal microbiota transplantation, response, inflammatory bowel disease

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic and relapsing intestinal disorder that is typically categorized into two subtypes, including ulcerative colitis (UC) and Crohn's disease (CD), and has become a global disease in the 21st century (1). Although the pathophysiological mechanisms of IBD remain unclear, increasing evidence suggests that the disease is caused by the interaction between complex genetic, environmental, and microbial factors, thereby triggering immune-mediated intestinal inflammation (2).

Previous studies have reported the alteration in gut microbiota composition (known as dysbiosis) in patients with IBD, which is characterized by the depletion of *Roseburia hominis*, *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Eubacterium rectale*, and enrichment of *Escherichia coli* (3, 4). Furthermore, patients with IBD exhibit a dramatic alteration in their gut microbiota-derived metabolite profiles compared to the healthy population (5). Based on these findings, therapeutic methods targeting microbiota or their metabolites, such as dietary optimization, probiotics, antibiotics, and fecal microbiota transplantation (FMT), have been applied in clinical practice (6, 7).

Fecal microbiota transplantation has already been recommended to treat recurrent *Clostridium difficile* infection (8). This provides supporting evidence for FMT as a potential treatment method for other intestinal diseases such as IBD. In recent years, there have been increasing studies of the efficacy of FMT for IBD treatment (9), but the clinical outcome is inconsistent among recipients, and the factors affecting its treatment response have been poorly investigated.

With the rapid development of microbiome sequencing technology, more and more researchers have focused on the use of microbiome as a predictive biomarker of clinical outcome and treatment response of FMT (10, 11). Thus, we conducted this systematic review to summarize the current findings on the relationship between microbiota and treatment response of FMT in patients with IBD.

MATERIALS AND METHODS

Search Strategy

A systematic search was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (12). We searched four databases: PubMed, Web of Science, Embase, and Cochrane Library from inception to 2 November 2020. The search terms covering expressions for fecal, microbiota, transplant, and IBD are listed in the **Supplementary Materials**.

Study Selection

Studies were included if they investigated the efficacy of FMT and baseline microbial features or dynamic alteration of the microbiome during FMT in both pediatric and adult patients with IBD.

Studies were excluded if they were: (1) reviews, guidelines, or comments, (2) animal studies, (3) studies that did not involve microbial data, and (4) studies that did not assess treatment response.

Data Extraction

After excluding studies whose title and abstract clearly did not meet our inclusion criteria, the full text of the remaining studies was reviewed to determine eligibility. The following information was extracted from eligible studies: authors' names, years of publication, country of origin, patient demographics, IBD types and disease activity, donor characteristics, FMT procedure, clinical outcome or treatment response of FMT, and microbial data.

Quality Assessment

The Newcastle–Ottawa scale (NOS) containing three criteria (selection, comparability, and exposure) was used to assess the quality of the included cohort studies, following the standard 9-point scale, and randomized controlled trials (RCTs) were assessed using the Cochrane risk of bias tool, which incorporate the evaluation of selection, performance, detection, attrition, and reporting bias (13).

RESULTS

Study Selection

After initial research, a total of 9,307 records were identified, which were reduced to 5,975 after the removal of internal and external duplicates. Titles and abstracts of 5,975 records were screened, 84 of which were retained for full-text review. Overall, a total of 30 articles or abstracts satisfied the inclusion criteria for this systematic review (**Figure 1**). The results of the quality assessment for cohort studies and RCTs are presented in **Supplementary Tables S1**, **S2**. The quality scores of the 20 studies ranged from 5 to 9 (moderate to high quality). The risk of bias was high in Sokol et al. and Kong et al. because their trials were single-blind, while the remaining studies were at low risk.

Study Characteristics

The characteristics of the included studies are presented in **Supplementary Table S3**. Eligible studies included two case reports (14, 15), one case series (16), 20 prospective cohort studies (17, 18), and seven RCTs, of which 29 studies reported on 978 patients, except for one study with no reported patient numbers. A total of 20 studies recruited only patients with UC, six studies recruited only patients with CD, and four studies recruited both the conditions.

Protocols of FMT

The scope of donor selection and donor stool preparation varied between studies (**Table 1**). Six studies used pooled donor stool (2–7 donors) to increase microbial diversity while the remaining ones used stool from a single donor. The ratios of stool weight to vehicle volume used for preparation ranged from 1:0.75 to 1:10, and the final volumes of fecal suspension for FMT were 100–500 ml per treatment. Particularly, the studies by Li et al. (11) and Zhang et al. (35) used washed microbiota transplantation (41). Antibiotic pretreatment was used in six studies (22, 26). The

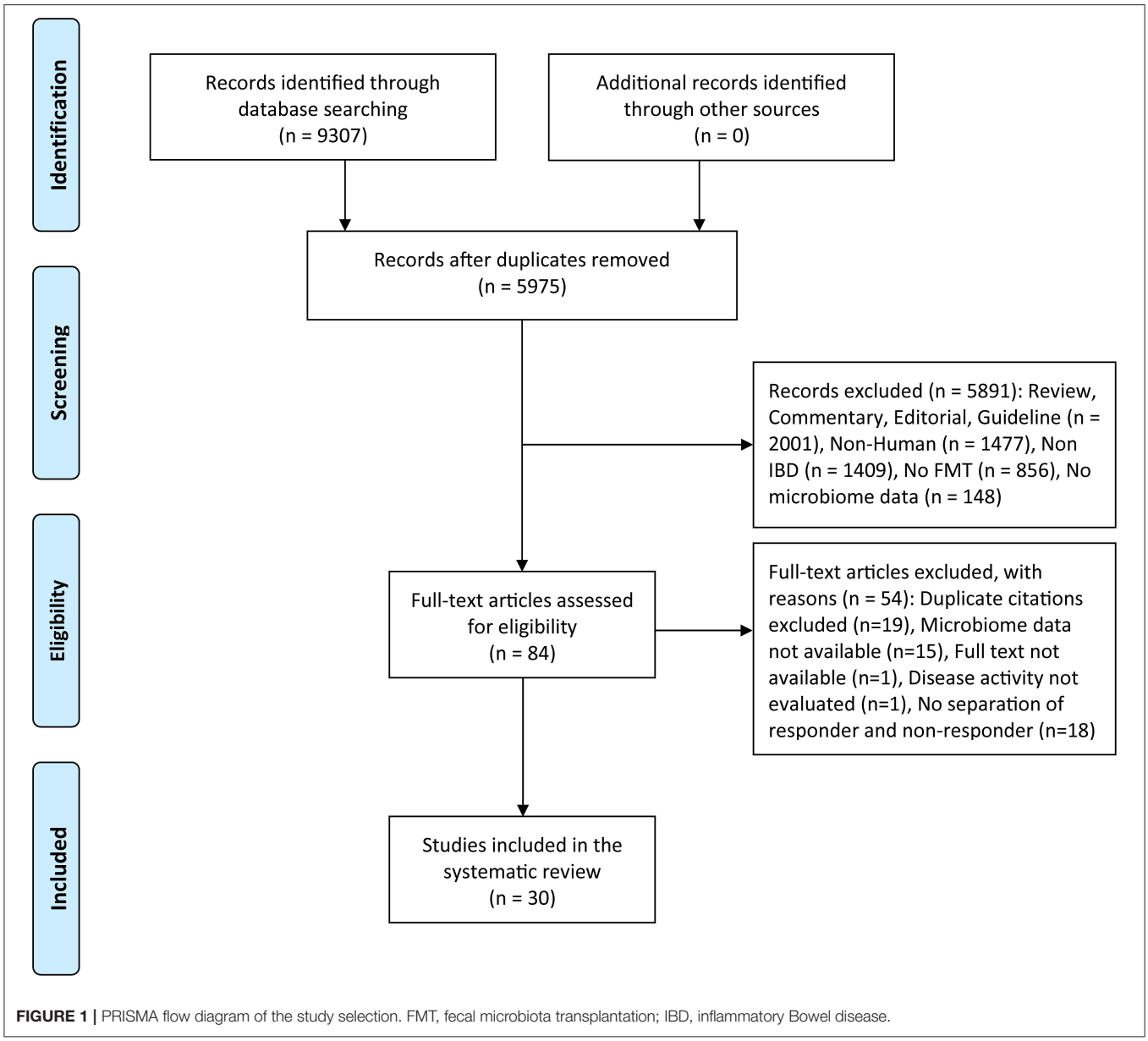


FIGURE 1 | PRISMA flow diagram of the study selection. FMT, fecal microbiota transplantation; IBD, inflammatory Bowel disease.

colonoscope was the most adopted route by researchers, and the infusion sites included the cecum, terminal ileum, and colon. The frequency of FMT varied between studies.

Clinical Outcomes

Clinical outcomes, including clinical response, clinical remission, and endoscopic remission, are shown in **Supplementary Table S4**. Follow-up after FMT varied between 1 and 35 months, and the most commonly used endpoint was 12 weeks. In cohort and RCT studies, 18 studies reported the clinical response rate of patients with UC ranging from 20 to 100%, and the clinical response rate of patients with CD reported in seven studies varied between 20 and 75%. The clinical remission rate of patients with UC and CD ranged from 0 to 71.4% and from 10 to 87.5%, respectively. Eight studies reported the endoscopic remission of patients with UC, ranging from 0 to 50%, while only one study on CD reported that no patients achieved endoscopic remission.

Microbial Sequencing Results

Differences in sample collection and sequencing are listed in **Supplementary Table S5**. Two studies used both stool and mucosal biopsy specimen for sequencing, and the remaining studies used stool samples. 16S rRNA sequencing was the most adopted method, and other methods included polymerase chain reaction (PCR) and terminal restriction fragment-length polymorphism (T-RFLP) analysis, HITChip, and metagenomic shotgun sequencing. In the case of 16S rRNA sequencing, the 16S rRNA variable regions used for DNA amplification,

TABLE 1 | Summary of stool preparation and delivery methods.

References	Donor relationship	Donor stool	Fresh/ frozen	Stool preparation	Dosage per treatment	Pre-antibiotics	Pre-medication	FMT route	Region of infusion	Number of infusions
Kao et al. (14)	Unrelated	Single donor	Fresh	1:4 of stool: saline	400 ml	None	None	Colonoscopy	Cecum	1
Shimizu et al. (15)	Related (father)	Single donor	Fresh	Stool diluted in 250 ml saline	250–300 ml	None	None	Colonoscopy for the first time, then enema	Throughout the colon (colonoscopy)	16 (daily for first 5 days, then every 2–4 weeks over 10 months)
Quagliariello et al. (16)	Related (father)	Single donor	Fresh	Stool diluted in saline at ratio 50 g/200 ml	NR	None	None	Colonoscopy	Cecum or duodenum-jejunum	1
Angelberger et al. (17)	Unrelated	Single donor*	Fresh	60 g mixed with 250 ml saline	Median: Nasojejunal infusion 24 g; Enema: 20 g	Metronidazole 500 mg bid for 5–10 days	Probiotics[$], pantoprazole	Nasojejunal tube and enema	Jejunum	3 (daily for 3 consecutive days)
Suskind et al. (18)	Related (parent)	Single donor	Fresh	30 g mixed with 100–200 ml saline	30 g	Rifaximin 200 mg tid for 3 days	Omeprazole	Nasogastric tube	Stomach	1
Vaughn et al. (19)	Unrelated	Single donor	Frozen	50 g mixed with 250 ml saline	250 ml	None	None	Colonoscopy	Terminal ileum to colon	1
Vermeire et al. (20)	Related (sibling or parent), unrelated (friend)	Single donor	Fresh	200 g homogenized with 400 ml saline	400 ml	None	None	Nasojejunal tube or rectal tube	Jejunum; rectum	2
Jacob et al. (21)	Unrelated	Pooled (2 donors)	Frozen	60 ml from each donor pooled	120 ml	None	None	Colonoscopy	Ileum and right colon	1
Ishikawa et al. (22)	Related (spouses or relatives)	Single donor	Fresh	150–250 g diluted with 350–500 ml saline	350–500 ml	Amoxicillin (1,500 mg/d), fosfomycin (3,000 mg/d), metronidazole (750 mg/d) for 2 weeks	None	Colonoscopy	Cecum and ascending colon (2/3 of the volume), transverse colon (1/3 of the volume)	1
Nishida et al. (23)	Related (relatives within the second degree of relationship)	Single donor	Fresh	150–200 g dissolved in 500 ml saline	500 ml	None	None	Colonoscopy	Cecum	1
Goyal et al. (24)	Family members, first-degree relatives, or trusted friends	Single donor	Fresh	150 g stool blended using 250–300 ml saline	Duodenum or jejunum: 20–30 ml; ileum and colon: 200–250 ml	Metronidazole or vancomycin 10 mg/kg tid for 5 days	Omeprazole, loperamide	Colonoscopy	Distal duodenum or proximal jejunum; ileum and right colon	1
Karakan et al. (25)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

(Continued)

TABLE 1 | Continued

References	Donor relationship	Donor stool	Fresh/ frozen	Stool preparation	Dosage per treatment	Pre-antibiotics	Pre-medication	FMT route	Region of infusion	Number of infusions
Kump et al. (26)	Related or unrelated	Single donor	Fresh	50 g stool diluted with 200–500 ml saline	250–500 ml	Vancomycin 250 mg qid, paromomycin 250 mg tid, nystatin 10 ml qid for 10 days	None	Colonoscopy for the first time, then sigmoidoscopy	Terminal ileum and right colon (colonoscopy); left colon (sigmoidoscopy)	5 (in 14 days intervals)
Nusbaum et al. (27)	Family members or friends	Single donor	Fresh	Stool blended in saline	240 ml maximum	None	None	Retention enema	NR	5 (daily for 5 days)
Cold et al. (28)	Unrelated	Pooled (4 donor)	Frozen	Stool homogenized with 500 ml saline, then concentrated and encapsulated	∼12 g	None	None	Capsules	Oral administration	25 capsules daily for 50 days
Fan et al. (29)	Unrelated	Pooled (2–3 donor)	NR	NR	NR	NR	NR	Capsules	Oral administration	3 days per week
Gogokhia et al. (30)	Unrelated	Pooled (2 donors)	Frozen	60 ml from each donor pooled	120 ml	None	None	Colonoscopy	Ileum and right colon	1
Gutin et al. (31)	Unrelated	NR	Frozen	NR	250 ml	Rifaximin 550 mg tid for 5 days[#]	None	Colonoscopy	Terminal ileum or neoterminal ileum	1
Chen et al. (32)	Unrelated	Single donor	Frozen	150–200 g stool dissolved in 1,000 ml saline	150 ml (∼50 cm^3 microbiota)	None	None	Transendoscopic enteral tubing (TET)	Entire colon	3
Li et al. (11)	Relatives or friends or unrelated	Single donor	Fresh or frozen	Preparation by automatic microbiota purification system	NR	None	None	Gastroscopy, colonic TET, mid-gut TET	Stomach, Ileocecum, distal duodenum	1
Ohmiya et al. (33)	NR	NR	Fresh	NR	NR	NR	NR	Colonoscopy (UC); oral enteroscopy (CD)	NR	1
Schierová et al. (34)	Unrelated	Single donor	Frozen	50 g dissolved in 150 ml saline	150 ml	None	None	Enema	NR	10 (5 times in the first week, then once a week for 5 weeks)
Zhang et al. (35)	Unrelated	NR	Fresh or frozen	Preparation by automatic microbiota purification system	NR	None	None	NR	NR	NR
Rossen et al. (36) and Fuentes et al. (37)	Partners, relatives, or volunteers	Single donor	Fresh	Median 120 g stool diluted in 500 ml saline	500 ml	None	None	Nasoduodenal tube	Duodenum	2 times at a 3-week interval

(Continued)

TABLE 1 | Continued

References	Donor relationship	Donor stool	Fresh/ frozen	Stool preparation	Dosage per treatment	Pre-antibiotics	Pre-medication	FMT route	Region of infusion	Number of infusions
Costello et al. (38)	Unrelated	Pooled (3–4 donors)	Frozen	pooled stool (25%) blended with saline (65%) and glycerol (10%)	Colonoscopic delivery: 50 g/200 ml; enema: 25 g/100 ml	None	Loperamide	Coloscopy for the first time, then enema	Right colon (colonoscopy)	3 (coloscopy for the first time, then enema 2 times in the following 7 days)
Sokol et al. (39) and Kong et al. (40)	Unrelated	Single donor	Fresh	50–100 g stool resuspended in 250–350 ml saline	300 ml	None	None	Colonoscopy	NR	1

CD, Crohn's disease; NR: not recorded; UC, ulcerative colitis.

[*] One patient in this study received fecal microbiota transplantation (FMT) from two different donors.

[#] Three patients in this study used rifaximin while others did not.

[$] Saccharomyces boulardii or Omnibiotic 6.

the sequencing and data analysis platform, and the reference database varied between studies.

Microbial Difference Between FMT Donors of Responders and Non-responders

A total of 15 studies reported the relationship between donor gut microbiota and the clinical response (**Table 2**). Microbial structural similarities between pre-FMT recipients and their donors were lower in responders than in non-responders by Goyal et al. (24) and Cold et al. (28). For post-FMT samples, six studies (19, 24, 27, 29, 34, 36) reported a significant increase in similarity to corresponding donors in responders compared to non-responders, and a case report by Kao et al. (14) also showed that the fecal microbial composition of the patient and the donor closely resembled each other after FMT. Furthermore, Cold et al. (28) found that the microbial composition of responders became closer to their donor than the nonresponders did.

Several studies compared the microbiota between the donors of responders (R-d) and non-responders (NR-d). Three studies (20, 25, 26) reported higher richness in R-d than in NR-d, while the study by Goyal et al. (24) showed no significant difference in richness between R-d and NR-d. The microbial structure of R-d and NR-d was significantly different in the studies by Jacob et al. (21) and Kump et al. (26), but not in the study by Goyal et al. (24).

In terms of microbial taxa difference, the abundance of *A. muciniphila* and *Runimococcuus.* spp. was elevated in R-d compared to NR-d in two studies consistently (25, 26), and other enriched bacteria phyla or genera included Actinobacteria, unclassified *Ruminococcaceae* (26), *Bifidobacterium* (23), *F. prausnitzii* (25), *Bacteroides fragilis*, and *Bacteroides finegoldii* (10). In addition, the relative abundance of *Lactobacillales*, *Clostridium cluster IV*, C*lostridium cluster XI* (23), and *Clostridium XIVa* (10) were higher in the feces of the donors of non-responders than that of the donors of responders. Particularly, one study reported that terpenoid backbone biosynthesis pathways in the microbiota were enriched in R-d (10).

Microbial Difference Between FMT Responders and Non-responders

α-Diversity

The majority of the included studies compared gut microbial diversity and composition between FMT responders and non-responders, by assessing α-diversity and bacterial abundance. Details of these findings are listed in **Table 3**. As for the α-diversity of pre-FMT samples, the results were discrepant in three studies, presenting higher diversity expressed by the number of OTUs and Shannon index (10), lower diversity reflected by observed OTUs (24) (difference not significant), or no difference (34) in the responders. In three of the seven studies comparing the α-diversity of post-FMT in responders to non-responders, the increasing degree in diversity was significantly greater for responders vs. non-responders (19, 24, 27), two studies showed increased values of α-diversity for responders than for non-responders (10, 36), and only one study reported no difference between responders and non-responders (23).

TABLE 2 | Association between donor microbiota and the response to FMT.

References	Microbial similarity between pre-FMT patients and donors	Microbial similarity between post-FMT patients and donors	Comparison between R-d and NR-d
Kao et al. (14)		↑	
Suskind et al. (18)		Not sure	
Vaughn et al. (19)		R > NR	
Vermeire et al. (20)			Richness: R-d > NR-d
Jacob et al. (21)			Significant difference of structure between R-d and NR-d
Nishida et al. (23)			*Bifidobacterium*: R-d > NR-d; *Lactobacillales*, *Clostridium cluster IV*, and *Clostridium cluster XI*: R-d < NR-d
Goyal et al. (24)	R < NR (difference not significant)	R > NR	α-diversity: R-d = NR-d; β- diversity: R-d = NR-d
Karakan et al. (25)			Richness: R-d > NR-d; *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Ruminococcus*: R-d > NR-d
Kump et al. (26)			Richness and diversity: RE-d > NR-d; significant difference of structure between RE-d and NR-d; Actinobacteria, unclassified *Ruminococcaceae*, an unclassified *Ruminococcus* and *Akkermansia muciniphila*: RE-d > NR-d
Nusbaum et al. (27)		R > NR	
Cold et al. (28)	R < NR	ΔR > ΔNR	
Fan et al. (29)		R > NR	
Schierová et al. (34)		R > NR	
Rossen et al. (36)		R > NR	

FMT, fecal microbiota transplantation; R, responders; NR, non-responders; R-d, donors of responders; NR-d, donors of non-responders; RE, remission.
Δ, alteration degree.

TABLE 3 | Comparison of α-diversity between responders and non-responders.

References	Pre-FMT	Post-FMT	α-diversity index
Vaughn et al. (19)		ΔR > ΔNR	Shannon
Nishida et al. (23)		R = NR; R-d = NR-d	Shannon
Goyal et al. (24)	R < NR (difference not significant)	ΔR > ΔNR	Observed OTUs
Nusbaum et al. (27)		ΔR > ΔNR	Species richness, Shannon, Inverse Simpson
Rossen et al. (36)		R ↑; NR no change	Shannon
Paramsothy et al. (10)	R > NR	R > NR	Number of OTUs, Shannon

FMT, fecal microbiota transplantation; R, responders; NR, non-responders.
Δ, alteration degree.

Baseline Microbiome Composition

Two of the included studies analyzed the association between response and baseline microbiome composition. The study performed by Goyal et al. (24) demonstrated that responders contained a higher relative abundance of *Fusobacterium* than non-responders at baseline, and Gutin et al. (31) observed that the baseline microbiome of responders had higher counts of *Enterobacteriaceae* and *Bifidobacterium* members, whereas non-responders had greater abundance of *Lachnospiraceae* and *Ruminococcaceae* members.

Differences in Microbiome Composition Between Responders and Non-responders

A number of differences were observed between responders and non-responders after FMT (**Table 4**). Several bacteria showed a relatively consistent trend in separate studies, in which the increased microorganisms included the phyla Bacteroidetes (22, 36), the family *Lachnospiraceae* (14, 27, 30, 31, 34), and the genera *Collinsella* (33, 34), *Bacteroides* (14, 15), *Blautia* (14, 34), *Faecalibacterium* (14, 15, 33, 34), *Eubacterium* (11, 15), *Clostridium clusters IV* (36, 42), *Roseburia* (14, 20, 27), and *Ruminococcus* (11, 30, 42). In contrast, the relative abundance of the genera *Enterococcus* (14, 37), *Lactobacillus* (14, 34), *Veillonella* (10, 37), and *Sutterella* (14, 42) was reported to decrease in responders. For the species level, responders had an increased abundance of the species *Ruminococcus bromii* (10, 16), *Eubacterium hallii* (10, 37), *Eubacterium ventriosum* (19, 37), and *F. prausnitzii* (17, 27, 32), and reduced abundance of species *Bacteroides vulgatus* (19, 37), *E. coli* (18, 30, 37), *Escherichia-Shigella* (29, 30), and *Sutterella wadsworthensis* (10, 37). A few of bacteria showed an opposite changing trend in their abundance, including the family *Ruminococcaceae* (33, 34) and

TABLE 4 | Microbial difference between responders and non-responders after FMT.

	Studies																					Total	
Microbial taxa	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**22**	**26**	**27**	**29**	**30**	**31**	**32**	**11**	**33**	**34**	**36**	**37**	**42**	**10**	↑	↓
Actinobacteria																							
Collinsella																↑(CD)	↑					2	0
Bacteroidetes								↑										↑				2	0
Bacteroides	↑	↑																				2	0
Bacteroides ovatus			↑	↑		↓													↓			2	2
Bacteroides vulgatus						↓													↓			0	2
Firmicutes																							
Lachnospiraceae	↑									↑		↑	↑			↓(UC)	↑					5	1
Ruminococcaceae																↓(UC)	↑					1	1
Christensenellaceae												↑					↓					1	1
Blautia	↑																↑					2	0
Faecalibacterium	↑	↑														↑(UC)	↑					4	0
Eubacterium		↑													↑							2	0
Clostridium clusters IV																		↑		↑		2	0
Clostridium clusters XIVa																		↑	↑		↓	2	1
Roseburia	↑						↑(UC)			↑												3	0
Enterococcus	↓																		↓			0	2
Lactobacillus	↓																↓					0	2
Ruminococcus												↑			↑					↑		3	0
Veillonella																			↓		↓	0	2
Dialister							↑(CD)		↓												↓	1	2
Ruminococcus bromii			↑																		↑	2	0
Ruminococcus gnavus			↓																↑			1	1
Eubacterium hallii																			↑		↑	2	0
Eubacterium ventriosum						↑													↑			2	0
Faecalibacterium prausnitzii				↑						↑				↑								3	0
Proteobacteria																							
Sutterella	↓																			↓		0	2
Escherichia		↑																			↓	1	1
Escheria coli					↓							↓							↓			0	3
Escherichia-Shigella											↓	↓										0	2
Sutterella wadsworthensis																			↓		↓	0	2

Only taxa reported by at least two separate studies are displayed.
CD, Crohn's disease; FMT, fecal microbiota transplantation; UC, ulcerative colitis.
↑, higher abundance in responders compared with non-responders; ↓, lower abundance in responders compared with non-responders.

TABLE 5 | Correlation between microbiota and clinical phenotypes.

References	Microbial taxa	Clinical phenotypes	Correlation
Angelberger et al. (17)	*Enterobacteriaceae*	Mayo score	+
Suskind et al. (18)	*E. coli*	Calprotectin and disease activity	+
Ishikawa et al. (22)	Bacteroidetes	Endoscopic sum score	−
Cold et al. (28)	An OTU belonging to *Faecalibacterium prausnitzii*	SCCAI	+
	α-diversity	F-calprotectin levels	−
Li et al. (11)	The differences of the relative abundance in genera *Eggerthella*, *Lactobacillus*, and *Ruminococcus* between pre-FMT and 5 days post-FMT	Clinical efficacy	+
Costello et al. (38)	*Anaerofilum pentosovorans*, *Bacteroides coprophilus*	Disease improvement	+
Sokol et al. (39)	Taxa belonging to Gammaproteobacteria and Clostridiales comprising *Ruminococcus gnavus*	Flare	+
	Ruminococcaceae, *Coprococcus*, *Desulfovibrio*	Maintenance of remission	+
Kong et al. (40)	Engraftment of Proteobacteria and Bacteroidetes	Relapse	+

FMT, fecal microbiota transplantation; SCCAI, Simple Clinical Colitis Activity Index. +, positive; –, negative.

Christensenellaceae (30, 34), the genus *Escherichia* (10, 15), and the species *Bacteroides ovatus* (16, 17, 19, 37) and *Ruminococcus gnavus* (16, 37).

Association Between Individual Bacteria and Clinical Phenotypes

A few studies assessed correlations between gut microbiota and clinical outcomes or disease biomarkers (**Table 5**). *Enterobacteriaceae* (17), *E. coli* (18), an OTU belonging to *F. prausnitzii* (28), taxa belonging to the class Gammaproteobacteria and the order Clostridiales comprising *Ruminococcus gnavus* (39), and engraftment of Proteobacteria and Bacteroidetes (40) were found to be correlated with higher disease severity or relapse in separate studies. In contrast, two studies showed a negative correlation between endoscopic sum score and Bacteroidetes (22), and F-calprotectin levels and α-diversity (28), respectively. Furthermore, three other studies found that certain bacteria benefited the clinical outcome. Li et al. (11) demonstrated that the differences of abundance in *Eggerthella*, *Lactobacillus*, and *Ruminococcus* between pre- and post-FMT were positively correlated with efficacy. In the trial by Costello et al. (38), increased abundance of *Anaerofilum pentosovorans* and *Bacteroides coprophilus* was strongly associated with disease improvement following FMT. In addition, *Ruminococcaceae*, *Coprococcus*, and *Desulfovibrio* were associated with the maintenance of remission after FMT (39).

TABLE 6 | Alterations of microbial gene pathways or metabolites.

References	Alterations of microbial gene pathways or metabolites
Vaughn et al. (19)	↑ in R: Pathways related to energy metabolism or components needed for bacterial cell surface or cell walls (serine and glutamine metabolic pathways, folic acid metabolic pathways, and lipid A biosynthetic pathways)
Nusbaum et al. (27)	Metabolomic profile of R shifts to donors after FMT; ↑ in R: Xanthine, oleic acid, butyric acid; ↓ in R: Putrescine, 5-aminovaleric acid, acetic acid
Fan et al. (29)	↑ in R: taurochenodeoxycholate and taurocholate
Ohmiya et al. (33)	↑ in R of CD: butyrate and secondary bile acids
Paramsothy et al. (10)	↑ in NR: heme biosynthesis, lipopolysaccharide/lipid A biosynthesis, peptidoglycan biosynthesis, ubiquinone and other terpenoid quinine biosynthesis, lysine biosynthesis, and oxidative phosphorylation pathways; ↑ in NR: heme, lysine; ↓ in NR: biotin, dehydrolithocholate
Costello et al. (38)	Stool SCFA concentrations were not associated with treatment effect
Kong et al. (40)	Relapsers had a depletion in community potential for anaerobic, energy metabolism, the NAD biosynthesis and transfer RNA charging pathways

CD, Crohn's disease; FMT, fecal microbiota transplantation; R, responders; NR, non-responders; SCFA, short chain fatty acids.

Differences in Bacterial Metabolic Pathways or Metabolites

Detailed findings of bacterial metabolic pathways or metabolites are provided in **Table 6**. Pathways related to increased energy metabolism or components needed for bacterial cell surface or cell walls were increased in responders after FMT compared to non-responders (19), while pathways related to the biosynthesis of Heme, lipopolysaccharide/lipid A, peptidoglycan, ubiquinone and lysine, and oxidative phosphorylation were increased in non-responders (10). Moreover, a study performed by Kong et al. revealed that relapsers after FMT have a depletion in community potential for anaerobic, energy metabolism, NAD biosynthesis, and transfer RNA charging pathways (40). Regarding bacterial metabolites, the metabolomic profile of responders shifts to donors after FMT in the study of Nusbaum et al. and, in particular, fecal butyrate acid increased in responders, which is consistent with the finding by the study of Ohmiya et al. (33). However, fecal butyrate acid and other short-chain fatty acids (SCFA) concentrations were not associated with treatment effect in another study (38).

DISCUSSION

Gut dysbiosis has drawn increasing attention for its role in the pathogenesis of IBD. Numerous studies have described the gut microbial features in patients with IBD (3), thus promoting the development of microbiota-targeted therapeutic methods, such as FMT. Given the heterogeneity of clinical outcomes in individual patients with IBD after receiving FMT, a better understanding of the factors that influence the response to FMT will help to optimize the treatment strategy. In this systematic review, we focused on the microbial distinction between FMT responders and non-responders, and the results showed several convergent findings.

First of all, the delivery route is a significant factor that influences treatment efficacy. The most used route was the colonoscope, while other routes included capsules, nasoduodenal tube, nasojejunal tube, transendoscopic enteral tubing (TET), and retention enema. Previous systemic review and meta-analysis have reported that the remission rate of patients with UC receiving FMT through lower gastrointestinal (GI) administration was much higher than that of upper GI administration (43, 44). It seems that the lower GI route has a trend of superiority over the upper GI route for the treatment of IBD, and this needs to be investigated in further research.

The numbers of infusions and follow-up duration also differed among the studies. There is no uniform conclusion on the lasting time of FMT effect. Li et al. (45) reported that the median time of maintaining clinical response to FMT in 69 patients with CD was 125 days in the first place. Among the 56 patients who received the second FMT, the time of maintaining clinical response was 176.5 days. Their data demonstrated that patients with CD should be given the second course of FMT within 4 months after the first FMT to maintain the clinical benefits of the first FMT.

Stool is a non-standardized material with heterogeneous microbial composition between individual donors, thus the donor stool is a key determinant for a successful FMT. Six of the included studies applied multi-donor stool preparation to increase microbial diversity and the possibility of recipients receiving therapeutically effective donor stool. When analyzing microbial features, we found that the structural difference between responders and donors was larger than that between non-responders and donors. However, responders had a higher increasing degree of microbial similarity to donors than non-responders. This perhaps means that the higher abundance of certain bacterial species in donors is conducive to FMT treatment. By comparing the microbial composition of R-d and NR-d, we observed that R-d had a higher richness and different microbial structure from donors of non-responders in most studies. This further supports the view that successful FMT was highly donor-dependent, and suggests us the necessity to incorporate the analysis of microbiota into the screening of donor stools in the future.

Microbial diversity is a crucial indicator of community stability and function. Decreased diversity was observed in many diseases compared to healthy controls, including IBD (3). In this review, we found that most of the studies reported higher diversity or a greater degree of increased diversity in responders than in non-responders, thus it can be speculated that the effective treatment may be a result of the restoration of microbial homeostasis.

The acquirement of baseline microbial features of patients is needed to predict the FMT outcome. However, only two studies compared the microbiome composition between responders and non-responders. Intriguingly, in addition to the probiotic genera *Bifidobacterium, Fusobacterium*, and *Enterobacteriaceae*, the two potentially pathogenic microorganisms, were also higher in abundance in responders' pre-FMT microbiome. *Fusobacterium* were capable of introducing host inflammatory or tumorigenic responses, predominantly by its unique FadA adhesin (46), and the family *Enterobacteriaceae* was associated with severe infectious diseases (47). These findings were consistent with the higher increasing degree of microbial similarity to donors in responders by Goyal et al. (24), and whether a bigger gap between responders and their donors might result in more effective treatment deserves further investigation.

Regarding the microbial alteration after FMT, we observed some common patterns. Responders presented an increase in relative abundances of SCFA-producing bacteria, such as the genera *Blautia*, *Faecalibacterium*, *Eubacterium, Roseburia*, and *Ruminococcus*, all of which were core genera in the healthy population worldwide (48). Among them, the important role of *Faecalibacterium* and *Roseburia* in IBD has been recognized in recent years. It has been generally shown that patients with IBD had a lower abundance of *F. prausnitzii* and, furthermore, active patients had a lower abundance of *F. prausnitzii* than patients in remission (49). In previous preclinical experiments, *F. prausnitzii* has been proven to efficiently alleviate intestinal inflammation, mainly by blocking nuclear factor kappa B (NF-κB) activation and pro-inflammatory cytokine production, and promoting anti-inflammatory IL-10 secretion (50). A recent study revealed that *F. prausnitzii*-derived butyrate exerted an anti-inflammatory effect by upregulating the expression of *Dact3*, a gene involved in the Wnt/JNK pathway (51). *Roseburia* is another butyrate-producing genus, and could also serve as a biomarker for IBD (4). In general, *Roseburia intestinalis* and *R. hominis* are the two most studied species associated with IBD. In our review, however, two studies reported an increase in the abundance of *Roseburia faecis* (17) and *Roseburia inulinivorans* (10) in responders, respectively. Apart from butyrate production, *Roseburia* could also affect the host by its flagellin (52). Furthermore, one study specially focused on the genus *Akkermansia* (35). The positive correlation between the abundance of *Akkermansia* in responders' and donors' demonstrated its successful colonization in the gut. Intriguingly, this study found a co-occurrence relationship between *Akkermansia* and *F. prausnitzii*. This suggests us that the combination of these next-generation probiotics could serve as a supplementary method of FMT, so as to increase the response rate.

The abundance of certain pathogenic bacteria belonging to the phylum Proteobacteria was decreased after FMT in responders. These bacteria included *E. coli* and *Escherichia-Shigella*. *E. coli* has proven to have an abnormal immune and proinflammatory response in IBD (53). In addition, *Enterococcus faecium* V583 could secrete proteases to induce epithelial cell permeability (54),

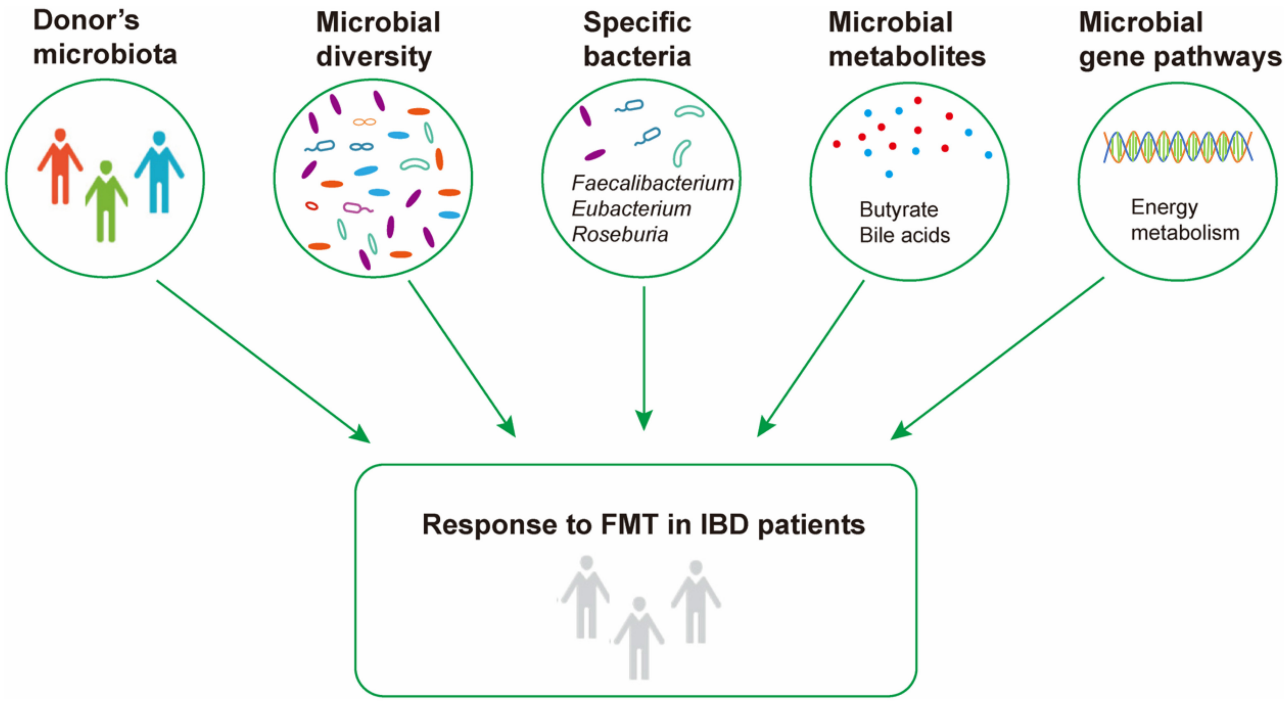


FIGURE 2 | Microbial factors influencing response to fecal microbiota transplantation in inflammatory bowel disease.

and promote intestinal cytokine expression. The elimination of these potential pathobionts may contribute to an effective response to FMT.

At the metabolic level, two studies reported increased butyrate concentrations in responders, which was consistent with the enrichment of butyrate-producing taxa in the studies. We also found an alteration of bile acids enriched in responders. Patients with IBD have reduced levels of lithocholic acid and deoxycholic acid (main secondary bile acids, SBA), and SBA supplementation could reduce intestinal inflammation (55). Although the results were divergent, microbial functional content analysis revealed differentially abundant pathways involved in energy metabolism and biosynthesis of virulence factors. Metabolic and functional alterations need to be further unraveled as studies on them are scarce.

Given the lack of a standardized procedure for FMT in patients with IBD, this systematic review had several limitations. Firstly, almost all studies only analyzed the microbiome from stool samples. However, mucosal microbiota may play a more important role due to their direct crosstalk with intestinal tissues. Hence, more studies concerning the mucosal microbiome associated with the response to FMT should be conducted in future research. Secondly, the different methods used for microbiota detection may lead to different conclusions about the microbial alteration. For example, the relative abundance of the potential probiotic species, *B. ovatus*, was reported to be increased in responders in two studies and decrease in the other two studies. These four studies used HITChip (37), whole-genome shotgun sequencing (19), pyrosequencing (17), and 16S amplicon sequencing (16), respectively, to assess the microbiota.

CONCLUSIONS

In conclusion, our systematic review revealed that the response to FMT was associated with gut microbiota and their metabolites, and the different results among different studies were probably attributed to the methodology of FMT, such as ways of delivery and number of infusions (**Figure 2**). The pre-FMT microbial features of recipients, the comparison of pre- and post-FMT microbiota, and the relationship between recipients and donors at baseline should be further investigated using uniform and standardized methods to develop the gut microbiome as a new biomarker for predicting the treatment effect of FMT, and perhaps presupplementation or depletion of specific bacterial taxa or metabolic molecule could enhance the curative effect of FMT.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JZ and YG: concept and design, database searching, literature screening, data interpretation, manuscript drafting, and final approval of manuscript. LD: concept and design, data interpretation, critical revision of manuscript, and final approval of manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by National Natural Science Foundation of China (Grant No. 82000510) and National Key R&D Program of China (Grant No. 2019YFA0905600).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.773105/full#supplementary-material

REFERENCES

1. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the[[sp]] 2[[/sp]]1st century: a systematic review of population-based studies. *Lancet.* (2018) 390:2769–78. doi: 10.1016/S0140-6736(17)32448-0
2. Chang JT. Pathophysiology of inflammatory bowel diseases. *N Engl J Med.* (2020) 383:2652–64. doi: 10.1056/NEJMra2002697
3. Pittayanon R, Lau JT, Leontiadis GI, Tse F, Yuan Y, Surette, M., et al. Differences in gut microbiota in patients with vs without inflammatory bowel diseases: a systematic review. *Gastroenterology.* (2019) 157:97–108. doi: 10.1053/j.gastro.2019.03.049
4. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, et al. A decrease of the butyrate-producing species Roseburia hominis and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut.* (2014) 63:1275–83. doi: 10.1136/gutjnl-2013-304833
5. Lavelle A, Sokol H. Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol.* (2020) 17:223–37. doi: 10.1038/s41575-019-0258-z
6. Jakubczyk D, Leszczynska K, Gorska S. The effectiveness of probiotics in the treatment of inflammatory bowel disease (IBD)-a critical review. *Nutrients.* (2020) 12:1973. doi: 10.3390/nu12071973
7. Glassner KL, Abraham BP, Quigley EMM. The microbiome and inflammatory bowel disease. *J Allergy Clin Immunol.* (2020) 145:16–27. doi: 10.1016/j.jaci.2019.11.003
8. Mcdonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical practice guidelines for clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis.* (2018) 66:987–94. doi: 10.1093/cid/ciy149
9. Costello SP, Soo W, Bryant RV, Jairath V, Hart AL, Andrews JM. Systematic review with meta-analysis: faecal microbiota transplantation for the induction of remission for active ulcerative colitis. *Aliment Pharmacol Ther.* (2017) 46:213–24. doi: 10.1111/apt.14173
10. Paramsothy S, Nielsen S, Kamm MA, Deshpande NP, Faith JJ, Clemente JC, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. *Gastroenterology.* (2019) 156:1440–54 e2. doi: 10.1053/j.gastro.2018.12.001
11. Li QQ, Ding X, Liu KJ, Marcella C, Liu XL, Zhang T, et al. Fecal microbiota transplantation for ulcerative colitis: the optimum timing and gut microbiota as predictors for long-term clinical outcomes. *Clin Transl Gastroenterol.* (2020) 11:e00224. doi: 10.14309/ctg.0000000000000224
12. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ.* (2009) 339:b2535. doi: 10.1136/bmj.b2535
13. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* (2011) 343:d5928. doi: 10.1136/bmj.d5928
14. Kao D, Hotte N, Gillevet P, Madsen K. Fecal microbiota transplantation inducing remission in Crohn's colitis and the associated changes in fecal microbial profile. *J Clin Gastroenterol.* (2014) 48:625–8. doi: 10.1097/MCG.0000000000000131
15. Shimizu H, Arai K, Abe J, Nakabayashi K, Yoshioka T, Hosoi K, et al. Repeated fecal microbiota transplantation in a child with ulcerative colitis. *Pediatr Int.* (2016) 58:781–5. doi: 10.1111/ped.12967
16. Quagliariello A, Del Chierico F, Reddel S, Russo A, Onetti Muda A, D'argenio P, et al. Fecal microbiota transplant in two ulcerative colitis pediatric cases: gut microbiota and clinical course correlations. *Microorganisms.* (2020) 8:1486. doi: 10.3390/microorganisms8101486
17. Angelberger S, Reinisch W, Makristathis A, Lichtenberger C, Dejaco C, Papay P, et al. Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal microbiota transplantation. *Am J Gastroenterol.* (2013) 108:1620–30. doi: 10.1038/ajg.2013.257
18. Suskind DL, Brittnacher MJ, Wahbeh G, Shaffer ML, Hayden HS, Qin X, et al. Fecal microbial transplant effect on clinical outcomes and fecal microbiome in active Crohn's disease. *Inflamm Bowel Dis.* (2015) 21:556–63. doi: 10.1097/MIB.0000000000000307
19. Vaughn BP, Vatanen T, Allegretti JR, Bai AP, Xavier RJ, Korzenik J, et al. Increased intestinal microbial diversity following fecal microbiota transplant for active Crohn's disease. *Inflamm Bowel Dis.* (2016) 22:2182–90. doi: 10.1097/MIB.0000000000000893
20. Vermeire S, Joossens M, Verbeke K, Wang J, Machiels K, Sabino J, et al. Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. *J Crohns Colitis.* (2016) 10:387–94. doi: 10.1093/ecco-jcc/jjv203
21. Jacob V, Crawford C, Cohen-Mekelburg S, Viladomiu M, Putzel GG, Schneider Y, et al. Single delivery of high-diversity fecal microbiota preparation by colonoscopy is safe and effective in increasing microbial diversity in active ulcerative colitis. *Inflamm Bowel Dis.* (2017) 23:903–11. doi: 10.1097/MIB.0000000000001132
22. Ishikawa D, Sasaki T, Osada T, Kuwahara-Arai K, Haga K, Shibuya T, et al. Changes in intestinal microbiota following combination therapy with fecal microbial transplantation and antibiotics for ulcerative colitis. *Inflamm Bowel Dis.* (2017) 23:116–25. doi: 10.1097/MIB.0000000000000975
23. Nishida A, Imaeda H, Ohno M, Inatomi O, Bamba S, Sugimoto M, et al. Efficacy and safety of single fecal microbiota transplantation for Japanese patients with mild to moderately active ulcerative colitis. *J Gastroenterol.* (2017) 52:476–82. doi: 10.1007/s00535-016-1271-4
24. Goyal A, Yeh A, Bush BR, Firek BA, sSiebold LM, Rogers MB, et al. Safety, Clinical response, and microbiome findings following fecal microbiota transplant in children with inflammatory bowel disease. *Inflamm Bowel Dis.* (2018) 24:410–21. doi: 10.1093/ibd/izx035
25. Karakan T, Karataş A. Donor microbiota as a determinant factor for response to FMT in patients with ulcerative colitis. *United Eur Gastroenterol J.* (2018) 6:A133–4.
26. Kump P, Wurm P, Gröchenig HP, Wenzl H, Petritsch W, Halwachs B, et al. The taxonomic composition of the donor intestinal microbiota is a major factor influencing the efficacy of faecal microbiota transplantation in therapy refractory ulcerative colitis. *Aliment Pharmacol Ther.* (2018) 47:67–77. doi: 10.1111/apt.14387
27. Nusbaum DJ, Sun F, Ren J, Zhu Z, Ramsy N, Pervolarakis N, et al. Gut microbial and metabolomic profiles after fecal microbiota transplantation in pediatric ulcerative colitis patients. *FEMS Microbiol Ecol.* (2018) 94:fiy133. doi: 10.1093/femsec/fiy133
28. Cold F, Browne PD, Günther S, Halkjaer SI, Petersen AM, Al-Gibouri Z, et al. Multidonor FMT capsules improve symptoms and decrease fecal calprotectin in ulcerative colitis patients while treated–an open-label pilot study. *Scand J Gastroenterol.* (2019) 54:289–96. doi: 10.1080/00365521.2019.1585939
29. Fan Y, Chen Q, Zhang B, Chen Z, Huang Q, Xu H, et al. Effect of multidonor intensive fecal microbiota transplantation by capsules for active uncreative colitis: a prospective trial. *Gut.* (2019) 68:A109–10. doi: 10.1136/gutjnl-2019-IDDFAbstracts.210
30. Gogokhia L, Crawford CV, Lima SF, Viladomiu M, Jacob V, Scherl E, et al. Transferable IGA-reactive microbiota stratify clinical response to fmt for ulcerative colitis. *Gastroenterology.* (2019) 156:S239. doi: 10.1016/S0016-5085(19)37397-4
31. Gutin L, Piceno Y, Fadrosh D, Lynch K, Zydek M, Kassam Z, et al. Fecal microbiota transplant for Crohn disease: a study evaluating safety,

efficacy, and microbiome profile. *United Eur Gastroenterol J.* (2019) 7:807–14. doi: 10.1177/2050640619845986
32. Chen HT, Huang HL, Xu HM, Luo QL, He J, Li YQ, et al. Fecal microbiota transplantation ameliorates active ulcerative colitis. *Exp Ther Med.* (2020) 19:2650–60. doi: 10.3892/etm.2020.8512
33. Ohmiya N, Osaki H, Jodai Y, Koyama K, Maeda K, Omori T, et al. Changes in fecal microbiota, short chain fatty acids, and bile acids after fecal microbiota transplantation for recurrent clostridioides difficile infection, ulcerative colitis, and Crohn's disease. *Gastroenterology.* (2020) 158:S483. doi: 10.1016/S0016-5085(20)31885-0
34. Schierova D, Brezina J, Mrazek J, Fliegerova KO, Kvasnova S, Bajer L, et al. Gut microbiome changes in patients with active left-sided ulcerative colitis after fecal microbiome transplantation and topical 5-aminosalicylic acid therapy. *Cells.* (2020) 9:2283. doi: 10.3390/cells9102283
35. Zhang T, Li P, Wu X, Lu G, Marcella C, Ji X, et al. Alterations of Akkermansia muciniphila in the inflammatory bowel disease patients with washed microbiota transplantation. *Appl Microbiol Biotechnol.* (2020). 104:10203–15. doi: 10.1007/s00253-020-10948-7
36. Rossen NG, Fuentes S, Van Der Spek MJ, Tijssen JG, Hartman JH, Duflou A, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology*. (2015) 149:110–8.e4. doi: 10.1053/j.gastro.2015.03.045
37. Fuentes S, Rossen NG, Van Der Spek MJ, Hartman JHA, Huuskonen L, Korpela K, et al. Microbial shifts and signatures of long-term remission in ulcerative colitis after faecal microbiota transplantation. *Isme J.* (2017) 11:1877–89. doi: 10.1038/ismej.2017.44
38. Costello SP, Hughes PA, Waters O, Bryant RV, Vincent AD, Blatchford P, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. *JAMA.* (2019) 321:156–64. doi: 10.1001/jama.2018.20046
39. Sokol H, Landman C, Seksik P, Berard L, Montil M, Nion-Larmurier I, et al. Fecal microbiota transplantation to maintain remission in Crohn's disease: a pilot randomized controlled study. *Microbiome.* (2020) 8:12. doi: 10.1186/s40168-020-0792-5
40. Kong L, Lloyd-Price J, Vatanen T, Seksik P, Beaugerie L, Simon T, et al. Linking strain engraftment in fecal microbiota transplantation with maintenance of remission in Crohn's disease. *Gastroenterology*. (2020). 159:2193–202. doi: 10.1053/j.gastro.2020.08.045
41. Zhang T, Lu G, Zhao Z, Liu Y, Shen Q, Li P, et al. Washed microbiota transplantation vs. manual fecal microbiota transplantation: clinical findings, animal studies and *in vitro* screening. *Protein Cell.* (2020) 11:251–66. doi: 10.1007/s13238-019-00684-8
42. Paramsothy S, Kamm MA, Kaakoush NO, Walsh AJ, Van Den Bogaerde J, Samuel D, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet.* (2017) 389:1218–28. doi: 10.1016/S0140-6736(17)30182-4
43. Shi Y, Dong Y, Huang W, Zhu D, Mao H, Su P. Fecal microbiota transplantation for ulcerative colitis: a systematic review and meta-analysis. *PLoS ONE.* (2016) 11:e0157259. doi: 10.1371/journal.pone.0157259
44. Paramsothy S, Paramsothy R, Rubin DT, Kamm MA, Kaakoush NO, Mitchell HM, et al. Faecal microbiota transplantation for inflammatory bowel disease: a systematic review and meta-analysis. *J Crohns Colitis.* (2017) 11:1180–99. doi: 10.1093/ecco-jcc/jjx063
45. Li P, Zhang T, Xiao Y, Tian L, Cui B, Ji G, et al. Timing for the second fecal microbiota transplantation to maintain the long-term benefit from the first treatment for Crohn's disease. *Appl Microbiol Biotechnol.* (2019) 103:349–60. doi: 10.1007/s00253-018-9447-x
46. Han YW. Fusobacterium nucleatum: a commensal-turned pathogen. *Curr Opin Microbiol.* (2015) 23:141–7. doi: 10.1016/j.mib.2014.11.013
47. Janda JM, Abbott SL. The changing face of the family Enterobacteriaceae (Order: "Enterobacterales"): new members, taxonomic issues, geographic expansion, and new diseases and disease syndromes. *Clin Microbiol Rev.* (2021) 34:e00174–20. doi: 10.1128/CMR.00174-20
48. Dehingia M, Devi KT, Talukdar NC, Talukdar R, Reddy N, Mande SS, et al. Gut bacterial diversity of the tribes of India and comparison with the worldwide data. *Sci Rep.* (2015) 5:18563. doi: 10.1038/srep18563
49. Zhao H, Xu H, Chen S, He J, Zhou Y, Nie Y. Systematic review and meta-analysis of the role of Faecalibacterium prausnitzii alteration in inflammatory bowel disease. *J Gastroenterol Hepatol.* (2021) 36:320–8. doi: 10.1111/jgh.15222
50. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA.* (2008) 105:16731–6. doi: 10.1073/pnas.0804812105
51. Lenoir M, Martin R, Torres-Maravilla E, Chadi S, Gonzalez-Davila P, Sokol H, et al. Butyrate mediates anti-inflammatory effects of Faecalibacterium prausnitzii in intestinal epithelial cells through Dact3. *Gut Microbes.* (2020) 12:1–16. doi: 10.1080/19490976.2020.1826748
52. Seo B, Jeon K, Moon S, Lee K, Kim WK, Jeong H, et al. Roseburia spp. abundance associates with alcohol consumption in humans and its administration ameliorates alcoholic fatty liver in mice. *Cell Host Microbe.* (2020) 27:25–40 e6. doi: 10.1016/j.chom.2019.11.001
53. Petersen AM, Halkjaer SI, Gluud LL. Intestinal colonization with phylogenetic group B2 Escherichia coli related to inflammatory bowel disease: a systematic review and meta-analysis. *Scand J Gastroenterol.* (2015) 50:1199–207. doi: 10.3109/00365521.2015.1028993
54. Maharshak N, Huh EY, Paiboonrungruang C, Shanahan M, Thurlow L, Herzog J, et al. Enterococcus faecalis gelatinase mediates intestinal permeability via protease-activated receptor 2. *Infect Immun.* (2015) 83:2762–70. doi: 10.1128/IAI.00425-15
55. Sinha SR, Haileselassie Y, Nguyen LP, Tropini C, Wang M, Becker LS, et al. Dysbiosis-induced secondary bile acid deficiency promotes intestinal inflammation. *Cell Host Microbe.* (2020) 27:659–70 e5. doi: 10.1016/j.chom.2020.01.021

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

TYPE Perspective
PUBLISHED 11 November 2022
DOI 10.3389/fmed.2022.1059148

OPEN ACCESS

EDITED BY
Angel Lanas,
University of Zaragoza, Spain

REVIEWED BY
Jean-Pierre Routy,
McGill University, Canada

*CORRESPONDENCE
Matthew R. Orr
orrma@oregonstate.edu

SPECIALTY SECTION
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

RECEIVED 30 September 2022
ACCEPTED 31 October 2022
PUBLISHED 11 November 2022

CITATION
Orr MR (2022) The biodiversity dose-response curve translates theory and practice from ecological restoration into research and clinical priorities for fecal microbiota transplantation.
Front. Med. 9:1059148.
doi: 10.3389/fmed.2022.1059148

The biodiversity dose-response curve translates theory and practice from ecological restoration into research and clinical priorities for fecal microbiota transplantation

Matthew R. Orr*

Department of Biology, Oregon State University-Cascades, Bend, OR, United States

Discoveries of the beneficial effects of gut microbiota have led to efforts to cultivate healthy gut flora to treat disease. The field of ecological restoration specializes on reestablishment of desired species in disturbed ecosystems, which suggests that it may be applicable to microbe restoration in the gut. Common language can lower barriers to interdisciplinary insights. Here I introduce the concept of a "biodiversity dose-response curve" to translate ideas from ecological restoration into research and clinical priorities for fecal microbiota transplantation (FMT). The curve is based on a relationship between ecosystem structure, measured as species diversity found in both nature and gut ecosystems, and ecosystem function, which are the measurable parameters that contribute to ecosystem and human health. I explain why the biodiversity dose-response curve may follow the ecological model of a "rivet-redundancy" relationship, in which the overlap of multiple organisms' functional contributions to a system mask the impact of initial losses of diversity, but, at a certain level of loss, function declines sharply. (Imagine an airplane that flies with a few rivets missing, until it loses enough to fail.) The biodiversity dose-response curve indicates that seemingly healthy individuals may be suboptimal donors; it highlights the importance of recipient diet in FMT success; and it introduces the concept of "passive restoration" into the field of gut medicine. These insights, which may help to explain low success rates of FMT in the treatment of non-*Clostridium difficile* conditions, are less apparent in the absence of interdisciplinary integration.

KEYWORDS

diet, biodiversity, interdisciplinary, dose-response, ecological restoration

Introduction

The germ theory of disease established a foundation for a focus on pathogen inhibition. In recent decades, a more holistic paradigm of disease has emerged that, like germ theory, assigns a principal role to microbes in the etiology of illness. In contrast to germ theory, however, this new paradigm points to the cultivation of beneficial microbe species as a cure for disease. Fecal microbiota transplantation (FMT) is a promising approach for cultivating beneficial species in the gut microbiome. Until now, however, FMT has operated principally to eliminate *Clostridium difficile* infection [CDI (1)]. To date there exists no regulatory approval for non-CDI FMT (1, 2), and guidelines for FMT emphasize preventing side effects over promoting cures. A better understanding of the factors that promote establishment of beneficial biota in recipients is a priority because their scarcity associates with variety of non-CDI diseases including obesity, diabetes, cancer, and inflammatory bowel disease (IBD) (3).

The need to cultivate and support beneficial gut microbe communities for human health raises the question of whether preexisting approaches from other areas of science may be of assistance. For example, general ecological models of community assembly lead to predictions for the selection of effective fecal donors (4). More specifically, the field of ecological restoration seeks to assist the recovery of ecosystems that have been damaged, degraded, or destroyed (5). Because a human and its microbes can be considered close equivalents to an ecosystem (6), interventions that reconstitute healthy gut microbial communities for the improved health of their host could be viewed as an exercise in medical ecological restoration.

The field of ecological restoration is a few decades older than the field of microbiome medicine. Both rose quickly once the reliance of human wellbeing on intact ecosystems was recognized. In ecological restoration this is measured in the currency of "ecosystem services," which are defined as benefits extracted by humans from nature (6). Rising recognition of ecosystem services, in concert with worsening degradation of the natural environment, led Wilson (7) to predict that the twenty first century would be "the era of restoration in ecology." It is unlikely that Wilson made his prediction with gut medicine in mind, but he might as well have. To respond to the rise of worldwide conditions such as *C. difficile* infection, obesity, and inflammatory bowel disease (IBD), medical researchers, like their peers in ecology, have begun to manipulate the health-related ecosystem benefits provided by beneficial species (6). As in the case of natural ecosystems, such interventions raise questions about how to optimize their effectiveness.

The similarity of goals between ecological restoration and microbiome medicine present not only opportunities but also challenges common in interdisciplinary research, defined as "the synergistic combination of two or more disciplines to achieve one research objective" (8). Funding barriers, institutional organization, domain specificity, and conceptual and methodological divides commonly impede interdisciplinary efforts (9). Accordingly, this perspective piece seeks to highlight common conceptual ground between restoration ecology and gut medicine by translating a fundamental concept in ecology—structure-function curves—into a common medical concept—medicinal dose-response curves—*via* the idea of a "biodiversity dose-response curve."

The biodiversity dose-response curve

A principal goal in ecology is to understand how the species composition of an ecosystem influences its function. One approach is to quantify an ecosystem metric, like biological diversity, and see how it relates to an ecosystem property, like biomass production, nutrient uptake, or decomposition (10). This line of research has led to the conclusion that species diversity and ecosystem function most often follow a "rivet-redundancy" relationship [(10); **Figure 1A**], in which the system is robust to initial species losses, like an airplane losing a few rivets, but can collapse if too many species disappear, like an airplane losing enough rivets to fall apart midflight. The shape of this relationship is considered important for biological conservation because it mandates caution in assuming that a superficially healthy system can afford ongoing species losses (11).

Multiple lines of evidence support the hypotheses that, as in nature, gut ecosystems exhibit a rivet-redundancy relationship between microbe diversity and host health (**Table 1**). Although perhaps esoteric for scientists outside of ecology, the relationship in **Figure 1B** can be viewed analogously to a more familiar concept in medicine: a dose-response curve, leading to the concept of a "biodiversity dose-response curve" (**Figure 1C**). The biodiversity dose-response curve supports two insights for FMT. First, an apparently healthy donor is not necessarily an appropriate donor. Seemingly healthy donors who are close to a precipitous drop in function due to low microbiota diversity (d1 in **Figures 1B,C**) create a high likelihood of FMT failure if engraftment is incomplete (r1 in **Figures 1B,C**), and engraftment often is incomplete (12). Thus, to insure against FMT failure from partial engraftment, it may be important for potential donors to lie as far to the right on the biodiversity dose-response curve as possible, indicating a robust donor species diversity (d2 in **Figures 1B,C**).

A second implication of the biodiversity dose-response curve is that high donor diversity should be complemented by treatments that optimize engraftment in recipients (shown as a smaller gap between d1 and r2 than d1 and r1 in **Figure 1C**). Engraftment success after FMT is comparable to the ecological priority of seedling establishment in natural ecosystems, which is often a limiting factor in restoration success (13). Seedlings

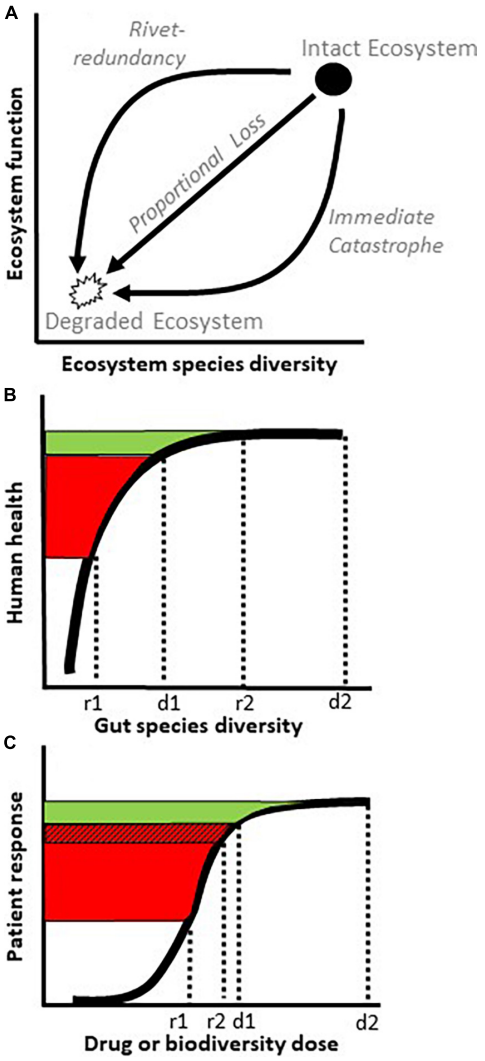


FIGURE 1
Curves relating species diversity with ecosystem function or host health. **(A)** Three ecological relationships between species diversity and ecosystem function. As an ecosystem transitions from an intact state with high diversity (filled circle) to a degraded state with low diversity (open polygon), its function (y axis) could decline *via* patterns of rivet-redundancy, proportional loss, or immediate catastrophe (arrows). **(B)** By virtue of its species redundancy, a rivet-redundancy relationship (thick curve), exhibits little increase in function (green) past a species diversity saturation point, d1. In terms of fecal microbiota transplantation (FMT), donors with species diversity d1 and d2 may exhibit roughly similar levels of health on the y axis (green), but incomplete engraftment of microbe diversity from donor d1 to recipient r1 risks a poor health outcome for the recipient (red). No similar risk exists from donor d2 to recipient r2 despite a similar or lower rate of engraftment. **(C)** In a medical dose-response relationship, drug efficacy flattens above dose d1 (green), similarly to rivet-redundancy in panel **(B)**. To increase the odds of FMT success, it may not only be beneficial to start with a high diversity donor (d2) but also to implement measures such as antibiotics, colon lavage, or an anti-inflammatory diet that reduce the risks of diversity loss during engraftment, shown here as a smaller gap between d1 and r2 than d1 and r1 and a smaller red hatched area than total red area [**(A,B)** adapted from (6)].

may fail to establish due to poor site conditions such as degraded soil or undesired competitors (14). Accordingly, restoration ecologists tend to focus on "site preparation," such as herbicides or watering, to remove unwanted competitors and improve seeding success (15). Analogous to the site prep of natural systems, site prep for FMT would include any gut intervention in a recipient that improves engraftment of donor biota, as discussed below.

Discussion

As applications of FMT shift from pathogen removal for CDI to include the establishment of beneficial biota to treat non-CDI diseases, it is paramount to identify and prioritize the factors that best support shifts to a healthy gut biota among FMT recipients. What is the evidence that the features identified by the biodiversity dose-response curve—donor diversity and recipient site prep—merit priority in research and clinical practice of FMT? In terms of the importance of donor diversity, remarkably few studies have assessed its association with remission of symptoms after FMT, and studies that have are retrospective, lack replication, and/or are poorly controlled for confounding factors. Despite such shortcomings, evidence supporting donor diversity for FMT success is accumulating (16–18), but much more remains to be learned.

A meta-analysis consisting of 226 triads of donors, pre-FMT recipients, and post-FMT recipients across eight different disease types found that engraftment success associated with clinical success after FMT (12). In the same way that site preparation for establishment of beneficial species in natural systems often focuses on removing competing weeds, recipient site prep for successful engraftment in FMT includes measures such as antibiotics and bowel lavage that reduce dysbiotic taxa. In terms of experimental support for recipient site prep, engraftment success was found to associate more strongly with administration of pre-FMT antibiotics than it did with disease severity (19). In addition, patients with infectious conditions treated with antibiotics exhibited better engraftment than those with non-communicable conditions who did not receive antibiotics (12), although this finding was confounded by different disease conditions. Community ecology models together with suggestive but not significant clinical results also support the hypothesis that competition from a recipient's resident microbes may reduce establishment of donor biota (4). More studies are needed to better understand the replicability of these findings and their relevance across different diseases.

Diet must also be considered for recipient site preparation. A gut disturbed by industrial, processed foods can be hostile to beneficial biota (6, 20). Viewed on the biodiversity dose-response curve, industrial diets may inhibit FMT by reducing the number and diversity, and therefore the "dose," of donor biota that establish in the recipient (r1 vs. r2 in **Figure 1C**). In

TABLE 1 Lines of evidence supporting a rivet-redundancy relationship between species diversity (structure) and health (function) in the human gut.

Area	Evidence
General evidence for structure-function relationships in the gut	Functions of natural ecosystems including decomposition, nutrient flows, and biomass share analogs in the gut including metabolism, energy harvest, and body mass index.
	In both nature and the gut, ecosystem structure is commonly measured using metrics of species diversity.
	Gut species diversity correlates positively with health-related functions including obesity, IBD, diabetes, autism, allergies, asthma, cancer, and anorexia.
Specific evidence that gut structure-function relationships are rivet-redundant	Different humans harbor the same microbe-mediated metabolic pathways despite differences in the microbe species present, suggesting functional redundancy among species.
	Subsets of the complete microbiota perform the same functions as a complete microbiota in both humans and mice.
	Genes performing gut functions are commonly exchanged among gut microbes.
	Hosts would be unlikely to evolve an overreliance on a single microbe "keystone" species whose loss could jeopardize host fitness.

For details and citations see (6) pp. 80–81.

other words, poor quality or processed food may inhibit FMT success analogously to food-drug interactions that reduce drug activity or inhibit drug bioavailability (21). Effects of diet on FMT success may be more difficult to study than antibiotics and lavage due to the challenge of patient dietary compliance, which is analogous to the challenges of obtaining stakeholder compliance in ecological restoration (6, 20).

Perhaps because it is more difficult to control patient diets than it is to administer antibiotics, lavage, or even FMT, very few studies have examined dietary influences on FMT. In the only such study that I know of, subjects placed on an ulcerative colitis exclusion diet (UCED) plus FMT did not differ after 8 weeks from subjects on UCED alone or FMT alone (22). However, the UCED diet mandated yogurt, even though dairy is linked to UC (23). Moreover, UCED commenced at the same time as FMT, which may not be early enough to induce meaningful taxonomic shifts (24) or physiological responses, such as recovery of the intestinal mucus layer or intestinal epithelial cells (25), to prepare recipient guts for engraftment. Finally, the study's low rate of patient responses is contradicted by a longer-term study in which FMT plus an anti-inflammatory diet that prohibited dairy was more effective than standard medical treatment in inducing both a clinical response and remission to UC (26). Much more needs to be done to better resolve effects of diet. A recent survey found that 71% of healthcare providers felt that diet was an important consideration for FMT, but they did not feel confident adding dietary protocols to FMT due to a lack of research to guide dietary advice (27).

Until proven otherwise, FMT without consideration of diet can be considered analogous to replanting sensitive species without removing the disturbances that facilitated noxious invaders in the first place (6). Viewed as such, ignoring diet in FMT violates a fundamental principle of ecological restoration: passive restoration, which removes disturbances such as livestock (analogous to removing fatty, sugary, and processed foods in the gut), must precede active restoration, which involves dynamic interventions such as weeding and herbicides (analogous to antibiotics and lavage) and species replantings (analogous to FMT). The principle of passive before active restoration is considered fundamental because active measures are less likely to succeed if the disturbances that caused degradation are permitted to persist.

A widespread failure to place passive restoration (i.e., diet) before active restoration (i.e., FMT) may help to explain a lack of evidence for long-term recipient microbiome changes after FMT in non-CDI diseases. A review of 24 non-CDI FMT research studies identified 19 studies that examined the duration of recipient microbiome changes. Of those, only three monitored recipients beyond 90 days post-treatment: one showed persistent changes for over a year and two reverted to no change after exhibiting an initial difference. Of the 16 studies that monitored for a shorter duration of 14–90 days, initial changes in recipient microbiomes either disappeared or became less significant over time in three studies (2). It is difficult to know how to interpret studies that do not demonstrate long term efficacy of FMT because failure to control for possible confounding effects of diet may increase the variability and reduce the magnitude of patient responses, leading to type II statistical errors. At least one study has attributed a failure to detect an FMT effect to low statistical power (4).

Restorationists tend to provide seedling support, such as by watering, for only a short duration of time due to practical considerations. If diet does influence engraftment of healthy microbiomes, research will be required to determine the degree to which short term dietary shifts are sufficient to support engraftment, or whether longer-term "lifestyle" changes before and/or after FMT are necessary. Such studies will require longer-term monitoring than most research on non-CDI diseases to date (2) as well as diet-without-FMT control groups, because changes in diet alone can be sufficient to alleviate IBD (20).

In summary, the biodiversity dose-response curve identifies factors likely to influence FMT success, beginning with diet as a form of passive restoration, followed by antibiotics or lavage for site prep, and finishing with high diversity donors to ensure sufficient engraftment above the threshold for system failure.

These theoretical priorities are supported by early research into the beneficial effects of donor gut microbiome diversity (16) and recipient "site prep" [lavage, antibiotics (12, 19)], with little and contradictory evidence for diet (22, 26). Additional research is needed to better understand the extent to which these factors improve clinical success in FMT, which until now has been higher for CDI, in which the priority is pathogen removal, than it has for non-CDI diseases requiring the sustained establishment and restoration of beneficial biota (1).

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

Acknowledgments

Kathryn Kocurek provided helpful comments on the manuscript.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Lai CY, Sung J, Cheng F, Tang W, Wong SH, Chan PKS, et al. Systematic review with meta-analysis: review of donor features, procedures and outcomes in 168 clinical studies of faecal microbiota transplantation. *Aliment Pharmacol Ther.* (2019) 49:354–63. doi: 10.1111/apt.15116

2. Green J, Davies J, Loughman A, Berk M, Hair C, Castle D, et al. FMT for the treatment of diseases other than clostridium difficile: a systematic review protocol. *Bipolar Disord.* (2019) 21:139–139.

3. Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. *Cell Host Microbe.* (2012) 12:611–22. doi: 10.1016/j.chom.2012.10.012

4. Xiao Y, Tulio Angulo M, Lao S, Weiss ST, Liu Y-Y. An ecological framework to understand the efficacy of fecal microbiota transplantation. *Nat Commun.* (2020) 11:3329. doi: 10.1038/s41467-020-17180-x

5. Society for Ecological Restoration International Science & Policy Working Group,. *The SER International Primer on Ecological Restoration*. (2004). Available online at: https://www.ser-rrc.org/resource/the-ser-international-primer-on/ (accessed August 28, 2022).

6. Orr MR, Kocurek KM, Young DL. Gut microbiota and human health: insights from ecological restoration. *Q Rev Biol.* (2018) 93:73–90. doi: 10.1086/698021

7. Wilson EO. *The Diversity of Life*. Cambridge, MA: Harvard University Press (1992). 340 p.

8. Daniel KL, McConnell M, Schuchardt A, Peffer ME. Challenges facing interdisciplinary researchers: findings from a professional development workshop. *PLoS One.* (2022) 17:e0267234. doi: 10.1371/journal.pone.0267234

9. MacLeod M. What makes interdisciplinarity difficult? Some consequences of domain specificity in interdisciplinary practice. *Synthese.* (2018) 195:697–720. doi: 10.1007/s11229-016-1236-4

10. Cardinale BJ, Matulich KL, Hooper DU, Byrnes JE, Duffy E, Gamfeldt L, et al. The functional role of producer diversity in ecosystems. *Am J Bot.* (2011) 98:572–92. doi: 10.3732/ajb.1000364

11. Ehrlich P, Walker B. Rivets and redundancy. *BioScience.* (1998) 48:387–8.

12. Ianiro G, Punčochář M, Karcher N, Porcari S, Armanini F, Asnicar F, et al. Variability of strain engraftment and predictability of microbiome composition after fecal microbiota transplantation across different diseases. *Nat Med.* (2022) 28:1913–23. doi: 10.1038/s41591-022-01964-3

13. Rinella MJ, James JJ. A modelling framework for improving plant establishment during ecological restoration. *Ecol Model.* (2017) 361:177–83. doi: 10.1016/j.ecolmodel.2017.08.005

14. Smith ME, Cavagnaro TR, Christmas MJ, Pound LM, Facelli JM. Site preparation impacts on soil biotic and abiotic properties, weed control, and native grass establishment. *Restor Ecol.* (2021) 29:e13297. doi: 10.1111/rec.13297

15. Letsch L. *Restoration Best Practices: Site Preparation Methods. Rogue Native Plant Partnership*. (2020). Available online at: https://www.roguenativeplants.org/restoration-best-practices-site-preparation-methods/ (accessed September 18, 2022).

16. Rees NP, Shaheen W, Quince C, Tselepis C, Horniblow RD, Sharma N, et al. Systematic review of donor and recipient predictive biomarkers of response to faecal microbiota transplantation in patients with ulcerative colitis. *EBioMedicine.* (2022) 81:104088. doi: 10.1016/j.ebiom.2022.104088

17. Kump P, Wurm P, Groechenig HP, Wenzl H, Petritsch W, Halwachs B, et al. The taxonomic composition of the donor intestinal microbiota is a major factor influencing the efficacy of faecal microbiota transplantation in therapy refractory ulcerative colitis. *Aliment Pharmacol Ther.* (2018) 47:67–77. doi: 10.1111/apt.14387

18. Vermeire S, Joossens M, Verbeke K, Wang J, Machiels K, Sabino J, et al. Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. *J Crohns Colitis.* (2016) 10:387–94. doi: 10.1093/ecco-jcc/jjv203

19. Podlesny D, Durdevic M, Paramsothy S, Kaakoush NO, Högenauer C, Gorkiewicz G, et al. Identification of clinical and ecological determinants of strain engraftment after fecal microbiota transplantation using metagenomics. *Cell Rep Med.* (2022) 3:100711. doi: 10.1016/j.xcrm.2022.100711

20. Orr MR, Kocurek KM, Bakos YJ, McDowell RC. A restoration ecology perspective on the treatment of inflammatory bowel disease. *Evol Med Public Health.* (2019) 1:217–20. doi: 10.1093/emph/eoz031

21. Bushra R, Aslam N, Khan AY. Food-drug interactions. *Oman Med J.* (2011) 26:77–83. doi: 10.5001/omj.2011.21

22. Sarbagili Shabat C, Scaldaferri F, Zittan E, Hirsch A, Mentella MC, Musca T, et al. Use of faecal transplantation with a novel diet for mild to moderate active ulcerative colitis: the CRAFT UC randomised controlled trial. *J Crohns Colitis.* (2022) 16:369–78. doi: 10.1093/ecco-jcc/jjab165

23. Judaki A, Hafeziahmadi M, Yousefi A, Havasian MR, Panahi J, Sayehmiri K, et al. Evaluation of dairy allergy among ulcerative colitis patients. *Bioinformation.* (2014) 10:693–6. doi: 10.6026/97320630010693

24. Leeming ER, Johnson AJ, Spector TD, Le Roy CI. Effect of diet on the gut microbiota: rethinking intervention duration. *Nutrients.* (2019) 11:2862. doi: 10.3390/nu11122862

25. Ma J, Piao X, Mahfuz S, Long S, Wang J. The interaction among gut microbes, the intestinal barrier and short chain fatty acids. *Anim Nutr.* (2021) 9:159–74. doi: 10.1016/j.aninu.2021.09.012

26. Kedia S, Virmani S, Vuyyuru SK, Kumar P, Kante B, Sahu P, et al. Faecal microbiota transplantation with anti-inflammatory diet (FMT-AID) followed by anti-inflammatory diet alone is effective in inducing and maintaining remission over 1 year in mild to moderate ulcerative colitis: a randomised controlled trial. *Gut.* (2022) 1–13. doi: 10.1136/gutjnl-2022-32781

27. Clancy AK, Gunaratne AW, Borody TJ. Dietary management for faecal microbiota transplant: an international survey of clinical and research practice, knowledge and attitudes. *Front Nutr.* (2021) 8:653653. doi: 10.3389/fnut.2021.653653

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 02 December 2022
DOI 10.3389/fmed.2022.1068567

OPEN ACCESS

EDITED BY
Angel Lanas,
University of Zaragoza, Spain

REVIEWED BY
Jean-Pierre Routy,
McGill University, Canada
Jing Ouyang,
Chongqing Public Health Medical Center, China

*CORRESPONDENCE
Dai Ishikawa
dai@juntendo.ac.jp

SPECIALTY SECTION
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

RECEIVED 13 October 2022
ACCEPTED 21 November 2022
PUBLISHED 02 December 2022

CITATION
Zhang X, Ishikawa D, Ohkusa T, Fukuda S and Nagahara A (2022) Hot topics on fecal microbiota transplantation for the treatment of inflammatory bowel disease. *Front. Med.* 9:1068567. doi: 10.3389/fmed.2022.1068567

Hot topics on fecal microbiota transplantation for the treatment of inflammatory bowel disease

Xiaochen Zhang[1], Dai Ishikawa[1,2]*, Toshifumi Ohkusa[3,4], Shinji Fukuda[2] and Akihito Nagahara[1,2]

[1]Department of Gastroenterology, Juntendo University School of Medicine, Tokyo, Japan, [2]Department of Regenerative Microbiology, Juntendo University School of Medicine, Tokyo, Japan, [3]Department of Microbiota Research, Juntendo University Graduate School of Medicine, Tokyo, Japan, [4]Department of Gastroenterology and Hepatology, The Jikei University Kashiwa Hospital, Chiba, Japan

Inflammatory bowel disease (IBD) is a chronic intestinal mucosal inflammatory disease with complex etiology. Traditional anti-inflammatory treatment regimens have yielded unsatisfactory results. As research continues to deepen, it has been found that the gut microbiota of patients with IBD is generally altered. The presence of microorganisms in the human gastrointestinal tract is inextricably linked to the regulation of health and disease. Disruption of the microbiotic balance of microbiota in the gastrointestinal tract is called dysbiosis, which leads to disease. Therefore, in recent years, the exploration of therapeutic methods to restore the homeostasis of the gut microbiota has attracted attention. Moreover, the use of the well-established fecal microbiota transplantation (FMT) regimen for the treatment of *Clostridioides difficile* infection has attracted the interest of IBD researchers. Therefore, there are an increasing number of clinical studies regarding FMT for IBD treatment. However, a series of questions regarding FMT in the treatment of IBD warrants further investigation and discussion. By reviewing published studies, this review explored hot topics such as the efficacy, safety, and administration protocol flow of FMT in the treatment of IBD. Different administration protocols have generally shown reassuring results with significant efficacy and safety. However, the FMT

treatment regimen needs to be further optimized. We believe that in the future, individual customized or standard FMT implementation will further enhance the relevance of FMT in the treatment of IBD.

KEYWORDS

fecal microbiota transplantation, inflammatory bowel disease, ulcerative colitis, Crohn's disease, pouchitis

Introduction

A growing number of studies have suggested that the presence of microbes in the human gastrointestinal tract is inextricably linked to the regulation of health and disease. Gut microbes ferment food into absorbable metabolites, synthesize essential vitamins, regulate the immune system, and act as a barrier to protect the gastrointestinal tract. Disruption of the gut microbiota balance, called dysbiosis, can lead to disease (1).

Inflammatory bowel disease (IBD) is an intestinal disease characterized by chronic inflammation of the intestinal mucosa that is prone to relapse. Common clinical types mainly include ulcerative colitis (UC), Crohn's disease (CD), and pouchitis. The etiology of IBD is complex and diverse, which may be related to multiple interactive influences, such as environmental, microbial, genetic, and immune factors (2, 3). Traditional IBD treatment regimens have primarily focused on reducing inflammation. Although this treatment regimen has been continuously developed and updated, there are still drawbacks, such as easy relapse, immune tolerance, and drug resistance (4). Therefore, researchers continue to explore more effective treatment measures. It is generally accepted that the gut microbiota of patients with IBD is altered (3). The exploration of therapeutics to restore gut microbiota homeostasis has gained attention in recent years because the qualitative and quantitative profiles of the gastrointestinal microbiota in patients with IBD vary significantly compared to healthy individuals. Fecal microbiota transplantation (FMT) is an advanced microbial therapy that restores the gut microbiota and corrects the dysbiosis of the microbiota by providing full-spectrum microorganisms of healthy individuals to the patient so that the patient can obtain a complete functional ecosystem (5). In the *Clostridioides difficile* infection (CDI) treatment guidelines published in the United States and Europe, it is stated that FMT is a strongly recommended regimen for CDI with multiple recurrences (6, 7), with an effective rate of 92% (8). FMT has been implemented in a variety of disease fields (9–11), especially in improving the response of anti-PD-1 immunotherapy in metastatic melanoma (12, 13). Openbiome (14), a non-profit organization in the United States, is committed to providing an internationally standardized public stool bank for microbial treatment of various diseases. This provides the basic guarantee for FMT treatment. However, the use of FMT for the treatment of IBD is still progressing toward clinical application. In this review, we summarized hot topics such as efficacy, safety, and implementation of FMT for the treatment of IBD.

Efficacy

Since the two cases of using FMT to treat patients with UC in 1989 proved effective (15, 16), researchers have been increasingly enthusiastic about exploring the use of FMT for IBD treatment.

Efficacy of fecal microbiota transplantation in ulcerative colitis therapy

To date, six double-blinded, randomized controlled trials (RCTs) on the efficacy of FMT-induced remission in UC have been published (**Table 1**; 17–22). Moayyedi et al. recruited 75 patients with mild-severe UC (38 received FMT and 37 received placebo) and demonstrated that patients who received fecal enemas from donors (24%) had significantly higher rates of clinical remission at week 7 than those in the placebo enema group (5%) (p = 0.03). Two years later, Paramsothy et al. reported the results of their study of 81 patients with mild-moderate UC. Forty-one patients were included in the FMT group and 40 in the placebo group. At week 8, steroid-free clinical and endoscopic remission were achieved in 11 (27%) patients, which was significantly higher than that in the control group (3 patients [8%]) (p = 0.021). In an article published in 2019, Costello et al. enrolled 73 mild-moderate UC patients (38 in the FMT group and 35 in the placebo group). At week 8, steroid-free clinical and endoscopic remission were achieved in 12 (32%) of them. The

TABLE 1 Efficacy of FMT on UC patients with six double-blind, randomized controlled trials.

References	Rossen et al. (17)	Moayyedi et al. (18)	Paramsothy et al. (19)	Costello et al. (20)	Haifer et al. (22)	Crothers et al. (21)
Number of patients	48 (FMT: 23, placebo: 25)	75 (FMT: 38, placebo: 37)	81 (FMT: 41, placebo: 40)	73 (FMT: 38, placebo: 35)	35 (FMT: 15, placebo: 20)	12 (FMT: 6, placebo: 6)
Patient criteria	Mild-moderate (11 ≥ SCCAI ≥ 4, MES ≥ 1)	Mild-severe (Mayo: 4–12, MES ≥ 1)	Mild-moderate (Mayo: 4–10, MES ≥ 1/PGA ≤ 2)	Mild-moderate (Mayo: 3–10, MES ≥ 2)	Mild-moderate (Mayo: 4–10, MES ≥ 1)	Mayo: 4–10, MES ≥ 1, RBC ≥ 1, SFS ≥ 1
Pre-treatment	Bowel lavage	None	Bowel lavage	Bowel lavage	Amoxicillin, doxycycline, and metronidazole.	Ciprofloxacin, metronidazole, and bowel lavage
Steroid	Concomitant (<10 mg)	Concomitant	Taper 2.5 mg/w to free	Taper 5 mg/w to free	Taper 2.5 mg/w to free	free
FMT	2 times	6 times	41 times	3 times	49 times	85 times
Donor	Single	Single	Multiple (3–7 donors)	Multiple (3–4 donors)	Single	Single
Stool	Fresh	Fresh/frozen	Frozen −80°C	Frozen −80°C	Lyophilized	Frozen −20°C
Primary endpoint (FMT vs. placebo)	CR + ER at week 12 30 vs. 20%, $p = 0.51$	CR + ER at week 7 24 vs. 5%, $p = 0.03$	CR + ER/Er at week 8 27 vs. 8%, $p = 0.02$	CR + ER at week 8 32 vs. 9%, $p = 0.03$	CR + ER/Er at week 8 53 vs. 15%, $p = 0.027$	CR at week 12 2/6 vs. 0/6, $p = 0.45$
Clinical remission (FMT vs. placebo)	30 vs. 32%, $p = 1.0$	24 vs. 5%, $p = 0.03$	44 vs. 20%, $p = 0.02$	47 vs. 17%, $p = 0.01$	73 vs. 25%, $p = 0.0045$	/

treatment effect was significantly better than that observed in the placebo group, with only three of the 35 with complete remission ($p = 0.03$). In 2021, Haifer et al. also published the results of a RCT. Of the 35 mild-moderate UC patients recruited, 15 received FMT and 20 received a placebo. At week 8, the expected steroid-free clinical and endoscopic remission were achieved in 53% ($n = 8$) of patients in the FMT group, a significantly higher rate of remission than that in the placebo group of 15% ($n = 3$) ($p = 0.027$). Although positive results continued to emerge, as early as 2015, Rossen et al. reported contrary results. In 48 patients with mild-moderate UC, only seven of 23 patients receiving FMT achieved clinical and endoscopic remission at week 12, and five of 25 patients receiving placebo achieved remission, a result that was not significantly different ($p = 0.51$). Moreover, Crothers et al. published the results of a study with a small sample size ($n = 12$) in 2021. In the 12th week, only two of six patients in the FMT group achieved steroid-free clinical remission, while none in the placebo group achieved remission. There was no significant difference between the two groups ($p = 0.45$).

El Hage Chehade et al. (23) conducted a meta-analysis of the different results of six double-blinded RCTs. A total of 324 patients were included in the analysis, and 30.43% of patients treated with FMT achieved clinical and endoscopic remission, significantly higher than 9.82% of patients in the placebo group who achieved clinical and endoscopic remission ($p < 0.00001$). In another non-double-blinded RCT (24), 90% of patients in the FMT group achieved the primary endpoint at week 8, compared with 50% in the placebo group. Considering the published conclusions so far, we believe that the efficacy of FMT for UC treatment is excellent.

Efficacy of fecal microbiota transplantation in Crohn's disease therapy

Cohort studies showed that FMT for CD treatment is generally effective (25–29). However, a few reports also showed a less obvious effect (30, 31).

Currently, only one RCT study has evaluated the clinical effect of FMT in CD (32). In 2020, Sokol et al. published a multicenter, single-blinded RCT study. Twenty-one patients who achieved clinical remission after 3 weeks of prednisolone therapy were randomly assigned to the FMT or placebo groups. No patients in either the FMT or placebo groups achieved the primary outcome of successful gut colonization with the donor microbiota at 6 weeks. The steroid-free clinical remission rates in the FMT and placebo groups were 87.5 and 44.4% at week 10 and 50 and 33.3% at week 24, respectively. Both results were not statistically significant. In 2021, a meta-analysis of FMT for CD treatment reported that the pooled rate of clinical remission in patients with CD reached 0.62, and that of clinical response was 0.79 (33).

Because CD lesions extend into the small intestine, determining the treatment response is expected to be more challenging than for UC. Moreover, it is expected that the response to FMT treatment will differ depending on the site of the lesion and whether it is a small or large bowel type. The results of using FMT for the treatment

of CD are still controversial; hence, more convincing RCT studies are required.

Efficacy of fecal microbiota transplantation in pouchitis therapy

Pouchitis is the most common complication of ileal pouch-anal anastomosis for refractory UC, with an incidence of up to 80% at 30-year follow-up (34). Some reports showed that 80% (35) of patients and 44% (36) with pouchitis achieved clinical remission after receiving FMT. A case report (37) also showed that antibiotic-refractory pouchitis improved significantly after FMT and persisted for more than 6 months. However, some other reports showed that (38–42) the efficacy was not very satisfactory, and no patient achieved clinical remission. Moreover, a recent RCT (43) report showed that FMT was not associated with relapse-free survival of pouchitis. In summary, the current results of the use of FMT in treating pouchitis are not satisfactory. Therefore, well-designed controlled studies are further needed.

Safety

For a new treatment regimen for IBD, the public is most concerned about safety and efficacy. Most patients experience only transient discomfort, such as diarrhea, abdominal pain, bloating, borborygmus, nausea, vomiting, and increase in C-reactive protein level (17, 21, 22, 25, 26, 29, 31, 32, 44–63), which are believed to be an immune response caused by the infused fecal microbiota. There are also a small number of patients who have narcolepsy, fatigue (61), skin pruritus (29, 52, 62), testicular pain, rectal abscess (18), perianal pain or fistula (26), blood in the stool (27, 57), herpes zoster (57), and other complaints (64). However, these symptoms have not been shown to be directly related to FMT. Serious adverse events of worsening colitis requiring colectomy and hospitalization have been reported in some patients (18–20, 22, 26, 30, 32, 45, 57, 65, 66). Some of these exacerbated conditions were observed in the placebo group, while those in the FMT group may have been associated with a change in treatment regimen or a disproportionate host immune response induced by the new microbiota of the incomplete mucosa and disease progression rather than FMT itself. In addition, the spread of infection is a problem that doctors are very concerned about. Cytomegalovirus infections (17, 67), and CDI (18, 51) have been reported in FMT for the treatment of IBD. However, Rossen et al. concluded that CMV infection was not associated with FMT because patients were randomly assigned to the placebo group (17). In addition, Suskind et al. speculated that *C. difficile* infection in two patients, which occurred 3 and 4 months after transplantation, may not be related to FMT because the feces used showed no abnormal results on microbiological examination (51). Some studies have also described the risk of bacteremia. However, most of the fever symptoms in patients suspected of bacteremia resolved spontaneously within a short period (17, 21, 25, 26, 28, 31, 45–47, 49, 50, 62, 63, 68–72). Blood cultures were used in some studies to test whether a patient had bacteremia but did not yield positive results (47, 50, 62). However, a report (73) described a patient with CD who had positive blood cultures for multidrug-sensitive *Escherichia coli* bacteremia after FMT. Moreover, Grewal et al. (66) reported a patient with UC progression and toxic megacolon after FMT, who died of sepsis after surgery. Although not treated for UC, in March 2020, the FDA issued a safety warning[1] that two patients with CDI were infected with drug-resistant *Escherichia coli* as a result of FMT treatment, and one died due to bacteremia (74). Despite occasional infections, rigorous donor screening is believed to reduce the risk of bacteremia and infectious disease transmission to almost zero.

Small bowel perforation (17), obstruction (26), and aspiration pneumonia (27, 31) caused by improper handling of routes of administration in the upper gastrointestinal tract (nasogastric, nasoduodenal, or nasojejunal tube) and lower gastrointestinal tract (transendocopic enteral tubing) have also been reported. This has caused severe pneumonia and intestinal bleeding leading to the death of a patient (27). The occurrence of these adverse events makes every doctor distressed, and the operation regimen is constantly improving. Moreover, a recent meta-analysis article analyzed published RCTs using FMT for various diseases and no significant difference in the incidence of serious adverse events was observed between the FMT and placebo groups (75). This suggests that FMT is a safe treatment modality.

Implementation

There is still no unified standard protocol of FMT. The protocol of FMT affects the efficacy, safety, and patient acceptance of the treatment.

Dose intensity and antibiotic pre-treatment

Fecal microbiota transplantation attempts to reverse dysbiosis by colonizing patients with healthy microbiota. It is now known that a single FMT treatment can restore the abnormal microbiota environment in most patients with CDI for several years (76, 77). However, according to the current

1 https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse-events-likely

study results, the effect of administration intensity on efficacy in patients with IBD is unstable.

Published articles showing the effect of a single FMT administration on clinical outcomes are controversial (69, 78). In addition, the lack of a control group in these articles makes it impossible to rule out other factors that may have contributed to the biased results. However, Mocanu et al. statistically analyzed that repeated FMT administrations were higher than single administrations in both clinical response (70 vs. 53%) and clinical remission rates (43 vs. 30%) (79).

Some researchers have conducted some double-blinded RCTs on multiple administrations of FMT. In 2015, Moayyedi et al. (18) published an article involving six administrations of FMT per patient. The remission rate of patients in the FMT group was significantly higher than that in the placebo group, which led to interest in the negative results of a study involving two administrations published by Rossen et al. (17) in the same year. Were the negative results of Rossen et al. related to the frequency of FMT use? The study by Paramsothy et al. (19), Haifer et al. (22), and Crothers et al. (21) performed 41, 49, and 85 FMTs on each patient, respectively, and the effect of using FMT was significantly better in the FMT group than in the placebo group. However, in 2019, Costello et al. (20) used a similar FMT implementation protocol as Paramsothy et al. (19); however, they only performed three FMT administrations, obtaining similar clinical outcomes as Paramsothy's 41-administration study. This result raises the question of if more than 40 administrations are meaningful. Furthermore, how many administrations can give the best results? In a subgroup analysis of the number of administrations by Paramsothy et al. the pooled proportion of patients with UC who received more than 10 administrations and achieved clinical remission was 49%, significantly higher than the remission rate (27%) for patients with UC who received fewer than 10 administrations (p = 0.001) (54). There have been reports that there was no significant difference in adverse events (both severe and common adverse events) between the FMT and placebo groups in RCT studies involving the use of either single or multiple FMT administrations (75). However, too many administrations of FMT will bring inconvenience and psychological burden to patients; therefore, getting the best therapeutic effect under the premise of the least number of administrations is a topic worthy of further study. To the best of our knowledge, in addition to the effectiveness of antibiotic cocktail therapy in the treatment of patients with UC (80, 81), recent studies have shown that pre-treatment with antibiotics prior to FMT can improve FMT treatment efficacy by aiding microbiota colonization (82). We have previously reported (53, 60, 83) a clinical remission rate of approximately 35% with combined antibiotic pretreatment prior to the use of a single FMT, which is higher than the clinical remission rate observed by using multiple FMTs as reported by Rossen et al. (30%) (17) and Moayyedi et al. (24%) (18). Moreover, a case report showed that patients with refractory CD who received a single dose of FMT after pre-treatment with antibiotics had significantly improved symptoms (84). More RCTs are needed to verify the potentiating ability of antibiotic pre-treatment on FMT.

Route administration

At present, the widely used FMT administration routes are mainly divided into upper gastrointestinal tract, lower gastrointestinal tract, and oral capsule-based FMT (**Figure 1**). There are meta-analysis statistics on the therapeutic effect of the FMT administration route on IBD, and the conclusions are inconsistent (54, 85). However, we believe it is challenging to assess the effect of the administration route on efficacy due to the use of different FMT protocols between studies. However, several routes of administration in the upper gastrointestinal tract (nasogastric, nasoduodenal, and nasojejunal tube) are inevitably affected by the distance from inflammation and the influence of proximal gastrointestinal secretions. Furthermore, in addition to the inherent risks of endoscopy, such as perforation, they may lead to symptoms such as aspiration pneumonia (31), vomiting (17, 31), runny nose, sore throat (59), and reflux (86).

Lower gastrointestinal administration routes mainly include enema and colonoscopy routes. Although patients can perform FMT with self-enema at home, possible related adverse events such as rectal abscess (18) and left-sided abdominal fullness (50) have been reported. The administration of FMT *via* colonoscopy has the advantage of transporting more stool to the site of inflammation (87). Moreover, it can detect the inflammatory state of the intestinal mucosa and compare the mucosal healing after treatment (88). However, frequent colonoscopies can also bring mental stress to patients. Therefore, an oral capsule-based FMT has recently attracted attention. In previous studies, oral capsule FMT was generally used as an adjunctive therapy (21, 89–91). A small sample-sized open-label study showed that oral capsule FMT can temporarily improve patients' quality of life and reduce calprotectin (92). A double-blinded RCT (22) report in 2021 showed that oral lyophilized capsule FMT combined with antibiotic pre-treatment was significantly more effective than the placebo treatment (p = 0.027). In addition, no significant difference in FMT maintenance between an enema and oral capsule delivery was observed (91). Therefore, oral FMT capsules are a promising drug delivery option for long-term use to maintain a stable gut microbiota structure (21). More RCTs on oral capsule-administered FMT with high acceptability are required.

There are also some less frequently used methods, such as transendocopic enteral tubing (26, 93) and ercutaneous endoscopic cecostomy (94). It is also important to choose a method acceptable to the patient because patient compliance is the key to treatment.

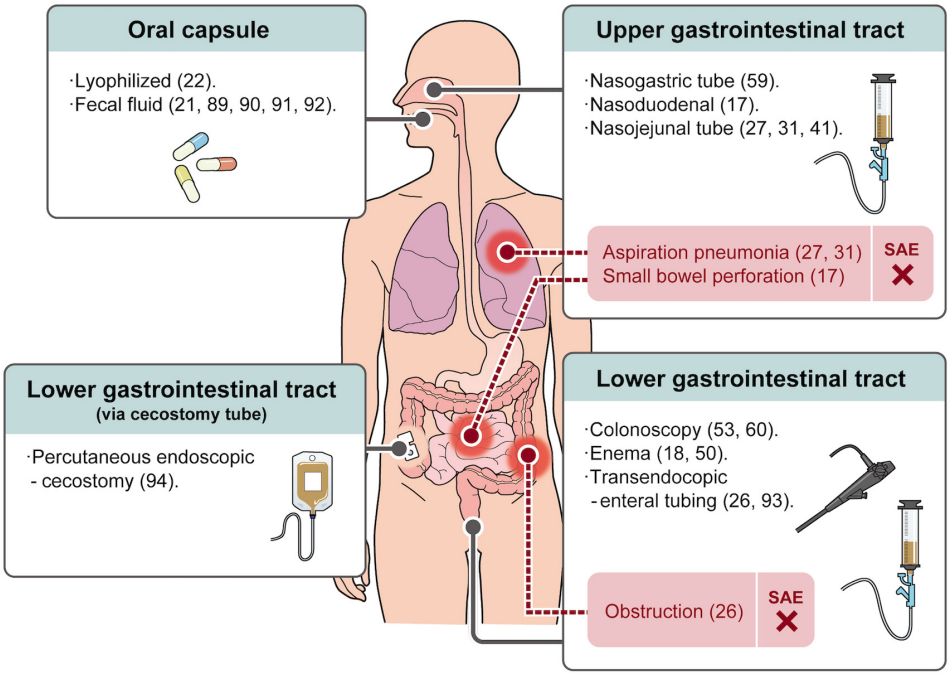


FIGURE 1
Route administration of FMT for IBD treatment and serious adverse events (SAE).

Treatment maintenance

Currently, the long-term maintenance effect of FMT in the treatment of IBD is unclear. Researchers have tried to maintain the diversity of gut microbiota through post-intervention of FMT to achieve the long-term efficacy of FMT in treating IBD. Repeating FMT several times after reaching remission to stabilize the intestinal environment is one method (95). An RCT study published in 2015 showed that of the nine patients who achieved clinical remission at week 7, eight were still in remission at week 52 with a monthly FMT interval (18). The article published by He et al. showed that the clinical remission rate (52%) after the initial FMT decreased slowly with the sustained remission rate after multiple FMT boosters, and 22.7% of patients were still in remission at 18 months (26). An RCT study randomly assigned 61 patients in remission to FMT to receive FMT or placebo administrations every 8 weeks for 48 weeks to determine the long-term maintenance effect of FMT. The results showed that FMT administration during the maintenance phase of UC patients could prolong the clinical, endoscopic, and histological remission of patients (96). It was further investigated that a second course of FMT consolidation therapy within 1 month could maintain the benefits of FMT in CD patients (65).

There are also attempts to maintain patient treatment outcomes in more light-hearted ways. For example, Wei et al. achieved the effect of slowing the loss of colonized microbiota by the oral administration of pectin that can be fermented into short-chain fatty acids and beneficial to intestinal microbiota (71). In our research group, we are conducting a double-blinded controlled RCT study to consolidate the efficacy of FMT in patients with UC by giving patients oral alginic acid (97). It is hoped that further research on the maintenance of efficacy will increase patients' expectations and confidence in FMT for the treatment of IBD.

Donor stool

The first major hurdle in FMT treatment is donor stool selection and preparation. Not only the transmission of pathogens can occur during FMT, as the impact of intestinal microbiota on patients with mental and endocrine diseases has been reported (9, 11). Hence, the screening of healthy fecal providers is currently a primary task. Many institutions also propose and continuously improve screening criteria according to the living background and the occurrence of epidemics in their respective regions (6, 14, 98, 99). Donor screening can be performed using questionnaires, blood tests, and stool tests. The basic questionnaire section should exclude infection risk factors such as HIV infection, exposure to viral hepatitis, high-risk sexual behavior, tattooing or piercing within 6 months, history of incarceration, travel history to areas endemic for infectious diseases, known history of infection, and risk factors for multi-drug resistant organisms. There are also potential microbiota-mediated conditions which should be determined,

such as whether the donor has gastrointestinal disease, atopic disease, autoimmune disease, chronic pain syndrome, malignancy, and surgical history, and questions about the donor's metabolic system, neurological system, mental, and medication conditions. Blood tests should mainly include complete blood count with differential, hepatic function, HIV, hepatitis, treponema pallidum, and parasite testing. Fecal testing should mainly include *C. difficile* toxin A/B, *Campylobacter*, *Salmonella*, *Shigella*, *Vibrio*, *Escherichia coli*, *Helicobacter pylori*, rotavirus, norovirus, adenovirus, COVID-19, and monkeypox. A more detailed screening should ensure patient safety but will reduce screening pass rates and increase screening costs. Therefore, maintaining a balance between the three methods is a question that needs to be considered. Of course, the relationship between FMT efficacy and donor feces is also a problem to be explored.

Relationship between patients and donor

As far as we know, there are mainly two ways to obtain feces: one is from relatives or friends recommended by the patient and the other is from undirected stranger donors. Since some ethical, esthetic, and psychological barriers can be avoided by accepting stool from a donor recommended by the patient, the patient may be more receptive to the treatment. In addition, we previously reported higher long-term non-relapse rates for the treatment of UC with the stools of siblings compared to the stools of parents and offspring ($p = 0.007$) (60). The gut microbiota of siblings may be similar to the healthy microbiota state of the patient before IBD (100), and species originally present in the recipient's microbiota are more likely to colonize the patient's intestinal mucosa stably.

However, a meta-analysis showed no difference in the efficacy of feces from undirected stranger donors or patient-recommended donors for patients with CDI (101). Compared with patient-recommended donors, the undirected donor format has the advantages of avoiding screening time and starting treatment quickly, protecting the privacy of donor candidates, and saving costs for serving multiple patients after the successful screening. Therefore, doctors are more inclined to use the undirected donation of stranger feces.

Fresh, frozen, or lyophilized stool

Using frozen stool can reduce the cost of FMT and increase the timeliness and safety of treatment. In addition, it has been reported that although freezing reduced the overall viability of the fecal microbiota by approximately 25%, the live microbiota composition was not significantly different from that of fresh feces (102). Cryopreservation of fecal samples for 6 months did not affect colony forming unit counts for some bacterial groups (*E. coli*, total coliforms, *Bifidobacteria*, total aerobes, *Lactobacilli*, or total anaerobic bacteria) (103). Therefore, frozen feces did not affect the efficacy of FMT in the treatment of CDI (103–105). However, there are meta-analysis statistics that the preservation status before FMT has an unstable impact on IBD (85, 106). UC patients treated with fresh donor stool had a lower pooled clinical remission rate (15%) than those with frozen stool (42%). Moreover, for CD patients, the remission rate for FMT with fresh stool was 36% higher than that with frozen stool (28%). Recently, the use of oral-fecal lyophilized capsules is a new method of drug delivery and storage. This delivery method requires that the capsules are always stored at −20°C and should not be directly transferred between refrigerators. If transfer is required, it should be kept on dry ice at all times to maintain the microbiota's viability (21). It is difficult to link these three stool processes before drug delivery to IBD efficacy without RCTs that control for other potentially confounding variables.

Donor microbiota characteristics

Donor biomarkers which are best for IBD have not been definitively reported. However, it has been reported that the microbial diversity of donor feces is associated with the efficacy of FMT in the treatment of IBD (31, 107). While testing the relationship between the abundance of the single donor's gut microbial species and the therapeutic effect, some studies have also attempted to transplant the mixed feces of multiple people into the patient's gut and achieved a significant effect compared to the placebo group (19, 20). However, there seems to be a super-donor phenomenon in the treatment of UC with FMT in previous studies. In 2015, Moayyedi et al. found that seven of nine patients with UC who achieved remission after FMT received stool from the same donor (18). Moreover, the efficacy rate of the multiple donors' fecal microbiota transplant containing the donor number D054 was higher than that in patients who received multiple donors' fecal transplant that did not contain the donor D054′s feces ($p = 0.054$) (19). From the current evidence, increasing the abundance of microbiota may not be the only condition for inducing remission. Further analysis of the study showed that a high abundance of specific species of *Bacteroides* (*B. fragilis* and *B. finegoldii*) in mixed donor feces was associated with the efficacy of FMT in patients with UC (108).

Fecal microbiota in patients with IBD is not only less diverse (109) but also often lacks commensal bacteria (110). For example, the bacterial phylum *Bacteroidota* (83, 111, 112), which produces zwitterionic capsular polysaccharides that suppress inflammation by regulating T cells, and *Bacillota*, which produces host-beneficial short chain fatty acids (SCFAs), are lacking. Therefore, some of the special bacteria carried in the guts of super-donors may colonize the guts of IBD patients if they supplemented the lost bacteria, and restoring the microbiota to a pre-morbid state could be beneficial. Reports

showed that the presence of the bacterial genus *Ruminococcus* in the feces of the donors was associated with the induction of remission (18, 107). UC patients who achieved long-term FMT maintenance response showed a similar profile of microbiota to donors, especially *Bacteroidetes* species (60). In accordance with our previous report that dysbiosis of fecal microbiota in patients with UC is associated with loss of *Bacteroides* species diversity (83), we identified a relative abundance of 12 key *Bacteroidetes* species inversely associated with UC activity (112). The proportion of *Bacteroidetes* in feces was significantly increased in patients who underwent FMT (53). Therefore, the enrichment of *Bacteriodetes* in donor feces is one of our future research directions. In addition, different reports have shown that the intestinal microbiota of patients with CD has undergone inconsistent changes, such as a decrease of *Bacillota* (113), *Bididobacterium* (114), *Enterobacteriaceae* (115), or *Lactobacillus* (116), or an increase of *Helicobacter* species (117). In patients with UC and pouchitis, decreases of *Roseburia hominis* and *Faecalibacterium prausnitzii* (118) and absence of *Streptococcus* species (119) were also found. Therefore, determining the change of intestinal microbiota in IBD patients is a prerequisite for FMT treatment for IBD that cannot be ignored.

Although the results of the current study have not been able to establish the best donor guidelines for FMT, we can predict that in the future, the stool for the treatment of IBD will be selective and even customized.

Conclusion

From the current research results, the effectiveness and safety of FMT in treating IBD are beyond doubt. However, the details of the entire execution process are still up for debate. New techniques for FMT are constantly being updated, and study has suggested that Sterile Fecal Filtrate Transfer (which only contains bacterial debris, proteins, antimicrobial compounds, metabolites, and oligonucleotides/DNA) can also eliminate symptoms and restore normal bowel habits in patients with CDI (120). It is unknown which substance in the gut produces this therapeutic effect. SCFA-producing bacteria are typically reduced in the gut of patients with IBD compared to healthy individuals (121). However, butyrate was increased in patients with UC who responded to FMT (122). Whether butyrate plays a major role in the treatment of FMT is unknown due to the lack of relevant clinical research data. Therefore, it is necessary to interpret the mechanism of FMT in the treatment of IBD from the perspectives of microbiology, immunology, and metabolism and propose a one-to-one customization scheme with a narrow-spectrum. Finally, while continuously optimizing the curative effect and maintaining the therapeutic outcome, it is essential to find the most acceptable route of administration for patients. In conclusion, more results from future studies are needed to obtain a perfect treatment of IBD using FMT.

Author contributions

All authors contributed to the generation of the concept, wrote and edited the manuscript, and approved the submitted version.

Funding

This research was funded by Japan Agency for Medical Research and Development (Issue tracking number: 22ae0121038h0002).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Heintz-Buschart A, Wilmes P. Human gut microbiome: function matters. *Trends Microbiol.* (2018) 26:563–74. doi: 10.1016/j.tim.2017.11.002

2. Nishida A, Nishino K, Sakai K, Owaki Y, Noda Y, Imaeda H. Can control of gut microbiota be a future therapeutic option for inflammatory bowel disease? *World J Gastroenterol.* (2021) 27:3317–26. doi: 10.3748/wjg.v27.i23.3317

3. Akutko K, Stawarski A. Probiotics, prebiotics and synbiotics in inflammatory bowel diseases. *J Clin Med.* (2021) 10:2466. doi: 10.3390/jcm10112466

4. Triantafillidis JK, Merikas E, Georgopoulos F. Current and emerging drugs for the treatment of inflammatory bowel disease. *Drug Des Dev Ther.* (2011) 5:185–210. doi: 10.2147/DDDT.S11290

5. Borody TJ, Paramsothy S, Agrawal G. Fecal microbiota transplantation: indications, methods, evidence, and future directions. *Curr Gastroenterol Rep.* (2013) 15:337. doi: 10.1007/s11894-013-0337-1

6. Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, et al. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut.* (2017) 66:569–80. doi: 10.1136/gutjnl-2016-313017

7. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical practice guidelines for Clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis.* (2018) 66:e1–48. doi: 10.1093/cid/cix1085

8. Quraishi MN, Widlak M, Bhala N, Moore D, Price M, Sharma N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. *Aliment Pharmacol Ther.* (2017) 46:479–93. doi: 10.1111/apt.14201

9. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology.* (2012) 143:913–6.e7. doi: 10.1053/j.gastro.2012.06.031

10. Halkjaer SI, Christensen AH, Lo BZS, Browne PD, Günther S, Hansen LH, et al. Faecal microbiota transplantation alters gut microbiota in patients with irritable bowel syndrome: results from a randomised, double-blind placebo-controlled study. *Gut.* (2018) 67:2107–15. doi: 10.1136/gutjnl-2018-316434

11. Kang DW, Adams JB, Coleman DM, Pollard EL, Maldonado J, McDonough-Means S, et al. Long-term benefit of microbiota Transfer Therapy on autism symptoms and gut microbiota. *Sci Rep.* (2019) 9:5821. doi: 10.1038/s41598-019-42183-0

12. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science.* (2021) 371:602–9. doi: 10.1126/science.abb5920

13. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science.* (2021) 371:595–602. doi: 10.1126/science.abf3363

14. OpenBiome. *The OpenBiome Quality and Safety Program*. Medford, MA: OpenBiome (2021).

15. Bennet JD, Brinkman M. Treatment of ulcerative colitis by implantation of normal colonic flora. *Lancet.* (1989) 1:164. doi: 10.1016/s0140-6736(89)91183-5

16. Borody TJ, George L, Andrews P, Brandl S, Noonan S, Cole P, et al. Bowel-flora alteration: a potential cure for inflammatory bowel disease and irritable bowel syndrome? *Med J Aust.* (1989) 150:604. doi: 10.5694/j.1326-5377.1989.tb136704.x

17. Rossen NG, Fuentes S, van der Spek MJ, Tijssen JG, Hartman JH, Duflou A, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology.* (2015) 149:110–8.e4. doi: 10.1053/j.gastro.2015.03.045

18. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology.* (2015) 149:102–9.e6. doi: 10.1053/j.gastro.2015.04.001

19. Paramsothy S, Kamm MA, Kaakoush NO, Walsh AJ, van den Bogaerde J, Samuel D, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet.* (2017) 389:1218–28. doi: 10.1016/S0140-6736(17)30182-4

20. Costello SP, Hughes PA, Waters O, Bryant RV, Vincent AD, Blatchford P, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. *JAMA.* (2019) 321:156–64. doi: 10.1001/jama.2018.20046

21. Crothers JW, Chu ND, Nguyen LTT, Phillips M, Collins C, Fortner K, et al. Daily, oral FMT for long-term maintenance therapy in ulcerative colitis: results of a single-center, prospective, randomized pilot study. *BMC Gastroenterol.* (2021) 21:281. doi: 10.1186/s12876-021-01856-9

22. Haifer C, Paramsothy S, Kaakoush NO, Saikal A, Ghaly S, Yang T, et al. Lyophilised oral faecal microbiota transplantation for ulcerative colitis (Lotus): a randomised, double-blind, placebo-controlled trial. *Lancet Gastroenterol Hepatol.* (2022) 7:141–51. doi: 10.1016/S2468-1253(21)00400-3

23. El Hage Chehade N, Ghoneim S, Shah S, Chahine A, Mourad FH, Francis FF, et al. Efficacy of fecal microbiota transplantation in the treatment of active ulcerative colitis: a systematic review and meta-analysis of double-blind randomized controlled trials. *Inflam Bowel Dis.* (2022) [Epub ahead of print]. doi: 10.1093/ibd/izac135

24. Fang H, Fu L, Li X, Lu C, Su Y, Xiong K, et al. Long-term efficacy and safety of monotherapy with a single fresh fecal microbiota transplant for recurrent active ulcerative colitis: a prospective randomized pilot study. *Microb Cell Fact.* (2021) 20:18. doi: 10.1186/s12934-021-01513-6

25. Cui B, Feng Q, Wang H, Wang M, Peng Z, Li P, et al. Fecal microbiota transplantation through mid-gut for refractory Crohn's disease: safety, feasibility, and efficacy trial results. *J Gastroenterol Hepatol.* (2015) 30:51–8. doi: 10.1111/jgh.12727

26. He Z, Li P, Zhu J, Cui B, Xu L, Xiang J, et al. Multiple fresh fecal microbiota transplants induces and maintains clinical remission in Crohn's disease complicated with inflammatory mass. *Sci Rep.* (2017) 7:4753. doi: 10.1038/s41598-017-04984-z

27. Xiang L, Ding X, Li Q, Wu X, Dai M, Long C, et al. Efficacy of faecal microbiota transplantation in Crohn's disease: a new target treatment? *Microb Biotechnol.* (2020) 13:760–9. doi: 10.1111/1751-7915.13536

28. Zou M, Jie Z, Cui B, Wang H, Feng Q, Zou Y, et al. Fecal microbiota transplantation results in bacterial strain displacement in patients with inflammatory bowel diseases. *FEBS Open Bio.* (2020) 10:41–55. doi: 10.1002/2211-5463.12744

29. Vaughn BP, Vatanen T, Allegretti JR, Bai A, Xavier RJ, Korzenik J, et al. Increased intestinal microbial diversity following fecal microbiota transplant for active Crohn's disease. *Inflamm Bowel Dis.* (2016) 22:2182–90. doi: 10.1097/MIB.0000000000000893

30. Gutin L, Piceno Y, Fadrosh D, Lynch K, Zydek M, Kassam Z, et al. Fecal microbiota transplant for Crohn disease: a study evaluating safety, efficacy, and microbiome profile. *U Eur Gastroenterol J.* (2019) 7:807–14. doi: 10.1177/2050640619845986

31. Vermeire S, Joossens M, Verbeke K, Wang J, Machiels K, Sabino J, et al. Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. *J Crohns Colitis.* (2016) 10:387–94. doi: 10.1093/ecco-jcc/jjv203

32. Sokol H, Landman C, Seksik P, Berard L, Montil M, Nion-Larmurier I, et al. Fecal microbiota transplantation to maintain remission in Crohn's disease: a pilot randomized controlled study. *Microbiome.* (2020) 8:12. doi: 10.1186/s40168-020-0792-5

33. Cheng F, Huang Z, Wei W, Li Z. Fecal microbiota transplantation for Crohn's disease: a systematic review and meta-analysis. *Tech Coloproctol.* (2021) 25:495–504. doi: 10.1007/s10151-020-02395-3

34. Lightner AL, Mathis KL, Dozois EJ, Hahnsloser D, Loftus EV, Raffals LE, et al. Results at UP to 30 years after ileal pouch-anal anastomosis for chronic ulcerative colitis. *Inflamm Bowel Dis.* (2017) 23:781–90. doi: 10.1097/MIB.0000000000001061

35. Stallmach A, Lange K, Buening J, Sina C, Vital M, Pieper DH. Fecal microbiota transfer in patients with chronic antibiotic-refractory pouchitis. *Am J Gastroenterol.* (2016) 111:441–3. doi: 10.1038/ajg.2015.436

36. Kousgaard SJ, Michaelsen TY, Nielsen HL, Kirk KF, Brandt J, Albertsen M, et al. Clinical results and microbiota changes after faecal microbiota transplantation for chronic pouchitis: a pilot study. *Scand J Gastroenterol.* (2020) 55:421–9. doi: 10.1080/00365521.2020.1748221

37. Fang S, Kraft CS, Dhere T, Srinivasan J, Begley B, Weinstein D, et al. Successful treatment of chronic Pouchitis utilizing fecal microbiota transplantation (FMT): a case report. *Int J Colorectal Dis.* (2016) 31:1093–4. doi: 10.1007/s00384-015-2428-y

38. Nishida A, Imaeda H, Inatomi O, Bamba S, Sugimoto M, Andoh A. The efficacy of fecal microbiota transplantation for patients with chronic pouchitis: a case series. *Clin Case Rep.* (2019) 7:782–8. doi: 10.1002/ccr3.2096

39. Herfarth H, Barnes EL, Long MD, Isaacs KL, Leith T, Silverstein M, et al. Combined endoscopic and oral fecal microbiota transplantation in patients with antibiotic-dependent pouchitis: low clinical efficacy due to low donor microbial engraftment. *Inflamm Intest Dis.* (2019) 4:1–6. doi: 10.1159/000497042

40. Landy J, Walker AW, Li JV, Al-Hassi HO, Ronde E, English NR, et al. Variable alterations of the microbiota, without metabolic or immunological change, following faecal microbiota transplantation in patients with chronic pouchitis. *Sci Rep.* (2015) 5:12955. doi: 10.1038/srep12955

41. Selvig D, Piceno Y, Terdiman J, Zydek M, Umetsu SE, Balitzer D, et al. Fecal microbiota transplantation in pouchitis: clinical, endoscopic, histologic, and microbiota results from a pilot study. *Dig Dis Sci.* (2020) 65:1099–106. doi: 10.1007/s10620-019-05715-2

42. Schmid M, Frick JS, Malek N, Goetz M. Successful treatment of pouchitis with vedolizumab, but not fecal microbiota transfer (FMT), after proctocolectomy in ulcerative colitis. *Int J Colorectal Dis.* (2017) 32:597–8. doi: 10.1007/s00384-017-2761-4

43. Karjalainen EK, Renkonen-Sinisalo L, Satokari R, Mustonen H, Ristimäki A, Arkkila P, et al. Fecal microbiota transplantation in chronic pouchitis: a

randomized, parallel, double-blinded clinical trial. *Inflamm Bowel Dis.* (2021) 27:1766–72. doi: 10.1093/ibd/izab001

44. Karolewska-Bochenek K, Grzesiowski P, Banaszkiewicz A, Gawronska A, Kotowska M, Dziekiewicz M, et al. A two-week fecal microbiota transplantation course in pediatric patients with inflammatory bowel disease. *Adv Exp Med Biol.* (2018) 1047:81–7. doi: 10.1007/5584_2017_123

45. Goyal A, Yeh A, Bush BR, Firek BA, Siebold LM, Rogers MB, et al. Safety, clinical response, and microbiome findings following fecal microbiota transplant in children with inflammatory bowel disease. *Inflamm Bowel Dis.* (2018) 24:410–21. doi: 10.1093/ibd/izx035

46. Vandenplas Y, Veereman G, van der Werff Ten Bosch J, Goossens A, Pierard D, Samsom JN. Fecal microbial transplantation in Early-onset colitis: caution advised. *J Pediatr Gastroenterol Nutr.* (2015) 61:e12–4. doi: 10.1097/MPG.0000000000000281

47. Kumagai H, Yokoyama K, Imagawa T, Inoue S, Tulyeu J, Tanaka M, et al. Failure of fecal microbiota transplantation in a three-year-old child with severe refractory ulcerative colitis. *Pediatr Gastroenterol Hepatol Nutr.* (2016) 19:214–20. doi: 10.5223/pghn.2016.19.3.214

48. Damman CJ, Brittnacher MJ, Westerhoff M, Hayden HS, Radey M, Hager KR, et al. Low Level Engraftment and Improvement following a Single Colonoscopic Administration of Fecal microbiota to Patients with Ulcerative Colitis. *PLoS One.* (2015) 10:e0133925. doi: 10.1371/journal.pone.0133925

49. Cui B, Li P, Xu L, Zhao Y, Wang H, Peng Z, et al. Step-up fecal microbiota transplantation strategy: a pilot study for steroid-dependent ulcerative colitis. *J Transl Med.* (2015) 13:298. doi: 10.1186/s12967-015-0646-2

50. Kunde S, Pham A, Bonczyk S, Crumb T, Duba M, Conrad H, et al. Safety, tolerability, and clinical response after fecal transplantation in children and young adults with ulcerative colitis. *J Pediatr Gastroenterol Nutr.* (2013) 56:597–601. doi: 10.1097/MPG.0b013e318292fa0d

51. Suskind DL, Singh N, Nielson H, Wahbeh G. Fecal microbial transplant via nasogastric tube for active pediatric ulcerative colitis. *J Pediatr Gastroenterol Nutr.* (2015) 60:27–9. doi: 10.1097/MPG.0000000000000544

52. Zhang T, Cui B, Li P, He Z, Long C, Wei L, et al. Short-term surveillance of cytokines and C-reactive protein cannot predict efficacy of fecal microbiota transplantation for ulcerative colitis. *PLoS One.* (2016) 11:e0158227. doi: 10.1371/journal.pone.0158227

53. Ishikawa D, Sasaki T, Osada T, Kuwahara-Arai K, Haga K, Shibuya T, et al. Changes in intestinal microbiota following combination therapy with fecal microbial transplantation and antibiotics for ulcerative colitis. *Inflamm Bowel Dis.* (2017) 23:116–25. doi: 10.1097/MIB.0000000000000975

54. Paramsothy S, Paramsothy R, Rubin DT, Kamm MA, Kaakoush NO, Mitchell HM, et al. Faecal microbiota transplantation for inflammatory bowel disease: a systematic review and meta-analysis. *J Crohns Colitis.* (2017) 11:1180–99. doi: 10.1093/ecco-jcc/jjx063

55. Baxter M, Colville A. Adverse events in faecal microbiota transplant: a review of the literature. *J Hosp Infect.* (2016) 92:117–27. doi: 10.1016/j.jhin.2015.10.024

56. Wang S, Xu M, Wang W, Cao X, Piao M, Khan S, et al. Systematic review: adverse events of fecal microbiota transplantation. *PLoS One.* (2016) 11:e0161174. doi: 10.1371/journal.pone.0161174

57. Wang H, Cui B, Li Q, Ding X, Li P, Zhang T, et al. The safety of fecal microbiota transplantation for Crohn's disease: findings from A long-term study. *Adv Ther.* (2018) 35:1935–44. doi: 10.1007/s12325-018-0800-3

58. Yang Z, Bu C, Yuan W, Shen Z, Quan Y, Wu S, et al. Fecal microbiota transplant via endoscopic delivering through small intestine and colon: no difference for Crohn's disease. *Dig Dis Sci.* (2020) 65:150–7. doi: 10.1007/s10620-019-05751-y

59. Suskind DL, Brittnacher MJ, Wahbeh G, Shaffer ML, Hayden HS, Qin X, et al. Fecal microbial transplant effect on clinical outcomes and fecal microbiome in active Crohn's disease. *Inflamm Bowel Dis.* (2015) 21:556–63. doi: 10.1097/MIB.0000000000000307

60. Okahara K, Ishikawa D, Nomura K, Ito S, Haga K, Takahashi M, et al. Matching between donors and ulcerative colitis patients is important for long-term maintenance after fecal microbiota transplantation. *J Clin Med.* (2020) 9:1650. doi: 10.3390/jcm9061650

61. Chen M, Liu XL, Zhang YJ, Nie YZ, Wu KC, Shi YQ. Efficacy and safety of fecal microbiota transplantation by washed preparation in patients with moderate to severely active ulcerative colitis. *J Dig Dis.* (2020) 21:621–8. doi: 10.1111/1751-2980.12938

62. Angelberger S, Reinisch W, Makristathis A, Lichtenberger C, Dejaco C, Papay P, et al. Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal microbiota transplantation. *Am J Gastroenterol.* (2013) 108:1620–30. doi: 10.1038/ajg.2013.257

63. Ren R, Sun G, Yang Y, Peng L, Zhang X, Wang S, et al. [A pilot study of treating ulcerative colitis with fecal microbiota transplantation]. *Zhonghua Nei Ke Za Zhi.* (2015) 54:411–5.

64. Blanchaert C, Strubbe B, Peeters H. Fecal microbiota transplantation in ulcerative colitis. *Acta Gastroenterol Belg.* (2019) 82:519–28.

65. Li P, Zhang T, Xiao Y, Tian L, Cui B, Ji G, et al. Timing for the second fecal microbiota transplantation to maintain the long-term benefit from the first treatment for Crohn's disease. *Appl Microbiol Biotechnol.* (2019) 103:349–60. doi: 10.1007/s00253-018-9447-x

66. Grewal CS, Sood A, Mehta V, Sood N, Midha V, Mahajan R, et al. Role of Fecal Microbiota Transplantation in Patients with Steroid Dependant Ulcerative Colitis: 2455. *Am J Gastroenterol.* (2016) 111:S1252–61. doi: 10.1038/ajg.2016.385

67. Hohmann EL, Ananthakrishnan AN, Deshpande V. Case records of the Massachusetts general hospital. case 25-2014. a 37-year-old man with ulcerative colitis and bloody diarrhea. *N Engl J Med.* (2014) 371:668–75. doi: 10.1056/NEJMcpc1400842

68. Uygun A, Ozturk K, Demirci H, Oger C, Avci IY, Turker T, et al. Fecal microbiota transplantation is a rescue treatment modality for refractory ulcerative colitis. *Medicine.* (2017) 96:e6479. doi: 10.1097/MD.0000000000006479

69. Jacob V, Crawford C, Cohen-Mekelburg S, Viladomiu M, Putzel GG, Schneider Y, et al. Single delivery of high-diversity fecal microbiota preparation by colonoscopy is safe and effective in increasing microbial diversity in active ulcerative colitis. *Inflamm Bowel Dis.* (2017) 23:903–11. doi: 10.1097/MIB.0000000000001132

70. Kump PK, Gröchenig HP, Lackner S, Trajanoski S, Reicht G, Hoffmann KM, et al. Alteration of intestinal dysbiosis by fecal microbiota transplantation does not induce remission in patients with chronic active ulcerative colitis. *Inflamm Bowel Dis.* (2013) 19:2155–65. doi: 10.1097/MIB.0b013e31829ea325

71. Wei Y, Gong J, Zhu W, Tian H, Ding C, Gu L, et al. Pectin enhances the effect of fecal microbiota transplantation in ulcerative colitis by delaying the loss of diversity of gut flora. *BMC Microbiol.* (2016) 16:255. doi: 10.1186/s12866-016-0869-2

72. Wei Y, Zhu W, Gong J, Guo D, Gu L, Li N, et al. Fecal microbiota transplantation improves the quality of life in patients with inflammatory bowel disease. *Gastroenterol Res Pract.* (2015) 2015:517597. doi: 10.1155/2015/517597

73. Quera R, Espinoza R, Estay C, Rivera D. Bacteremia as an adverse event of fecal microbiota transplantation in a patient with Crohn's disease and recurrent Clostridium difficile infection. *J Crohns Colitis.* (2014) 8:252–3. doi: 10.1016/j.crohns.2013.10.002

74. DeFilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N Engl J Med.* (2019) 381:2043–50. doi: 10.1056/NEJMoa1910437

75. Chen C, Chen L, Sun D, Li C, Xi S, Ding S, et al. Adverse events of intestinal microbiota transplantation in randomized controlled trials: a systematic review and meta-analysis. *Gut Pathog.* (2022) 14:20. doi: 10.1186/s13099-022-00491-3

76. Staley C, Kaiser T, Vaughn BP, Graiziger C, Hamilton MJ, Kabage AJ, et al. Durable long-term bacterial engraftment following encapsulated fecal microbiota transplantation to treat clostridium difficile infection. *mBio.* (2019) 10:e01586–19. doi: 10.1128/mBio.01586-19

77. Jalanka J, Mattila E, Jouhten H, Hartman J, de Vos WM, Arkkila P, et al. Long-term effects on luminal and mucosal microbiota and commonly acquired taxa in faecal microbiota transplantation for recurrent Clostridium difficile infection. *BMC Med.* (2016) 14:155. doi: 10.1186/s12916-016-0698-z

78. Mizuno S, Nanki K, Matsuoka K, Saigusa K, Ono K, Arai M, et al. Single fecal microbiota transplantation failed to change intestinal microbiota and had limited effectiveness against ulcerative colitis in Japanese patients. *Intest Res.* (2017) 15:68–74. doi: 10.5217/ir.2017.15.1.68

79. Mocanu V, Rajaruban S, Dang J, Kung JY, Deehan EC, Madsen KL. Repeated fecal microbial transplantations and antibiotic pre-treatment are linked to improved clinical response and remission in inflammatory bowel disease: a systematic review and pooled proportion meta-analysis. *J Clin Med.* (2021) 10:959. doi: 10.3390/jcm10050959

80. Nishikawa Y, Sato N, Tsukinaga S, Uchiyama K, Koido S, Ishikawa D, et al. Long-term outcomes of antibiotic combination therapy for ulcerative colitis. *Ther Adv Chronic Dis.* (2021) 12:20406223211028790. doi: 10.1177/20406223211028790

81. Ohkusa T, Kato K, Terao S, Chiba T, Mabe K, Murakami K, et al. Newly developed antibiotic combination therapy for ulcerative colitis: a double-blind placebo-controlled multicenter trial. *Am J Gastroenterol.* (2010) 105:1820–9. doi: 10.1038/ajg.2010.84

82. Ji SK, Yan H, Jiang T, Guo CY, Liu JJ, Dong SZ, et al. Preparing the gut with antibiotics enhances gut microbiota reprogramming efficiency by promoting

xenomicrobiota colonization. *Front Microbiol.* (2017) 8:1208. doi: 10.3389/fmicb.2017.01208

83. Ishikawa D, Sasaki T, Takahashi M, Kuwahara-Arai K, Haga K, Ito S, et al. The microbial composition of Bacteroidetes species in ulcerative colitis is effectively improved by combination therapy with fecal microbiota transplantation and antibiotics. *Inflamm Bowel Dis.* (2018) 24:2590–8. doi: 10.1093/ibd/izy266

84. Kao D, Hotte N, Gillevet P, Madsen K. Fecal microbiota transplantation inducing remission in Crohn's colitis and the associated changes in fecal microbial profile. *J Clin Gastroenterol.* (2014) 48:625–8. doi: 10.1097/MCG.0000000000000131

85. Fang H, Fu L, Wang J. Protocol for fecal microbiota transplantation in inflammatory bowel disease: a systematic review and meta-analysis. *BioMed Res Int.* (2018) 2018:8941340. doi: 10.1155/2018/8941340

86. Wang Y, Ren R, Sun G, Peng L, Tian Y, Yang Y. Pilot study of cytokine changes evaluation after fecal microbiota transplantation in patients with ulcerative colitis. *Int Immunopharmacol.* (2020) 85:106661. doi: 10.1016/j.intimp.2020.106661

87. Narula N, Kassam Z, Yuan Y, Colombel JF, Ponsioen C, Reinisch W, et al. Systematic review and meta-analysis: fecal microbiota transplantation for treatment of active ulcerative colitis. *Inflamm Bowel Dis.* (2017) 23:1702–9. doi: 10.1097/MIB.0000000000001228

88. Allegretti J, Eysenbach LM, El-Nachef N, Fischer M, Kelly C, Kassam Z. The current landscape and lessons from fecal microbiota transplantation for inflammatory bowel disease: past, present, and future. *Inflamm Bowel Dis.* (2017) 23:1710–7. doi: 10.1097/MIB.0000000000001247

89. Adler E, Tabaa A, Kassam Z, Zydek M, Terdiman J, El-Nachef N. Capsule-delivered fecal microbiota transplant is safe and well tolerated in patients with ulcerative colitis. *Dig Dis Sci.* (2019) 64:2452–4. doi: 10.1007/s10620-019-05596-5

90. Chu ND, Crothers JW, Nguyen LTT, Kearney SM, Smith MB, Kassam Z, et al. Dynamic colonization of microbes and their functions after fecal microbiota transplantation for inflammatory bowel disease. *mBio.* (2021) 12:e00975–21. doi: 10.1128/mBio.00975-21

91. Smith BJ, Piceno Y, Zydek M, Zhang B, Syriani LA, Terdiman JP, et al. Strain-resolved analysis in a randomized trial of antibiotic pretreatment and maintenance dose delivery mode with fecal microbiota transplant for ulcerative colitis. *Sci Rep.* (2022) 12:5517. doi: 10.1038/s41598-022-09307-5

92. Cold F, Browne PD, Günther S, Halkjaer SI, Petersen AM, Al-Gibouri Z, et al. Multidonor FMT capsules improve symptoms and decrease fecal calprotectin in ulcerative colitis patients while treated - An open-label pilot study. *Scand J Gastroenterol.* (2019) 54:289–96. doi: 10.1080/00365521.2019.1585939

93. Chen HT, Huang HL, Xu HM, Luo QL, He J, Li YQ, et al. Fecal microbiota transplantation ameliorates active ulcerative colitis. *Exp Ther Med.* (2020) 19:2650–60. doi: 10.3892/etm.2020.8512

94. Ni X, Fan S, Zhang Y, Wang Z, Ding L, Li Y, et al. Coordinated hospital-home fecal microbiota transplantation via percutaneous endoscopic cecostomy for recurrent steroid-dependent ulcerative colitis. *Gut Liver.* (2016) 10:975–80. doi: 10.5009/gnl15456

95. Li Q, Zhang T, Ding X, Xiang L, Cui B, Buch H, et al. Enhancing patient adherence to fecal microbiota transplantation maintains the long-term clinical effects in ulcerative colitis. *Eur J Gastroenterol Hepatol.* (2020) 32:955–62. doi: 10.1097/MEG.0000000000001725

96. Sood A, Mahajan R, Singh A, Midha V, Mehta V, Narang V, et al. Role of faecal microbiota transplantation for maintenance of remission in patients with ulcerative colitis: a pilot study. *J Crohns Colitis.* (2019) 13:1311–7. doi: 10.1093/ecco-jcc/jjz060

97. Ishikawa D, Zhang X, Nomura K, Seki N, Haraikawa M, Haga K, et al. A randomized placebo-controlled trial of combination therapy with post-triple-antibiotic-therapy fecal microbiota transplantation and alginate for ulcerative colitis: protocol. *Front Med.* (2022) 9:779205. doi: 10.3389/fmed.2022.779205

98. Costello SP, Tucker EC, La Brooy J, Schoeman MN, Andrews JM. Establishing a fecal microbiota transplant service for the treatment of clostridium difficile infection. *Clin Infect Dis.* (2016) 62:908–14. doi: 10.1093/cid/civ994

99. Zhang X, Ishikawa D, Nomura K, Fukuda N, Haraikawa M, Haga K, et al. Donor screening revisions of fecal microbiota transplantation in patients with ulcerative colitis. *J Clin Med.* (2022) 11:1055. doi: 10.3390/jcm11041055

100. Korpela K, Helve O, Kolho KL, Saisto T, Skogberg K, Dikareva E, et al. Maternal fecal microbiota transplantation in Cesarean-born infants rapidly restores normal gut microbial development: a proof-of-concept study. *Cell.* (2020) 183:324–34.e5. doi: 10.1016/j.cell.2020.08.047

101. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. *Am J Gastroenterol.* (2013) 108:500–8. doi: 10.1038/ajg.2013.59

102. Papanicolas LE, Choo JM, Wang Y, Leong LEX, Costello SP, Gordon DL, et al. Bacterial viability in faecal transplants: which bacteria survive? *EBiomedicine.* (2019) 41:509–16. doi: 10.1016/j.ebiom.2019.02.023

103. Costello SP, Conlon MA, Vuaran MS, Roberts-Thomson IC, Andrews JM. Faecal microbiota transplant for recurrent Clostridium difficile infection using long-term frozen stool is effective: clinical efficacy and bacterial viability data. *Aliment Pharmacol Ther.* (2015) 42:1011–8. doi: 10.1111/apt.13366

104. Lee CH, Steiner T, Petrof EO, Smieja M, Roscoe D, Nematallah A, et al. Frozen *vs* fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent Clostridium difficile infection: a randomized clinical trial. *JAMA.* (2016) 315:142–9. doi: 10.1001/jama.2015.18098

105. Satokari R, Mattila E, Kainulainen V, Arkkila PE. Simple faecal preparation and efficacy of frozen inoculum in faecal microbiota transplantation for recurrent Clostridium difficile infection–An observational cohort study. *Aliment Pharmacol Ther.* (2015) 41:46–53. doi: 10.1111/apt.13009

106. Takahashi M, Ishikawa D, Sasaki T, Lu YJ, Kuwahara-Arai K, Kamei M, et al. Faecal freezing preservation period influences colonization ability for faecal microbiota transplantation. *J Appl Microbiol.* (2019) 126:973–84. doi: 10.1111/jam.14167

107. Kump P, Wurm P, Gröchenig HP, Wenzl H, Petritsch W, Halwachs B, et al. The taxonomic composition of the donor intestinal microbiota is a major factor influencing the efficacy of faecal microbiota transplantation in therapy refractory ulcerative colitis. *Aliment Pharmacol Ther.* (2018) 47:67–77. doi: 10.1111/apt.14387

108. Paramsothy S, Nielsen S, Kamm MA, Deshpande NP, Faith JJ, Clemente JC, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. *Gastroenterology.* (2019) 156:1440–54.e2. doi: 10.1053/j.gastro.2018.12.001

109. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut.* (2006) 55:205–11. doi: 10.1136/gut.2005.073817

110. Stojek M, Jabłońska A, Adrych K. The role of fecal microbiota transplantation in the treatment of inflammatory bowel disease. *J Clin Med.* (2021) 10:4055. doi: 10.3390/jcm10184055

111. Neff CP, Rhodes ME, Arnolds KL, Collins CB, Donnelly J, Nusbacher N, et al. Diverse intestinal bacteria contain putative zwitterionic capsular polysaccharides with anti-inflammatory properties. *Cell Host Microbe.* (2016) 20:535–47. doi: 10.1016/j.chom.2016.09.002

112. Nomura K, Ishikawa D, Okahara K, Ito S, Haga K, Takahashi M, et al. Bacteroidetes species Are correlated with disease activity in ulcerative colitis. *J Clin Med.* (2021) 10:1749. doi: 10.3390/jcm10081749

113. Fujimoto T, Imaeda H, Takahashi K, Kasumi E, Bamba S, Fujiyama Y, et al. Decreased abundance of Faecalibacterium prausnitzii in the gut microbiota of Crohn's disease. *J Gastroenterol Hepatol.* (2013) 28:613–9. doi: 10.1111/jgh.12073

114. Joossens M, Huys G, Cnockaert M, De Preter V, Verbeke K, Rutgeerts P, et al. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. *Gut.* (2011) 60:631–7. doi: 10.1136/gut.2010.223263

115. Seksik P, Rigottier-Gois L, Gramet G, Sutren M, Pochart P, Marteau P, et al. Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. *Gut.* (2003) 52:237–42. doi: 10.1136/gut.52.2.237

116. Favier C, Neut C, Mizon C, Cortot A, Colombel JF, Mizon J. Fecal beta-D-galactosidase production and Bifidobacteria are decreased in Crohn's disease. *Dig Dis Sci.* (1997) 42:817–22. doi: 10.1023/a:1018876400528

117. Bartels LE, Jepsen P, Christensen LA, Gerdes LU, Vilstrup H, Dahlerup JF. Diagnosis of *Helicobacter* Pylori Infection is Associated with Lower Prevalence and Subsequent Incidence of Crohn's Disease. *J Crohns Colitis.* (2016) 10:443–8. doi: 10.1093/ecco-jcc/jjv229

118. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, et al. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut.* (2014) 63:1275–83. doi: 10.1136/gutjnl-2013-304833

119. Segal JP, Oke S, Hold GL, Clark SK, Faiz OD, Hart AL. Systematic review: ileoanal pouch microbiota in health and disease. *Aliment Pharmacol Ther.* (2018) 47:466–77. doi: 10.1111/apt.14454

120. 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.e7. doi: 10.1053/j.gastro.2016.11.010

121. Dong LN, Wang M, Guo J, Wang JP. Role of intestinal microbiota and metabolites in inflammatory bowel disease. *Chin Med J.* (2019) 132:1610–4. doi: 10.1097/CM9.0000000000000290

122. Fuentes S, Rossen NG, van der Spek MJ, Hartman JH, Huuskonen L, Korpela K, et al. Microbial shifts and signatures of long-term remission in ulcerative colitis after faecal microbiota transplantation. *ISME J.* (2017) 11:1877–89. doi: 10.1038/ismej.2017.44

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 05 December 2022
DOI 10.3389/fmed.2022.1077275

OPEN ACCESS

EDITED BY
Angel Lanas,
University of Zaragoza, Spain

REVIEWED BY
Fangyan Wang,
Wenzhou Medical University, China
Mihai Covasa,
Western University of Health Sciences, United States

*CORRESPONDENCE
E. Marleen Kemper
m.kemper@apotheeka15.nl

SPECIALTY SECTION
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

RECEIVED 22 October 2022
ACCEPTED 21 November 2022
PUBLISHED 05 December 2022

CITATION
Wortelboer K, Koopen AM, Herrema H, de Vos WM, Nieuwdorp M and Kemper EM (2022) From fecal microbiota transplantation toward next-generation beneficial microbes: The case of *Anaerobutyricum soehngenii*. *Front. Med.* 9:1077275. doi: 10.3389/fmed.2022.1077275

From fecal microbiota transplantation toward next-generation beneficial microbes: The case of *Anaerobutyricum soehngenii*

Koen Wortelboer[1,2,3,4], Annefleur M. Koopen[2,3,5], Hilde Herrema[1,2,3], Willem M. de Vos[1,6,7], Max Nieuwdorp[1,2,3,5,8] and E. Marleen Kemper[4]*

[1]Department of Experimental Vascular Medicine, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, Netherlands, [2]Amsterdam Cardiovascular Sciences, Diabetes and Metabolism, Amsterdam, Netherlands, [3]Amsterdam Gastroenterology Endocrinology Metabolism, Endocrinology, Metabolism and Nutrition, Amsterdam, Netherlands, [4]Department of Pharmacy, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, Netherlands, [5]Department of Vascular Medicine, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, Netherlands, [6]Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands, [7]Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland, [8]Diabetes Center, Department of Internal Medicine, Amsterdam UMC, Location VUMC, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

The commensal gut microbiota is important for human health and well-being whereas deviations of the gut microbiota have been associated with a multitude of diseases. Restoration of a balanced and diverse microbiota by fecal microbiota transplantation (FMT) has emerged as a potential treatment strategy and promising tool to study causality of the microbiota in disease pathogenesis. However, FMT comes with logistical challenges and potential safety risks, such as the transfer of pathogenic microorganisms, undesired phenotypes or an increased risk of developing disease later in life. Therefore, a more controlled, personalized mixture of cultured beneficial microbes might prove a better alternative. Most of these beneficial microbes will be endogenous commensals to the host without a long history of safe and beneficial use and are therefore commonly referred to as next-generation probiotics (NGP) or live biotherapeutic products (LBP). Following a previous FMT study within our group, the commensal butyrate producer *Anaerobutyricum* spp. (previously named *Eubacterium hallii)* was found to be associated with improved insulin-sensitivity in subjects with the metabolic syndrome. After the preclinical testing with *Anaerobutyricum soehngenii* in mice models was completed, the strain was produced under controlled conditions and several clinical studies evaluating its safety and

efficacy in humans were performed. Here, we describe and reflect on the development of *A. soehngenii* for clinical use, providing practical guidance for the development and testing of NGPs and reflecting on the current regulatory framework.

KEYWORDS

***Anaerobutyricum soehngenii*, *Eubacterium hallii*, next-generation probiotic, live biotherapeutic product, fecal microbiota transplantation**

Introduction

The commensal gut microbiota play an important role in human health and well-being, regulating host metabolism, shaping our immune system and preventing pathogen colonization (1–3). However, disruption of the intestinal microbiota has been implicated in several diseases, such as gastrointestinal disorders, metabolic disorders and even autoimmune diseases (4, 5). Over the past decades, fecal microbiota transplantation (FMT) has emerged as a potential treatment strategy for such disorders by restoring a balanced and diverse microbiota (6). In addition, FMT has enabled researchers to study causality of the gut microbiota in disease pathogenesis (7, 8). Even though FMT has shown promising results in several diseases (9), the therapy is currently only indicated for the treatment of recurrent *Clostridioides difficile* infections (10). Furthermore, FMT faces several logistical challenges such as donor screening and (anaerobic) sample processing and storage (11, 12). In addition, there are potential safety risks with FMT, such as the potential transfer of pathogenic microorganisms missed during donor screening (13). Other potential risks include the potential transfer of unwanted phenotypes such as obesity or an increased risk of developing disease later in life such a colorectal cancer (14–16).

Due to these limitations and risks of FMT, a more controlled, personalized mixture of beneficial microbes might prove a better alternative. Traditional probiotics are believed to be beneficial for the host health by supporting a balanced microbiota, contributing to the health of the digestive tract and immune system and counteracting pathogenic bacteria through various mechanisms (17–19). However, even though decades of extensive studies have led to numerous prophylactic and therapeutic health claims (20, 21), clinical trials of high methodological quality report conflicting results and debatable conclusions (22). In addition, the majority of the probiotics currently sold on the market contain microorganisms from the *Lactobacillus* and *Bifidobacterium* genera, while these genera constitute only a minor proportion of the human intestinal microbiota (23, 24).

With increasing knowledge of the gut microbiota through affordable genome and metagenome sequencing and the development of better culturing techniques, the list of endogenous microbes with potential health benefits has dramatically increased. Since these microbes are endogenous to the host, they are more likely to engraft and be metabolically active. Even though most of these commensal microbes are still at an early stage of mechanistic investigation, there have been several reports of beneficial microbes restoring the balance of the intestinal ecosystem and improving disease phenotype (25–30). These microorganisms without a long history of safe and beneficial use are commonly referred to as next-generation probiotics (NGP) or live biotherapeutic products (LBP) (31).

Previously, our group performed a randomized controlled trial studying the effects of lean donor FMT in human obese, insulin resistant subjects (32). In line with an improved insulin sensitivity, we observed an increased abundance of the commensal *Anaerobutyricum* spp. [previously named *Eubacterium hallii* (33)] in the small intestine upon allogenic FMT compared to autologous FMT. We thus set out to further study and develop this potential beneficial microbe and focused on *Anaerobutyricum soehngenii* L2-7 among others since it was best characterized (34–36). After confirming a dose-dependent improvement of insulin sensitivity and safety of *A. soehngenii* in a mouse model (37), the strain was produced under controlled conditions and tested in a dose-escalating phase I/II clinical trial (38). Here, we describe the development of *A. soehngenii*, from the identification and production to the first clinicals trial in humans. In addition, we provide a practical roadmap for the development and testing of similar NGPs and reflect on the current regulatory framework.

Definition of next-generation probiotics and live biotherapeutic products

The traditional probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"(39). These microbes have a long history of use and are regarded as safe, having a Generally Regarded as Safe (GRAS) status in the United States

or a Qualified Presumption of Safety (QPS) status in the European Union (40). In contrast, NGPs are microorganisms without a long history of safe and beneficial use, that like traditional probiotics, confer a health benefit on the host when administered in adequate amounts (31). In 2012 the United States Food and Drug Administration (FDA) introduced the term live biotherapeutic products (LBP), defined as "a biological product that: (1) contains live organisms, such as bacteria; (2) is applicable to the prevention, treatment, or cure of disease or condition of human beings; and (3) is not a vaccine" (41). This FDA guidance statement was followed up in the European Union in 2019, where LBPs were defined as "medicinal products containing live micro-organisms (bacteria or yeasts) for human use" in the European Pharmacopeia (Ph. Eur.) (42). However, since LBPs comprise besides the microorganism also the formulation of the final product and are defined as a medicinal product, this term should not be systematically used to replace NGPs. The term NGP is more extensive, including both the microorganisms present in LBPs and those currently being investigated, not formulated in a final product yet (31). In addition, NGPs could be employed both as a food supplement like traditional probiotics or as a medicinal product in the prevention, treatment, or cure of disease. Finally, genetically modified microorganisms can be viewed a NGPs as well, although the route to market as an LBP is most likely. **Figure 1** schematically depicts the various definitions.

Discovery and isolation of *Anaerobutyricum soehngenii*

In line with the worsening global obesity pandemic, the incidence of the metabolic syndrome has dramatically increased, predisposing individuals to developing cardiovascular diseases and type 2 diabetes (43). Dybiosis of the gut microbiota, defined as a perturbation of the composition and function, has been associated with the emergence of metabolic syndrome (44–46). To further investigate a causal role of the gut microbiota in metabolic syndrome, we previously infused fecal microbiota from lean healthy donors to male subjects with metabolic syndrome (32). Six weeks after the infusion of donor microbiota, peripheral insulin sensitivity increased along with levels of butyrate-producing bacteria, as compared to the autologous FMT group. Among these butyrate-producing bacteria, *Anaerobutyricum* spp. were more abundant in the small intestine, pointing toward a potential role in regulating insulin sensitivity through butyrate production. Since insulin resistant metabolic syndrome subjects are characterized by reduced levels of short-chain fatty acid (SCFA)-producing bacteria (47, 48) and oral supplementation with butyrate improved insulin resistance and dyslipidemia in diet-induced obese mice (49, 50), we concluded that *A. soehngenii* could be a promising NGP to improve insulin-resistance.

Isolated from the feces of an infant in 1996 (34), *A. soehngenii* strain L2-7, previously designated *E. hallii*, is a strict anaerobic, Gram-positive, catalase negative bacterium within the family *Lachnospiraceae* (33). *A. soehngenii* is part of the core microbiota of the human gastrointestinal tract (51, 52). In contrast to other well-known butyrate-producing species such as *Roseburia* and *Faecalibacterium* spp. that produce butyrate from sugars, *A. soehngenii* has the capacity to utilize D- and L-lactate in the presence of acetate instead (53). In addition, the genome contains bile acid sodium symporter and choloylglycine hydrolase genes, suggesting that *A. soehngenii* can affect host bile acid metabolism (54).

The *A. soehngenii* strain (previously *E. hallii* L2-7^T) was obtained from collaborators in the UK (34, 55) and is available from the DSMZ (Deutsche Sammlung van Mikroorganismen und Zellkulturen) as DSM 17630. The strain was cultured routinely under anaerobic conditions using a previously published protocol (33). Next, we thoroughly characterized the strain. First, the complete genome was sequenced (54), leading to a better understanding of the genetic potential underlying its metabolic capabilities. Next, optimum growth temperature and pH were determined, as well as the tolerability to oxygen. Cell morphology, motility and spore formation were studied using an (electron) microscope and the resistance to heat inactivation and antibiotic susceptibility were determined. Fermentation end products on various carbohydrates were measured and the resistance to bile acids was determined. Finally, the cellular fatty acid contents and the type of peptidoglycan membrane were determined. The results of this thorough characterization led to the reclassification of the previously designated *E. hallii* type strain L2-7^T to *A. soehngenii* type strain L2-7^T (33).

The metabolic features of *A. soehngenii* were further characterized by proteomic profiling, revealing the complete pathway of butyrate production from sucrose, sorbitol and lactate (56). This analysis identified a new gene cluster, *lctABCDEF*, which was induced upon growth on D,L-lactate plus acetate. Comparative genomics showed this gene cluster to be highly conserved in only *Anaerobutyricum* and *Anaerostipes* spp., suggesting *A. soehngenii* is adapted to a lifestyle of lactate plus acetate utilization in the human gastrointestinal tract (56). The capability to convert potentially harmful D- and L-lactate (57, 58) to the beneficial SCFA butyrate (59) confirmed that *A. soehngenii* was a promising NGP for further preclinical development.

Learning points and directions

There are two strategies commonly being employed for the development of NGPs. The first method is to associate the presence of a specific strain with a health phenotype and explore whether that strain has a causal effect on the disease phenotype. To date, many NGP candidates have been identified

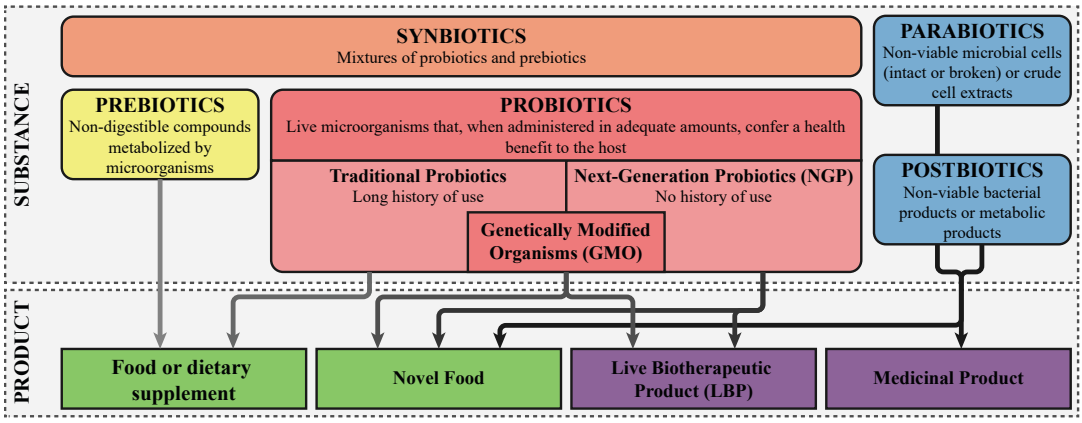


FIGURE 1
Definitions of probiotics, next-generation probiotics, and live biotherapeutic products. The different "biotics" are colored orange, here denoted as the active substance. The final products are colored green, with the darker green corresponding with products that are considered drugs, while the lighter green falls within the food and food supplements regulation.

using sequencing technologies to select strains with a depleted abundance in diseased subjects or strains that are associated with successful FMT treatment (60). The second strategy is to adopt a well-characterized probiotic strain and genetically modify the strain to confer a health benefit, e.g., through production and delivery of bioactive molecules (23). The latter approach will lead to a genetically modified organism (GMO) that is subject to specific regulations in various parts of the world, such as in the EU (61–63).

Regardless of the strategy used to identify or generate the NGP, before any health benefits can be studied *in vivo* the candidate strains need to be fully characterized *in vitro* (64). **Figure 2** summarizes the most important characteristics which have to be assessed besides genotyping and phenotyping the strain. In addition, the strain origin and subsequent manipulation or genetic modifications have to be documented. If there are any antimicrobial resistance genes or virulence genes present, the potential for transmission to other microorganisms of the human microbiota should be assessed, as well as measures taken to mitigate this risk. When the NGP is intended to be used in diseased persons with e.g., epithelial barrier damage of immunosuppression, the risk for bacterial translocation should be determined. A thorough strain characterization is critical for the assessment of the potential safety issues concerning the use of the NGP in healthy or diseased humans.

Preclinical development of *Anaerobutyricum soehngenii*

After *in vitro* testing of *A. soehngenii*, we moved to an animal model to assess safety and efficacy of the strain on insulin sensitivity. First, we manufactured a preclinical batch of *A. soehngenii* under anaerobic conditions as previously described (33). In short, cultures were grown under anaerobic conditions to the end of the exponential phase, concentrated by anaerobic centrifugation, washed with phosphate-buffered saline (PBS) and finally diluted in 10% glycerol to concentrations of 10^6, 10^8 and 10^{10} colony-forming units (CFU) in 100 µl. Purity was assessed by 16S rRNA sequencing and microscopic evaluation of cellular morphology. Viability was assessed by most probable number (MPN) analysis and confirmed by microscopic analysis. Samples were directly stored at −80°C and used within 6 months of production, during which time viability was stable. In addition, some of these samples were tested for stability during 2 years to support the product development for the clinical trial.

Next, we performed a dose-finding study in male diabetic (db/db) mice to test the safety and efficacy of orally administered *A. soehngenii* on insulin sensitivity and lipid metabolism (37). Mice were treated daily with *A. soehngenii* or placebo (10% glycerol) for up to 4 weeks, during which time no adverse events were observed (normal vital signs). A significant improvement on insulin sensitivity was observed during the insulin tolerance test, which was strongest for the 10^8 CFU dose. This was accompanied by a decrease in hepatic fat and a reduced expression of the *Fasn* and *Acc1* genes, both involved in lipogenesis.

To confirm these findings and further dissect the therapeutic mechanism of *A. soehngenii*, a second study with db/db mice was performed independently by the lab of prof. Bäckhed (Gothenburg) (37). Mice were treated with either 10^8 CFU of *A. soehngenii* or heat-inactivated *A. soehngenii* for 4 weeks. An increase in resting energy expenditure was observed after active *A. soehngenii* treatment, while bodyweight remained identical. In addition, active *A. soehngenii* increased fecal butyrate levels and modified bile acid metabolism as compared to the heat-inactivated *A. soehngenii*. These two mouse studies

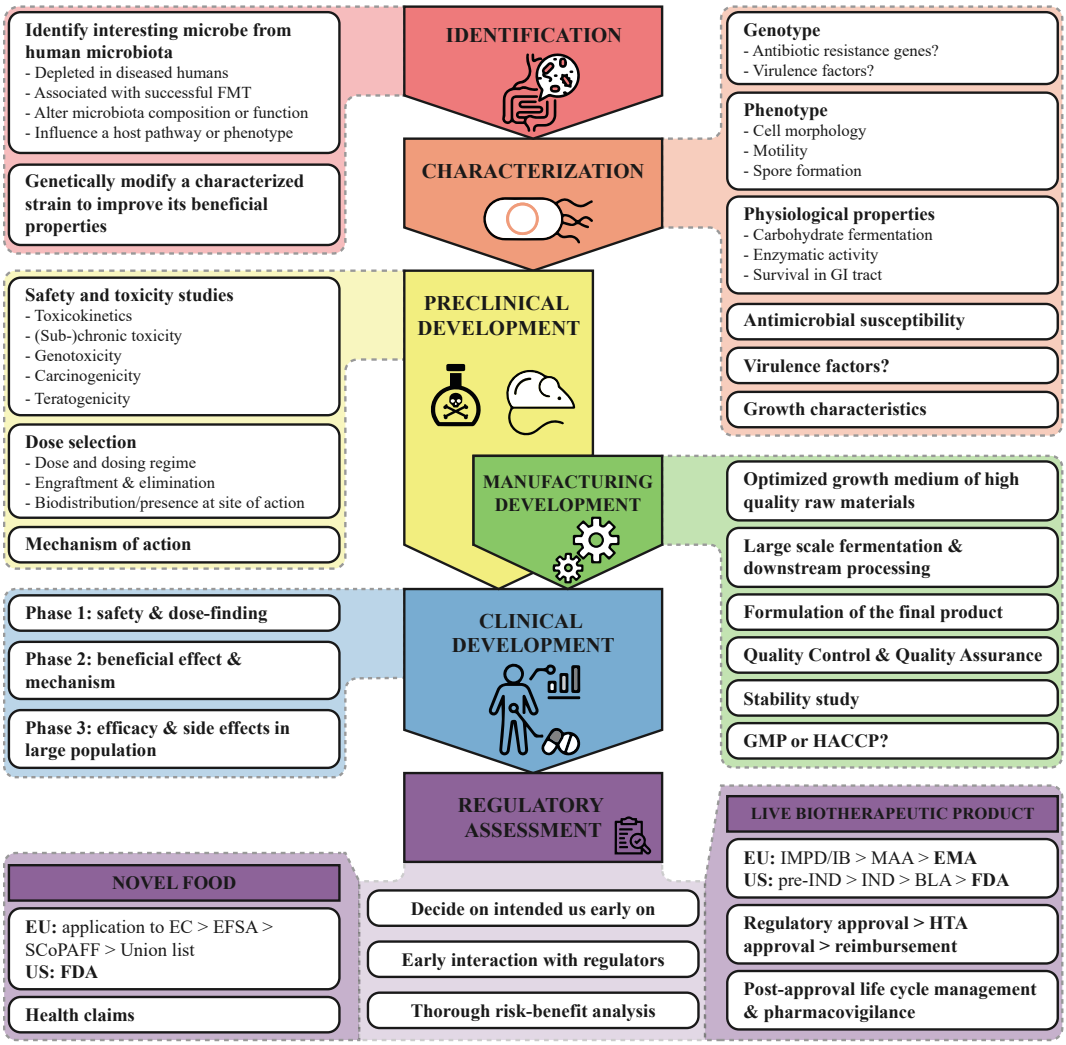


FIGURE 2
Roadmap for the development of NGP. Important points to consider for the development of NGPs are summarized from the identification to the regulatory assessment. BLA, Biologics License Application; EC, European Commission; EFSA, European Food Safety Authority; EMA, European Medicines Agency; EU, European Union; FDA, Food and Drug Administration; GI, gastrointestinal; GMP, Good Manufacturing Practices; HACCP, Hazard Analysis and Critical Control Points; HTA, Health Technology Assessment; IB, Investigators Brochure; IMPD, Investigational Medicinal Product Dossier; IND, Investigational New Drug; SCoPAFF, Standing Committee on Plants, Animals, Food and Feed, and US, United States.

have shown that treatment with *A. soehngenii* is safe and exerts beneficial effects on metabolism, potentially mediated by butyrate production and changes in bile acid metabolism. These data were used to obtain ethical approval for the clinical studies that we performed in humans.

More recently, a toxicological safety evaluation for *A. soehngenii* CH106, a tetracycline-sensitive derivative from *A. soehngenii* type strain L2-7^T, has been performed to show that the intake at the recommended dosages is safe (65). As required by the European Food Safety Authority (EFSA) and FDA for safety assessment of new nonabsorbable food ingredients, *A. soehngenii* was assessed for genotoxic potential and subchronic toxicity (66, 67). Both the bacterial reverse mutation and *in vitro* mammalian cell micronucleus tests showed no genotoxic effects. Furthermore, the 90-day subchronic toxicity in rats did not find any adverse events related to the feeding with *A. soehngenii*, not even at the highest dose (5×10^{11} CFU/kg body weight/day) exceeding human recommended daily intake more than 100-fold (65). These findings support that oral intake of *A. soehngenii* as food supplement is safe.

Learning points and directions

During the preclinical development, adequate information on pharmacological and toxicological properties should be

generated to support the proposed clinical trial(s). However, safety and toxicity studies with NGPs are challenging. Since the product generally does not reach the systemic circulation, but its metabolites or its activity could directly or indirectly influence physiological functions in the body, efficacy and toxicity are not necessarily related to the dosage. In addition, other factors such as the human physiology and microbiota composition might influence the safety and efficacy. Furthermore, since most NGPs have coevolved with the human host, the holobiont concept, it is difficult to translate the results from animal studies to the human setting (68–70). Therefore, it is highly recommended to combine *in vitro*, *ex vivo* and *in vivo* models to establish a global safety profile adapted to the risks within the intended population. It is common to perform the safety and toxicity studies according to the Organization for Economic Co-operation and Development (OECD) principles for Good Laboratory Practice (GLP). However, due to the need for innovative methods and models (e.g., an artificial model of the human gastrointestinal tract) which may not be validated nor at GLP level, this might prove difficult (71).

For food ingredients and dietary supplements, the EFSA advices a tiered approach for toxicological studies (67). This tiered approach evaluates the toxicokinetics, genotoxicity, subchronic and chronic toxicity, carcinogenicity and teratogenicity of the NGP, balancing data requirements against the risk. This approach was used as well for the toxicological safety evaluation for *A. soehngenii* CH106 (65). If the NGP is intended to be used as medicinal product in a diseased population, it is important that safety for the targeted population is demonstrated. **Figure 2** summarizes the most important issues that have to be addressed, such as the effect of dosage and duration of treatment on toxic response and the teratogenic, carcinogenic and genotoxic potential.

Manufacture of *Anaerobutyricum soehngenii* suitable for clinical testing

Before we could orally administer *A. soehngenii* to humans, a product suitable for a clinical trial had to be manufactured. At the time of approval by the independent ethics committee (2014), A. soehngenii was regarded as a probiotic and had to comply with the Dutch "Warenwet" (72), which was in line with the EU regulations for dietary supplements (73). This meant the manufacturing had to be performed according to Hazard Analysis and Critical Control Point (HACCP) standards (74). Therefore, we contracted a third-party manufacturer, which was ISO 9001 accredited and had ample experience with the fermentation of probiotic strains for clinical intervention studies under HACCP standards.

Growth medium

First of all, the growth medium was further optimized for large scale production of a food-grade product. The composition was based on previous experience (33), whereby (1) laboratory chemicals were converted to food-grade sources, (2) only animal-free components were used (no heme or meat peptone), (3) complexity was reduced (removal/reduction of trace minerals, vitamins, carbon sources and organic acids) and (4) the biomass yield was further improved. Raw materials were sourced from audited, reliable suppliers to ensure high quality. Before fermentation, the growth medium was prepared and sterilized inside a large fermenter system, which was made completely anaerobic by nitrogen (N2) flush.

Fermentation

Fermentation was performed in four sequential steps, which are depicted in **Figure 3**. First, a small volume of food-grade medium was inoculated with a carefully prepared frozen seed stock of *A. soehngenii*. The same strain was used in the animal studies and had therefore been well characterized, was viable, pure and free of any bacterial of viral contaminants. After 24 h of fermentation at 37°C, the culture was used to inoculate 1 L of medium, which was again fermented for another 18 h. Then, this secondary seed culture was used to inoculate 30 L of medium in a small fermenter, which was fermented for 17 h and which acted as a test run for the large-scale fermentation. Finally, 290 L of medium in the large fermenter was inoculated with 10 L of inoculum of the small fermenter. Both small and large fermenters were controlled for temperature, pH and oxygen level and the optical density (OD) of the culture was used to determine the fermentation time (between 14 and 18 h). After 16 h of fermentation in the large fermenter, *A. soehngenii* grew to an OD of approximately 10.

Concentration and washing

Using hollow fiber membranes (Koch membrane systems; HF3043-25-43-PM500; HF3043-16-106-PM500) and diafiltration with PBS, the cells were concentrated and washed. The fermentate was cooled to 10°C, pumped through the anaerobic membrane unit and concentrated to 40–50 L within 3 h. During the second phase diafiltration was performed to reduce the levels of medium components and fermentation products. Wash buffer was sterilized using ultra-high temperature, de-aerated and directly added to the returning cell flow into the fermenter. After 6 h, the cells were concentrated about 20-fold to 15 L and 99.8% of medium compounds were discarded to waste, leaving solely 2.9% of medium components in the final concentrate. Finally, 9 L of

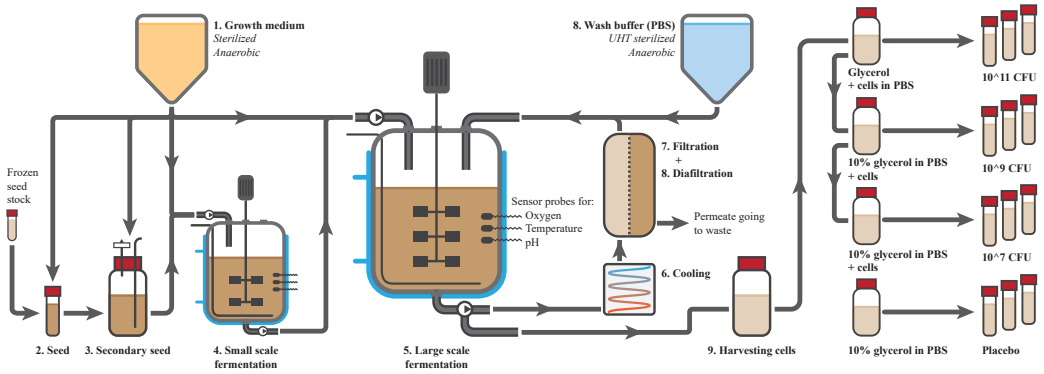


FIGURE 3
Preparation of Anaerobutyricum soehngenii. Production started with the production of a sterile anoxic growth medium (1), which was used for the seeding (2 and 3) and fermentations (4 and 5) of *A. soehngenii*. The fermentations were well controlled for temperature, presence of oxygen and pH amongst others. After the final fermentation, cells were cooled (6), concentrated (7) and washed by diafiltration (8). Cells were harvested from the fermenter (9), diluted with glycerol (10% final concentration) to 10^{10} CFU/ml and dispensed over 10 ml vials that were labeled and directly frozen. The highest concentration was used to make dilutions for the lower doses.

product could be harvested from the system into a sterile, N2-flushed container of 10 L.

Preparation of end-product

Four different batches were produced for the clinical study, consisting of 600 tubes with 10 mL *A. soehngenii* in concentrations of 10^6, 10^8, and 10^{10} CFU/ml in PBS + 10% glycerol and one placebo batch with only 10% glycerol in PBS. For every batch 7 L bottles were prepared with glycerol and PBS for further dilution, which were autoclaved, cooled and flushed with N2. From the 9 L harvested concentrate, the necessary volume was added to these bottles to obtain the correct concentration. Bottles were placed on ice, under continuous stirring and N2 flush. The 10 mL tubes were first filled with N2, followed by 10 mL of product using a dosing-tube-pump. Tubes were immediately closed, labeled and placed in a freezer at −30°C within 10 min of filling. All filling was performed inside a disinfected laminar flow cabinet.

Quality control

During the manufacturing, there was a continuous monitoring of temperature, pH and oxygen level. In addition, the cell count and OD were determined at every step during the process, as well as the absence of any contaminants. Since anaerobes are hard to enumerate quantitatively on agar plates, an MPN analysis was performed under anaerobic conditions to obtain the number of viable cells and cell morphology was assessed microscopically. All above quality controls were performed for the packaged vials, which complied with the standards for human consumption. Table 1 shows the specifications that were defined for the intermediates and final product.

Subsequently, the stability of the produced vials was tested every 6 months. After production, the vials were given a "best before" date of 6 months, which is required by law for food products in the Netherlands. This gave us the opportunity to extend the expiration date of the vials if the viability and purity criteria were met. Table 2 shows the potency and purity of the vials with the highest dose *A. soehngenii* during a 3-year time period.

Learning points and directions

Producing a strain at industrial scale sets different requirements for strains and culture media than laboratory scale culturing (75). Therefore, when a strain qualifies as potential NGP, steps should be taken to see if the strain can be cultured at an industrial scale. The strict conditions necessary for culturing NGPs are one of the technical challenges, such as the need for specific nutrition, the absence of oxygen, a stable temperature and a suitable pH (24). In addition, longer hold times, sheer stress from pumping, the downstream purification processes and storage may negatively impact the viability of the bacterial cells. Next, the strains have to be incorporated into a product, such as capsules, a powder or liquid suspension. Since most NGPs are strict anaerobes or facultative anaerobes, the exposure to oxygen should be kept to a minimum. To this end, oxygen permeability into containers should be reduced and antioxidants could be added to reduce the redox potential (76). Upon ingestion of the product, NGPs have to survive the harsh environment of the gastrointestinal tract. Enteric-coated capsules and microencapsulation are useful strategies to protect

TABLE 1 Specifications for the *Anaerobutyricum soehngenii* intermediates and final product.

Test	Method	Acceptance criteria	Intermediate (I), product (P), or stability (S)
Identity	Genome sequencing	Confirm strain is *A. soehngenii L2-7*	I*
	Microscopy (visual observation)	Complies with phenotypic characteristics *A. soehngenii L2-7*	I, P
Potency	Culturing/MPN	10^10 CFU/ml	P, S
Purity	Microbial contamination	*Salmonella* spp.: absent *Listeria monocytogenes*: absent *Enterobacteriaceae*: <10 CFU/ml Coagulase-negative *Staphylococci*: <10 CFU/ml *Bacillus cereus*: <10 CFU/ml	I, P, S
Other	pH	6.0–7.0	I, P
	Storage	Vial with 10 ml suspension, stored at −20°C	P
	Labeling	According to GMP annex 13	P

*The complete genome of the strain used for seeding has been completely sequenced. CFU, colony-forming unit; GMP, good manufacturing practice.

TABLE 2 Results of stability testing (potency and purity) of *A. soehngenii*.

Storage time (months)		6	12	18	24	30	36
Potency	MPN (CFU/ml)	1.0E+09	1.0E+09	1.0E+09	1.0E+10	1.0E+09	1.0E+09
	Microscopy	Normal	Normal	Normal	Normal	Normal	Normal
Purity	*Salmonella* spp.	Absent	Absent	Absent	Absent	Absent	Absent
	Listeria monocytogenes	Absent	Absent	Absent	Absent	Absent	Absent
	Enterobacteriaceae (CFU/ml)	<10	<10	<10	<10	<10	<10
	Coagulase-negative *Staphylococci* (CFU/ml)	<10	<10	<10	<10	<10	<10
	Bacillus cereus (CFU/ml)	<10	<10	<10	<10	<10	<10

MPN, most probable number; CFU, colony-forming unit.

the bacteria and deliver them to their site of action (77, 78). Ultimately, manufacturing needs to result in a robust and stable product that will allow for delivery of the NGP in sufficient numbers for an efficacious dose until the expiration date (75).

For medicinal products or LBPs, production according to Good Manufacturing Practices (GMP) is required (41). For foods and dietary supplements, production in HACCP-certified plants is the standard (74). Regardless, quality control and quality assurance programs needs to be in place to ensure a consistent quality of ingredients and final product and to secure a reliable production process (75). The manufacturing process of the strain should be clearly documented, from the raw materials used, the cell bank system, growth and harvesting of the cells, purification and downstream processing to the in-process testing. Likewise, the manufacturing of the final product has to be thoroughly described, including production records and instructions for formulation, filling, labeling and packaging. For both the strain and product manufacturing, the risks for cross-contamination with other products produced in the same rooms or with the same contact equipment has to be assessed. Specifications for the strain and product have to be described, including a description of sampling procedures and the validated test methods. These specifications should describe the identity, potency, purity, contamination, appearance and, if applicable, additional tests for percentage of viable cells, particulate matter, pyrogens, pH and residual moisture. Furthermore, stability data has to be generated, demonstrating the product is stable for the planned duration of use with regards to potency and contamination. For frozen products, the influence of multiple freeze-thaw cycles should be assessed, while for lyophilized products the shelf life after reconstitution should be explored. Finally, the impact of the product on the environment needs to be assessed, especially when the strain is genetically modified, pathogenic, ecologically more fit than the wildtype, or difficult to eradicate.

Clinical trials with *Anaerobutyricum soehngenii*

Safety/dose-finding trial

To validate the murine data in a human setting, we set up a single-blinded, phase I/II dose-escalation trial to determine safety and efficacy of *A. soehngenii* in obese, insulin-resistant subjects (38). In this study, 27 obese Caucasian males with the metabolic syndrome were included and assigned to receive

A. soehngenii in increasing dose of 10^7, 10^9, or 10^{11} cells/day for 28 days. While subjects were blinded for their respective treatment dose, first 9 subjects had to successfully complete the study protocol on the lowest dose before the dose was escalated to a higher concentration. Subjects stored the frozen vials with *A. soehngenii* at −20°C at home and every day a single 10 mL vial was thawed, mixed with 100 mL of milk and consumed orally. The milk was added to increase the pH in the stomach and thereby protect the living cells during gastrointestinal passage (79). The primary outcome was safety and in addition the impact on insulin sensitivity and lipolysis was assessed after 4 weeks of treatment.

Treatment with *A. soehngenii* up to 10^{11} cells/day was well tolerated without any serious adverse events (38). When all treatment groups were combined, the fecal abundance of *A. soehngenii* correlated with an improved peripheral insulin sensitivity, accompanied by beneficial changes in the bile acid profile. Unexpectedly, no increase in fecal butyrate levels was observed, which could be explained by the volatility of SCFAs and the assays' detection limits making butyrate difficult to measure. The increase in (fecal) *A. soehngenii* abundance was transient and mostly gone 2 weeks after cessation. The viability of the administered strain was negatively affected by stomach acid and oxygen. However, *A. soehngenii* was partially able to survive the gastrointestinal passage as indicated by the highest replication signal in the feces of subjects that received the highest dose. The viability (and therapeutic efficacy) could be further improved by protecting the strain better from the acidic and oxygenic environment through encapsulation and/or freeze-drying.

Different administration method and mode of action

To further elucidate the mode of action of *A. soehngenii* in humans, a randomized placebo-controlled crossover trial was performed in which the strain was directly administered in the duodenum, thereby circumventing the stomach acid and reducing the exposure to oxygen (80). Since the small intestine plays a central role in glucosensing, regulation of insulin sensitivity/secretion and glucose homeostasis, it was hypothesized that a direct duodenal infusion of *A. soehngenii* could further enhance the therapeutic effect (81). Again, obese subjects with the metabolic syndrome (N = 12) were included and randomized to a single nasoduodenal infusion with the highest dose of *A. soehngenii* (10^{11} cells) or placebo (10% glycerol in PBS). After 6 h, a duodenal biopsy and mixed meal test was performed. In addition, subject monitored their 24-h glucose and collected several fecal samples. After a 4-week washout period subjects switched to the other treatment arm, which was determined long enough to lose the strain during the first trial.

Again, this study showed that administration of *A. soehngenii* was safe and well-tolerated. Treatment with the strain increased postprandial excursion of insulinotropic hormone glucagon-like peptide 1 (GLP-1), which was accompanied by a reduced glucose variability (80). Given that *A. soehngenii* has the capacity to produce butyrate (51, 53) and fecal levels of butyrate tended to be higher following *A. soehngenii* treatment (80), the increased GLP-1 secretion could be the result of butyrate activating the G protein-coupled receptor 43 (GPR43) on intestinal L cells (82). In addition, since *A. soehngenii* expresses a bile acid sodium symporter and bile acid hydrolases (54) and plasma levels of secondary bile acids were elevated (80), the increased GLP-1 expression could also be the consequence of Takeda G protein- coupled receptor 5 (TGR5) activation by secondary bile acids (83). Moreover, treatment with *A. soehngenii* led to a decreased duodenal expression of the nuclear farnesoid X receptor (FXR) and its target gene *OSTa*, which may also account for an increased GLP-1 availability (84, 85). Finally, the improvement in glucose variability could be explained by the insulin-sensitizing effects of GLP-1 as well as butyrate (49, 86).

Furthermore, *A. soehngenii* altered the duodenal transcription of 73 genes, most prominently inducing the expression of *REG1B* along with *REG1A*, which encode for generating islet-derived protein 1A/B (80). Being strongly expressed within Paneth cells at the base of intestinal crypts, Reg1A and Reg1B are secreted in the lumen and probably act locally, possibly by inducing progenitor or L- cell hyperplasia (80). Moreover, Induction of *REG1B* was found to correlate with both an increased GLP-1 secretion and a reduced glucose variability 24 h after administration of *A. soehngenii* (80). Treatment with a single dose of *A. soehngenii* did not impact the microbiota composition or diversity, as was also seen in the previous studies. In addition, the abundance of fecal *A. soehngenii* was not altered over time, excluding microbiota-mediated carry-over effects at time of crossover (80).

Learning points and directions

The main objective of the first clinical studies is to establish safety and to define the appropriate dosage range and regimen based on the tolerability of the product (64). This includes the determination of the minimal effective dose or an optimal effective dose range and, if possible, the maximal safe dose. Besides dosing, the focus should be on obtaining safety data to identify common product-associated adverse events. These early clinical studies are commonly performed in healthy volunteers, although inclusion of patients could be more appropriate, for example when the NGP should correct dysbiosis (64). Risk mitigation measures to ensure the safety of study participants should be taken into account, such as sequential enrollment, dose escalation and monitoring by an independent data

monitoring committee. Furthermore, it is expedient to monitor for translocation, inflammation and infection and to establish persistence of NGP and its effects after the final administration.

It is important to account for other confounding factors that influence the function or composition of the microbiota, such as age (87, 88), diet (89), lifestyle (90) and environmental factors (91, 92). In this respect, studies with a placebo-controlled cross-over design are very useful as they can limit the influence of such extrinsic and intrinsic confounding factors, thereby allowing for a smaller sample size. Needless to say, blinding is very important and the washout period should be carefully considered. Increasingly, the baseline microbiota composition is incorporated in the screening criteria as well, looking for example for the presence of specific bacterial groups or clustering within specific enterotypes (93). This will lead to more comparable study groups and can optimize the efficacy of the intervention when a specific bacterial group is involved in the mechanism of action.

Regulatory framework next-generation probiotics

According to the definition of probiotics by the FAO and WHO, probiotics can be classified as both a dietary supplement and a drug, while there is a profound regulatory difference. Similarly, products with NGPs can reach the market as a food, dietary supplement or drug depending on the intended use. In the EU, foods are regulated by the EFSA and drugs by the EMA, while in the US the FDA deals with both categories. When the intended use is related to the prevention, alleviation or cure of disease, the product will be considered a medicinal product or medical device. In contrast, an orally ingested product with claims relating to enhancement of physiological function or reduction of a disease risk factor could be classified as a functional food or food supplement. Furthermore, topically applied products with a purely cosmetic function could be assessed as a cosmetic. To ensure regulatory compliance, it is important to decide on the indented use and consequent regulatory classification prior to preclinical studies and manufacturing (71).

Functional food or dietary supplement

In the European Union, "food" is defined as "any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans." Foods and food ingredients are further subdivided into different categories, such as conventional food, food supplements and novel foods, among others. Each of these categories is regulated accordingly, with general requirements and provisions regarding to labeling, presentation and advertising (73, 94). When NGPs are intended for use as food or dietary supplement, they are most likely considered a novel food, since new strains have not been widely consumed within the EU before May 1997 (95). However, if the NGP has been genetically modified, it will be regulated as a genetically modified food (61).

For an NGP to reach the market as a novel food, it needs to be authorized and included in the Union list (95). One of the most important conditions is that the NGP does not pose a risk to human health, which has to be supported by scientific evidence. This consists of a comprehensive risk assessment, combining biological and toxicological studies in the context of anticipated human exposure to evaluate the potential risk to human health (96). In addition, an application should contain detailed descriptions of the NGP, the manufacturing process, the composition of the product, analytical methods used, labeling and conditions for intended use (95).

Many safety-related aspects have been shown to be common at the species level, which has led to the QPS list of the EFSA, expressing a species-based safety evaluation for microbes used as food (40). If the NGP as a species can be unambiguously identified to a QPS group, the developer does not need to perform detailed tolerance and toxicology studies. However, most NGPs will not belong to a QPS group and must be evaluated by the EFSA to ensure safety (95). Besides safety, the product must not contribute to the spread of antimicrobial resistance in the food chain or environment, requiring phenotypic and genotypic assessment of antimicrobial resistance.

Any health claims for NGPs have to be submitted to a national competent authority and will be passed on to the EFSA for scientific evaluation (97). Even the statement "contains probiotics/prebiotics" is considered a health claim in the EU (93). For a health claim to be accepted, a proper characterization of the NGP is required, as well as a proven beneficial health effect and causal relationship supported by high-quality studies (98).

Live biotherapeutic product

Since 2012 and 2019 quality requirements for LBPs have been clarified by the FDA and EDQM (41, 42), where LBPs are described as medicinal products containing live microorganisms for human use. Other than these quality requirements, there is currently no specific LBP regulation. However, since LBPs contain live microorganisms, they are considered biological medicinal products and as such have to comply with the legislative and regulatory framework. In absence of a specific LBP subcategory, developers will have to rely on the regulatory concepts available for the other

subcategories of biological medicinal products. One of these concepts is a thorough risk-benefit analysis based on quality, safety and efficacy data obtained from preclinical and clinical studies. Cordaillat-Simmons et al. and Rouanet et al. previously elaborated on what a thorough risk-benefit analysis should include (64, 71). Other relevant guidelines for the design of preclinical and clinical studies are the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline on general consideration for clinical trials (ICHE8) (99), the Committee for Medicinal products for Human Use (CHMP) guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products (100), and the CHMP guideline on Human Cell-Based Medicinal Products (101).

For an LBP to reach the market in the EU, marketing authorization has to granted through a centralized or a national route. Under the centralized authorization procedure, EMA's CHMP carries out the scientific assessment, whereafter the European Commission takes a legally binding decision based on EMA's recommendation. To date, no LBPs have reached the EU market, which is partly due to the lack of a defined regulatory framework. Recently, Paquet et al. published their experiences with both the EMA and FDA leading up to their first-in-human trial (102). They described several key considerations for the development and (non-) clinical testing of LBPs based on points raised by the competent authorities. Furthermore, they highlighted the importance of early interaction with the competent authorities to discuss uncertainties and reduce risks in the absence of clear guidelines.

Concluding remarks

Above we described our experience with the development of *A. soehngenii* as an NGP and provided several (regulatory) directions. **Figure 2** summarizes these points and provides a schematic roadmap for developing NGPs. With the increasing knowledge on our intestinal microbiota, more and more potential NGPs will be discovered and developed, either as novel food/supplement or as LBP. It is important that these new strains are well characterized, of high quality and safe. Though difficult and complex, a thorough safety assessment for NGPs is very important, especially since efficacy and toxicity are not necessarily related to the dosage. Furthermore, since this is a relatively young field and currently no specific LBP regulation, talking to regulators in early stages of development can help to mitigate risks and clarify any uncertainties. This requires a clear view on the route to market (food or drug) early in the development.

We illustrated the development of NGPs with the strict anaerobe *A. soehngenii* as example. Identified as potential beneficial microbe after an FMT intervention, this microbe showed promising results in both preclinical *in vitro* and *in vivo* studies as well as in humans. Treatment with *A. soehngenii* was found to be safe and well tolerated. It showed promising effects on improving insulin sensitivity, increased GLP-1 secretion and reduced glucose variability. These effects are potentially mediated through the production of butyrate and secondary bile acids. By protecting the strain better from the acidic and oxygenic environment, e.g., through lyophilization and encapsulation, the viability and thereby therapeutic efficacy could potentially be increased. This NGP is currently being further developed as a food supplement.

Author contributions

KW wrote the first draft of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

Funding

KW was supported by a Novo Nordisk Foundation CAMIT grant 2018 (28232). HH was supported by a Senior Fellowship of the Dutch Diabetes Research Foundation (2019.82.004). WV was supported by the Netherlands Organization for Scientific Research (Spinoza Award and SIAM Gravity Grant 024.002.002). MN was supported by a personal ZONMW-VICI grant 2020 (09150182010020) and a Le Ducq consortium grant (17CVD01).

Conflict of interest

MN and WV are founders and scientific advisors of Caelus Health that is commercializing *A. soehngenii*.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Cani P. Gut microbiota-at the intersection of everything? *Nat Rev Gastroenterol Hepatol.* (2017) 14:321–2. doi: 10.1038/nrgastro.2017.54

2. Scheithauer T, Rampanelli E, Nieuwdorp M, Vallance B, Verchere C, van Raalte D, et al. Gut microbiota as a trigger for metabolic inflammation in obesity and type 2 diabetes. *Front Immunol.* (2020) 11:571731. doi: 10.3389/fimmu.2020.571731

3. Piewngam P, Zheng Y, Nguyen T, Dickey S, Joo H, Villaruz A, et al. Pathogen elimination by probiotic *Bacillus* via signalling interference. *Nature.* (2018) 562:532–7. doi: 10.1038/s41586-018-0616-y

4. de Vos W, Tilg H, Van Hul M, Cani P. Gut microbiome and health: mechanistic insights. *Gut.* (2022) 71:1020–32. doi: 10.1136/gutjnl-2021-326789

5. Miyauchi E, Shimokawa C, Steimle A, Desai M, Ohno H. The impact of the gut microbiome on extra-intestinal autoimmune diseases. *Nat Rev Immunol.* (2022). [Epub ahead of print]. doi: 10.1038/s41577-022-00727-y

6. Wortelboer K, Nieuwdorp M, Herrema H. Fecal microbiota transplantation beyond *Clostridioides* difficile infections. *EBioMed.* (2019) 44:716–29. doi: 10.1016/j.ebiom.2019.05.066

7. Hanssen N, de Vos W, Nieuwdorp M. Fecal microbiota transplantation in human metabolic diseases: from a murky past to a bright future? *Cell Metab.* (2021) 33:1098–110. doi: 10.1016/j.cmet.2021.05.005

8. Meijnikman A, Gerdes V, Nieuwdorp M, Herrema H. Evaluating Causality of gut microbiota in obesity and diabetes in humans. *Endocr Rev.* (2018) 39:133–53. doi: 10.1210/er.2017-00192

9. Waller K, Leong R, Paramsothy S. An update on fecal microbiota transplantation for the treatment of gastrointestinal diseases. *J Gastroenterol Hepatol.* (2022) 37:246–55. doi: 10.1111/jgh.15731

10. Baunwall S, Terveer E, Dahlerup J, Erikstrup C, Arkkila P, Vehreschild M, et al. The use of faecal microbiota transplantation (FMT) in Europe: a europe-wide survey. *Lancet Reg Heal Eur.* (2021) 9:100181. doi: 10.1016/j.lanepe.2021.100181

11. Keller J, Ooijevaar R, Hvas C, Terveer E, Lieberknecht S, Högenauer C, et al. A standardised model for stool banking for faecal microbiota transplantation: a consensus report from a multidisciplinary UEG working group. *United Eur Gastroenterol J.* (2021) 9:229–47. doi: 10.1177/2050640620967898

12. Dubois N, Read C, O'Brien K, Ling K. Challenges of screening prospective stool donors for fecal microbiota transplantation. *Biol Res Nurs.* (2021) 23:21–30. doi: 10.1177/1099800420941185

13. Park S, Seo G. Fecal microbiota transplantation: is it safe? *Clin Endosc.* (2021) 54:157–60. doi: 10.5946/ce.2021.072

14. Nooij S, Ducarmon Q, Laros J, Zwittink R, Norman J, Smits W, et al. Fecal microbiota transplantation influences procarcinogenic *Escherichia coli* in recipient recurrent clostridioides difficile patients. *Gastroenterology.* (2021) 161:1218–1228e5. doi: 10.1053/j.gastro.2021.06.009

15. Alang N, Kelly C. Weight gain after fecal microbiota transplantation. *Open Forum Infect Dis.* (2015) 2:ofv004. doi: 10.1093/ofid/ofv004

16. Cold F, Svensson C, Petersen A, Hansen L, Helms M. Long-term safety following faecal microbiota transplantation as a treatment for recurrent clostridioides difficile infection compared with patients treated with a fixed bacterial mixture: results from a retrospective cohort study. *Cells.* (2022) 11:435. doi: 10.3390/cells11030435

17. Ritchie M, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PLoS One.* (2012) 7:e34938. doi: 10.1371/journal.pone.0034938

18. Ashraf R, Shah N. Immune system stimulation by probiotic microorganisms. *Crit Rev Food Sci Nutr.* (2014) 54:938–56. doi: 10.1080/10408398.2011.619671

19. Hütt P, Shchepetova J, Lõivukene K, Kullisaar T, Mikelsaar M. Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. *J Appl Microbiol.* (2006) 100:1324–32. doi: 10.1111/j.1365-2672.2006.02857.x

20. Sniffen J, McFarland LV, Evans C, Goldstein E. Choosing an appropriate probiotic product for your patient: An evidence-based practical guide. *PLoS One.* (2018) 13:e0209205. doi: 10.1371/journal.pone.0209205

21. Agamennone V, Krul C, Rijkers G, Kort R. A practical guide for probiotics applied to the case of antibiotic-associated diarrhea in the Netherlands. *BMC Gastroenterol.* (2018) 18:103. doi: 10.1186/s12876-018-0831-x

22. Suez J, Zmora N, Segal E, Elinav E. The pros, cons, and many unknowns of probiotics. *Nat Med.* (2019) 25:716–29. doi: 10.1038/s41591-019-0439-x

23. O'Toole P, Marchesi J, Hill C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nat Microbiol.* (2017) 2:1–6. doi: 10.1038/nmicrobiol.2017.57

24. El Hage R, Hernandez-Sanabria E, Van de Wiele T. Emerging trends in "smart probiotics": functional consideration for the development of novel health and industrial applications. *Front Microbiol.* (2017) 8:1–11. doi: 10.3389/fmicb.2017.01889

25. Ulsemer P, Toutounian K, Kressel G, Goletz C, Schmidt J, Karsten U, et al. Impact of oral consumption of heat-treated *Bacteroides* xylanisolvens DSM 23964 on the level of natural TFα-specific antibodies in human adults. *Benef Microbes.* (2016) 7:485–500. doi: 10.3920/BM2015.0143

26. Shimbo I, Yamaguchi T, Odaka T, Nakajima K, Koide A, Koyama H, et al. Effect of clostridium butyricum on fecal flora in *Helicobacter* pylori eradication therapy. *World J Gastroenterol.* (2005) 11:7520–4. doi: 10.3748/wjg.v11.i47.7520

27. Limaye S, Haddad R, Cilli F, Sonis S, Colevas A, Brennan M, et al. Phase 1b, multicenter, single blinded, placebo-controlled, sequential dose escalation study to assess the safety and tolerability of topically applied AG013 in subjects with locally advanced head and neck cancer receiving induction chemotherapy. *Cancer.* (2013) 119:4268–76. doi: 10.1002/cncr.28365

28. Seki H, Shiohara M, Matsumura T, Miyagawa N, Tanaka M, Komiyama A, et al. Prevention of antibiotic-associated diarrhea in children by *Clostridium butyricum* MIYAIRI. *Pediatr Int.* (2003) 45:86–90. doi: 10.1046/j.1442-200x.2003.01671.x

29. Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, et al. Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat Med.* (2019) 25:1096–103. doi: 10.1038/s41591-019-0495-2

30. Perraudeau F, McMurdie P, Bullard J, Cheng A, Cutcliffe C, Deo A, et al. Improvements to postprandial glucose control in subjects with type 2 diabetes: a multicenter, double blind, randomized placebo-controlled trial of a novel probiotic formulation. *BMJ Open Diabetes Res Care.* (2020) 8:1–10. doi: 10.1136/bmjdrc-2020-001319

31. Martín R, Langella P. Emerging health concepts in the probiotics field: streamlining the definitions. *Front Microbiol.* (2019) 10:1047. doi: 10.3389/fmicb.2019.01047

32. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte R, Bartelsman J, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology.* (2012) 143:913–916e7. doi: 10.1053/j.gastro.2012.06.031

33. Shetty S, Zuffa S, Bui T, Aalvink S, Smidt H, De Vos W. Reclassification of *Eubacterium hallii* as *Anaerobutyricum hallii* gen. nov., comb. nov., and description of *Anaerobutyricum soehngenii* sp. nov., a butyrate and propionate-producing bacterium from infant faeces. *Int J Syst Evol Microbiol.* (2018) 68:3741–6. doi: 10.1099/ijsem.0.003041

34. Barcenilla A, Pryde S, Martin J, Duncan S, Stewart C, Henderson C, et al. Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol.* (2000) 66:1654–61. doi: 10.1128/aem.66.4.1654-1661.2000

35. Muñoz-Tamayo R, Laroche B, Walter E, Doré J, Duncan S, Flint H, et al. Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species. *FEMS Microbiol Ecol.* (2011) 76:615–24. doi: 10.1111/j.1574-6941.2011.01085.x

36. Belenguer A, Duncan S, Calder A, Holtrop G, Louis P, Lobley G, et al. Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. *Appl Environ Microbiol.* (2006) 72:3593–9. doi: 10.1128/AEM.72.5.3593-3599.2006

37. Udayappan S, Manneras-Holm L, Chaplin-Scott A, Belzer C, Herrema H, Dallinga-Thie G, et al. Oral treatment with *Eubacterium hallii* improves insulin sensitivity in db/db mice. *NPJ Biofilms Microb.* (2016) 2:9. doi: 10.1038/npjbiofilms.2016.9

38. Gilijamse P, Hartstra AV, Levin E, Wortelboer K, Serlie M, Ackermans M, et al. Treatment with *Anaerobutyricum soehngenii*: a pilot study of safety and dose–response effects on glucose metabolism in human subjects with metabolic syndrome. *NPJ Biofilms Microb.* (2020) 6:16. doi: 10.1038/s41522-020-0127-0

39. Hill C, Guarner F, Reid G, Gibson G, Merenstein D, Pot B, et al. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol.* (2014) 11:506–14. doi: 10.1038/nrgastro.2014.66

40. Panel E, Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, et al. Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 13: suitability of taxonomic units notified to EFSA until september 2020. *EFSA J Eur Food Saf Auth.* (2021) 19:e06377. doi: 10.2903/j.efsa.2021.6377

41. Unites States Food and Drug Administration. Early clinical trials with live biotherapeutic products: chemistry, manufacturing, and control information. *Guid Ind.* (2016) 2016:1–18.

42. European Pharmacopoeia Commission. *3053E general monograph on live biotherapeutic products. European pharmacopoeia 10.5*. (2019). Available online at: https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-10th-edition (accessed September 1, 2022).

43. Stefan N, Birkenfeld A, Schulze M. Global pandemics interconnected - obesity, impaired metabolic health and COVID-19. *Nat Rev Endocrinol.* (2021) 17:135–49. doi: 10.1038/s41574-020-00462-1

44. Hartstra AV, Bouter K, Backhed F, Nieuwdorp M. Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care.* (2015) 38:159–65. doi: 10.2337/dc14-0769

45. Atzeni A, Galié S, Muralidharan J, Babio N, Tinahones F, Vioque J, et al. Gut microbiota profile and changes in body weight in elderly subjects with overweight/obesity and metabolic syndrome. *Microorganisms.* (2021) 9:346. doi: 10.3390/microorganisms9020346

46. Del Chierico F, Manco M, Gardini S, Guarrasi V, Russo A, Bianchi M, et al. Fecal microbiota signatures of insulin resistance, inflammation, and metabolic syndrome in youth with obesity: a pilot study. *Acta Diabetol.* (2021) 58:1009–22. doi: 10.1007/s00592-020-01669-4

47. Karlsson F, Tremaroli V, Nookaew I, Bergström G, Behre C, Fagerberg B, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature.* (2013) 498:99–103. doi: 10.1038/nature12198

48. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature.* (2013) 500:541–6. doi: 10.1038/nature12506

49. Gao Z, Yin J, Zhang J, Ward R, Martin R, Lefevre M, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes.* (2009) 58:1509–17. doi: 10.2337/db08-1637

50. Henagan T, Stefanska B, Fang Z, Navard A, Ye J, Lenard N, et al. Sodium butyrate epigenetically modulates high-fat diet-induced skeletal muscle mitochondrial adaptation, obesity and insulin resistance through nucleosome positioning. *Br J Pharmacol.* (2015) 172:2782–98. doi: 10.1111/bph.13058

51. Louis P, Young P, Holtrop G, Flint H. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. *Environ Microbiol.* (2010) 12:304–14. doi: 10.1111/j.1462-2920.2009.02066.x

52. Shetty S, Hugenholtz F, Lahti L, Smidt H, de Vos W. Intestinal microbiome landscaping: insight in community assemblage and implications for microbial modulation strategies. *FEMS Microbiol Rev.* (2017) 41:182–99. doi: 10.1093/femsre/fuw045

53. Duncan S, Louis P, Flint H. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl Environ Microbiol.* (2004) 70:5810–7. doi: 10.1128/AEM.70.10.5810-5817.2004

54. Shetty S, Ritari J, Paulin L, Smidt H, De Vos W. Complete genome sequence of *Eubacterium hallii* strain L2-7. *Genome Ann.* (2017) 5:17. doi: 10.1128/genomeA.01167-17

55. Hold G, Schwiertz A, Aminov R, Blaut M, Flint H. Oligonucleotide probes that detect quantitatively significant groups of butyrate-producing bacteria in human feces. *Appl Environ Microbiol.* (2003) 69:4320–4. doi: 10.1128/AEM.69.7.4320-4324.2003

56. Shetty S, Boeren S, Bui T, Smidt H, de Vos W. Unravelling lactate-acetate and sugar conversion into butyrate by intestinal *Anaerobutyricum* and *Anaerostipes* species by comparative proteogenomics. *Environ Microbiol.* (2020) 22:4863–75. doi: 10.1111/1462-2920.15269

57. Ten Bruggencate S, Bovee-Oudenhoven I, Lettink-Wissink M, Katan M, van der Meer R. Dietary fructooligosaccharides affect intestinal barrier function in healthy men. *J Nutr.* (2006) 136:70–4. doi: 10.1093/jn/136.1.70

58. Seheult J, Fitzpatrick G, Boran G. Lactic acidosis: an update. *Clin Chem Lab Med.* (2017) 55:322–33. doi: 10.1515/cclm-2016-0438

59. Nogal A, Valdes A, Menni C. The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. *Gut Microbes.* (2021) 13:1–24. doi: 10.1080/19490976.2021.1897212

60. Bui T, de Vos W. Next-generation therapeutic bacteria for treatment of obesity, diabetes, and other endocrine diseases. *Best Pract Res Clin Endocrinol Metab.* (2021) 2021:101504. doi: 10.1016/j.beem.2021.101504

61. European Union. Regulation (EC) No 1829/2003 of the European parliament and of the council of 22 september 2003 on genetically modified food and feed. *Off J Eur Union.* (2003) 268:1–23.

62. European Union. Directive 2009/41/EC of the European parliament and of the council of 6 may 2009 on the contained use of genetically modified micro-organisms. *Off J Eur Union.* (2009) 125:75–97.

63. European Union. Directive 2001/18/EC of the European parliament and of the council of 12 march 2001 on the deliberate release into the environment of genetically modified organisms and repealing council directive 90/220/EEC. *Off J Eur Commun.* (2001) 106:1–39.

64. Rouanet A, Bolca S, Bru A, Claes I, Cvejic H, Girgis H, et al. Live biotherapeutic products, a road map for safety assessment. *Front Med.* (2020) 7:1–13. doi: 10.3389/fmed.2020.00237

65. Seegers J, Gül I, Hofkens S, Brosel S, Schreib G, Brenke J, et al. Toxicological safety evaluation of live *Anaerobutyricum soehngenii* strain CH106. *J Appl Toxicol.* (2022) 42:244–57. doi: 10.1002/jat.4207

66. Unites States Food and Drug Administration. *Guidance for Industry and Other Stakeholders: toxicological Principles for the Safety Assessment of Food Ingredients. Redbook 2000.* (2007). Available online at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-and-other-stakeholders-redbook-2000 (accessed September 1, 2022).

67. Aquilina G, Castle L, Engel K, Fowler P, Jose M, Fernandez F, et al. Guidance for submission for food additive evaluations. *EFSA J.* (2012) 10:2760. doi: 10.2903/J.EFSA.2012.2760

68. Nguyen T, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? *Dis Model Mech.* (2015) 8:1–16. doi: 10.1242/dmm.017400

69. Turner PV. The role of the gut microbiota on animal model reproducibility. *Anim Model Exp Med.* (2018) 1:109–15. doi: 10.1002/ame2.12022

70. Hugenholtz F, de Vos W. Mouse models for human intestinal microbiota research: a critical evaluation. *Cell Mol Life Sci.* (2018) 75:149–60. doi: 10.1007/s00018-017-2693-8

71. Cordaillat-Simmons M, Rouanet A, Pot B. Live biotherapeutic products: the importance of a defined regulatory framework. *Exp Mol Med.* (2020) 52:1397–406. doi: 10.1038/s12276-020-0437-6

72. Dutch Government. *Warenwetbesluit bereiding en behandeling van levensmiddelen. Staatsblad*. (1992). Available online at: https://wetten.overheid.nl/BWBR0005758/2021-07-01 (accessed September 1, 2022).

73. European Union. Regulation (EC) No 178/2002 of the European parliament and of the council of 28 january 2002 laying down the general principles and requirements of food law, establishing the european food safety authority and laying down procedures in matters of food saf. *Off J Eur Union.* (2002) 31:1–24.

74. European Union. REGULATION (EC) No 852/2004 of the European parliament and of the council of 29 april 2004 on the hygiene of foodstuffs. *Off J Eur Commun.* (2004) 139:1–54.

75. Fenster K, Freeburg B, Hollard C, Wong C, Rønhave Laursen R, Ouwehand A. The production and delivery of probiotics: a review of a practical approach. *Microorganisms.* (2019) 7:83. doi: 10.3390/microorganisms7030083

76. Shah N, Ding W, Fallourd M, Leyer G. Improving the stability of probiotic bacteria in model fruit juices using vitamins and antioxidants. *J Food Sci.* (2010) 75:M278–82. doi: 10.1111/j.1750-3841.2010.01628.x

77. Heidebach T, Först P, Kulozik U. Microencapsulation of probiotic cells for food applications. *Crit Rev Food Sci Nutr.* (2012) 52:291–311. doi: 10.1080/10408398.2010.499801

78. Assadpour E, Jafari S. Advances in spray-drying encapsulation of food bioactive ingredients: from microcapsules to *Nanocapsules*. *Annu Rev Food Sci Technol.* (2019) 10:103–31. doi: 10.1146/annurev-food-032818-121641

79. Tompkins T, Mainville I, Arcand Y. The impact of meals on a probiotic during transit through a model of the human upper gastrointestinal tract. *Benef Microbes.* (2011) 2:295–303. doi: 10.3920/BM2011.0022

80. Koopen A, Witjes J, Wortelboer K, Majait S, Prodan A, Levin E, et al. Duodenal *Anaerobutyricum soehngenii* infusion stimulates GLP-1 production, ameliorates glycaemic control and beneficially shapes the duodenal transcriptome in metabolic syndrome subjects: a randomised double-blind placebo-controlled cross-over study. *Gut.* (2021) 2021:323297. doi: 10.1136/gutjnl-2020-323297

81. Greiner T, Bäckhed F. Microbial regulation of GLP-1 and L-cell biology. *Mol Metab.* (2016) 5:753–8. doi: 10.1016/j.molmet.2016.05.012

82. Tolhurst G, Heffron H, Lam Y, Parker H, Habib A, Diakogiannaki E, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes.* (2012) 61:364–71. doi: 10.2337/db11-1019

83. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab.* (2009) 10:167–77. doi: 10.1016/j.cmet.2009.08.001

84. Ducastel S, Touche V, Trabelsi M, Boulinguiez A, Butruille L, Nawrot M, et al. The nuclear receptor FXR inhibits glucagon-like peptide-1 secretion in response to microbiota-derived short-chain fatty acids. *Sci Rep.* (2020) 10:174. doi: 10.1038/s41598-019-56743-x

85. Trabelsi M, Daoudi M, Prawitt J, Ducastel S, Touche V, Sayin S, et al. Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. *Nat Commun.* (2015) 6:7629. doi: 10.1038/ncomms8629

86. Jiang Y, Wang Z, Ma B, Fan L, Yi N, Lu B, et al. GLP-1 improves adipocyte insulin sensitivity following induction of endoplasmic reticulum stress. *Front Pharmacol.* (2018) 9:1168. doi: 10.3389/fphar.2018.01168

87. Yatsunenko T, Rey F, Manary M, Trehan I, Dominguez-Bello M, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature.* (2012) 486:222–7. doi: 10.1038/nature11053

88. Greenhalgh K, Meyer K, Aagaard K, Wilmes P. The human gut microbiome in health: establishment and resilience of microbiota over a lifetime. *Environ Microbiol.* (2016) 18:2103–16. doi: 10.1111/1462-2920.13318

89. Healey G, Murphy R, Butts C, Brough L, Whelan K, Coad J. Habitual dietary fibre intake influences gut microbiota response to an inulin-type fructan prebiotic: a randomised, double-blind, placebo-controlled, cross-over, human intervention study. *Br J Nutr.* (2018) 119:176–89. doi: 10.1017/S0007114517003440

90. Barton W, Penney N, Cronin O, Garcia-Perez I, Molloy M, Holmes E, et al. The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level. *Gut.* (2018) 67:625–33. doi: 10.1136/gutjnl-2016-313627

91. Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, et al. Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host Microbe.* (2018) 24:133–145e5. doi: 10.1016/j.chom.2018.06.005

92. Song S, Lauber C, Costello E, Lozupone C, Humphrey G, Berg-Lyons D, et al. Cohabiting family members share microbiota with one another and with their dogs. *Elife.* (2013) 2:e00458. doi: 10.7554/eLife.00458

93. Swann J, Rajilic-Stojanovic M, Salonen A, Sakwinska O, Gill C, Meynier A, et al. Considerations for the design and conduct of human gut microbiota intervention studies relating to foods. *Eur J Nutr.* (2020) 59:3347–68. doi: 10.1007/s00394-020-02232-1

94. European Union. Regulation (EU) No 1169/2011 of the European parliament and of the council of 25 october 2011 on the provision of food information to consumers, amending regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European parliament and of the council, an. *Off J Eur Union.* (2011) 304:18–63.

95. European Union. Regulation (EU) 2015/2283 of the European parliament and of the council of 25 november 2015 on novel foods, amending regulation (EU) No 1169/2011 of the European parliament and of the council and repealing regulation (EC) No 258/97 of the European parliam. *Off J Eur Union.* (2015) 327:1–22.

96. European Union. Commission implementing regulation (EU) 2017/2469 of 20 december 2017 laying down administrative and scientific requirements for applications referred to in article 10 of regulation (EU) 2015/2283 of the European parliament and of the council on novel foo. *Off J Eur Union.* (2017) 351:64–71.

97. European Union. Regulation (EC) No 1924/2006 of the European parliament and of the council of 20 december 2006 on nutrition and health claims made on foods. *Off J Eur Union.* (2006) 404:9–25. doi: 10.2147/JPR.S160660

98. Martínez S, Siani A. Health claims made on food in the EU: the edge between scientific knowledge and regulatory requirements. *Trends Food Sci Technol.* (2017) 69:315–23. doi: 10.1016/j.tifs.2017.01.005

99. Committee for Human Medicinal Products (CHMP). ICH guideline E8 (R1) on general considerations for clinical studies. *EMA.* (2021) 2021:1–25.

100. Committee for Human Medicinal Products (CHMP). Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products. *EMA.* (2017) 2017:1–22.

101. Committee for Human Medicinal Products (CHMP). Guideline on human cell-based medicinal products. *EMA.* (2008) 2008:1–25.

102. Paquet J, Claus S, Cordaillat-Simmons M, Mazier W, Rawadi G, Rinaldi L, et al. Entering first-in-human clinical study with a single-strain live biotherapeutic product: input and feedback gained from the EMA and the FDA. *Front Med.* (2021) 8:716266. doi: 10.3389/fmed.2021.716266

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 08 December 2022
DOI 10.3389/fmed.2022.1060581

OPEN ACCESS

EDITED BY
Angel Lanas,
University of Zaragoza, Spain

REVIEWED BY
Jing Ouyang,
Chongqing Public Health Medical Center, China
Tetyana Falalyeyeva,
Taras Shevchenko National University of Kyiv, Ukraine

*CORRESPONDENCE
Mihai Covasa
mcovasa@westernu.edu

SPECIALTY SECTION
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

RECEIVED 03 October 2022
ACCEPTED 21 November 2022
PUBLISHED 08 December 2022

CITATION
Hamamah S, Gheorghita R, Lobiuc A, Sirbu I-O and Covasa M (2022) Fecal microbiota transplantation in non-communicable diseases: Recent advances and protocols. *Front. Med.* 9:1060581. doi: 10.3389/fmed.2022.1060581

Fecal microbiota transplantation in non-communicable diseases: Recent advances and protocols

Sevag Hamamah[1], Roxana Gheorghita[2,3], Andrei Lobiuc[2], Ioan-Ovidiu Sirbu[3,4] and Mihai Covasa[1,2]*

[1]Department of Basic Medical Sciences, College of Osteopathic Medicine, Western University of Health Sciences, Pomona, CA, United States, [2]Department of Medicine and Biomedical Sciences, College of Medicine and Biological Science, University of Suceava, Suceava, Romania, [3]Department of Biochemistry, Victor Babeş University of Medicine and Pharmacy Timisoara, Timişoara, Romania, [4]Center for Complex Network Science, Victor Babeş University of Medicine and Pharmacy Timisoara, Timişoara, Romania

Fecal microbiota transplant (FMT) is a therapeutic method that aims to restore normal gut microbial composition in recipients. Currently, FMT is approved in the USA to treat recurrent and refractory *Clostridioides difficile* infection and has been shown to have great efficacy. As such, significant research has been directed toward understanding the potential role of FMT in other conditions associated with gut microbiota dysbiosis such as obesity, type 2 diabetes mellitus, metabolic syndrome, neuropsychiatric disorders, inflammatory bowel disease, irritable bowel syndrome, decompensated cirrhosis, cancers and graft-versus-host disease. This review examines current updates and efficacy of FMT in treating conditions other than *Clostridioides difficile* infection. Further, protocols for administration of FMT are also discussed including storage of fecal samples in stool banks, inclusion/exclusion criteria for donors, fecal sample preparation and methods of treatment administration. Overall, understanding the mechanisms by which FMT can manipulate gut microbiota to provide therapeutic benefit as well as identifying potential adverse effects is an important step in clarifying its long-term safety and efficacy in treating multiple conditions in the future.

KEYWORDS

microbiota transplant, obesity, metabolic disease, inflammatory bowel disease, irritable bowel syndrome, cirrhosis, cancer, FMT protocol

Introduction

In recent years, there has been an increasing interest in the role of the gut microbiota in health and disease. The term "gut microbiota" refers to all bacteria, archaea, microeukaryotes and viruses that co-exist within the human gastrointestinal (GI) tract (1), while the gut microbiome refers to the collective genomic composition of these microorganisms. Currently, it is estimated that human tract hosts over 100 trillion microorganisms, with a microbiome of approximately 3.3 million unique genes, far surpassing the complexity of the human genome that contains 23,000 genes (2). While initial studies analyzing fetal amniotic fluid suggested no detectable microbial community in the prenatal period (3), recent data provides compelling evidence

demonstrating that gut microbial colonization occurs *in utero* (4). After colonization, the gut microbiota develops continuously throughout childhood and adolescence and at the age of 3, it is assumed to closely resemble that of an adult (5). Throughout a individual's lifetime, the composition of these microorganisms is influenced by a variety of factors including gender, race/ethnicity, location in the GI tract, age and diet. For example, notable differences in gut microbiota species were observed when comparing the microbiota of children who consume healthier, mainly plant carbohydrates, as opposed to children that are adherent to a Westernized diet (6), indicating a heavy influence of lifestyle measures on gut microbiota.

Gut sequencing studies have indicated that richness and diversity of microorganisms in the intestinal tract is closely correlated with human health (7), as colonization of certain bacterial species are shown to be of benefit to the host. Collectively, gut bacteria have been shown to have important roles including, but not limited to, regulating inflammation (8), maintaining gut barrier integrity (9), facilitating digestion, improving insulin sensitivity (10), and enhancing brain health (11). Further, key gut microbiota metabolites, most prominently short-chain fatty acids (SCFA) produced primarily by symbiotic bacterial species, mediate a myriad of these favorable effects on human health (12). The concentration of these SCFA is directly influenced by the relative abundances and deficiencies of certain gut bacterial species. Two main bacterial phyla, Firmicutes and Bacteroidetes, predominate the human gut, accounting for 90% of the species that reside there (13). As such, the Firmicutes/Bacteroidetes ratio, has been often used as a marker to identify correlations with the onset of diseases such as obesity, type 2 diabetes mellitus (T2DM), inflammatory bowel disease and colorectal cancer (14, 15). Imbalances in the intestinal microbiota, also called dysbiosis, play a key role in changes in the Firmicutes/Bacteroidetes ratio, with decreasing microbial diversity, contributing to disease onset. Although numerous studies have shown that the microbiome can recover after certain aggressions, some disturbances may persist leading to negative health outcomes (16). Therefore, significant research has been directed toward understanding the mechanisms by which gut microbiota exert their effects and innovating therapeutic modalities to manipulate these microorganisms in a way that will benefit their host (17, 18).

One such therapeutic modality that has garnered significant interest in the last few decades is fecal microbiota transplant (FMT). FMT aims to restore microbial diversity that is diminished as a result of dysbiosis by delivering fecal microorganisms from a healthy person to a patient. Currently, FMT is primarily indicated in treating recurrent and refractory *Clostridium difficile* infection (CDI) with study findings showing better outcomes than antibiotic treatments (19). Due to its success in treating recurrent CDI, many ongoing studies are investigating the benefits of FMT in non-communicable diseases including metabolic diseases, neuropsychiatric conditions, inflammatory bowel conditions, decompensated cirrhosis, cancers, and graft-versus-host disease (20–24). Collectively, these non-communicable diseases contribute significantly to worldwide morbidity and mortality and often present comorbidly, further worsening patient outcomes and severity of disease (25). Therefore, understanding the safety of, and mechanisms by which, targeted microbiota therapies like FMT restore pathogenic changes can assist in assessing treatment efficacy and help work toward optimizing its' therapeutic benefits.

Overall, the procedure is deemed to be safe with serious side effects being unusual (26). However, the protocols referring to donor selection methods and the methodology used for fecal transplantation are not consistently or uniformly applied. In many countries, the legislation for using FMT is not well regulated at the national level and most facilities that implement FMT procedures use their own guidelines. The Food and Drug Administration (FDA) and national authority regulations consider stool samples to be drugs and suggest their strict oversight in clinical trials due to risks of accidental pathogen transmission and development of antibiotic resistance (27). Although FMT therapy is constantly simplifying and improving, it remains a complex and expensive procedure, due to the donor selection process, which includes some specific analyses, as well as complex training and administration techniques. Therefore, uniform questionnaires and methodologies to screen donors have been developed to eliminate risks of pathogens and ensure safety prior to transplantation.

In this review, we present the emerging evidence of FMT as a therapeutic modality to improve and restore deleterious effects on gut microbial composition and its resulting effects on the development of pathological conditions beyond recurrent CDI including obesity, diabetes mellitus, metabolic syndrome, neuropsychiatric disorders, inflammatory bowel conditions, cirrhosis, cancers, and graft-versus-host disease. Then, we provide a summary of the guidelines for fecal sample collection and administration involving the donor selection process with inclusion/exclusion criteria, preparation of fecal samples and patient preparation. Lastly, we briefly discuss the risks and benefits of the various methods by which FMT can be administered. Overall, this review highlights recent advances in FMT while providing an outline by which clinicians and scientists can follow when preparing for FMT administration.

Fecal microbiota transplant and obesity

Over the past several decades, there has been dramatic increases in the prevalence of obesity and its associated metabolic disorders, including type 2 diabetes (T2DM) and metabolic syndrome (28). Cumulatively, these diseases involve significant healthcare costs, with high levels of morbidity and

mortality (29). While these diseases are closely associated to human genetics and lifestyle changes, the intestinal microorganisms and their collective genome are now recognized to play an emerging role in their pathogenesis (30). Certain metagenomic sequencing patterns are associated with the phenotype of obesity. In general, health-promoting bacteria like *Lactobacillus, Bifidobacterium, Akkermansia* are reduced, while opportunistic pathogens in the *Enterobacteriaceae, Desulfovibrionaceae,* and *Streptococcaceae* families are elevated (31). These patterns are responsible for changes in the body weight of individuals, suggesting that the modulation of the intestinal microbiome is dynamically correlated with the metabolic phenotype of the human host. Therefore, FMT has been studied as a therapeutic method to replenish beneficial gut microbiota to potentially reverse or prevent further fat accumulation (21). Though it is well-supported that FMT exhibits sustained gut microbial composition changes in obese patients, there is ambiguity in whether the therapeutic modality is actually effective in decreasing body weight (32). In a randomized clinical trial assessing the effects of FMT on adolescents, there was no observed effect of FMT on weight loss at 12 weeks, however, a reduction in abdominal adiposity was detected (33). It should be noted however that *post hoc* analysis of the same patients at 26 weeks with co-existing metabolic syndrome revealed a significant benefit, with 78% resolution of metabolic abnormalities as compared to 23% in the placebo group.

There has also been controversy on whether FMT can induce an obese phenotype by implanting gut microbiota of overweight individuals into lean recipients. In a case study of a patient with CDI undergoing a successful FMT intervention, it was found that the recipient of the stool sample from an overweight donor later developed an obese phenotype (34). Further, FMT studies using twins discordant for obesity, and transfer of microbiota from obese mice significantly increases weight gain and adiposity (35). However, a more recent study evaluating weight gain in patients treated with a single FMT for recurrent CDI found an increased BMI post-FMT. However, the weight gain was not significant, and the increase in BMI was attributed to a return to baseline from the initial weight loss experienced during the active CDI (36). Several studies looked at lifestyle interventions in conjunction with FMT treatment to assess treatment efficacy. For example, dietary and exercise interventions, in addition to FMT in obese patients, results in more advantageous changes in recipient gut microbiota and lipid profile versus FMT alone (20). These improvements were associated with increases in *Lactobacillus* and *Bifidobacterium,* as well as reductions in total cholesterol, as well as low density lipoproteins (LDL). In another study, patients underwent Mediterranean diet-based weight loss programs for 6 months, followed by a weight regain phase from month 6 to 14. Fecal samples were collected during the weight loss period and autologous FMT was performed during the weight gain phase (37). The results showed that autologous FMT with samples obtained during the weight loss period may preserve weight loss and help maintain glycemic control. Still, it is unclear whether most of the benefits observed in this study are a result of dietary and exercise interventions or FMT, though it is likely that lifestyle modifications optimize the therapeutic effects of FMT. Overall, the current literature does not provide clear evidence of the efficacy of FMT in humans as a treatment for reducing BMI directly. It is possible that the length of these studies do not provide enough time for FMT to influence weight changes or that other lifestyle factors are interfering with direct assessment of FMT-related outcomes. However, some studies support the therapeutic role of FMT on metabolic abnormalities and obesity-related sequelae including T2DM and metabolic syndrome, which will be discussed in the next section.

Fecal microbiota transplant effects on diabetes and metabolic syndrome

There is promising evidence that FMT can exert positive therapeutic effects by attenuating the development and progression of T2DM, T1DM and metabolic syndrome. These metabolic diseases are characterized by a high degree of inflammation, which may eventually lead to insulin resistance and metabolic endotoxemia through damage to the protective intestinal mucosa (38). Induction of a chronic inflammatory state results from an uninterrupted release of cytokines, which damages insulin-sensitive cells in the liver, muscles, and adipose tissue (39). Sequencing studies of gut flora in diabetics has shown particular changes that have been attributed to increase gut permeability and susceptibility to chronic inflammatory states (40). For example, diabetic patients have lower colonies of *Akkermansia muciniphila* compared to healthy controls. *Akkermansia muciniphila* is a Gram-negative bacterium that improves glucose tolerance and insulin resistance. More specifically, *Akkermansia* is found to decrease metabolic endotoxemia by reducing plasma LPS levels and reinforcing the gut barrier, thus exerting its beneficial effects on T2DM (41). Other studies have also shown that the microbiota of T2DM patients show relative deficiencies in *Clostridium, Roseburia,* and *Faecalibacterium prausnitzii*, which are species associated with production of butyrate (42). As such, FMT has been shown to promote the growth of butyrate producing bacteria such as *Roseburia intestinalis* and *Eubacterium hallii*, thus conferring beneficial effect on metabolic diseases (43). Butyrate is a SCFA that is associated with improved insulin sensitivity and attenuates progression of T2DM (42).

It is also important to note that most patients with T2DM take medications to lower blood glucose levels, such as metformin, which have been shown to exert positive effects on gut microbial composition (44, 45). Thus, when FMT is

combined with drug administration, the beneficial effect of transplantation from healthy donors to T2DM patients as a direct result from the FMT treatment may be difficult to assess. Most studies assessing the efficacy of FMT are conducted in animal models, with fewer studies in patients with T2DM. For example, a recent study evaluating clinical responses to FMT of 17 human participants, showed that 11 of them (64%) had statistically significant decrease in hemoglobin A1c (HbA1c) and blood glucose, while post-prandial C-peptide, a measure correlated with serum insulin, was elevated (46). Microbiota analysis revealed increases of the genus *Anaerotruncus*, which has been associated with increased insulin resistance (47). The individuals harboring increased abundance of *Anaerotruncus* exhibited a better clinical response to FMT intervention (46), indicating that this bacterial genus may be a marker of treatment efficacy in diabetics. Results from another recently conducted study indicated that FMT-induced gut microbiota changes were correlated with improvements in blood glucose in T2DM (48). Importantly, FMT increased the genus *Bifidobacterium* concentrations, shown to have multiple benefits on metabolic health, while reducing *Desulfovibrio* and *Bilophila,* two sulfate-reducing genera associated with increased inflammation and elevated blood glucose.

Similarly, several studies have reported positive effects of FMT in patients with T1DM, which has also been associated with dysbiosis of the gut microbiota (49). For example, in T1DM patients receiving three FMT treatments over the span of 4 months, FMT halted progression of the disease by preventing a decline in residual beta-cell function (50). Specifically, plasma metabolites 1-arachidonoyl-GPC and 1-myristoyl-2-arachidonoyl-GPC were associated with beta-cell preservation, while *Prevotella* was inversely related with beta-cell function. At 12 months post-FMT, stimulated C-peptide serum levels was observed to be at a level similar to the ones measured prior to treatment, indicating the efficacious role of microbiota transplant. In a separate study, Xie et al. reported a case of a 24-year-old patient with T1DM, with severe malnutrition and recurrent abdominal pain, nausea and vomiting, which are symptoms consistent with diabetic ketoacidosis (51). FMT treatment significantly relieved patient's nausea and vomiting, while also showing gradual improvements in nutritional status and blood glucose control as measured by HbA1c and fasting blood glucose. These clinical improvements were accompanied by drastic improvements in the microbiota composition, that resembled that of the healthy donor. Further, a recent study conducted in a T1DM-induced mice model has shown significant benefits of FMT on male fertility such as improved deficits in spermatogenesis and semen quality (52). This effect was attributed to *Lactobacillus* spp. that were more abundant in the treatment group, leading to increase production of *n*-3 polyunsaturated fatty acid docosahexaenoic acid and eicosapentaenoic acid in the testes, which likely mediate the beneficial effects. Taken together, these findings suggest that FMT administration in patients with T1DM is effective in improving the progression of the disease, its' metabolic parameters as well as systemic complications that result from disease onset.

In addition to its beneficial effects in improving T1DM and T2DM, FMT has been shown to alleviate symptoms associated with diabetic kidney disease (53). For example, FMT treatment improved multiple parameters including amelioration of insulin resistance, prevention of weight gain as well as reduction of tumor necrosis factor-α (TNF-α) and albuminuria in a mouse model. Intestinal structural integrity was maintained while the abundance of succinate consuming *Odoribacteraceae* bacteria family was increased compared with untreated mice. The succinate consumption capacity of *Odoribacteraceae* is known to cause mitochondrial damage-associated molecular pattern (DAMP), with reductions in the bacterial family being implicated in various inflammatory diseases (54). However, the possible influence of other factors on metabolic outcomes such as lifestyle, pharmacological drugs, in particular metformin, sodium-glucose cotransporter-2 inhibitors, GLP-1 receptor agonists and lipid-lowering drugs, should all be considered when interpreting these results.

In addition to T2DM, FMT has been shown to restore deficits seen in both human and animal model studies of metabolic syndrome (55). For example, in a rat model of fructose-induced metabolic syndrome, FMT reduced metabolic syndrome markers including inflammation and oxidative stress (56). The fructose diet increased *Coprococcus* and *Ruminococcus* levels, both of which were normalized after FMT treatment. *Ruminococcus* is a mucin-degrading species that is associated with pro-inflammatory markers especially when in excess (57). Therefore, some of the anti-inflammatory effects observed in the study may be attributed to reduction in *Ruminococcus* species *via* FMT. In another study that evaluated the effects of FMT on 26 patients with metabolic syndrome, 65% of them showed improved insulin sensitivity 6 weeks after treatment (58), an effect associated with *Bifidobacterium*-induced increases in acetate (59). The specific mechanisms by which FMT exert its benefits on metabolic syndrome are not completely known, however, allogenic microbiota transplant showed improvements of insulin sensitivity *via* methylation of actin-filament associated protein 1 (AFAP1) gene (60), a gene that is associated with altered glucose metabolism. Additionally, FMT recipients with metabolic syndrome showed that treatment helps promote a bacteriophage environment that is similar to that of healthy individuals (61). Taken together, these studies provide strong evidence for FMT in improving insulin sensitivity and glucose metabolism in metabolic disorders (**Figure 1**).

Fecal microbiota transplant in neuropsychiatric disorders

Fecal microbiota transplant has been shown to exert a myriad of beneficial effects on psychiatric, neurologic,

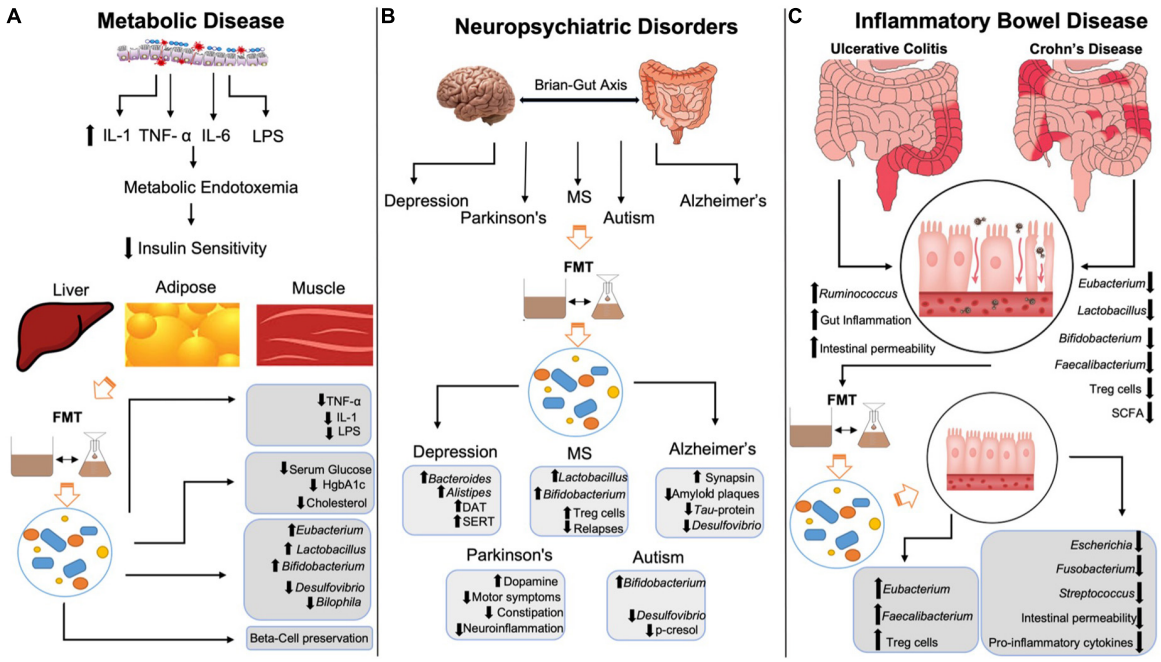


FIGURE 1
Mechanisms by which FMT restores negative changes in metabolic disease, neuropsychiatric conditions and inflammatory bowel disease. **(A)** LPS-mediated increases in IL-1, TNF-α, and IL-6 leads to metabolic endotoxemia decreasing insulin sensitivity in liver, adipose, and muscle tissue. FMT reduces LPS, IL-1 and TNF-α, lowers serum glucose, HgbA1c and cholesterol levels and preserves beta-cells in T1DM patients. FMT increases *Eubacterium, Lactobacillus,* and *Bifidobacterium* spp., while reducing *Desulfovibrio* and *Bilophila* spp. **(B)** FMT increases dopamine transporter and serotonin transporter expression, while also increasing *Bacteroides* and *Alistipes* in patients in depression. FMT increase *Lactobacillus, Bifidobacterium* and Treg activity in multiple sclerosis patients, while reducing relapses/flares. FMT increases synapsin, amyloid plaques and Tau-protein phosphorylation in Alzheimer's disease while reducing *Desulfovibrio* spp. FMT increases dopamine while decreasing neuroinflammation, motor and non-motor symptoms in Parkinson's disease. FMT increases *Bifidobacterium* spp. and reduces *p*-cresol and *Desulfovibrio* spp. in autism spectrum disorder. **(C)** Inflammatory bowel disease is characterized by increased gut inflammation, intestinal permeability and *Ruminococcus* spp. with decreased *Eubacterium, Lactobacillus, Bifidobacterium, Faecalibacterium,* Treg activity and SCFA. FMT restores *Eubacterium* and *Faecalibacterium,* increases Treg activity, while decreasing *Escherichia* spp., *Fusobacterium, Streptococcus,* intestinal permeability, and pro-inflammatory cytokines. IL-1, interleukin-1; TNF-α, tumor necrosis factor α; IL-6, interleukin-6; LPS, lipopolysaccharides; FMT, fecal microbiota transplant; HgbA1c, hemoglobin A1c; MS, multiple sclerosis; DAT, dopamine transporter; SERT, serotonin transporter; Treg, T-regulatory; SCFA, short-chain fatty acids.

neurodevelopmental, and neurodegenerative disorders (62) (**Figure 1**). The bidirectional communication between the brain and the gut, known as the microbiota-gut-brain (MGB) axis is a pivotal component of the neuropsychiatric changes observed after modification of gut microbiota composition. The MGB axis has been shown to influence concentrations of many neuropeptides and neurotransmitters that contribute to altered brain chemistry and disease onset including serotonin (5-HT), dopamine (DA), norepinephrine (NE), epinephrine (Epi) as well as their precursors, receptors, and metabolites (63). Gut microbiota exert effects on the brain neurochemistry *via* neuroactive metabolites such as SCFAs activating vagal afferents, neuroendocrine control of the hypothalamic-pituitary-adrenal axis and pro-inflammatory cytokine mediated inflammation, to name a few (11). As such, FMT has been studied in the setting of neuropsychiatric imbalance to assess the impact of gut microbiota on these pathways and to provide therapeutic benefits to patients.

Mood disorders such as major depressive disorder (MDD), anxiety and bipolar disorders (BD) are multifactorial disorders that are etiologically complex. The lifetime prevalence of generalized anxiety disorder is 33.7% (64), while MDD is 16% and BD is approximately 5% (65). Due to their impact on the global population, significant efforts have been directed toward understanding the role of gut microbiota in the pathogenesis of psychiatric conditions to develop and optimize treatment modalities, including FMT. When evaluating the effects of FMT treatment in mice studies, microbiota from donor stress-induced mice that was transplanted into germ-free mice caused increased anxiety and depression like behaviors and decreased intestinal 5-HT concentrations compared to control animals (66). Both donor stress-induced mice and their microbiota recipients had low levels of *Lactobacillus* and increased *Akkermansia*. *Akkermansia*, when in adequate concentrations, plays an important role in degrading the mucin layer, however, when increased, it can lead to mucin

degradation resulting in increased intestinal permeability and susceptibility to endotoxemia (67). Indeed, stress-induced mice have increased neuroinflammation with elevated pro-inflammatory cytokines like interferon-γ (IFN- γ) and tumor necrosis factor-α (TNF-α). Further, the dopamine transporter (DAT) and serotonin transporter (SERT) binding capacities are increased in human subjects with metabolic syndrome undergoing FMT with oral capsules (22). DAT and SERT facilitate reuptake of DA and 5-HT, respectively, to increase neurotransmitter availability in the synaptic cleft. Therefore, the increased bioavailability of these two key neurotransmitters that are heavily implicated in mood disorders, may be a mechanism by which FMT exerts beneficial effects. Additionally, FMT administration to individuals with irritable bowel syndrome (IBS) not only alleviated IBS symptoms but also significantly reduced both depression and anxiety scores (68). Similarly, in the case study reported by Xie et al. (51) and discussed above, the patient with T1DM who underwent FMT treatment also had comorbid depression and treated with duloxetine. Interestingly, during the follow-up post-FMT, the patient no longer experienced depression symptoms. These findings were attributed to alterations in the gut flora that were related to depression, including *Alistipes onderdonkii*, *Bacteroides uniformis*, and *Parabacteroides distasonis*. Further, a recent case report evaluated the effect of FMT in two patients as an adjunctive treatment for depression (69). After 4 weeks post treatment, both patients reported improvement in their MDD symptoms, with one patient reporting benefits up to 8 weeks. Interestingly, the second patient developed a *Bacteroides* enterotype, a species known for its beneficial effects on improving mood *via* production of large quantities of gamma-aminobutyric acid (GABA) (70). Taken together, these findings support the data demonstrating the ability of microbiota transplant to ameliorate symptoms of mood disorders that can be used as a comprehensive treatment to potentially treat multiple comorbidities.

Recent studies have also shown that the intestinal microbiota is involved in the pathogenesis of schizophrenia (71). For example, Zheng et al. (71) have shown that individuals with schizophrenia exhibit reduced microbial diversity and altered microbial composition, notably a decrease of species from the families *Lachnospiraceae* and *Ruminococcaceae*. In the same study, fecal transfer of microbiota obtained from patients with schizophrenia into germ-free mice resulted in increased inhibitory transmitter levels and displayed schizophrenia-like behaviors including increased startle response, locomotor hyperactivity and decreased anxiety and depressive behaviors. These findings are supported by more recent studies showing that healthy mice inoculated with microbiota from patients with schizophrenia developed schizophrenia-like behaviors such as cognitive impairment and psychomotor hyperactivity through increases in the tryptophan degradation pathway, a marker of psychosis onset (72, 73). These changes were accompanied by increased dopamine and 5-HT, in the prefrontal cortex and hippocampus, respectively. Since schizophrenia-like symptoms can be induced through FMT, future studies should be directed toward evaluating the effects of restoring normal gut microbiota in schizophrenic patients *via* microbiota transplant.

Fecal microbiota transplant has also been studied in the context of neurodevelopmental conditions like autism spectrum disorder (ASD), which is characterized by repetitive behaviors with impaired social interactions and communication. Children with ASD have specific plasma and fecal metabolites which are normalized by FMT treatment (74). For example, *p*-cresol sulfate, a fecal metabolite is elevated in children with ASD, an effect that was restored by FMT treatment. *P*-cresol is a harmful microbial metabolite that can cause DNA damage, cell-cycle alterations as well as induce symptoms of autism (75). Recent evidence using a mouse model support the beneficial effects of FMT on reducing *p*-cresol concentrations and rescuing behaviors associated with ASD such as social behavioral deficits and repetitive mannerisms (76). Similarly, FMT performed in 18 children with ASD significantly improved behavioral and GI symptoms up to 8 weeks after treatment (77). This was associated with changes in key bacterial species such as *Bifidobacterium, Prevotella,* and *Desulfovibrio* which persisted for 8 weeks until the end of the study. Importantly, in a follow-up study of the same 18 children, the beneficial effects of FMT in improving behavioral symptoms associated with ASD lasted up to 2 years following treatment (78). Although these trials used small sample size, the findings suggest that FMT is a promising therapy for ASD.

Microbiota transfer trials have also been conducted in the setting of neurologic conditions such as Multiple Sclerosis and Guillain Barre syndrome. For example, transplantation of gut microbiota from intermittent fasting mice, resulted in elevated regulatory T cell (T-reg) activity and increased beneficial species like *Lactobacillus* and *Bifidobacterium,* ameliorated experimental autoimmune encephalomyelitis-induced in MS mice models (79). MS is an demyelinating disease of the CNS that is autoimmune-mediated (80). Therefore, increased T-reg activity after FMT suggests that FMT may modulate the immune system through altering the gut microbial composition. Indeed, a case study of a patient with secondary progressive MS also showed benefits of FMT on disease stability (81). MS is characterized by disease relapses causing flares and disease associated symptoms. This particular patient had recurrent CDI, and seven relapses of MS in the span of 3 years, with worsening neurologic symptoms of balance, bladder function and weakness in extremities. Following FMT treatment *via* rectal enema, the patient did not report any relapses during a 10-year follow-up and had improved functional scores associated with MS severity. Conversely, transplantation of gut microbiota from MS patients into mice induced an MS-like autoimmune disease with less regulatory cytokine production than controls, indicating

a critical role of gut microbiota derived influences on MS pathophysiology and its beneficial effects on MS patients (82).

Patients with neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) may also benefit from microbiota transplant (83). AD is characterized by extracellular aggregation of amyloid plaques and intracellular misfolded tau proteins, which lead to progressive impairments in memory and cognitive decline (84). Recent studies have shown that transfer of fecal microbiota obtained from a rodent model of AD into healthy mice induces symptoms consistent with AD including memory impairment and decreased neurogenesis (85). Gut bacterial dysbiosis and resulting changes in metabolite profile led to an activation of microglia, the macrophages of the CNS. For example, microglia produce Tumor Necrosis Factor-α (TNF-α) and Interleukin-1 (IL-1), to promote neuroinflammation leading to irreversible neuronal damage, a finding characteristic of neurodegenerative disorders (86). In the study described above, transplanting the gut microbes from an AD mouse model potentiated the action of microglia in healthy animals by affecting neurogenesis leading to memory loss. Conversely, transplant of healthy gut microbiota to an AD mouse model decreased Tau-protein phosphorylation and reduced amyloid plaques (87). These effects were associated with significant decrease in key bacterial species from the *Desulfovibrionaceae* family associated with memory loss, as well as increases in other neuroprotective butyrate-producing species. These changes were accompanied by increased synapsin I expression with associated increases in synaptic plasticity and has been found to mitigate mitochondrial damage and memory loss in AD (88). Further, PD that is characterized by dopaminergic neuronal degeneration in the substantia nigra, has been shown to have distinct gut enterotypes, with FMT being proposed as a potential therapeutic modality. For example, in a recent case series of six patients with PD who underwent FMT *via* colonoscopy, it was shown that both motor and non-motor symptom improved in five patients (89). Thus, optimizing gut microbial composition in PD helps to improve dopamine signaling throughout the body, therefore FMT may exert its benefits *via* these pathways (11, 15).

Fecal microbiota transplant, inflammatory bowel disease, and irritable bowel syndrome

Inflammatory bowel disease (IBD) includes Crohn's disease and Ulcerative colitis, both of which are characterized by recurrent bouts of intestinal inflammation and their own unique clinical sequelae. Considering the contribution of the gut microbiota to inflammatory states, it is not surprising that certain genera of gut microbiota have been shown to contribute or protect against IBD. For example, pro-inflammatory bacterial species within the *Ruminococcus* genus are elevated in IBD, while SCFA-producing bacterial genera like *Bifidobacterium, Lactobacillus, Eubacterium,* and *Faecalibacterium* are reduced (90). Therefore, targeted microbiota therapy *via* FMT has been studied extensively in the context of IBD (**Figure 1**).

Ulcerative colitis (UC) is a subset of IBD, characterized by continuous lesions starting from the rectum and extending to the proximal colon (91). Several clinical studies have assessed the efficacy of FMT as a treatment for UC in human subjects. For example, out of 43 patients with UC who received colonoscopic FMT infusion of multidonor samples, 11 patients showed steroid-free clinical remission at 8 weeks, a 19% increase relative to controls (92). Further, FMT *via* colonoscopy administered to 38 individuals with mild to moderate UC resulted in a 23% increase in remission rates at 8-week follow up, with 5 out of 12 patients who achieved remission at 8 weeks exhibited no relapse up to 1 year (24). Though it is important to note that out of 38 patients who received FMT treatment, 3 exhibited serious adverse events including worsening colitis, CDI, resulting in colectomy and pneumonia. A recent study, similarly, evaluating FMT efficacy in 15 UC patients found that, at 8 weeks post FMT, 53% patients in the trial group reported corticosteroid-free remission, compared to only 15% in controls (93). Out of the 10 patients evaluated during a 56-week maintenance phase, four patients continued to have remission by the end of the study. Worsening colitis was again the most common serious adverse effect with two patients developing the condition.

Additionally, microbiota transplant has been recently studied, for the first time, in nine pediatric patients ranging from 4 to 17 years old (94). Out of the nine patients that were treated with FMT and completed the study, eight showed clinical improvement with five patients having clinical remission at 30 weeks, as measured by a Pediatric UC Activity Index score of under 15. However, three patients in the FMT and one patient in the placebo group developed worsening colitis requiring hospitalization and IV methylprednisolone treatment. Since adverse effects have been reported, more recent studies have evaluated both the long-term safety and efficacy of FMT in UC patients. In one prospective pilot study, 10 FMT-treated UC patients were followed over a course of 6–38 months (95). Mayo scores, a marker for UC disease severity, were decreased up to 8 weeks, however this was not statistically significant beyond 6 months. One patient developed Ebstein-Barr Virus within 2 weeks of microbiota transplant, however, no other adverse effects were reported at the time of follow-up, up to 38 months. Important gut microbiota changes after FMT included an increase in the phylum Bacteroidetes, improving the *Firmicutes*/*Bacteroidetes* ratio, with decreases in harmful genera such as *Escherichia*. Long-term efficacy was also assessed by using oral FMT capsules as an adjunctive treatment to FMT *via* colonoscopy (96). The results suggested that using a combination of the two methods of microbiota transplant decreased cytokine production by mucosal associated

invariant T (MAIT) cells, up to 36-week follow-up. MAIT cells have been found to be activated in response to active ulcerative colitis, releasing regulatory cytokines such as IL-17 (97). Therefore, reduced MAIT cell activity correlates with the state of remission in ulcerative colitis patients, indicating that FMT may help prevent relapses. Similarly, targeting increased T regulatory cell (Treg) activity is of great interest in IBD (98). Indeed, results from a recent study suggest that FMT introduction of *Faecalibacterium* in UC patients alleviates inflammation by increasing Treg activity, along with decreasing fecal calprotectin, a clinical marker for intestinal inflammation. Taken together, there is a promising body of evidence supporting treatment of UC with FMT in humans, however, further studies need to assess long-term efficacy and safety measures to minimize serious adverse effects before regularly using this therapeutic modality for UC. It is also important to understand and control the factors that predispose disease recurrence in both UC and CD, including anemia, hypoalbuminemia, low peripheral blood lymphocytes and immunosuppression as it may require extra caution with using FMT as a therapeutic intervention (99). Lastly, it should be noted that in UC, FMT *via* colonoscopy appears to be the most effective method as lesions usually begin in the rectum and the distal colon (94).

Crohn's disease, in contrast to UC, presents with inflammatory lesions that can be present in a discontinuous manner along the entirety of the GI tract, with beneficial outcomes observed in FMT studies that have shown remission in patients up to 24 weeks (100). For example, in 27 patients who received two rounds of FMT one week apart *via* endoscopy and colonoscopy, clinical remission was observed in 18 patients, as measured by serum testing and endoscopy after 8 weeks (101). Importantly, clinically significant difference was observed between the two FMT modalities (endoscopy and colonoscopy). Patients displayed increased microbial richness and diversity, specifically with increases in *Roseburia, Eubacterium,* and *Faecalibacterium,* and reduced *Fusobacterium* and *Streptococcus* after treatment. Interestingly, timing a second FMT intervention in Crohn's patients who benefited from the first treatment may be of therapeutic value since administration of a second FMT within 4 months of the initial intervention helped maintain clinical benefits (102).

In addition to the intestinal inflammatory conditions, described above, IBS is an unrelated disease, diagnosed clinically and marked primarily by altered bowel habits, either constipation or diarrhea. More recently, IBS has been associated with changes in gut microbiota and microbiota-derived metabolites such as SCFA, bile acids and neurotransmitters like serotonin which is present in abundance within the GI tract (103). SCFA-producing bacterial genus *Bifidobacterium* rich donors have been found to be a key indicator in the response to FMT treatment in IBS patients (104). For example, in a study with 10 IBS patients, six patients achieved a positive clinical response, all of which had donor samples with more *Bifidobacterium*. Similarly, in another fecal transplantation study evaluating 142 IBS patients, the SCFA, butyrate which is inversely correlated with disease symptoms and severity, was found to be significantly increased (105). Therefore, increased SCFA production in recipients after FMT treatment is strongly correlated with treatment efficacy. Other recent studies have assessed the treatment response to FMT in IBS patients. For example, in a study assessing FMT efficacy in 17 patients, 10 were considered responders as measured by the IBS severity index (106). Importantly, in all three of the studies described above no major adverse effects were reported with only some mild self-limiting abdominal, diarrhea or constipation, which are characteristic of IBS at baseline. Further, antibiotic treatment with Ciprofloxacin/Metronidazole or Rifaximin prior to FMT was found to hinder its effects in moderate to severe IBS (107). 15% of patients had improved IBS severity with FMT alone, while the antibiotic treated groups were below 5%. As such, it is important to take the use of antibiotics into account before treating with FMT. Additionally, a recent study has evaluated the efficacy of microbiota transplant in treating IBS with comorbid depression and anxiety (68). A 3-course treatment of FMT *via* oral capsules at 1, 8, and 12 weeks showed improved IBS severity scores and significantly reduced Hamilton anxiety and depression scores at 12-week follow-up, providing more insight into the versatile therapeutic effects of FMT. A summary of the mechanisms by which FMT restores changes in metabolic, neuropsychiatric and inflammatory bowel disease is presented in **Figure 1**.

Fecal microbiota transplant, cirrhosis, and hepatic encephalopathy

Cirrhosis develops from long-term liver damage, leading to progressively worsening fibrosis of liver tissue thus preventing normal liver functions. In recent years, significant research has been directed at understanding the microbiota-gut-liver axis, which has been shown to be involved in normal and pathophysiological liver functions (108). Among the proposed mechanisms of microbiota involvement in the onset of cirrhosis is bacterial translocation through intestinal barrier alterations, systemic inflammation, and small intestinal bacterial overgrowth (109). Often, complications of cirrhosis like hepatic encephalopathy and secondary bacterial peritonitis are treated with antibiotics, however, resistance to antibiotic genes is associated with poorer outcomes. Hepatic Encephalopathy (HE) is an indication of decompensated liver cirrhosis that results from excess ammonia buildup leading to altered mental status. Importantly, ammonia producing gut microbes contribute to this process and standard of care includes clearing the ammonia and depleting the culprit bacteria through two medications, lactulose and rifaximin, respectively (110).

Theoretically, FMT can introduce beneficial bacteria *via* the gut-liver axis to outcompete ammonia producing microbiota and improve antibiotic resistance. For example, studies have found that FMT can restore antibiotic induced gut microbial dysbiosis (111). In decompensated cirrhosis patients, standard lactulose/rifaximin therapy followed by microbiota transplant with enriched *Lachnospiraceae* and *Ruminococcaceae* resulted in increased SCFA and bile acids with increased microbial richness and diversity. FMT was also found to reduce antibiotic resistance genes, specifically against rifamycin, vancomycin, and beta-lactamases in individuals with decompensated cirrhosis (112). Further, oral capsule FMT was correlated with decreased lipopolysaccharide (LPS) activity and reduced interleukin-6 (IL-6) (113), two inflammatory mediators that can worsen cirrhosis. As such, FMT intervention can improve antibiotic treatment response by lessening the accumulation of resistant bacteria and reduce the overgrowth of harmful bacteria to prevent against LPS-mediated endotoxemia in patients with cirrhosis.

Fecal microbiota transplant has also been studied in patients with recurrent hepatic encephalopathy (HE) as a complication of decompensated cirrhosis. In a study of 10 patients with recurrent HE, cirrhosis severity, cognitive status, liver function and white blood cells were measured in response to FMT without antibiotic pre-treatment compared with the standard of care (SOC) of antibiotic treatment alone (114). FMT donor's microbiota was enriched with *Ruminococcaceae*, *Bifidobacteriaceae,* and *Lactobacillaceae*, an effect that was observed post-treatment. There was no significant improvement in Model for End Stage Liver Disease (MELD) scores, a measure of cirrhosis severity, however, the SOC worsened MELD scores. FMT treated patients with HE exhibited better cognitive outcomes compared to baseline without significant change compared with SOC group. Importantly, during the 5-month course of the study, no hospitalizations related to altered mental status were observed in the FMT treated individuals, while one was observed in the SOC group. Taken together, these findings suggest that FMT can be an effective treatment in treating cirrhosis and its complications, though more large-scale and longer-term studies are needed.

Fecal microbiota transplant and cancer

The influence of gut microbiota in tumorigenic pathways has been studied extensively over the years. Several mechanisms by which microbiota can induce carcinogenesis have been put forward, including but not limited to alterations of checkpoint inhibitors, breakdown of gut associated lymphoid tissue and secretion of toxic metabolites (115). For example, intestinal dysbiosis can increase formation of deoxycholic acid, a secondary bile acid with involvement in carcinogenesis *via* increases in tumor cell proliferation and vascular endothelial growth factor receptor expression (116). Conversely, certain gut microbial metabolites have also been shown to ameliorate tumorigenesis. For example, *Bacteroides fragilis* mitigates progression of UC into colorectal cancer through its anti-inflammatory effects (117). This species exerts anti-inflammatory effect by increasing butyrate production and inhibiting activation the NLRP3 inflammasome, a key pro-inflammatory mediator. *Lactobacillus* spp. have also been shown to suppress cell proliferation and inhibit tumorigenesis in a mouse model (118). Therefore, FMT may alleviate the deleterious effects of some factors involved in the progression and development of cancer with a potential role as an adjunct therapy in the future.

Interestingly, two recent studies have found that FMT may improve the response to monoclonal antibody therapy in patients with advanced melanoma (23, 119). Melanoma, in advanced stages, can metastasize and lead to a lack of immune destruction of abnormal cells by T cells after bypassing the programmed cell death-1 (PD-1) immune checkpoint (120). Therefore, targeting the bypassed immune checkpoint inhibitor with anti-programmed cell death protein (Anti-PD1) immunotherapy is effective in long-term treatment, however, anti-PD1 refractory melanoma do exist. In a recent study, combining FMT with anti-PD1 therapy was found to overcome resistance to refractory melanoma (23). Clinical benefits were observed in response to the joint therapy with 6 of 15 patients showing increased CD8 + T cell activation and decreased interleukin-8 myeloid cells, a finding consistent with increased clinical response to anti-PD1 therapy (121). Importantly, gut sequencing studies revealed increased *Bifidobacterium* spp. after FMT treatment, a species associated with synergistic effects on immune checkpoint inhibitors including anti-PD1. Further, a similar study supports these findings by showing that FMT may enhance response to immune checkpoint inhibitor therapy in patients with refractory and metastatic melanoma (119). Study findings show that 3 out of the 10 patients in the clinical trial showed response to the dual therapy with an up-regulation in the immune system activity as measured by T-cell activation, MHCII complex expression and interferon- γ signaling pathways.

Further, chemotherapy treatments are known to cause immunosuppression, leading to infections that require antibiotic therapy. Therefore, in addition to worsening systemic manifestations of cancer, gut microbiota dysbiosis can ensue and FMT may serve as a potential intervention to mitigate complications (122). For example, in 25 patients with acute myeloid leukemia on aggressive antibiotics and chemotherapy, FMT restored microbial richness and diversity, with decreased abundances of pro-inflammatory families *Enterobacteriaceae*, *Enterococcaceae,* and *Veillonellaceae*. No serious adverse events were reported in the study besides mild self-limiting abdominal symptoms indicating treatment safety

and its potential adjunctive role in eradicating multi-drug resistance bacteria in cancer patients. Additionally, a case report on a patient with acute lymphocytic leukemia showed similar value on the enhancing effects of gut microbiota in cancer patients who are immunosuppressed (123). In this case, immunosuppressive therapy led to the development of recurrent CDI, which was efficaciously treated with FMT. As such, FMT is a promising therapeutic intervention that may be used in conjunction with cancer immunotherapy to achieve optimal clinical outcomes in refractory cases. Considering that lifetime prevalence of colorectal cancer in long-standing IBD of 30 years is up to 18% (124) and that patients with cirrhosis have a sevenfold increase in risk for developing hepatocellular carcinoma (125), FMT may serve as a preventive measure against carcinogenesis by preventing progression of CD, UC, and cirrhosis.

Fecal microbiota transplant and graft-versus-host disease

Graft-versus-host disease (GvHD) is an immunologically mediated condition which can result after hematopoietic stem cell transplant (HSCT) when donor bone marrow attacks graft stem cells (126). Interestingly, gut microbiota have been associated with the pathogenesis of GvHD through mechanisms including immune cell and gut microbiota cross-talk across intestinal epithelial cells, stimulation of dendritic cells and Treg cell suppression (127, 128). It is also shown that gut microbiota-derived metabolites such as butyrate and riboflavin are markedly reduced in GvHD (129), with exogenous butyrate administration being shown to attenuate GvHD disease severity by improving intestinal epithelial cells and barrier integrity. Further, MAIT cells, a T-cell subset that is responsive to gut microbiota-derived riboflavin metabolites and present in GvHD target organs, are shown to suppress activity in GvHD through associated decreases in intestinal barrier integrity and IL-17-mediated Th17 expansion (130). More specifically, analysis of colon tissue and stool of MAIT cell-deficient MR1 and IL-17 deficient mice were found to have similar changes in gut microbiota (131). As mentioned earlier, FMT studies on UC patients has been shown to help achieve clinical remission by reducing MAIT cell cytokine production (96), providing a potential role for FMT in GvHD through similar mechanisms. For example, recent longitudinal analysis of FMT performed in a 14-year old GvHD patient showed sustained decreases in *Enterococcus* to undetectable levels over a 3-day period after the FMT (132), while *Faecalibacterium* and *Bacteroides* became more abundant in the patient's gut. Interestingly, another recent study has shown that *Faecalibacterium* has been associated with high MAIT levels, while *Enterococcus* is correlated with low MAIT levels (133). Overall, these findings suggest that FMT can optimize gut microbial composition to restore MAIT cell function and T-regulatory cell imbalance to exert benefits in GvHD patients.

Due to these findings showing significant involvement of gut microbiota in GvHD, the efficacy and safety of FMT as a therapeutic intervention has been studied. For example, in a study evaluating the effects of FMT on grade IV steroid-refractory GI tract GvHD, the FMT group showed higher rates of clinical remission just 2–3 weeks after treatment and increased the mean survival time to over 432 days as compared to controls (134). These findings were associated with overall increases in the Bacteroidetes/Firmicutes ratio while also increasing other symptoms such as diarrhea and abdominal pain. Of the 23 patients that underwent FMT, two experienced adverse effects including thrombocytopenia and a cardiac event within 7 days of receiving treatment. It is also important to mention that GvHD is a complex pathology and other medications such as immunosuppressants and antibiotics were used concurrently in both the study and control groups, though their effects may vary on an individual basis. Still, the significant improvements in event-free survival as well as overall survival, suggest that FMT administration in GvHD may serve as a viable therapeutic intervention for steroid-refractory GvHD. Another smaller scale study of four patients with steroid resistant acute GvHD reported three complete response and one partial response without adverse events (135). Importantly, changes in gut microbial composition include increases in Faecalibacterium, Bifidobacterium, and Bacteroides, with decreases in Streptococcus, another bacterial species associated with low MAIT cell activity (133). FoxP3 + CD4 + T cells assays showed similar trends in four patients, further supporting the role of effector Treg cells in achieving therapeutic effect in GvHD (135). Further, a larger scale study examining the use of FMT in patients with GvHD, supports the use of microbiota transplant to decolonize antibiotic-resistant bacteria seen in 11 out of 14 patients (136). As such, reduction of the prevalence of antibiotic resistant bacteria may aid treatment of GvHD, should antibiotic treatment be necessary. However, this study does report serious adverse effects though most unrelated to FMT treatment. Septic shock was reported in two patients and Norovirus in another patient, both of which were deemed to be related to FMT, though it should be noted that these patients were severely ill at baseline. Lastly, studies have implemented FMT prior to HSCT to evaluate efficacy in preventing the prevalence and severity of GvHD, however no significant difference in overall survival was found in pre-FMT treatment as compared to controls over a 20-month period (137), indicating that post-HSCT FMT treatment may be more efficacious in clinical outcomes. Overall, there is strong evidence for the use of FMT in controlling the disease severity of GvHD after HSCT.

Similarities and differences between fecal microbiota transplant studies

Although the studies described in prior subsections evaluate the efficacy of FMT in different non-communicable diseases (**Table 1**), there are mechanistic similarities in observed benefits as well as trends in gut microbiota profile that correspond to better treatment outcomes. Favorable microbial changes consist of increases in butyrogenic species such as *Faecalibacterium, Eubacterium, Roseburia, Butyrivibrio,* and *Blautia* as well as other beneficial bacteria that produce butyrate precursors like Acetyl-CoA such as *Lactobacillus, Bifidobacterium,* and *Bacteroides* (20, 33, 48, 51, 79, 101, 114, 135, 138). Butyrate strengthens intestinal barrier integrity by inducing AMPK activity to increase tight junction protein expression and improve transepithelial electrical resistance (139, 140). Further, butyrate has been shown to control inflammation by inducing apoptosis of neutrophils, inhibiting mast cell degranulation in the gut and reducing pro-inflammatory cytokines such as IL-6, IL-1 and TNF-α which are elevated in LPS-induced endotoxemia (141, 142). Butyrate also reduces neuroinflammation by upregulating zonulin, occludin, and claudin-5, which are brain tight junction proteins that reduce blood-brain barrier permeability (143). As mentioned, inflammatory states and metabolic endotoxemia contribute significantly to the pathogenesis of metabolic disease, IBD, neuropsychiatric conditions, cancers and GvHD. Therefore, it is not surprising that studies showing therapeutic benefits exerted by FMT share similarities that involve increased butyrogenic species in treatment-responsive individuals with non-communicable diseases. Similarly, the studies discussed in this manuscript show trends in bacterial genera that are reduced in FMT-responsive individuals including *Escherichia, Streptococcus, Desulfovibrio,* and *Bilophila*. Collectively, these species chronically upregulate inflammatory processes through LPS-mediated endotoxemia and reduction of the relative abundances of butyrogenic species, contributing to the development of disease states (144, 145). In addition to trends in gut microbial changes, there are other mechanistic similarities by which FMT may exert its therapeutic effects. For example, four separate studies evaluating the effects of FMT in MS, UC, advanced melanoma and GvHD identify increased MAIT cell activity to the quantity of Treg cells, an important factor in treatment-responsive individuals (23, 79, 96, 134), Further, the incorporation of FMT into the treatment plan of patients with HE and GvHD in adjunction to current regimen can help reduce antibiotic resistance genes to further increase efficacy of standard of care treatments (112, 136). As such, creating targeted changes in gut microbiota to improve gut inflammation and bacterial resistance can help improve treatment-responsiveness to both FMT and concurrent treatment that patients may receive.

Though trends of certain bacteria correlating with better disease outcomes were present, these were not consistent in all studies and disease conditions. In T1DM patients, elevation of *Desulfovibrio piger* spp. was correlated with preservation of Beta-cell function (50). Similarly, *Desulfovibrio* was found to be elevated after FMT in children with ASD (77). This is contrary to findings shown in other non-communicable diseases like T2DM, AD, PD, IBS and obesity, that associate elevated *Desulfovibrio* with worse treatment-responsiveness (48, 87, 106). Similarly, variable changes were found in mucin-degrading species, such as *Akkermansia* and *Ruminococcus* (56, 58, 87, 92, 106), as the beneficial effects of these species are concentration dependent (146, 147). Therefore, the post-FMT effects of these bacteria may be specific to both disease and bacterial species, and it is important to consider the relative concentrations to the total microbial diversity within an individual's gut.

Further, variations in study designs and delivery methods also exist between the studies. For example, some studies evaluate the efficacy of FMT in conjunction with the standard of care or lifestyle interventions (20, 48) while others evaluate the effects of FMT alone particularly in studies evaluating FMT efficacy in metabolic disorders. This makes it difficult to separate the true therapeutic effect of FMT from the effect of lifestyle interventions as gut microbiota are shown to be largely affected by environmental factors, including diet. Also, it is important to note, that due to the severity of some diseases, other treatments were not discontinued during the study, so improvements in patient conditions could involve a combination between FMT and the standard of care (23, 134, 135). Additionally, certain studies used multiple FMT treatments with maintenance therapy (24, 77, 92, 96, 101, 105, 112), while others assess the efficacy of a single FMT treatment (58, 69, 95, 104), with multiple FMTs or maintenance therapies reporting more sustained changes in gut microbiota in the long-term. Preferred delivery methods amongst different diseases were mostly similar, however, varied amongst different diseases. For example, in metabolic diseases, GvHD, CD and depression, FMT was administered through endoscopic approaches or oral capsules (20, 46, 69, 101, 132, 135), while studies evaluating UC and IBS preferred colonoscopy or rectal enema as the distal colon is the most affected (92, 94, 95, 104, 106). The efficacy, advantages and disadvantages of the various delivery methods are further discussed in the following sections.

Factors for a successful transplant

Donor selection process

Though FMT is found to be generally effective, it must be performed in a standardized and efficient manner to allow

TABLE 1 Comparison between FMT studies.

Disease studied	Study description	Observed effect	Adverse effects	Gut microbiota alterations	Citation
Obesity	Oral capsule FMT to obese adolescents (n = 42) vs. sham treatment (n = 45)	No effect on BMI. Reduced abdominal adiposity observed at 12 weeks	Loose stools, abdominal pain, nausea, vomiting, bloody stools	↑*Faecalibacterium prausnitzii, Alistipes, Bacteroides* ↓ *Escherichia coli*	(33)
	Endoscopic FMT on obese patients. FMT (n = 20) vs. FMT + lifestyle intervention (LSI) (n = 21) vs. sham FMT treatment (n = 20)	No significant weight loss in FMT only and sham FMT groups. Reduced liver stiffness, total and LDL cholesterol with weight loss in the FMT + LSI group at 24 weeks	Nausea, vomiting, abdominal pain No FMT related serious adverse effects	FMT alone: ↑ *Faecalibacterium, Roseburia, Eubacterium* FMT + LSI: ↑ *Bifidobacterium, Lactobacillus*	(20)
Type 2 diabetes mellitus (T2DM)	Transendoscopic enteric tube FMT treatment (n = 17) on T2DM patients	64% with significant decrease in HgbA1c, blood glucose and uric acid with elevated C-peptide at 12 weeks	none	↑ *Anaerotruncus, Rikenenellaceae*	(46)
	Diet only (n = 8) vs. Diet + Oral capsule FMT group (n = 8) on T2DM patients	Both groups showed decreased blood glucose and weight loss after 90 days with FMT accelerating the effect	None	↑ *Bifidobacterium, Lactobacillus* ↓ *Desulfovibrio, Bilophila*	(48)
Type 1 diabetes mellitus (T1DM)	Allogenic FMT (n = 11) vs. Autologous FMT (n = 10) in T1DM patients	Preserved C-peptide levels and beta-cell function at 12 months	None	*Desulfovibrio piger* concentrations predicted beta-cell function	(50)
	Nasojejunal FMT on a 24-year-old patient with T1DM and depression	Improved blood glucose, HgbA1c, constipation, nutritional status Depression symptoms resolved	None	↑ *Bifidobacterium, Blautia, Faecalibacterium, Bacteroides, Eubacterium, Streptococcus* ↓*Alistipes, Escherichia, Parabacteroides*	(51)
Diabetic kidney disease (DKD)	Rectal probe FMT into a mouse model with T2DM and DKD	No weight gain Reduced insulin resistance, TNF-α and albuminuria		↑ *Odoribacteraceae*	(53)
Metabolic syndrome	Oral gavage FMT in metabolic syndrome induced rodent model	Decreased LPS, TNF-α and oxidative stress post-FMT		↓ *Ruminococcus, Coprococcus*	(56)
	Allogenic FMT (n = 26) vs. Autologous FMT (n = 12) on Metabolic syndrome patients	Improved insulin sensitivity and decreased HgbA1c at 6 weeks post-FMT with no significant difference at 18 weeks	None	↑ *Lactobacillus, Butyrivibrio, Akkermansia* ↓ *Eubacterium ventriosum, Ruminococcus torques*	(58)
Major depressive disorder (MDD)	Oral capsule FMT on MDD patients (n = 2)	Both with improved depressive symptoms after 4 weeks and one up to 8 weeks	No serious adverse effects	↑ *Bacteroides, Butyrivibrio, Faecalibacterium* Variable: *Alistipes* spp.	(69)
Autism spectrum disorder (ASD)	Oral or rectal FMT on children with ASD (n = 18)	80% with improved GI symptoms Behavioral deficits improved over an 8-week period	Vomiting (n = 1)	↑ *Bifidobacterium, Prevotella, Desulfovibrio*	(77)
Multiple sclerosis (MS)	FMT into a mouse model of MS *via* oral gavage	Reduced myelin antigen-specific lymphocytic proliferation, disease severity and spinal cord pathology Increased number of T regulatory cells		↑ *Lactobacillus* spp., *Bifidobacterium pseudolongum, Bacteroides fragilis*	(79)
	Rectal enema FMT in a 61-year-old with secondary progressive MS	Disease stability achieved for 10 years after single FMT Functional composite MS scores improved over 10 years	None	Not assessed	(81)
Alzheimer's disease (AD)	Intragastric FMT on a mouse model of AD	Reduced Tau-protein phosphorylation and amyloid plaques		↑ Bacteroidetes, *Alloprevotella* ↓ *Akkermansia, Desulfovibrio*	(87)
Parkinson's disease (PD)	FMT treatment for PD patients (n = 6) *via* various delivery methods	Five patients with improvement of motor and non-motor symptoms as early as 4 weeks with significant improvement at 24 weeks	One unspecified adverse event requiring hospitalization	Not assessed	(89)

(Continued)

TABLE 1 (Continued)

Disease studied	Study description	Observed effect	Adverse effects	Gut microbiota alterations	Citation
Ulcerative colitis (UC)	Single FMT *via* colonoscopy and 5 enema FMT per week for 8 weeks (n = 42) vs. placebo (n = 43) in UC patients	19% increase in remission rates at 8 weeks follow up in the FMT group	Self-limiting GI symptoms in 78% Serious adverse events (n = 2)	↑ *Prevotella, Bacteroides Barnesiella, Parabacteroides, Clostridium* cluster IV, *Ruminococcus, Blautia* associated with remission *Fusobacterium* and *Sutterella* associated with lack of remission	(92)
	Prepared pooled donor FMT (n = 38) vs. autologous FMT (n = 35) *via* colonoscopy in UC patients followed by 2 enemas over 7 days	23% increase in steroid-free remission relative to controls at 8 weeks 5/12 patients remained in remission for 1 year	Worsening colitis (n = 1) *C. Difficile* infection requiring colectomy (n = 1) Pneumonia (n = 1)	↑ *Anaerofilum pentosovorans, Bacteroides coprophilus, Alistipes indistinctus, Odoribacter splanchnicus* ↓ *Anaerostipescaccae, Clostridium aldenense*	(24)
	Rectal enema FMT (n = 9) vs. placebo (n = 6) in pediatric UC patients	Eight patients with clinical improvement measured by the pediatric UC activity index 5 patients with remission at 30 weeks follow up	Development of *C. Difficile* infection (n = 2) *Patients already had history of CDI	↑ *Alistipes* spp. ↓ *Escherichia* spp.	(94)
	FMT *via* colonoscopy (n = 10) vs. control (n = 10) in UC patients	40% improvement in Mayo scores in the FMT treatment group up to 8 weeks but no significant difference to controls at 24 weeks	Ebstein-Barr virus infection	↑ Bacteroidetes, *Prevotella* ↓ Proteobacteria, *Escherichia* spp.	(95)
	Oral capsule FMT after colonoscopic FMT vs. sham oral placebo after colonoscopic FMT	Daily encapsulated therapy extended the durability of FMT-induced changes in gut microbiota Decreased cytokine production by mucosal invariant T cells (MAIT)	Nausea, fever Worsening colitis (n = 2)	Similar community-level changes in gut microbiota between donor and recipients	(96)
Crohn's disease (CD)	Endoscopic FMT followed by colonoscopic FMT one week later in CD patients (n = 27)	Clinical remission in 18 patients	No serious adverse effects	↑ *Roseburia, Eubacterium, Faecalibacterium, Bacteroides* ↓ *Fusobacterium, Streptococcus, Clostridium*	(101)
Irritable bowel syndrome (IBS)	FMT *via* colonoscopy in patients with IBS (n = 10)	Six patients exhibited improved stool form at 4 weeks Hamilton anxiety and depression scores improved irrespective of IBS response	None	↑*Bifidobacterium* The genus was strongly associated with clinical response to FMT	(104)
	FMT *via* colonoscopy in patients with refractory IBS (n = 17)	10 patients showed improved IBS severity index scores of 50 or more points after 12 weeks	Abdominal distention for 2 days after FMT	↑*Akkermansia, Neisseria* ↓ *Desulfovibrio, Delftia*	(106)
	FMT *via* colonoscopy of 30 g samples (n = 37) vs. 60 g sample (n = 40) in IBS patients already responsive to first FMT	32/37 patients-maintained response to FMT in 1 year 35/40 patients-maintained response to FMT in 1 year	Diverticulitis (n = 2)	↑*Eubacterium biforme, Parabacteroides, Bacteroides, Prevotella, Alistipes*	(105)
Hepatic encephalopathy (HE)	Rifaximin/Lactulose followed by rectal enema or oral capsule FMT in cirrhotic patients (n = 20)	Increase SCFA and bile acids Reduction in antibiotic resistance genes	Lower HE related complications in FMT group	↑ *Lachnospiraceae, Ruminococcaceae*	(112)
	Single enema FMT in patients with recurrent HE (n = 10) vs. Standard of care (SOC) (n = 10)	MELD scores remained stable but higher than SOC group FMT treated groups had no HE related hospitalizations while the SOC group had five	No FMT-related adverse effects	↑ *Bifidobacteriaceae Ruminococcaceae, Lactobacillaceae*	(114)

(Continued)

TABLE 1 (Continued)

Disease studied	Study description	Observed effect	Adverse effects	Gut microbiota alterations	Citation
Advanced melanoma	FMT *via* colonoscopy in addition to pembrolizumab in patients with PD-1-refractory-melanoma ($n = 15$)	Six patients showed clinical improvement Increased CD8 + T cell and MAIT cell activation and decreased IL-8 expressing myeloid cells	Hypothyroidism (17.6%)	↑*Bifidobacteriaceae, Ruminococcaceae, Lachnospiraceae* ↓ *Bacteroidaceae, Sutterellaceae*	(23)
	Oral capsule FMT in patients with PD-1-refractory-melanoma ($n = 10$)	Three patients showed clinical response (two partial and one complete)	Mild bloating ($n = 1$)	↑*Enterococcaceae* ↓*Veillonella atypica*	(119)
Acute myeloid leukemia (AML)	FMT treated AML patients ($n = 25$) vs. standard of care ($n = 20$)	FMT is a safe and effective treatment to restore microbiota concentration in AML patients	*Escherichia coli* sepsis (3 months after FMT)	↑*Ruminococceacae, Lachnospiraceae* ↓*Veillonellaceae, Enterococcaceae*	(122)
Graft-versus-host disease (GvHD)	FMT *via* nasojejunal tube to IV steroid refractory GI tract GvHD patients ($n = 23$) vs. controls ($n = 18$)	Higher rates of clinical remission in just 2–3 weeks Increased mean survival to over 432 days compared to controls	Thrombocytopenia ($n = 1$) Cardiac event ($n = 1$)	↑ Bacteroidetes, Firmicutes ↓ Proteobacteria	(134)
	Nasoduodenal tube FMT in GvHD patients ($n = 4$)	Complete response in three patients and partial response in one patient	Paroxysmal atrial fibrillation ($n = 1$)	↑*Faecalibacterium, Bifidobacterium, Bacteroides, Lactobacillus* ↓*Streptococcus*	(135)
	Four FMTs *via* endoscopy in 1 month in a 14-year-old with stage 4 GvHD	Favorable alterations in gut microbiota are present post-FMT in a GvHD patient	None	↑*Faecalibacterium, Bacteroides* ↓ *Enterococcus*	(132)

for the provision of safe and correct treatment (148). This is extremely important because patients who need care are often elderly, with comorbidities, which may require urgency in transplantation. Biological sample banks have been developed to facilitate the standardization of the FMT process and ensure the availability and supply of fecal samples on request (149). The existence of these cryogenic biological banks also regulates the availability of willing and healthy donors that meet specific criteria. Although individual donor samples are regularly used in FMT treatment, it has been found that combining fecal samples of multiple donors to create a so called "super donor" augments clinical response to treatment (150). For example, engraftment from both a male and female donor increased microbial diversity, provided more significant enterotype shifts and enhanced metabolic potential of the gut microbial community. More recently, engraftment of the donor microbiota assessed by the strain specific single nucleotide variation in bacterial *rrn* operons has been correlated with improvements in the metabolic health of recipients (151). These methods, however, can be labor intensive and require detailed analysis of fecal samples, which can be performed in a cost-effective manner in organizations with large sample banks and proper equipment. As such, standardized sample banks can optimize and personalize samples from multiple donors to achieve maximal efficacy for patients.

Biological sample banks can be set up directly in individual treatment centers, or they can exist in the form of organizations, such as those in the United States. Until recently, patients who were selected for such treatment usually resorted to fecal samples collected from family members or friends. This approach poses several issues, especially when there is a possibility of donor coercion and ethical and confidentiality concerns regarding the screening of known donors (152). Additionally, family members may carry similar gut microbial profiles as genetic components of certain pathologies and similar environmental factors such as diet and age may yield a similar gut microbial profile to the recipient (153). Though not preferred, FMT may be obtained from related donors, if need be, as there is significant variation in gut microbiota even between family (154). Although the donors with healthy gut microbiota tend to be younger than recipients, age-matching fecal samples can be important, if possible, as variations of microbial composition have been reported in different stages of life (155). Moreover, strict exclusion criteria can be more easily applied to voluntary donors in the community than to those targeted by beneficiaries, as there are more potential candidates without perceived personal obligation between beneficiaries and donors. Further, there is also evidence from safety blood transfusions studies that recipient-directed donors are more likely to be tested positive for infectious disease than unrelated voluntary donors (152), which may also be applied to FMT transmitted infections. It has been found that each stool donation can provide enough fecal samples for up to 8 FMT

treatments, thus biological sample banks can be resourceful and maximize donations (156).

Even with the presence of biological sample banks, donor recruitment is an expensive and lengthy process and therefore identifying a target population is recommended to increase donor probability of meeting the inclusion/exclusion criteria (Table 2). This, in itself, presents challenges considering that in 3-year clinical trial only 25% of willing donors, out of 114 candidates assessed in the study were eligible to donate (157). Similarly, in another study, only 12 of 116 (10%) potential donors were eligible to donate fecal samples (158). To maximize the efficiency of the process,

TABLE 2 Donor inclusion/exclusion criteria.

Inclusion criteria
Age: 18–50 ani (Children under 18 can only donate with parental consent)
BMI 18.5–30 kg/m^2
Should feel good at the time of donation and are similar to age as recipient, if possible
Exclusion criteria
High risk behavior
○ Use of drugs or other injections without a prescription
○ Exposure to HIV, HBV, or HCV in the last 12 months
○ Unprotected sexual contact or prostitution in the last 12 months
○ Tattoos and piercings made in the last 6 months
○ Incarceration
○ Risk factors for Creutzfeldt-Jakob disease
○ Chronically poor diet
○ Homelessness
○ Pregnancy
○ Frequent activities involving animal (to exclude the risk of transmission of zoonotic infections)
○ Diarrhea (more than three stools per day) among close contacts members (including children) within 4 weeks before donation
○ Person is in a vulnerable group, unable to take care of him/her or unable to protect him/her from significant harm or exploitation
Current contagious diseases
○ Fever, vomiting, diarrhea, or other symptoms of infection in the last 4 weeks
○ Vaccinations or injections in the last 8 weeks
○ Blood transfusion, accidental sting with needles exposed to another person's blood or biological fluids in the last 12 months
○ International travel to countries with poor hygiene, in the last 6 months
Other conditions
○ Family members with active gastrointestinal infections
○ Antibiotic treatment in the last 3 months
○ Organ/tissue transplantation
○ *Helicobacter pylori* induced ulcers
○ Gastrointestinal diseases, celiac disease, irritable bowel syndrome, chronic constipation, gastrointestinal tumors, or major gastrointestinal tract surgery
○ Family history of colorectal cancer (more than 2 grade two relatives have/have had the disease)
○ Autoimmune disease
○ Treatment with immunomodulatory drugs
○ Other cancers and active chemotherapy for other diseases
○ History of metabolic syndrome, obesity (BMI > 30 kg/m^2) or malabsorption
○ Chronic pain syndrome or other neurodegenerative diseases
○ Diabetes
○ Autism
○ Cardiovascular disease, stroke
○ Active or history of mental illness; depression requiring treatment
○ Systemic autoimmunity or atopic diseases
○ Anterior prosthetic implant (e.g., metal heart valve, joint replacement, ventricular-peritoneal shunt, cardiac stent)
○ Allergy to tested antibiotics
○ Known contagious disease or at least 2 weeks after complete recovery from infectious diseases (e.g., chickenpox)

the inclusion-exclusion questionnaire is administered followed by the medical examination of the volunteers. The use of a strict protocol for FMT increases cure rate such as seen in the recurrent CDI community-based university hospital study where 86% primary cure rate was observed (159). Therefore, instructions and protocols for fecal sample donation emphasize the importance of extremely rigorous methods for donor selection. Most candidates are excluded after this first screening, thus avoiding the costs of subsequent blood and stool tests. The risk of transmitting an infection through this procedure is minimized by the multi-step screening process. It is also known that several psychiatric, neurological, neurodegenerative, autoimmune, or malignant disorders are associated with certain degrees of dysbiosis and potential donors identified with these disorders should be excluded after screening. To qualify as a donor, potential participants should be interviewed to identify high-risk behaviors and tested for blood and stool samples to exclude any potential infectious agents (**Figure 2**).

Inclusion/Exclusion questionnaire

For prospective donors, a physician or nurse will perform a routine medical check-up and evaluate the inclusion/exclusion criteria. Recently, several measures against SARS-CoV-2 have been included as viral particles have been found in the stool of COVID-19 patients and can likely be transmitted (160). As such, prior to any initial assessment or testing, the donor will complete the questionnaire to eliminate the risk of COVID-19 and will be mandatorily tested by RT-PCR or nasopharyngeal exudate to eliminate the risk of SARS-CoV-2 infection. If the potential donor has symptoms associated with COVID-19, it is excluded from the next steps of the donation process until isolation period has passed and RT-PCR negative tests obtained. This criterion extends beyond COVID-19 and any current contagious illness such as those with upper respiratory infections who should not donate fecal samples until they are cleared.

There are several important criteria within the inclusion/exclusion questionnaire. Individuals with history of conditions that have been associated with gut microbial dysbiosis should be excluded This include those discussed in prior subsections like metabolic syndrome, T2DM, neuropsychiatric conditions, IBD, IBS, malnutrition and cancer. Patients with autoimmune diseases and atopic conditions such as asthma and eczema should also be excluded as these conditions have associated changes in gut microbiota and can potentially predispose recipients to new allergic reactions (161). Further, patients on immunomodulatory drugs or chemotherapy are part of the exclusion criteria as the resulting immunosuppression can lead to opportunistic infections that can be transferred to recipients.

High-risk behaviors are another important part of initial screening and should be taken seriously. These behaviors include use of injection drugs, recent tattoos or piercings, incarceration, recent travel to countries with poor hygiene, homelessness, high-risk sex behaviors and those in vulnerable groups (162). Individuals in these categories unfortunately are at higher risk for transmissible infection and should not donate fecal samples. Further, after initial screening, stool and blood testing should be performed to rule out several transmissible conditions. Blood testing evaluates routine labs like complete blood counts, liver function tests, rate of erythrocyte sedimentation, electrolytes, urea and creatine, as well as transmissible diseases such as human immunodeficiency virus, hepatitis, syphilis and human T-cell lymphocytic virus (158). Although these conditions are primarily viral, FMT has been shown to transfer viral communities among donors and recipients and therefore screening prior to treatment is imperative (163). Fecal testing includes screening for *C. difficile* toxin, *cryptosporidium* antigen, a fecal ova/cyst/parasite panel, norovirus immunoassay, rotavirus immunoassay, adenovirus assay and routine bacterial culture for enteric pathogens (158). Stool testing for the presence of antibiotic resistant bacteria, especially those associated with higher mortality rates such as methicillin-resistant *Staphylococcus aureus,* Vancomycin-resistant *Enterococcus,* carbapenamase-producing *Enterobacteriaceae* and extended-spectrum beta-lactamase *Escherichia coli*, should be completed as up to 55% of qualified donors have had multidrug-resistant organisms (164). Failure to screen for these bacterial species have been related to transfer of antibiotic resistance to recipients resulting in bacteremia and even death (165). Importantly, in 2019 more rigorous screening protocols were added for asymptomatic *Helicobacter pylori,* a leading cause of peptic ulcer disease, which was detected in up to 44% *via* nested PCR (164). As such, urea breath test, the gold standard for *Helicobacter pylori* diagnosis, is recommended in stool testing.

Criteria for obtaining and processing fecal samples

For use for microbial transplantation, feces must be collected correctly and safely. An important step in ensuring the success of a FMT is the quality of the sample delivered to the beneficiary. Therefore, it is important that the procedure for obtaining samples for FMT contains a set of regulations, including access to high quality facilities, with standard operating procedures that allow the safe processing of samples by trained staff.

After the completion of the screening and the identification of the donors, the stool samples are collected from the donor within a maximum of one month from the analysis. It is recommended that, before donation, people involved in this process take a mild laxative to facilitate the elimination of stool the next day (166). Samples will be collected using a specific kit and should be free of water, urine or blood. Donors have the option to donate to the default location for collection or at

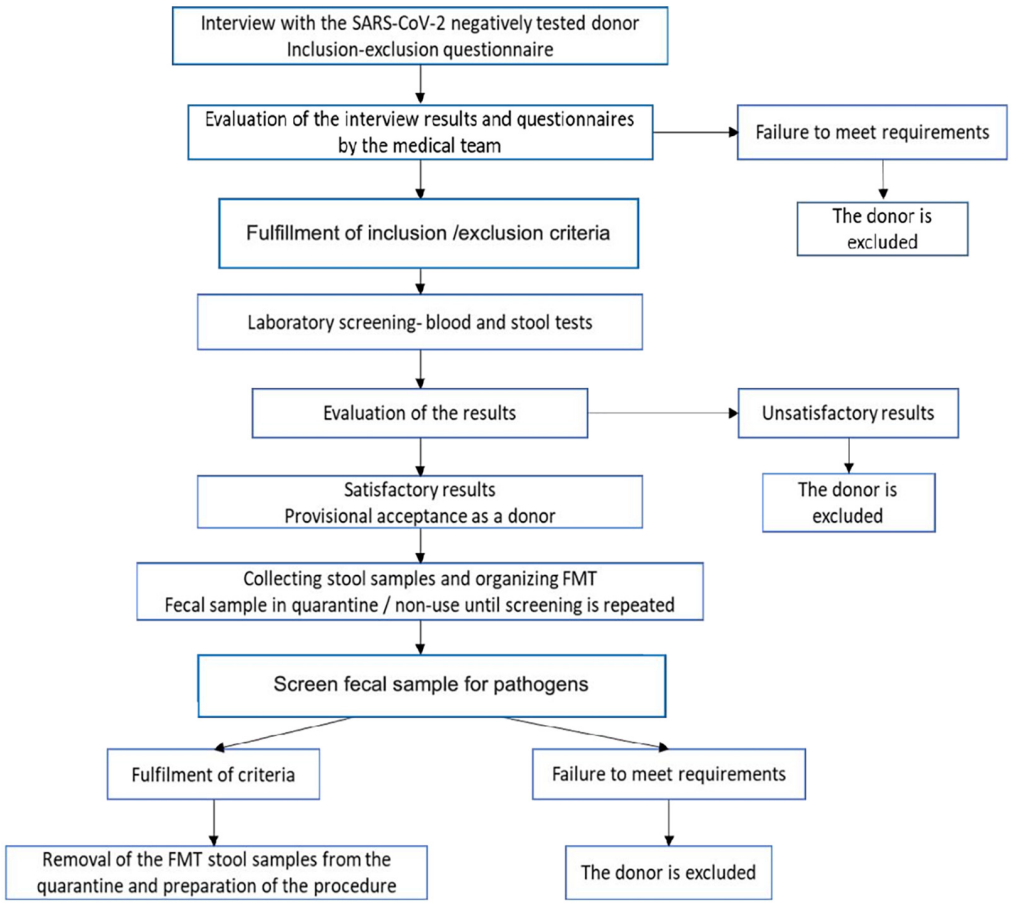


FIGURE 2
Donor selection and sample preparation flow chart. Patient first undergo screening for SARS-CoV-2, then are assessed with the inclusion-exclusion questionnaire. Donors excluded if the criteria are not met. If inclusion/exclusion criteria met, donors will undergo laboratory blood and stool testing for antibodies/antigens. Donors excluded if they test positive for any antigens/antibodies that can be transmitted through FMT. If blood and stool testing negative, fecal samples will be collected and stored for use in −80°C Celsius freezers. Prior to administration, fecal samples should be checked again for pathogens to ensure safety. If final screening criteria fulfilled, fecal sample is removed from isolation and prepared for the procedure.

home; for collection at home, the donor is required to follow an additional set of instructions that involves an important step which is maintaining the sample in a cooled area or with ice packs, and the obligation of returning the stool sample to collection centers, within 1 h after defecation. Subsequently, the stool sample can be stored for up to 8 h at 4°C, without affecting the bacterial flora (167). Studies have shown that fecal samples contain viable bacteria even after 6 months of storage in at least −80°C and, in many cases, cryogenic samples were as effective as freshly harvested ones (168).

Generally, for FMT, a minimum of 50 g stool sample is required for successful transplant, though studies have shown efficacy with 30 g (169). This stool sample is combined with saline and glycerol in a stool, saline, and glycerol ratio of 25, 65, and 10%, respectively (170). The proportion has been well established so that the amount of stool in suspension has a suitable viscosity so that it can be manipulated and transplanted into the colon, using the biopsy tube of the colonoscope (171). In addition to the ability to homogenize the stool sample, glycerin is required to maintain bacterial viability in frozen biological samples (172). The procedure requires homogenizing the fecal sample with saline and glycerin for 1 min, using a rotary blender (**Figure 3**). The blender mixing process produces a fine suspension that can be loaded into a catheter-syringe and inserted into the patient's colon through the biopsy channel of the colonoscope (170, 173). If a blender or autoclave is not available, the suspension can be prepared by manually mixing the stool sample, saline and glycerol in a special bag, used only for this purpose. Similarly, the stool can be homogenized directly in the storage bag with a spatula or in a bottle (174). Although these methods are easier, they can result in suspensions with large particles, which will block the syringe at the time of transplantation; therefore,

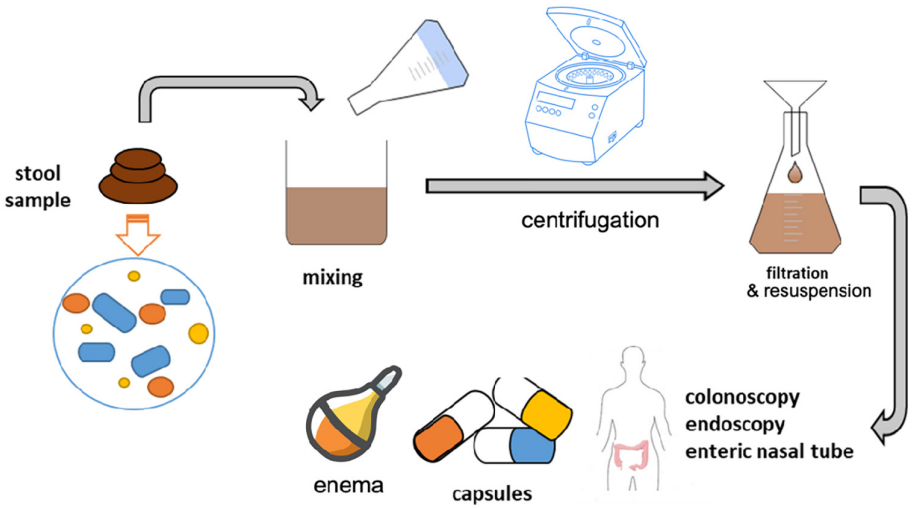


FIGURE 3
Fecal sample preparation: After testing for contaminants and pathogenic species, stool samples are mixed with saline and glycerol with a final ratio of 25% stool, 65% saline, and 10% glycerol. Mixing is done through a blender or centrifugation to homogenize the sample with the adequate ratio of substrates. The mixture is then filtered and resuspended until it is fully homogenized. The homogenized solution is stored in cryotolerant containers at −80°C for FMT use. When ready for use, the solution is loaded into capsules or delivered through colonoscopy, endoscopy, enteric nasal tube or enema.

in order to eliminate this risk, it is necessary to filter the suspension. After homogenization, the sample is divided into cryotolerant containers, which is stored at −80°C (175). When storing samples, it is advisable to use larger containers than the amount of homogenized liquid as cryogenic solutions may increase in volume (173). Another preservation method involves filtering and centrifuging the obtained suspension, followed by resuspension of the concentrated formula in saline and 12.5% glycerol for cryoprotection of frozen formulas (170). Medical personnel performing the stool preparation operation for fecal transplantation must wear disposable microbiological protective equipment including masks, gloves, insulating suits, etc. The procedure will be performed in the hood or, if possible, in an anaerobic environment, in order to protect the anaerobic bacteria. Further, continuous, and efficient sanitation of the equipment involved in the fecal sample preparation process is essential to avoid cross-contamination.

A secured document will be completed for each donor, and it will include information about the donor, contact details, screening results, and identification number (173). If the donor is unknown to the patient, the general data protection regulation (GDPR) recommendations for anonymization will be followed. The information kept confidential is necessary to identify the traceability of evidence in the event of the recipient's illness and to properly record evidence and donors. Containers with stool samples will have the number and date of collection written on the labels. Research has shown that frozen fecal material is shown to be as effective as freshly collected samples, therefore samples should not be refrozen after defrosting.

Patient preparation for fecal microbiota transplant

The preparation of patients for FMT also involves administration of antibiotics at least 3 days before the procedure (166), with stoppage of antibiotics at least 24–48 h prior to transplantation. Further, it is important to understand the effects of the medications that the patient is on that may affect bowel habits and increase the likelihood of complications from the procedure. In addition to stopping antibiotics, iron-containing supplements and anti-coagulants should be stopped if delivery route presents a risk of bleeding. The delivery routes are discussed in more detail in the following subsection. Preparation is dependent on delivery methods. The FMT administration team will be required to provide the patient with the risks and benefits of the procedure with discussion of possible complications correlated with each specific delivery route. The patient can then provide informed consent and sign the consent form. If FMT is delivered by colonoscopy, the bowel is prepared in advance with polyethylene glycol to improve the visualization of the colon (166). Those undergoing FMT administration *via* flexible sigmoidoscopy may also benefit from a bowel lavage. Further, bowel preparation may be useful to clear out *C. difficile* as well for upper GI administration, however studies have shown that other routes of administration can be effective without it (176). The standard dose is set by each institution or medical team but varies from 50 to 100 g of donated fecal material, diluted in 250–500 ml infused.

10.3389/fmed.2022.1060581

Fecal microbiota transplant delivery methods

Fecal microbiota transplant can be performed using invasive procedures, such as colonoscopy, sigmoidoscopy, endoscopy, or can be administered by retention enema, ingestion of capsules and nasal tubes (**Table 3**).

The most effective mechanism of FMT administration is *via* colonoscopy with success rates described to be between 84 and 93%, with a recent meta-analysis reporting a cure rate of 95% (177). Besides bowel preparation, the procedure is almost always performed with sedation and does present low risks for complications including intestinal perforation, bleeding and side effects associated with anesthesia (178). Contraindications to colonoscopy include recent surgeries, recent myocardial infarction, hemodynamic instability, recent bowel injury (179). It is recommended that the fecal sample is deposited in the right colon, if possible. Peristaltic contractions will move the fecal sample along the colon and gut microbiota contained within the sample will be distributed throughout the gut (166). Even with the risks associated with colonoscopy, it is the most preferred invasive method due to the ability to perform colon screening simultaneously (180).

Sigmoidoscopy also allows for deposition of the fecal sample within the colon, however only the left colon can be accessed *via* this delivery route (181). Sigmoidoscopy presents similar risks of complications including intestinal perforation and bleeding, but the procedure can be performed with sedation, so risks associated with anesthesia are not pertinent. Although this method is not used often, a recent case report shows a successful case of FMT treatment *via* sigmoidoscopy on a patient with ischemic colitis secondary to CDI (182). This delivery method may be important if patients have severe right colon disease or obstructions proximal to the hepatic flexure.

Endoscopy provides an invasive method of fecal sample delivery through the upper GI tract into the proximal duodenum (183). The risks associated with endoscopy are similar to those of colonoscopy, with intestinal perforation, bleeding and side effects associated with anesthesia being the most common, although they are rare in general. Further, introduction of samples into sedated patients poses a risk for aspiration as well, therefore patients should be kept upright after the procedure (184). A previous study using endoscopy to infuse fecal samples to recipients showing an 81% cure rate after the first infusion and 94% cure rate after multiple duodenal infusions *via* endoscopy (185). Although repeated infusions showed similar efficacy to colonoscopy, the need for sedation and risks of repeated procedure makes this method less efficacious in comparison.

Administration of fecal samples *via* retention enema is another viable option with high cure rates for recurrent CDI of 87%, though found to be less effective than colonoscopy and capsules (177). Enemas have minimal risks of complications and can be repeated, which increases efficacy. It is important to instruct patients to resist the urge to defecate and retain the enema for as long as possible. Studies have also recommended that retention enemas may be a good adjunctive FMT treatment in addition to upper GI administration (186).

TABLE 3 Fecal microbiota transplant (FMT) delivery methods with advantages and disadvantages.

Delivery methods	Advantages	Disadvantages
Nasal tube	Delivery without sedation Low costs Useful if patient unable to swallow	Risk of vomiting and aspiration Lowest efficacy
Endoscopy	– It can be performed safely in patients at risk of post-colonoscopy complications	– Discomfort associated with administration Risk of vomiting and aspiration Risk associated with the procedure Requires sedation
Capsules	Non-invasive process Time efficiency Convenient administration High cure rates Can be repeated easily	Risk of vomiting and aspiration Capsules can be large with higher mass
Colonoscopy	Most effective method Can deliver fecal sample to the right colon Most preferred invasive method Can screen for other etiologies simultaneously	Risks for intestinal perforation and bleeding Needs sedation Need for a board-certified gastroenterologist More costly Important to stop anti-coagulants Contraindications Requires bowel preparation
Sigmoidoscopy	No sedation required Can screen for distal 1/3rd colonic pathologies simultaneously	Risk for intestinal perforation and bleeding Inability to use the area on the right side of the colon Need for a board-certified gastroenterologist Important to stop anti-coagulants Bowel preparation recommended
Retention enema	Low costs High tolerability Without sedation Easily repeated Can be done in pediatric patients that cannot have a colonoscopy	– Retention difficulties in some cases Inability to use the area on the right side of the colon

Oral capsules, a highly preferred method by patients, as it is the least invasive with high levels of efficacy of up to 92% (177). Several studies have compared the cure rates of colonoscopy with oral capsules, particularly in treating recurrent CDI (187). Benefits of oral capsules include ease of administration and repeated treatments; however, a single treatment can require up to 30 capsules (188). Capsule shells are resistant to gastric acid; thus, proton pump inhibitors are not required though they can be effective in facilitating treatment (189). Adverse effects are much fewer than invasive methods with rare cases of nausea and vomiting reported (187). Finally, FMT with nasal gastric/duodenal tube is another option, though it has been shown to have the lowest cure rates for CDI at approximately 78% (177). Although this method is not preferred, it can be indicated when patients are unable to swallow oral capsules.

Challenges, limitations, and future perspective

Fecal microbiota transplant has been proven effective in the treatment of numerous diseases. Initially tested and used for the treatment of recurrent and refractory CDI, the advantages of its use have broadened its applicability, with ongoing clinical trials to assess efficacy in non-communicable diseases including obesity, type 2 diabetes mellitus, metabolic syndrome, neuropsychiatric disorders, inflammatory bowel disease, IBS, decompensated cirrhosis, GvHD and even cancers, as discussed throughout this review. Though generally safe, there are adverse events reported throughout the literature. As such, regulated stool banks have been developed with specific inclusion/exclusion criteria and protocols for sample preparation and FMT administration to limit transfer of unwanted pathogens. Still, there remains a lot that is unknown and missing knowledge gaps that has prevented this therapeutic modality from obtaining FDA approval for treatments beyond CDI.

Although gut sequencing technology is continuously advancing, the gut microbiota comprises a vast amount of species, with a considerable amount of the bacterial species and their role and functions still being unknown (190). This is a significant limitation as it is possible that some of these species have a major impact on the variable outcomes seen between studies. Likewise, significant efforts and progress have been made in identifying key bacterial species that are correlated with better outcomes in FMT. It is clear, however, that the overall response to treatment involves a complex interplay between the gut microbial composition and the host (190). These unknown factors add an extra layer of variability, hence the need for detecting and uncovering the functions of other bacterial species, yet unknown, that may exert a major role in health and disease, whether alone or *via* bacterial competition and host interaction. Further, due to the large variations between donors, stool samples that are heterogeneous, the results may be less reproducible and long-term outcomes may be transient (191). To eliminate variability in fecal samples, recent studies have suggested that synthetic microbiota communities may be the future of FMT (191, 192). By creating a structurally controlled bacterial community, similar samples can be reproduced, pathogenic microorganisms can be eliminated and bacteria that is deemed to be beneficial can be cultured at a larger scale. With continuous technological advances, it is possible that fecal sample preparation may be standardized through synthetic microbial communities to provide a balance of optimal gut microbiota concentrations for recipients.

Overall, the data within the scientific literature for FMT for treatment of a variety of conditions is promising. Though longer-term evaluations exist, most studies have assessed efficacy of treatment for 6 months or less and using small samples. Further, changes in gut microbiota are highly dependent on environmental factors including diet and geographic locations which can enhance efficacy or prevent the desired response to FMT treatment (193). As such, future studies should take these factors into account to define long-term safety of the treatment more clearly and provide lifestyle recommendations that can be used in conjunction with FMT to maximize its therapeutic benefits.

Author contributions

SH: conceptualization, writing original draft, and editing. RG: conceptualization, resources, writing original draft, and editing. AL: writing – review and editing. I-OS: conceptualization and writing – review. MC: conceptualization, writing – review, and supervision. All authors made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Funding

This work was supported by the project titled "The analysis of interrelationship between gut microbiota and the host with applications in the prevention and control of type 2 diabetes" co-financed by European Regional Development Fund through Competitiveness Operational Program under the contract number 120/16.09.2016.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. *Microbiol Mol Biol Rev.* (2017) 81: e36–17.

2. Cryan J, O'Riordan K, Sandhu K, Peterson V, Dinan T. The gut microbiome in neurological disorders. *Lancet Neurol.* (2020) 19:179–94.

3. Lim E, Rodriguez C, Holtz L. Amniotic fluid from healthy term pregnancies does not harbor a detectable microbial community. *Microbiome.* (2018) 6:87.

4. Bi Y, Tu Y, Zhang N, Wang S, Zhang F, Suen G, et al. Multiomics analysis reveals the presence of a microbiome in the gut of fetal lambs. *Gut.* (2021) 70:853–64. doi: 10.1136/gutjnl-2020-320951

5. Radjabzadeh D, Boer C, Beth S, van der Wal P, Kiefte-De Jong J, Jansen M, et al. Diversity, compositional and functional differences between gut microbiota of children and adults. *Sci Rep.* (2020) 10:1040.

6. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet J, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA.* (2010) 107:14691–6. doi: 10.1073/pnas.1005963107

7. Lozupone C, Stombaugh J, Gordon J, Jansson J, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature.* (2012) 489:220–30.

8. Al Bander Z, Nitert M, Mousa A, Naderpoor N. The gut microbiota and inflammation: An overview. *Int J Environ Res Public Health.* (2020) 17:7618.

9. Paone P, Cani P. Mucus barrier, mucins and gut microbiota: the expected slimy partners? *Gut.* (2020) 69:2232–43. doi: 10.1136/gutjnl-2020-322260

10. Guevara-Cruz M, Godinez-Salas E, Sanchez-Tapia M, Torres-Villalobos G, Pichardo-Ontiveros E, Guizar-Heredia R, et al. Genistein stimulates insulin sensitivity through gut microbiota reshaping and skeletal muscle AMPK activation in obese subjects. *BMJ Open Diabetes Res Care.* (2020) 8:e000948. doi: 10.1136/bmjdrc-2019-000948

11. Hamamah S, Aghazarian A, Nazaryan A, Hajnal A, and Covasa M. Role of microbiota-gut-brain axis in regulating dopaminergic signaling. *Biomedicines.* (2022) 10:436.

12. Martin-Gallausiaux C, Marinelli L, Blottière H, Larraufie P, Lapaque N. SCFA: mechanisms and functional importance in the gut. *Proc Nutr Soc.* (2021) 80:37–49.

13. Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, Navarrete P, et al. The Firmicutes/Bacteroidetes Ratio: A relevant marker of gut dysbiosis in obese patients? *Nutrients.* (2020) 12:1474. doi: 10.3390/nu12051474

14. Zhang Y, Zhang Q, Wang Y, Zhang W, Hu H, Wu H, et al. A comparison study of age and colorectal cancer-related gut bacteria. *Front Cell Infect Microbiol.* (2021) 11:606490. doi: 10.3389/fcimb.2021.606490

15. Hamamah S, Hajnal A, Covasa M. Impact of nutrition, microbiota transplant and weight loss surgery on dopaminergic alterations in Parkinson's Disease and Obesity. *Int J Mol Sci.* (2022) 23:7503. doi: 10.3390/ijms23147503

16. Iizuka K, Yabe D. The role of metagenomics in precision nutrition. *Nutrients.* (2020) 12:1668.

17. Vendrik K, Ooijevaar R, de Jong P, Laman J, van Oosten B, van Hilten J, et al. Fecal microbiota transplantation in neurological disorders. *Front Cell Infect Microbiol.* (2020) 10:98. doi: 10.3389/fcimb.2020.00098

18. Stojanov S, Berlec A, Štrukelj B. The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. *Microorganisms.* (2020) 8:1715. doi: 10.3390/microorganisms8111715

19. Xu Q, Zhang S, Quan J, Wu Z, Gu S, Chen Y, et al. The evaluation of fecal microbiota transplantation vs vancomycin in a Clostridioides difficile infection model. *Appl Microbiol Biotechnol.* (2022) 106:6689–700. doi: 10.1007/s00253-022-12154-z

20. Ng S, Xu Z, Mak J, Yang K, Liu Q, Zuo T, et al. Microbiota engraftment after faecal microbiota transplantation in obese subjects with type 2 diabetes: a 24-week, double-blind, randomised controlled trial. *Gut.* (2022) 71:716–23. doi: 10.1136/gutjnl-2020-323617

21. Aron-Wisnewsky J, Clément K, Nieuwdorp M. Fecal microbiota transplantation: A future therapeutic option for obesity/diabetes? *Curr Diab Rep.* (2019) 19:51. doi: 10.1007/s11892-019-1180-z

22. Hartstra A, Schüppel V, Imangaliyev S, Schrantee A, Prodan A, Collard D, et al. Infusion of donor feces affects the gut-brain axis in humans with metabolic syndrome. *Mol Metab.* (2020) 42:101076. doi: 10.1016/j.molmet.2020.101076

23. Davar D, Dzutsev A, McCulloch J, Rodrigues R, Chauvin J, Morrison R, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science.* (2021) 371:595–602. doi: 10.1126/science.abf3363

24. Costello S, Hughes P, Waters O, Bryant R, Vincent A, Blatchford P, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: A randomized clinical trial. *JAMA.* (2019) 321:156–64.

25. Mendenhall E, Kohrt B, Norris S, Ndetei D, Prabhakaran D. Non-communicable disease syndemics: poverty, depression, and diabetes among low-income populations. *Lancet.* (2017) 389:951–63.

26. Green J, Davis J, Berk M, Hair C, Loughman A, Castle D, et al. Efficacy and safety of fecal microbiota transplantation for the treatment of diseases other than *Clostridium* difficile infection: a systematic review and meta-analysis. *Gut Microbes.* (2020) 12:1–25.

27. Sachs R, Edelstein C. Ensuring the safe and effective FDA regulation of fecal microbiota transplantation. *J Law Biosci.* (2015) 2:396–415. doi: 10.1093/jlb/lsv032

28. Golden A. Obesity's Impact. *Nurs Clin North Am.* (2021) 56:xiii–xiv.

29. Blüher M. Obesity: global epidemiology and pathogenesis. *Nat Rev Endocrinol.* (2019) 15:288–98.

30. Asadi A, Shadab Mehr N, Mohamadi M, Shokri F, Heidary M, Sadeghifard N, et al. Obesity and gut-microbiota-brain axis: A narrative review. *J Clin Lab Anal.* (2022) 36:e24420. doi: 10.1002/jcla.24420

31. Cornejo-Pareja I, Muñoz-Garach A, Clemente-Postigo M, Tinahones F. Importance of gut microbiota in obesity. *Eur J Clin Nutr.* (2019) 72(Suppl 1):26–37.

32. Allegretti J, Kassam Z, Mullish B, Chiang A, Carrellas M, Hurtado J, et al. Effects of fecal microbiota transplantation with oral capsules in obese patients. *Clin Gastroenterol Hepatol.* (2020) 18:855–63.

33. Leong K, Jayasinghe T, Wilson B, Derraik J, Albert B, Chiavaroli V, et al. Effects of fecal microbiome transfer in adolescents with obesity: The gut bugs randomized controlled trial. *JAMA Netw Open.* (2020) 3:e2030415.

34. Alang N, Kelly C. Weight gain after fecal microbiota transplantation. *Open Forum Infect Dis.* (2015) 2:ofv004.

35. Ridaura V, Faith J, Rey F, Cheng J, Duncan A, Kau A, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science.* (2013) 341:1241214. doi: 10.1126/science.1241214

36. Fischer M, Kao D, Kassam Z, Smith J, Louie T, Sipe B, et al. Stool donor body mass index does not affect recipient weight after a single fecal microbiota Transplantation for *Clostridium* difficile Infection. *Clin Gastroenterol Hepatol.* (2018) 16:1351–3.

37. Rinott E, Youngster I, Yaskolka Meir A, Tsaban G, Zelicha H, Kaplan A, et al. Effects of diet-modulated autologous fecal microbiota transplantation on weight regain. *Gastroenterology.* (2021) 160:158–73. doi: 10.1053/j.gastro.2020.08.041

38. Sbierski-Kind J, Mai K, Kath J, Jurisch A, Streitz M, Kuchenbecker L, et al. Association between subcutaneous adipose tissue inflammation, insulin resistance, and calorie restriction in obese females. *J Immunol.* (2020) 205:45–55.

39. Esser N, Legrand-Poels S, Piette J, Scheen A, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res Clin Pract.* (2014) 105:141–50.

40. Ma Q, Li Y, Li P, Wang M, Wang J, Tang Z, et al. Research progress in the relationship between type 2 diabetes mellitus and intestinal flora. *Biomed Pharmacother.* (2019) 117:109138.

41. Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, et al. Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat Med.* (2019) 25:1096–103. doi: 10.1038/s41591-019-0495-2

42. Liu F, Li P, Chen M, Luo Y, Prabhakar M, Zheng H, et al. Fructooligosaccharide (FOS) and Galactooligosaccharide (GOS) Increase *Bifidobacterium* but reduce butyrate producing bacteria with adverse glycemic metabolism in healthy young population. *Sci Rep.* (2017) 7:11789. doi: 10.1038/s41598-017-10722-2

43. Paramsothy S, Nielsen S, Kamm M, Deshpande N, Faith J, Clemente J, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. *Gastroenterology.* (2019) 156:1440–54.

44. Tong X, Xu J, Lian F, Yu X, Zhao Y, Xu L, et al. Structural alteration of gut microbiota during the amelioration of human type 2 diabetes with hyperlipidemia by metformin and a traditional chinese herbal formula: a multicenter, randomized, open label clinical trial. *mBio.* (2018) 9:e2392–2317. doi: 10.1128/mBio.02392-17

45. Wu H, Esteve E, Tremaroli V, Khan M, Caesar R, Mannerås-Holm L, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med.* (2017) 23:850–8. doi: 10.1038/nm.4345

46. Ding D, Yong H, You N, Lu W, Yang X, Ye X, et al. Prospective study reveals host microbial determinants of clinical response to fecal microbiota transplant therapy in type 2 diabetes patients. *Front Cell Infect Microbiol.* (2022) 12:820367. doi: 10.3389/fcimb.2022.820367

47. Khorraminezhad L, Leclercq M, O'Connor S, Julien P, Weisnagel S, Gagnon C, et al. Dairy product intake modifies gut microbiota composition among hyperinsulinemic individuals. *Eur J Nutr.* (2021) 60:159–67. doi: 10.1007/s00394-020-02226-z

48. Su L, Hong Z, Zhou T, Jian Y, Xu M, Zhang X, et al. Health improvements of type 2 diabetic patients through diet and diet plus fecal microbiota transplantation. *Sci Rep.* (2022) 12:1152. doi: 10.1038/s41598-022-05127-9

49. Calabrese C, Valentini A, Calabrese G. Gut microbiota and Type 1 diabetes mellitus: The effect of mediterranean diet. *Front Nutr.* (2020) 7:612773. doi: 10.3389/fnut.2020.612773

50. de Groot P, Nikolic T, Pellegrini S, Sordi V, Imangaliyev S, Rampanelli E, et al. Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. *Gut.* (2021) 70:92–105. doi: 10.1136/gutjnl-2020-322630

51. Xie Y, Jing X, Chen X, Chen L, Zhang S, Cai X. Fecal microbiota transplantation treatment for type 1 diabetes mellitus with malnutrition: a case report. *Ther Adv Chronic Dis.* (2022) 13:20406223221117449. doi: 10.1177/20406223221117449

52. Hao Y, Feng Y, Yan X, Chen L, Zhong R, Tang X, et al. Gut microbiota-testis axis: FMT improves systemic and testicular micro-environment to increase semen quality in type 1 diabetes. *Mol Med.* (2022) 28:45. doi: 10.1186/s10020-022-00473-w

53. Bastos R, Simplício-Filho A, Sávio-Silva C, Oliveira L, Cruz G, Sousa E, et al. Fecal microbiota transplant in a pre-clinical model of type 2 diabetes mellitus, obesity and diabetic kidney disease. *Int J Mol Sci.* (2022) 23:3842. doi: 10.3390/ijms23073842

54. Serena C, Ceperuelo-Mallafré V, Keiran N, Queipo-Ortuño M, Bernal R, Gomez-Huelgas R, et al. Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. *ISME J.* (2018) 12:1642–57.

55. de Groot P, Frissen M, de Clercq N, Nieuwdorp M. Fecal microbiota transplantation in metabolic syndrome: History, present and future. *Gut Microbes.* (2017) 8:253–67. doi: 10.1080/19490976.2017.1293224

56. Di Luccia B, Crescenzo R, Mazzoli A, Cigliano L, Venditti P, Walser J, et al. Rescue of fructose-induced metabolic syndrome by antibiotics or faecal transplantation in a rat model of obesity. *PLoS One.* (2015) 10:e0134893. doi: 10.1371/journal.pone.0134893

57. Hall A, Yassour M, Sauk J, Garner A, Jiang X, Arthur T, et al. A novel *Ruminococcus gnavus* clade enriched in inflammatory bowel disease patients. *Genome Med.* (2017) 9:103. doi: 10.1186/s13073-017-0490-5

58. Kootte R, Levin E, Salojärvi J, Smits L, Hartstra A, Udayappan S, et al. Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metab.* (2017) 26:611–9. doi: 10.1016/j.cmet.2017.09.008

59. Yamaguchi Y, Adachi K, Sugiyama T, Shimozato A, Ebi M, Ogasawara N, et al. Association of intestinal microbiota with metabolic markers and dietary habits in patients with type 2 diabetes. *Digestion.* (2016) 94:66–72.

60. van der Vossen E, Bastos D, Stols-Gonçalves D, de Goffau M, Davids M, Pereira J, et al. Effects of fecal microbiota transplant on DNA methylation in subjects with metabolic syndrome. *Gut Microbes.* (2021) 13:1993513. doi: 10.1080/19490976.2021.1993513

61. Manrique P, Zhu Y, van der Oost J, Herrema H, Nieuwdorp M, de Vos W, et al. Gut bacteriophage dynamics during fecal microbial transplantation in subjects with metabolic syndrome. *Gut Microbes.* (2021) 13:1–15. doi: 10.1080/19490976.2021.1897217

62. Kim Y, Shin C. The microbiota-gut-brain axis in neuropsychiatric disorders: Pathophysiological mechanisms and novel treatments. *Curr Neuropharmacol.* (2018) 16:559–73. doi: 10.2174/1570159X15666170915141036

63. Mittal R, Debs L, Patel A, Nguyen D, Patel K, O'Connor G, et al. Neurotransmitters: The critical modulators regulating gut-brain axis. *J Cell Physiol.* (2017) 232:2359–72.

64. Bandelow B, Michaelis S. Epidemiology of anxiety disorders in the 21st century. *Dialogues Clin Neurosci.* (2015) 17:327–35.

65. Rakofsky J, Rapaport M. Mood Disorders. *Continuum (Minneap Minn).* (2018) 24:804–27.

66. Li N, Wang Q, Wang Y, Sun A, Lin Y, Jin Y, et al. Fecal microbiota transplantation from chronic unpredictable mild stress mice donors affects anxiety-like and depression-like behavior in recipient mice *via* the gut microbiota-inflammation-brain axis. *Stress.* (2019) 22:592–602. doi: 10.1080/10253890.2019.1617267

67. Macchione I, Lopetuso L, Ianiro G, Napoli M, Gibiino G, Rizzatti G, et al. Akkermansia muciniphila: key player in metabolic and gastrointestinal disorders. *Eur Rev Med Pharmacol Sci.* (2019) 23:8075–83.

68. Guo Q, Lin H, Chen P, Tan S, Wen Z, Lin L, et al. Dynamic changes of intestinal flora in patients with irritable bowel syndrome combined with anxiety and depression after oral administration of enterobacteria capsules. *Bioengineered.* (2021) 12:11885–97. doi: 10.1080/21655979.2021.1999374

69. Doll J, Vázquez-Castellanos J, Schaub A, Schweinfurth N, Kettelhack C, Schneider E, et al. Fecal Microbiota Transplantation (FMT) as an adjunctive therapy for depression-case report. *Front Psychiatry.* (2022) 13:815422. doi: 10.3389/fpsyt.2022.815422

70. Strandwitz P, Kim K, Terekhova D, Liu J, Sharma A, Levering J, et al. GABA-modulating bacteria of the human gut microbiota. *Nat Microbiol.* (2019) 4:396–403.

71. Zheng P, Zeng B, Liu M, Chen J, Pan J, Han Y, et al. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. *Sci Adv.* (2019) 5:eaau8317.

72. Zhu F, Guo R, Wang W, Ju Y, Wang Q, Ma Q, et al. Transplantation of microbiota from drug-free patients with schizophrenia causes schizophrenia-like abnormal behaviors and dysregulated kynurenine metabolism in mice. *Mol Psychiatry.* (2020) 25:2905–18. doi: 10.1038/s41380-019-0475-4

73. Barry S, Clarke G, Scully P, Dinan T. Kynurenine pathway in psychosis: evidence of increased tryptophan degradation. *J Psychopharmacol.* (2009) 23:287–94.

74. Kang D, Adams J, Vargason T, Santiago M, Hahn J, Krajmalnik-Brown R. Distinct fecal and plasma metabolites in children with autism spectrum disorders and their modulation after microbiota transfer therapy. *mSphere.* (2020) 5:e314–20. doi: 10.1128/mSphere.00314-20

75. Al Hinai E, Kullamethee P, Rowland I, Swann J, Walton G, Commane D. Modelling the role of microbial p-cresol in colorectal genotoxicity. *Gut Microbes.* (2019) 10:398–411. doi: 10.1080/19490976.2018.1534514

76. Bermudez-Martin P, Becker J, Caramello N, Fernandez S, Costa-Campos R, Canaguier J, et al. The microbial metabolite p-Cresol induces autistic-like behaviors in mice by remodeling the gut microbiota. *Microbiome.* (2021) 9:157. doi: 10.1186/s40168-021-01103-z

77. Kang D, Adams J, Gregory A, Borody T, Chittick L, Fasano A, et al. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome.* (2017) 5:10.

78. Kang D, Adams J, Coleman D, Pollard E, Maldonado J, McDonough-Means S, et al. Long-term benefit of microbiota transfer therapy on autism symptoms and gut microbiota. *Sci Rep.* (2019) 9:5821. doi: 10.1038/s41598-019-42183-0

79. Cignarella F, Cantoni C, Ghezzi L, Salter A, Dorsett Y, Chen L, et al. Intermittent fasting confers protection in CNS autoimmunity by altering the gut microbiota. *Cell Metab.* (2018) 27:1222–35. doi: 10.1016/j.cmet.2018.05.006

80. Tollár J, Nagy F, Tóth B, Török K, Szita K, Csutorás B, et al. Exercise effects on multiple sclerosis quality of life and clinical-motor symptoms. *Med Sci Sports Exerc.* (2020) 52:1007–14. doi: 10.1249/MSS.0000000000002228

81. Makkawi S, Camara-Lemarroy C, Metz L. Fecal microbiota transplantation associated with 10 years of stability in a patient with SPMS. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e459. doi: 10.1212/NXI.0000000000000459

82. Berer K, Gerdes L, Cekanaviciute E, Jia X, Xiao L, Xia Z, et al. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc Natl Acad Sci USA.* (2017) 114:10719–24. doi: 10.1073/pnas.1711233114

83. Xu H, Huang H, Zhou Y, Zhao H, Xu J, Shou D, et al. Fecal microbiota transplantation: A new therapeutic attempt from the gut to the brain. *Gastroenterol Res Pract.* (2021) 2021:6699268. doi: 10.1155/2021/6699268

84. Tiwari S, Atluri V, Kaushik A, Yndart A, Nair M. Alzheimer's disease: pathogenesis, diagnostics, and therapeutics. *Int J Nanomed.* (2019) 14:5541–54.

85. Kim N, Jeon S, Ju I, Gee M, Do J, Oh M, et al. Transplantation of gut microbiota derived from Alzheimer's disease mouse model impairs memory function and neurogenesis in C57BL/6 mice. *Brain Behav Immun.* (2021) 98:357–65. doi: 10.1016/j.bbi.2021.09.002

86. Muzio L, Viotti A, Martino G. Microglia in neuroinflammation and neurodegeneration: From understanding to therapy. *Front Neurosci.* (2021) 15:742065. doi: 10.3389/fnins.2021.742065

87. Sun J, Xu J, Ling Y, Wang F, Gong T, Yang C, et al. Fecal microbiota transplantation alleviated Alzheimer's disease-like pathogenesis in APP/PS1 transgenic mice. *Transl Psychiatry.* (2019) 9:189. doi: 10.1038/s41398-019-0525-3

88. Wang S, Ichinomiya T, Terada Y, Wang D, Patel H, Head B. Synapsin-Promoted Caveolin-1 overexpression maintains mitochondrial morphology and function in PSAPP Alzheimer's Disease Mice. *Cells.* (2021) 10:2487. doi: 10.3390/cells10092487

89. Segal A, Zlotnik Y, Moyal-Atias K, Abuhasira R, Ifergane G. Fecal microbiota transplant as a potential treatment for Parkinson's disease - A case series. *Clin Neurol Neurosurg.* (2021) 207:106791. doi: 10.1016/j.clineuro.2021.106791

90. Mentella M, Scaldaferri F, Pizzoferrato M, Gasbarrini A, Miggiano G. Nutrition, IBD and gut microbiota: A review. *Nutrients.* (2020) 12:944.

91. Ordás I, Eckmann L, Talamini M, Baumgart D, Sandborn W. Ulcerative colitis. *Lancet.* (2012) 380:1606–19.

92. Paramsothy S, Kamm M, Kaakoush N, Walsh A, van den Bogaerde J, Samuel D, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet.* (2017) 389:1218–28.

93. Haifer C, Paramsothy S, Kaakoush N, Saikal A, Ghaly S, Yang T, et al. Lyophilised oral faecal microbiota transplantation for ulcerative colitis (LOTUS): a randomised, double-blind, placebo-controlled trial. *Lancet Gastroenterol Hepatol.* (2022) 7:141–51. doi: 10.1016/S2468-1253(21)00400-3

94. Pai N, Popov J, Hill L, Hartung E, Grzywacz K, Moayyedi P. Results of the first pilot randomized controlled trial of fecal microbiota transplant in pediatric ulcerative colitis: Lessons, limitations, and future prospects. *Gastroenterology.* (2021) 161:388–93. doi: 10.1053/j.gastro.2021.04.067

95. Fang H, Fu L, Li X, Lu C, Su Y, Xiong K, et al. Long-term efficacy and safety of monotherapy with a single fresh fecal microbiota transplant for recurrent active ulcerative colitis: a prospective randomized pilot study. *Microb Cell Fact.* (2021) 20:18. doi: 10.1186/s12934-021-01513-6

96. Crothers J, Chu N, Nguyen L, Phillips M, Collins C, Fortner K, et al. Daily, oral FMT for long-term maintenance therapy in ulcerative colitis: results of a single-center, prospective, randomized pilot study. *BMC Gastroenterol.* (2021) 21:281. doi: 10.1186/s12876-021-01856-9

97. Haga K, Chiba A, Shibuya T, Osada T, Ishikawa D, Kodani T, et al. MAIT cells are activated and accumulated in the inflamed mucosa of ulcerative colitis. *J Gastroenterol Hepatol.* (2016) 31:965–72.

98. Huang C, Mei Q, Lou L, Huang Z, Fu Y, Fan J, et al. Ulcerative colitis in response to fecal microbiota transplantation *via* modulation of gut microbiota and Th17/Treg Cell Balance. *Cells.* (2022) 11:1851. doi: 10.3390/cells11111851

99. Zhao D, Ye C, Zhang S, Lv X, Yang B. Analysis of risk factors for early clinical recurrence of inflammatory bowel disease after fecal microbiota transplantation. *Am J Transl Res.* (2021) 13:12875–86.

100. Sokol H, Landman C, Seksik P, Berard L, Montil M, Nion-Larmurier I, et al. Fecal microbiota transplantation to maintain remission in Crohn's disease: a pilot randomized controlled study. *Microbiome.* (2020) 8:12. doi: 10.1186/s40168-020-0792-5

101. Yang Z, Bu C, Yuan W, Shen Z, Quan Y, Wu S, et al. Fecal microbiota transplant *via* endoscopic delivering through small intestine and colon: No difference for Crohn's Disease. *Dig Dis Sci.* (2020) 65:150–7. doi: 10.1007/s10620-019-05751-y

102. Li P, Zhang T, Xiao Y, Tian L, Cui B, Ji G, et al. Timing for the second fecal microbiota transplantation to maintain the long-term benefit from the first treatment for Crohn's disease. *Appl Microbiol Biotechnol.* (2019) 103:349–60. doi: 10.1007/s00253-018-9447-x

103. Xiao L, Liu Q, Luo M, Xiong L. Gut microbiota-derived metabolites in irritable bowel syndrome. *Front Cell Infect Microbiol.* (2021) 11:729346. doi: 10.3389/fcimb.2021.729346

104. Mizuno S, Masaoka T, Naganuma M, Kishimoto T, Kitazawa M, Kurokawa S, et al. *Bifidobacterium*-rich fecal donor may be a positive predictor for successful fecal microbiota transplantation in patients with irritable bowel syndrome. *Digestion.* (2017) 96:29–38. doi: 10.1159/000471919

105. El-Salhy M, Kristoffersen A, Valeur J, Casen C, Hatlebakk J, Gilja O, et al. Long-term effects of fecal microbiota transplantation (FMT) in patients with irritable bowel syndrome. *Neurogastroenterol Motil.* (2022) 34:e14200.

106. Hamazaki M, Sawada T, Yamamura T, Maeda K, Mizutani Y, Ishikawa E, et al. Fecal microbiota transplantation in the treatment of irritable bowel syndrome: a single-center prospective study in Japan. *BMC Gastroenterol.* (2022) 22:342. doi: 10.1186/s12876-022-02408-5

107. Singh R, Chang H, Yan D, Lee K, Ucmak D, Wong K, et al. Influence of diet on the gut microbiome and implications for human health. *J Transl Med.* (2017) 15:73.

108. Sharma S, Suk K, Kim D. Significance of gut microbiota in alcoholic and non-alcoholic fatty liver diseases. *World J Gastroenterol.* (2021) 27:6161–79.

109. Maslennikov R, Ivashkin V, Efremova I, Poluektova E, Shirokova E. Gut-liver axis in cirrhosis: Are hemodynamic changes a missing link? *World J Clin Cases.* (2021) 9:9320–32. doi: 10.12998/wjcc.v9.i31.9320

110. Rahimi R, Brown K, Flamm S, Brown R Jr. Overt hepatic encephalopathy: Current pharmacologic treatments and improving clinical outcomes. *Am J Med.* (2021) 134:1330–8. doi: 10.1016/j.amjmed.2021.06.007

111. Bajaj J, Kakiyama G, Savidge T, Takei H, Kassam Z, Fagan A, et al. Antibiotic-Associated disruption of microbiota composition and function in cirrhosis is restored by fecal transplant. *Hepatology.* (2018) 68:1549–58.

112. Bajaj J, Shamsaddini A, Fagan A, Sterling R, Gavis E, Khoruts A, et al. Fecal microbiota transplant in cirrhosis reduces gut microbial antibiotic resistance genes: Analysis of two trials. *Hepatol Commun.* (2021) 5:258–71. doi: 10.1002/hep4.1639

113. Bajaj J, Salzman N, Acharya C, Takei H, Kakiyama G, Fagan A, et al. Microbial functional change is linked with clinical outcomes after capsular fecal transplant in cirrhosis. *JCI Insight.* (2019) 4:e133410. doi: 10.1172/jci.insight.133410

114. Bajaj J, Kassam Z, Fagan A, Gavis E, Liu E, Cox I, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. *Hepatology.* (2017) 66:1727–38.

115. Park E, Chelvanambi M, Bhutiani N, Kroemer G, Zitvogel L, Wargo J. Targeting the gut and tumor microbiota in cancer. *Nat Med.* (2022) 28:690–703.

116. Song X, An Y, Chen D, Zhang W, Wu X, Li C, et al. Microbial metabolite deoxycholic acid promotes vasculogenic mimicry formation in intestinal carcinogenesis. *Cancer Sci.* (2022) 113:459–77. doi: 10.1111/cas.15208

117. Shao X, Sun S, Zhou Y, Wang H, Yu Y, Hu T, et al. *Bacteroides fragilis* restricts colitis-associated cancer *via* negative regulation of the NLRP3 axis. *Cancer Lett.* (2021) 523:170–81. doi: 10.1016/j.canlet.2021.10.002

118. Sugimura N, Li Q, Chu E, Lau H, Fong W, Liu W, et al. *Lactobacillus gallinarum* modulates the gut microbiota and produces anti-cancer metabolites to protect against colorectal tumourigenesis. *Gut.* (2021) 71:2011–21. doi: 10.1136/gutjnl-2020-323951

119. Baruch E, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science.* (2021) 371:602–9. doi: 10.1126/science.abb5920

120. Carlino M, Larkin J, Long G. Immune checkpoint inhibitors in melanoma. *Lancet.* (2021) 398:1002–14.

121. Schalper K, Carleton M, Zhou M, Chen T, Feng Y, Huang S, et al. Elevated serum interleukin-8 is associated with enhanced intratumor neutrophils and reduced clinical benefit of immune-checkpoint inhibitors. *Nat Med.* (2020) 26:688–92. doi: 10.1038/s41591-020-0856-x

122. Malard F, Vekhoff A, Lapusan S, Isnard F, D'Incan-Corda E, Rey J, et al. Gut microbiota diversity after autologous fecal microbiota transfer in acute myeloid

leukemia patients. *Nat Commun.* (2021) 12:3084. doi: 10.1038/s41467-021-23376-6

123. de Castro C Jr., Ganc A, Ganc R, Petrolli M, Hamerschlack N. Fecal microbiota transplant after hematopoietic SCT: report of a successful case. *Bone Marrow Transplant.* (2015) 50:145. doi: 10.1038/bmt.2014.212

124. Stidham R, Higgins P. Colorectal cancer in inflammatory bowel disease. *Clin Colon Rectal Surg.* (2018) 31:168–78.

125. Tarao K, Nozaki A, Ikeda T, Sato A, Komatsu H, Komatsu T, et al. Real impact of liver cirrhosis on the development of hepatocellular carcinoma in various liver diseases-meta-analytic assessment. *Cancer Med.* (2019) 8:1054–65. doi: 10.1002/cam4.1998

126. Ramachandran V, Kolli S, Strowd L. Review of graft-versus-host disease. *Dermatol Clin.* (2019) 37:569–82.

127. Fredricks D. The gut microbiota and graft-versus-host disease. *J Clin Invest.* (2019) 129:1808–17.

128. Biliński J, Jasiński M, Basak G. The role of fecal microbiota transplantation in the treatment of acute graft-versus-host disease. *Biomedicines.* (2022) 10:837.

129. Mathewson N, Jenq R, Mathew A, Koenigsknecht M, Hanash A, Toubai T, et al. Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nat Immunol.* (2016) 17:505–13.

130. Bhattacharyya A, Hanafi L, Sheih A, Golob J, Srinivasan S, Boeckh M, et al. Graft-derived reconstitution of mucosal-associated invariant T cells after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* (2018) 24:242–51. doi: 10.1016/j.bbmt.2017.10.003

131. Varelias A, Bunting M, Ormerod K, Koyama M, Olver S, Straube J, et al. Recipient mucosal-associated invariant T cells control GVHD within the colon. *J Clin Invest.* (2018) 128:1919–36. doi: 10.1172/JCI91646

132. Zhang F, Zuo T, Yeoh Y, Cheng F, Liu Q, Tang W, et al. Longitudinal dynamics of gut bacteriome, mycobiome and virome after fecal microbiota transplantation in graft-versus-host disease. *Nat Commun.* (2021) 12:65. doi: 10.1038/s41467-020-20240-x

133. Gao M, Hong Y, Zhao X, Pan X, Sun Y, Kong J, et al. The potential roles of mucosa-associated invariant T cells in the pathogenesis of gut graft-versus-host disease after hematopoietic stem cell transplantation. *Front Immunol.* (2021) 12:720354. doi: 10.3389/fimmu.2021.720354

134. Zhao Y, Li X, Zhou Y, Gao J, Jiao Y, Zhu B, et al. Safety and efficacy of fecal microbiota transplantation for grade IV steroid refractory GI-GvHD Patients: Interim Results From FMT2017002 Trial. *Front Immunol.* (2021) 12:678476. doi: 10.3389/fimmu.2021.678476

135. Kakihana K, Fujioka Y, Suda W, Najima Y, Kuwata G, Sasajima S, et al. Fecal microbiota transplantation for patients with steroid-resistant acute graft-versus-host disease of the gut. *Blood.* (2016) 128:2083–8.

136. Bilinski J, Lis K, Tomaszewska A, Grzesiowski P, Dzieciatkowski T, Tyszka M, et al. Fecal microbiota transplantation in patients with acute and chronic graft-versus-host disease-spectrum of responses and safety profile. Results from a prospective, multicenter study. *Am J Hematol.* (2021) 96:E88–91. doi: 10.1002/ajh.26077

137. Doki N, Suyama M, Sasajima S, Ota J, Igarashi A, Mimura I, et al. Clinical impact of pre-transplant gut microbial diversity on outcomes of allogeneic hematopoietic stem cell transplantation. *Ann Hematol.* (2017) 96:1517–23. doi: 10.1007/s00277-017-3069-8

138. Devaux C, Million M, Raoult D. The butyrogenic and lactic bacteria of the gut microbiota determine the outcome of allogenic hematopoietic cell transplant. *Front Microbiol.* (2020) 11:1642. doi: 10.3389/fmicb.2020.01642

139. Siddiqui M, Cresci G. The immunomodulatory functions of butyrate. *J Inflamm Res.* (2021) 14:6025–41.

140. Peng L, Li Z, Green R, Holzman I, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly *via* activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr.* (2009) 139:1619–25. doi: 10.3945/jn.109.104638

141. Wang C, Wu H, Lin F, Gong R, Xie F, Peng Y, et al. Sodium butyrate enhances intestinal integrity, inhibits mast cell activation, inflammatory mediator production and JNK signaling pathway in weaned pigs. *Innate Immun.* (2018) 24:40–6. doi: 10.1177/1753425917741970

142. Aoyama M, Kotani J, Usami M. Butyrate and propionate induced activated or non-activated neutrophil apoptosis *via* HDAC inhibitor activity but without activating GPR-41/GPR-43 pathways. *Nutrition.* (2010) 26:653–61.

143. Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M, et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med.* (2014) 6:263ra158.

144. Mirsepasi-Lauridsen H, Vallance B, Krogfelt K, Petersen A. *Escherichia coli* pathobionts associated with inflammatory bowel disease. *Clin Microbiol Rev.* (2019) 32:e60–18. doi: 10.1128/CMR.00060-18

145. Figliuolo V, Dos Santos L, Abalo A, Nanini H, Santos A, Brittes N, et al. Sulfate-reducing bacteria stimulate gut immune responses and contribute to inflammation in experimental colitis. *Life Sci.* (2017) 189:29–38. doi: 10.1016/j.lfs.2017.09.014

146. Henke M, Kenny D, Cassilly C, Vlamakis H, Xavier R, Clardy J. *Ruminococcus gnavus*, a member of the human gut microbiome associated with Crohn's disease, produces an inflammatory polysaccharide. *Proc Natl Acad Sci USA.* (2019) 116:12672–7. doi: 10.1073/pnas.1904099116

147. Zhou Q, Pang G, Zhang Z, Yuan H, Chen C, Zhang N, et al. Association between gut *Akkermansia* and metabolic syndrome is dose-dependent and affected by microbial interactions: A cross-sectional study. *Diabetes Metab Syndr Obes.* (2021) 14:2177–88. doi: 10.2147/DMSO.S311388

148. Cammarota G, Ianiro G, Kelly C, Mullish B, Allegretti J, Kassam Z, et al. International consensus conference on stool banking for faecal microbiota transplantation in clinical practice. *Gut.* (2019) 68:2111–21. doi: 10.1136/gutjnl-2019-319548

149. Terveer E, van Beurden Y, Goorhuis A, Seegers J, Bauer M, van Nood E, et al. How to: Establish and run a stool bank. *Clin Microbiol Infect.* (2017) 23:924–30. doi: 10.1016/j.cmi.2017.05.015

150. Wilson B, Vatanen T, Jayasinghe T, Leong K, Derraik J, Albert B, et al. Strain engraftment competition and functional augmentation in a multi-donor fecal microbiota transplantation trial for obesity. *Microbiome.* (2021) 9:107. doi: 10.1186/s40168-021-01060-7

151. Benítez-Páez A, Hartstra A, Nieuwdorp M, Sanz Y. Species- and strain-level assessment using rrn long-amplicons suggests donor's influence on gut microbial transference *via* fecal transplants in metabolic syndrome subjects. *Gut Microbes.* (2022) 14:2078621. doi: 10.1080/19490976.2022.2078621

152. Jain R, Gupta G. Family/friend donors are not true voluntary donors. *Asian J Transfus Sci.* (2012) 6:29–31.

153. Song S, Lauber C, Costello E, Lozupone C, Humphrey G, Berg-Lyons D, et al. Cohabiting family members share microbiota with one another and with their dogs. *Elife.* (2013) 2:e00458. doi: 10.7554/eLife.00458

154. Schloss P, Iverson K, Petrosino J, Schloss S. The dynamics of a family's gut microbiota reveal variations on a theme. *Microbiome.* (2014) 2:25. doi: 10.1186/2049-2618-2-25

155. O'Toole P, Jeffery I. Gut microbiota and aging. *Science.* (2015) 350:1214–5.

156. Hamilton M, Weingarden A, Sadowsky M, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent *Clostridium* difficile infection. *Am J Gastroenterol.* (2012) 107:761–7. doi: 10.1038/ajg.2011.482

157. Ianiro G, Porcari S, Bibbò S, Giambò F, Quaranta G, Masucci L, et al. Donor program for fecal microbiota transplantation: A 3-year experience of a large-volume Italian stool bank. *Dig Liver Dis.* (2021) 53:1428–32. doi: 10.1016/j.dld.2021.04.009

158. Paramsothy S, Borody T, Lin E, Finlayson S, Walsh A, Samuel D, et al. Donor recruitment for fecal microbiota transplantation. *Inflamm Bowel Dis.* (2015) 21:1600–6.

159. Duarte-Chavez R, Wojda T, Zanders T, Geme B, Fioravanti G, Stawicki S. Early results of fecal microbial transplantation protocol implementation at a community-based university hospital. *J Glob Infect Dis.* (2018) 10:47–57. doi: 10.4103/jgid.jgid_145_17

160. Gu J, Han B, Wang J. COVID-19: Gastrointestinal manifestations and potential fecal-oral transmission. *Gastroenterology.* (2020) 158:1518–9.

161. Lee S, Lee E, Park Y, Hong S. Microbiome in the Gut-Skin Axis in Atopic Dermatitis. *Allergy Asthma Immunol Res.* (2018) 10:354–62.

162. Li S, Sen S, Schneider J, Xiong K, Nusbacher N, Moreno-Huizar N, et al. Gut microbiota from high-risk men who have sex with men drive immune activation in gnotobiotic mice and *in vitro* HIV infection. *PLoS Pathog.* (2019) 15:e1007611. doi: 10.1371/journal.ppat.1007611

163. Chehoud C, Dryga A, Hwang Y, Nagy-Szakal D, Hollister E, Luna R, et al. Transfer of viral communities between human individuals during fecal microbiota transplantation. *mBio.* (2016) 7:e00322.

164. Seo H, Chin H, Kim Y, Moon H, Kim K, Nguyen L, et al. Laboratory aspects of donor screening for fecal microbiota transplantation at a korean fecal microbiota bank. *Ann Lab Med.* (2021) 41:424–8.

165. DeFilipp Z, Bloom P, Torres Soto M, Mansour M, Sater M, Huntley M, et al. Drug-Resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N Engl J Med.* (2019) 381:2043–50.

166. Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, et al. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut.* (2017) 66:569–80. doi: 10.1136/gutjnl-2016-313017

167. Ott S, Musfeldt M, Timmis K, Hampe J, Wenderoth D, Schreiber S. *In vitro* alterations of intestinal bacterial microbiota in fecal samples during storage. *Diagn Microbiol Infect Dis.* (2004) 50:237–45. doi: 10.1016/j.diagmicrobio.2004.08.012

168. Prakash O, Nimonkar Y, Shouche Y. Practice and prospects of microbial preservation. *FEMS Microbiol Lett.* (2013) 339:1–9.

169. Satokari R, Mattila E, Kainulainen V, Arkkila P. Simple faecal preparation and efficacy of frozen inoculum in faecal microbiota transplantation for recurrent *Clostridium* difficile infection–an observational cohort study. *Aliment Pharmacol Ther.* (2015) 41:46–53. doi: 10.1111/apt.13009

170. Nicco C, Paule A, Konturek P, Edeas M. From donor to patient: Collection, preparation and cryopreservation of fecal samples for fecal microbiota transplantation. *Diseases* (2020) 8:9.

171. Costello S, Conlon M, Vuaran M, Roberts-Thomson I, Andrews J. Faecal microbiota transplant for recurrent *Clostridium* difficile infection using long-term frozen stool is effective: clinical efficacy and bacterial viability data. *Aliment Pharmacol Ther.* (2015) 42:1011–8. doi: 10.1111/apt.13366

172. O'Donnell M, Rea M, O'Sullivan Ó, Flynn C, Jones B, McQuaid A, et al. Preparation of a standardised faecal slurry for ex-vivo microbiota studies which reduces inter-individual donor bias. *J Microbiol Methods.* (2016) 129:109–16. doi: 10.1016/j.mimet.2016.08.002

173. Costello S, Tucker E, La Brooy J, Schoeman M, Andrews J. Establishing a fecal microbiota transplant service for the treatment of *Clostridium* difficile infection. *Clin Infect Dis.* (2016) 62:908–14. doi: 10.1093/cid/civ994

174. Ekekezie C, Perler B, Wexler A, Duff C, Lillis C, Kelly C. Understanding the scope of do-it-yourself fecal microbiota transplant. *Am J Gastroenterol.* (2020) 115:603–7. doi: 10.14309/ajg.0000000000000499

175. Jones J, Reinke S, Ali A, Palmer D, Christophersen C. Fecal sample collection methods and time of day impact microbiome composition and short chain fatty acid concentrations. *Sci Rep.* (2021) 11:13964. doi: 10.1038/s41598-021-93031-z

176. Lee C, Steiner T, Petrof E, Smieja M, Roscoe D, Nematallah A, et al. Frozen vs fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent *Clostridium* difficile infection: A randomized clinical trial. *JAMA.* (2016) 315:142–9. doi: 10.1001/jama.2015.18098

177. Ramai D, Zakhia K, Fields P, Ofosu A, Patel G, Shahnazarian V, et al. Fecal Microbiota Transplantation (FMT) with colonoscopy is superior to enema and nasogastric tube while comparable to capsule for the treatment of recurrent *Clostridioides* difficile infection: A systematic review and meta-analysis. *Dig Dis Sci.* (2021) 66:369–80. doi: 10.1007/s10620-020-06185-7

178. Ko C. Colonoscopy risks: What is known and what are the next steps? *Gastroenterology.* (2018) 154:473–5. doi: 10.1053/j.gastro.2018.01.010

179. Nahas S, Oliveira Filho D, Araújo S, Lourenção J, Sobrado Júnior C, Nahas C, et al. [Colonoscopy: indications, contraindications and complications]. *Rev Hosp Clin Fac Med Sao Paulo.* (1998) 53:91–9.

180. Agrawal M, Aroniadis O, Brandt L, Kelly C, Freeman S, Surawicz C, et al. The long-term efficacy and safety of fecal microbiota transplant for recurrent, severe, and complicated *Clostridium* difficile infection in 146 elderly individuals. *J Clin Gastroenterol.* (2016) 50:403–7. doi: 10.1097/MCG.0000000000000410

181. Randel K, Schult A, Botteri E, Hoff G, Bretthauer M, Ursin G, et al. Colorectal cancer screening with repeated fecal immunochemical test versus sigmoidoscopy: Baseline results from a randomized trial. *Gastroenterology.* (2021) 160:1085–96. doi: 10.1053/j.gastro.2020.11.037

182. Kang, S, Gweon T, Hwang H, Baeg M. A Case of ischemic colitis complicated by *Clostridioides* difficile infection treated with fecal microbiota transplantation. *Clin Endosc.* (2022) 199:1–4. doi: 10.5946/ce.2021.199

183. Gkouvatsos K, Mathys P, Bastid C, Frossard J, Lepilliez V, Bichard P. [Innovations in endoscopy]. *Rev Med Suisse.* (2019) 15:1478–82.

184. Thomson A, Tye-Din J, Tonga S, Scott J, McLaren C, Pavli P, et al. Aspiration in the context of upper gastrointestinal endoscopy. *Can J Gastroenterol.* (2007) 21:223–5.

185. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal E, de Vos W, et al. Duodenal infusion of donor feces for recurrent *Clostridium* difficile. *N Engl J Med.* (2013) 368:407–15. doi: 10.1056/NEJMoa1205037

186. Rupawala A, Gachette D, Bakhit M, Jimoh L, Kelly C. Management of severe and severe/complicated *Clostridoides* difficile infection using sequential fecal microbiota transplant by retention enema. *Clin Infect Dis.* (2021) 73:716–9. doi: 10.1093/cid/ciab041

187. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan G, et al. Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent *Clostridium* difficile infection: A randomized clinical trial. *JAMA.* (2017) 318:1985–93. doi: 10.1001/jama.2017.17077

188. Gulati M, Singh S, Corrie L, Kaur I, Chandwani L. Delivery routes for faecal microbiota transplants: Available, anticipated and aspired. *Pharmacol Res.* (2020) 159:104954. doi: 10.1016/j.phrs.2020.104954

189. Youngster I, Russell G, Pindar C, Ziv-Baran T, Sauk J, Hohmann E. Oral, capsulized, frozen fecal microbiota transplantation for relapsing *Clostridium* difficile infection. *JAMA.* (2014) 312:1772–8. doi: 10.1001/jama.2014.13875

190. Sung J, Wong S. What is unknown in using microbiota as a therapeutic? *J Gastroenterol Hepatol.* (2022) 37:39–44.

191. Khan R, Roy N, Ali H, Naeem M. Fecal microbiota transplants for inflammatory bowel disease treatment: synthetic- and engineered communities-based microbiota transplants are the future. *Gastroenterol Res Pract.* (2022) 2022:9999925. doi: 10.1155/2022/9999925

192. Vázquez-Castellanos J, Biclot A, Vrancken G, Huys G, Raes J. Design of synthetic microbial consortia for gut microbiota modulation. *Curr Opin Pharmacol.* (2019) 49:52–9. doi: 10.1016/j.coph.2019.07.005

193. Wilson A, Koller K, Ramaboli M, Nesengani L, Ocvirk S, Chen C, et al. Diet and the human gut microbiome: An international review. *Dig Dis Sci.* (2020) 65:723–40.

frontiers | Frontiers in Medicine

TYPE Mini Review
PUBLISHED 09 January 2023
DOI 10.3389/fmed.2022.1093329

OPEN ACCESS

EDITED BY
Angel Lanas,
University of Zaragoza, Spain

REVIEWED BY
Thomas Louie,
University of Calgary, Canada

*CORRESPONDENCE
Teena Chopra
✉ tchopra@med.wayne.edu

SPECIALTY SECTION
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

RECEIVED 08 November 2022
ACCEPTED 15 December 2022
PUBLISHED 09 January 2023

CITATION
Chopra T, Hecht G and Tillotson G (2023) Gut microbiota and microbiota-based therapies for *Clostridioides difficile* infection. *Front. Med.* 9:1093329. doi: 10.3389/fmed.2022.1093329

Gut microbiota and microbiota-based therapies for *Clostridioides difficile* infection

Teena Chopra[1]*, Gail Hecht[2] and Glenn Tillotson[3]

[1]Division of Infectious Diseases, Wayne State University, Detroit, MI, United States, [2]Department of Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, IL, United States, [3]GST Micro LLC, Henrico, VA, United States

Clostridioides difficile infection poses significant clinical challenges due to its recurrent nature. Current antibiotic management does not address the underlying issue, that of a disturbed gastrointestinal microbiome, called dysbiosis. This provides a supportive environment for the germination of *C. difficile* spores which lead to infection and toxin production as well as an array of other health conditions. The use of microbiome restoration therapies such as live biotherapeutics can reverse dysbiosis and lead to good clinical outcomes. Several such therapies are under clinical investigation.

KEYWORDS

microbiota, microbiome, fecal microbiota transplant, *Clostridioides difficile* infection, *Clostridium difficile*, recurrent CDI

1. Introduction

Clostridioides difficile infection (CDI) is an urgent threat, both for the patient and healthcare professionals. CDI is one of the most common healthcare-associated infections and aggressive action is required to combat this threat (1). There are an estimated 467,400 cases of healthcare- and community-associated CDI cases annually in the United States and a cumulative incidence of 8 per 100,000 person-years in the European Union (2, 3). The estimated direct medical cost of CDI in the US is $5.4 billion (2014 dollars) (4).

Patients with CDI often present with watery diarrhea and abdominal pain, but symptoms can also include fever, hypotension, or ileus in more severe cases (5); complications can include sepsis or colectomy/ileostomy (6–11). Testing for CDI is recommended for patients who have unexplained, new onset diarrhea (at least 3 unformed stools over ≥24 h) using a nucleic acid amplification test alone or as part of an algorithm that includes glutamate dehydrogenase or stool toxin test (12). The current recommended treatment regimen for an initial episode of CDI is fidaxomicin (200 mg BID q10d) or vancomycin (125 mg, QID q10d) as an acceptable alternative (13).

Unfortunately, in approximately 25% of cases, CDI recurs within 1–2 months of the initial infection (6, 7, 14, 15). Recurrence is often associated with more severe disease, increased costs, and hypervirulent strains of *C. difficile* (16–19). After a first

recurrence, patients are substantially more likely to have a subsequent recurrence, with approximately 50–60% of these patients experiencing multiply recurrent CDI (6, 7, 20, 21).

2. Gut dysbiosis and *Clostridioides difficile* infection

Initial episodes of CDI are almost always precipitated by antibiotic use, so much so that it has the strongest association of any identified risk factor for CDI (6, 7, 22, 23). Other common risk factors for CDI include older age, use of gastric acid suppressants, comorbid conditions such as kidney disease and cardiovascular disease, and recent healthcare exposure (24–27).

Clostridioides difficile is found in the gut of some healthy individuals and is kept in check, residing in a dormant spore state, by a healthy gut microbiota (28). Underlying the pathophysiology of CDI is disruption of the gut microbiota, or gut dysbiosis. Dysbiosis has been defined as "any change to the composition of resident commensal communities relative to [those] found in healthy individuals" (29). This can include a loss of beneficial microbes, reduced diversity of gut species, or expansion of a pathogenic species (29). In patients with CDI, the gut microbiota exhibits a loss of diversity, which can worsen with recurrent CDI (30). With gut dysbiosis, *C. difficile* spores can germinate and produce exotoxins, disrupting the intestinal mucosa and causing CDI-associated diarrhea (31, 32).

The inciting dysbiosis for CDI can arise for several reasons. Antibiotics that are considered significant disruptors of the gut microbiota also have the strongest association with developing CDI (33–37). Older age brings changes in the gut microbiota, which could be influenced by a change in diet, lifestyle, or immune senescence (30, 38, 39). Patients taking chronic gastric acid suppressants, who are often older, show significant increases in gut *Enterococcus*, *Streptococcus*, and *Staphylococcus* species (40).

Clostridioides difficile spores require a germinant to transform from the spore state to the growing, vegetative cell, in the form of specific bile acids. Primary bile acids are synthesized by hepatocytes and transformed into secondary bile acids by certain members of the healthy gut microbiota (28, 41). Bile acids derived from cholic acid promote the germination of *C. difficile* spores, while bile acids derived from chenodeoxycholic acid (CDCA) inhibit germination (41). In addition, vegetative cell growth of *C. difficile* is inhibited by CDCA. In animal studies and in humans, hosts with higher levels of secondary bile acids were more resistant to developing CDI, whereas hosts with higher levels of primary bile acids were more susceptible (41).

Perhaps counterintuitively, CDI is treated with antibiotics. While antibiotics may eliminate the initial infection, they alter the composition of the gut microbiota, including widespread reduction in diversity by commonly-used vancomycin (29, 30). With the continued burden of recurrent CDI, that does not appear to be lessening with increased infection control measures or changes in antimicrobial prescribing, a non-antibiotic approach may offer an alternative means of addressing the disease (2).

3. Restoring the gut microbiota in *Clostridioides difficile* infection

Given the underlying state of gut dysbiosis that fosters CDI, an ideal goal for patients with CDI is eubiosis, or restoring the gut microbiota to a healthy state (28, 29). Microbiota-based therapies have been investigated by Western medicine as a treatment for gut dysbiosis since the 1950s (42). Since then, their use has increased steadily, in parallel with our understanding of gut microbiota disruption as an underlying cause of CDI as well as many other gastrointestinal disorders.

Fecal microbiota transplantation (FMT) is the delivery of intestinal microbiota from a healthy donor to a recipient to mitigate disease by modifying the structure and/or function of the gut microbiota (43, 44). FMT is currently recommended in the CDI treatment guidelines as an option at the second or subsequent recurrence (12, 45). In addition to CDI treatment, including FMT, changes to underlying risk factors should be considered for their effect on the gut microbiota, such as discontinuing gastric acid suppressants or altering systemic antimicrobial therapy for a non-CDI infection.

The goal of FMT is to restore the gut microbiota to a healthy state and replace dysbiotic microbes with taxa/species that are associated with healthy host microbiota (46, 47). The expectation is that reintroduced healthy species will engraft and out-compete *C. difficile*, thus eliminating dysbiosis and providing colonization resistance (48). FMT can return metabolite levels and profiles, including bile acids and short-chain fatty acids, to a healthy state, presumably as a result of enzymatic activity provided by normal host microbiota (48).

Reduced presence of *Bacteroides* spp. appears to be associated with negative consequences for GI disorders, including CDI (49). Bacteria in the phyla Bacteroidetes are abundant in healthy gut microbiota and likely play a key role in bacterial metabolism and the gut environment (28). The presence of *Bacteroides* spp. and their surface proteins and metabolites may activate the host immune system to limit entry and proliferation of potential pathogens or exert an antibacterial effect (50, 51).

The initial literature regarding FMT for CDI was primarily case reports and retrospective cohort studies as the therapy was being investigated (52–54). While these studies often showed positive patient outcomes, namely prevention of CDI recurrence for several months after treatment, by nature of their study design the resulting data were prone to selection bias. More recently, prospective and randomized controlled

TABLE 1 Pathogen screening on RBX2660.

Pathogens		Multi-drug resistant organisms
Clostridioides difficile A/B	*Plesiomonas shigelloides*	Extended spectrum beta-lactamase (ESBL)
Enteroaggregative *E. coli* (EAEC)	*Campylobacter* species	Vancomycin-resistant Enterococci (VRE)
Enterotoxigenic *E. coli* (ETEC)	*Salmonella* species	Carbapenem-resistant Enterobacterales
Enteropathogenic *E. coli* (EPEC)	Vibrio species/cholerae	Methicillin-resistant *Staphylococcus aureus* (MRSA)
Entamoeba histolytica	*Yersinia enterocolitica*	
Astrovirus	Shiga-like-toxin-producer *E. coli* (STEC) Shigella/Enteroinvasive *E. coli* (EIEC)	
Sapovirus (Genogroups I, II, IV, V)	*Giardia lamblia*	
Listeria culture	Norovirus GI/GII	
Cryptosporidium	Rotavirus A	
Cyclospora	Adenovirus F40/41	
Cystoisospora		
Ova and Parasite exam		
Aeromonas		

trials of FMT have been completed, generally demonstrating FMT as a safe and effective therapy for CDI with treatment success rates of ∼75% (55–57). A recent prospective, real-world observational study of medically complex patients receiving FMT for CDI reported 78% (4,195/5,344) of patients exhibited clinical cure, with 3.6% of patients experiencing a serious adverse event (58). FMT has also been shown to decrease mortality in patients with refractory severe or fulminant CDI (59).

Performing FMT can be operationally challenging, including costs and logistical concerns around screening donors and processing stool (58, 60). Additionally, there is no standard protocol for FMT composition, route of delivery, number of infusions, or dosage, variables that could all affect treatment outcomes (61).

4. Approaches to restoring the gut microbiota

Live biotherapeutic products (LBPs) have been developed as an extension of the initial FMT studies, in part as a way to standardize products and outcomes being measured. LBPs contain live microbes that are able to prevent, treat or cure a disease (62). Several LBPs have been or are currently being studied for CDI. The goal of treatment with LBPs for CDI is similar to FMT, namely to restore the gut microbiota to a healthier state (63).

LBPs that are currently in late-stage development differ in their approach toward product composition and delivery. SER-109 (Seres Pharmaceuticals, Lexington MA) is an oral capsule (4 capsules once daily q3d) containing spores of ∼50 specific species of only Firmicutes that are isolated from healthy donors (64). SER-109 was designed on the premise that Firmicutes can compete metabolically with *C. difficile* for essential nutrients and bile acids (63). While a phase 2 study of SER-109 did not show a significant difference versus placebo in patients with multiply recurrent CDI, in those patients who did show SER-109 engraftment by microbiome analysis, there was also a significant increase in secondary bile acids (65). From a phase 3 study of patients who had 3 or more episodes of CDI, the treatment success after SER-109 was 88% (recurrence rate of 12%) (66).

RBX2660 (Ferring Pharmaceuticals, Parsippany NJ) is a biologically-sourced, broad consortium microbiota-based live biotherapeutic product (LBP) that is processed from the stool of healthy donors, standardized and administered rectally (67). The product was approved on November 30, 2022 by the FDA as Rebyota as a live biotherapeutic for the treatment of recurrent *C. difficile* infection (REBYOTA | FDA) RBX2660 is screened for 29 different species of pathogens as shown in **Table 1**. Results from a phase 3 trial of RBX2660, analyzed with a Bayesian hierarchical model formally incorporating data from a phase 2b trial, showed a treatment success rate of 70.6% (68). Long-term data (up to 24 months) after treatment with RBX2660 in a phase 2 trial showed durable treatment success, with more than 90% of treatment responders remaining CDI-free at 6, 12, and 24 months (69, 70). Microbiota analyses from this phase 2 trial also showed a highly dysbiotic composition before treatment, which converged toward the RBX2660 composition within 7 days after treatment (69, 71). Taxa that were restored to predominance after RBX2660 included Bacteroidia and Clostridia while gammaproteobacteria and bacilli, the deleterious organisms, were reduced. Administration of RBX2660 delivery is via enema, without the need for bowel

preparation or colonoscopy and can be used in patients who are not able to take an oral product.

CP101 (Finch Therapeutics, Somerville, MA, USA) is an oral capsule (10 capsules taken once) delivering a full-spectrum microbiota product that showed 75% efficacy in preventing CDI recurrence in a phase 2 trial (72). A phase 3 trial of CP101 is currently recruiting patients (NCT05153499). Several other microbiota-based products in earlier stages of development have been or are currently being investigated for CDI (63).

The negative physical effects of gut dysbiosis are clear, but emerging evidence also points to psychological effects as well. Psychological consequences of CDI are reported by ∼70% of people who have active or previous infection (73). From an analysis of Medicare Fee-for-service beneficiaries, within a 12-month period after an initial CDI episode, approximately 15–20% of the cohort had newly diagnosed psychiatric conditions (anxiety, depression, delirium) (7). After receiving a microbiota-based LBP for CDI treatment in a phase 2 trial setting, participants exhibited statistically significant and clinically meaningful improvements in the mental component score of the SF-36 assessment of quality of life (QoL) (74). From a phase 3 randomized, controlled trial, using a the CDiff32, a CDI-specific measure of QoL, patients receiving an LBP reported significant improvements in mental health-related QoL as early as week 1, which continued throughout the 8-week blinded study period (75). While definitive mechanisms linking changes in the gut microbiota to mental state have not been determined, it is clear that there is a link (76).

5. Discussion

A healthy gut microbiota is associated with many aspects of health and resistance to CDI as well as other diseases. Restoring healthy gut microbial communities can help break the vicious cycle of recurrence in CDI patients. The outcomes of treatment with live biotherapeutic products have been measured in terms of short- and long-term clinical observations and microbiome changes, which modify the metabolic processes in the gut and elicit positive changes in mental aspects associated with CDI. The availability of regulated standardized products will be welcome additions to the armamentarium against *C. difficile* infections.

Author contributions

TC and GT conceived the idea for the work, wrote the original draft, revised and edited the manuscript. GH wrote the original draft and revised and edited the manuscript. All authors agreed to be accountable for the content of the work and approved the final version for publication.

Funding

The authors report no funding for this report. Publication fees were funded by Ferring Pharmaceuticals Inc. (Parsippany, NJ, USA).

Acknowledgments

Medical writing and editorial support were provided by Agnella Izzo Matic, Ph.D., CMPP (AIM Biomedical, LLC) and funded by Ferring Pharmaceuticals Inc.

Conflict of interest

TC is a speaker for Abbvie Inc., Cepheid, Ferring Pharmaceuticals Inc., and Pfizer Inc.; and a consultant for Cepheid, Ferring Pharmaceuticals Inc., Pfizer Inc., and Shionogi Inc. GT is a consultant to Ferring Pharmaceuticals Inc., Spero Therapeutics, and a speaker for Hikma Pharmaceuticals and was employed by GST Micro LLC. GH is a consultant to Ferring Pharmaceuticals Inc., and BioK+.

Publisher's note

References

1. Centers for Disease Control and Prevention. *Antibiotic Resistance Threats in the United States, 2019*. Atlanta, GA: US Department of Health and Human Services (2019).

2. Guh A, Mu Y, Winston L, Johnston H, Olson D, Farley M, et al. Trends in U.S. burden of *Clostridioides difficile* infection and outcomes. *N Engl J Med.* (2020) 382:1320–30. doi: 10.1056/NEJMoa1910215

3. Balsells E, Shi T, Leese C, Lyell I, Burrows J, Wiuff C, et al. Global burden of *Clostridium difficile* infections: a systematic review and meta-analysis. *J Glob Health.* (2019) 9:010407. doi: 10.7189/jogh.09.010407

4. Desai K, Gupta S, Dubberke E, Prabhu V, Browne C, Mast T. Epidemiological and economic burden of *Clostridium difficile* in the United States: estimates from

a modeling approach. *BMC Infect Dis.* (2016) 16:303. doi: 10.1186/s12879-016-1610-3

5. Gerding D, File TMJ, McDonald L. Diagnosis and treatment of *Clostridium difficile* infection (CDI). *Infect Dis Clin Pr (Baltim Md).* (2016) 24:3–10. doi: 10.1097/IPC.0000000000000350

6. Feuerstadt P, Boules M, Stong L, Dahdal D, Sacks N, Lang K, et al. Clinical complications in patients with primary and recurrent *Clostridioides difficile* infection: a real-world data analysis. *SAGE Open Med.* (2021) 9:1–8. doi: 10.1177/20503121209867 33

7. Feuerstadt P, Nelson W, Teigland C, Dahdal D. Clinical burden of recurrent *Clostridioides difficile* infection in the medicare population: a real-world claims analysis. *Antimicrob Steward Heal Epidemiol.* (2022) 2:e60. doi: 10.1017/ash.2022.2

8. Falcone M, Russo A, Iraci F, Carfagna P, Goldoni P, Vullo V, et al. Risk factors and outcomes for bloodstream infections secondary to *Clostridium difficile* infection. *Antimicrob Agents Chemother.* (2016) 60:252–7. doi: 10.1128/AAC.01927-15

9. Ianiro G, Murri R, Sciumè G, Impagnatiello M, Masucci L, Ford A, et al. Incidence of bloodstream infections, length of hospital stay and survival in patients with recurrent *Clostridioides difficile* infection treated with fecal microbiota transplantation or antibiotics: a prospective cohort study. *Ann Intern Med.* (2019) 171:695–702. doi: 10.7326/M18-3635

10. Kasper A, Nyazee H, Yokoe D, Mayer J, Mangino J, Khan Y, et al. A multicenter study of *Clostridium difficile* infection-related colectomy, 2000–2006. *Infect Control Hosp Epidemiol.* (2012) 33:470–6. doi: 10.1086/665318

11. Rodrigues R, Barber G, Ananthakrishnan AN. A comprehensive study of costs associated with recurrent *Clostridium difficile* infection. *Infect Control Hosp Epidemiol.* (2017) 38:196–202. doi: 10.1017/ice.2016.246

12. McDonald L, Gerding D, Johnson S, Bakken J, Carroll K, Coffin S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the infectious diseases society of America (IDSA) and society for healthcare epidemiology of America (SHEA). *Clin Infect Dis.* (2018) 66:e1–48. doi: 10.1093/cid/cix1085

13. Johnson S, Lavergne V, Skinner A, Gonzales-Luna A, Garey K, Kelly C, et al. Clinical practice guideline by the infectious diseases society of America (IDSA) and society for healthcare epidemiology of America (SHEA): 2021 focused update guidelines on management of *Clostridioides difficile* infection in adults. *Clin Infect Dis.* (2021) 73:e1029–44. doi: 10.1093/cid/ciab549

14. Leffler D, Lamont J. *Clostridium difficile* infection. *N Engl J Med.* (2015) 372:1539–48. doi: 10.1056/NEJMra1403772

15. Lessa F, Mu Y, Bamberg W, Beldavs Z, Dumyati G, Dunn J, et al. Burden of *Clostridium difficile* infection in the United States. *N Engl J Med.* (2015) 372:825–34. doi: 10.1056/NEJMoa1408913

16. Song J, Kim Y. Recurrent *Clostridium difficile* infection: risk factors, treatment, and prevention. *Gut Liver.* (2019) 13:16–24. doi: 10.5009/gnl18071

17. Shields K, Araujo-Castillo RV, Theethira T, Alonso C, Kelly C. Recurrent *Clostridium difficile* infection: from colonization to cure. *Anaerobe.* (2015) 34:59–73. doi: 10.1016/j.anaerobe.2015.04.012

18. Appaneal H, Caffrey A, Beganovic M, Avramovic S, LaPlante K. Predictors of *Clostridioides difficile* recurrence across a national cohort of veterans in outpatient, acute, and long-term care settings. *Am J Heal Pharm.* (2019) 76:581–90. doi: 10.1093/ajhp/zxz032

19. Nour Abou Chakra C, Pepin J. Risk factors for recurrence, complications and mortality in *Clostridium difficile* infection: a systematic review. *PLoS One.* (2014) 9:e98400. doi: 10.1371/journal.pone.0098400

20. Ma G, Brensinger C, Wu Q, Lewis J. Increasing incidence of multiply recurrent *Clostridium difficile* infection in the United States: a cohort study. *Ann Intern Med.* (2017) 167:152–8. doi: 10.7326/M16-2733

21. Sheitoyan-Pesant C, Abou Chakra C, Pépin J, Marcil-Héguy A, Nault V, Valiquette L. Clinical and healthcare burden of multiple recurrences of *Clostridium difficile* infection. *Clin Infect Dis.* (2016) 62:574–80. doi: 10.1093/cid/civ958

22. Davies K, Lawrence J, Berry C, Davis G, Yu H, Cai B, et al. Risk factors for primary *Clostridium difficile* infection; results from the observational study of risk factors for *Clostridium difficile* infection in hospitalized patients with infective diarrhea (ORCHID). *Front Public Heal.* (2020) 8:293. doi: 10.3389/fpubh.2020.00293

23. Eze P, Balsells E, Kyaw M, Nair H. Risk factors for *Clostridium difficile* infections – an overview of the evidence base and challenges in data synthesis. *J Glob Heal.* (2017) 7:010417. doi: 10.7189/jogh.07.010417

24. Collins C, Ayturk M, Flahive J, Emhoff T Jr., Frederick AJ, Santry H. Epidemiology and outcomes of community acquired *Clostridium difficile* infections in medicare beneficiaries. *J Am Coll Surg.* (2014) 218:1141–7.e1. doi: 10.1016/j.jamcollsurg.2014.01.053

25. Isidro J, Mendes A, Serrano M, Henriques A, Oleastro M. *Overview of Clostridium difficile Infection: Life Cycle, Epidemiology, Antimicrobial Resistance and Treatment. Clostridium Difficile – A Comprehensive Overview*. (2017). doi: 10.5772/intechopen.69053

26. Smits W, Lyras D, Lacy D, Wilcox M, Kuijper E. *Clostridium difficile* infection. *Nat Rev Dis Prim.* (2016) 2:16020. doi: 10.1038/nrdp.2016.20

27. Khanna S, Gupta A, Baddour L, Pardi D. Epidemiology, outcomes, and predictors of mortality in hospitalized adults with *Clostridium difficile* infection. *Intern Emerg Med.* (2016) 11:657–65. doi: 10.1007/s11739-015-1366-6

28. Theriot C, Young V. Interactions between the gastrointestinal microbiome and *Clostridium difficile*. *Annu Rev Microbiol.* (2015) 69:445–61. doi: 10.1146/annurev-micro-091014-104115

29. Petersen C, Round J. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol.* (2014) 16:1024–33. doi: 10.1111/cmi.12308

30. Shin J, Warren C. Collateral damage during antibiotic treatment of C. difficile infection in the aged host: insights into why recurrent disease happens. *Gut Microbe.* (2017) 8:504–10. doi: 10.1080/19490976.2017.1323616

31. Fernández-García L, Blasco L, López M, Tomás M. *Clostridium difficile Infection: Pathogenesis, Diagnosis and Treatment. Clostridium Difficile – A Comprehensive Overview*. (2017). doi: 10.5772/67754

32. Abt M, McKenney P, Pamer E. *Clostridium difficile* colitis: pathogenesis and host defence. *Nat Rev Microbiol.* (2016) 14:609–20. doi: 10.1038/nrmicro.2016.108

33. Brown K, Langford B, Schwartz K, Diong C, Garber G, Daneman N. Antibiotic prescribing choices and their comparative C. difficile infection risks: a longitudinal case-cohort study. *Clin Infect Dis.* (2021) 72:836–44. doi: 10.1093/cid/ciaa124

34. Brown K, Khanafer N, Daneman N, Fisman D. Meta-analysis of antibiotics and the risk of community-associated *Clostridium difficile* infection. *Antimicrob Agents Chemother.* (2013) 57:2326–32. doi: 10.1128/AAC.02176-12

35. Tabak Y, Srinivasan A, Yu K, Kurtz S, Gupta V, Gelone S, et al. Hospital-level high-risk antibiotic use in relation to hospital-associated *Clostridioides difficile* infections: retrospective analysis of 2016–2017 data from US hospitals. *Infect Control Hosp Epidemiol.* (2019) 40:1229–35. doi: 10.1017/ice.2019.236

36. Pepin J, Saheb N, Coulombe M, Alary M, Corriveau M, Authier S, et al. Emergence of fluoroquinolones as the predominant risk factor for *Clostridium difficile*-associated diarrhea: a cohort study during an epidemic in Quebec. *Clin Infect Dis.* (2005) 41:1254–60. doi: 10.1086/496986

37. Bhalodi A, van Engelen T, Virk H, Wiersinga W. Impact of antimicrobial therapy on the gut microbiome. *J Antimicrob Chemotheri.* (2019) 74:i6–15. doi: 10.1093/jac/dky530

38. Nagpal R, Mainali R, Ahmadi S, Wang S, Singh R, Kavanagh K, et al. Gut microbiome and aging: physiological and mechanistic insights. *Nutr Heal Aging.* (2018) 4:267–85. doi: 10.3233/NHA-170030

39. Donskey C. *Clostridium difficile* in older adults. *Infect Dis Clin N Am.* (2017) 31:743–56. doi: 10.1016/j.idc.2017.07.003

40. Imhann F, Bonder M, Vila A, Fu J, Mujagic Z, Vork L, et al. Proton pump inhibitors affect the gut microbiome. *Gut.* (2016) 65:740–8. doi: 10.1136/gutjnl-2015-310376

41. Aguirre A, Sorg J. Gut associated metabolites and their roles in *Clostridioides difficile* pathogenesis. *Gut Microbe.* (2022) 14:2094672. doi: 10.1080/19490976.2022.2094672

42. Eiseman B, Silen W, Bascom G, Kauvar A. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery.* (1958) 44:854–9.

43. U Food and Drug Administration. *Enforcement Policy Regarding Investigational New Drug Requirements for Use of Fecal Microbiota for Transplantation to Treat Clostridium difficile Infection Not Responsive to Standard Therapies*. (2020). Available online at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/enforcement-policy-regarding-investigational-new-drug-requirements-use-fecal-microbiota (accessed December 21, 2022).

44. Kelly C, Kim A. The AGA's fecal microbiota transplantation national registry: an important step toward understanding risks and benefits of microbiota therapeutics. *Gastroenterology.* (2017) 152:681–4. doi: 10.1053/j.gastro.2017.01.028

45. van Prehn J, Reigadas E, Vogelzang E, Bouza E, Hristea A, Guery B, et al. European society of clinical microbiology and infectious diseases: 2021 update on the treatment guidance document for *Clostridioides difficile* infection

in adults. *Clin Microbiol Infect.* (2021) 27:S1–21. doi: 10.1016/j.cmi.2021.09.038

46. Kaakoush N. Fecal transplants as a microbiome-based therapeutic. *Curr Opin Microbiol.* (2020) 56:1–8. doi: 10.1016/j.mib.2020.05.008

47. Khoruts A, Staley C, Sadowsky M. Faecal microbiota transplantation for *Clostridioides difficile*: mechanisms and pharmacology. *Nat Rev Gastroenterol Hepatol.* (2021) 18:67–80. doi: 10.1038/s41575-020-0350-4

48. Segal J, Mullish B, Quraishi M, Iqbal T, Marchesi J, Sokol H. Mechanisms underpinning the efficacy of faecal microbiota transplantation in treating gastrointestinal disease. *Ther Adv Gastroenterol.* (2020) 13:1–14. doi: 10.1177/1756284820946904

49. Shin A, Preidis G, Shulman R, Kashyap P. The gut microbiome in adult and pediatric functional gastrointestinal disorders. *Clin Gastroenterol Hepatol.* (2019) 17:256–74. doi: 10.1016/j.cgh.2018.08.054

50. Wexler H. *Bacteroides*: the good, the bad, and the nitty-gritty. *Clin Microbiol Rev.* (2007) 20:593–621. doi: 10.1128/CMR.00008-07

51. Yekani M, Baghi H, Naghili B, Vahed S, Sóki J, Memar M. To resist and persist: important factors in the pathogenesis of *Bacteroides fragilis*. *Microb Pathog.* (2020) 149:104506. doi: 10.1016/j.micpath.2020.104506

52. Perler B, Chen B, Phelps E, Allegretti J, Fischer M, Ganapini V, et al. Long-term efficacy and safety of fecal microbiota transplantation for treatment of recurrent *Clostridioides difficile* infection. *J Clin Gastroenterol.* (2020) 54:701–6. doi: 10.1097/MCG.0000000000001281

53. Bafeta A, Yavchitz A, Riveros C, Batista R, Ravaud P. Methods and reporting studies assessing fecal microbiota transplantation a systematic review. *Ann Intern Med.* (2017) 167:34–9. doi: 10.7326/M16-2810

54. Drekonja D, Reich J, Gezahegn S, Greer N, Shaukat A, MacDonald R, et al. Fecal microbiota transplantation for *Clostridium difficile* infection a systematic review. *Ann Intern Med.* (2015) 162:630–8. doi: 10.7326/M14-2693

55. Tariq R, Pardi D, Bartlett M, Khanna S. Low cure rates in controlled trials of fecal microbiota transplantation for recurrent *Clostridium difficile* infection: a systematic review and meta-analysis. *Clin Infect Dis.* (2019) 68:1351–8. doi: 10.1093/cid/ciy721

56. Saha S, Mara K, Pardi D, Khanna S. Long-term safety of fecal microbiota transplantation for recurrent *Clostridioides difficile* infection. *Gastroenterology.* (2021) 160:1961–9. doi: 10.1053/j.gastro.2021.01.010

57. Quraishi M, Widlak M, Bhala N, Moore D, Price M, Sharma N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory *Clostridium difficile* infection. *Aliment Pharmacol Ther.* (2017) 46:479–93. doi: 10.1111/apt.14201

58. Osman M, Budree S, Kelly C, Panchal P, Allegretti J, Kassam Z, et al. Effectiveness and safety of fecal microbiota transplantation for *Clostridioides difficile* infection: results from a 5,344 patient cohort study. *Gastroenterology.* (2022) 163:319–22. doi: 10.1053/j.gastro.2022.03.051

59. Cheng Y, Phelps E, Nemes S, Rogers N, Sagi S, Bohm M, et al. Fecal microbiota transplant decreases mortality in patients with refractory severe or fulminant *Clostridioides difficile* infection. *Clin Gastroenterol Hepatol.* (2020) 18:2234.e–43.e. doi: 10.1016/j.cgh.2019.12.029

60. Gundacker N, Morrow C, Rodriguez M. Letter: a simple out-patient faecal microbiota transplant technique. *Aliment Pharmacol Ther.* (2016) 44:101. doi: 10.1111/apt.13648

61. Ianiro G, Maida M, Burisch J, Simonelli C, Hold G, Ventimiglia M, et al. Efficacy of different faecal microbiota transplantation protocols for *Clostridium difficile* infection: a systematic review and meta-analysis. *United Eur Gastroenterol J.* (2018) 6:1232–44. doi: 10.1177/2050640618780762

62. United States Food and Drug Administration. *Early Clinical Trials with live Biotherapeutic Products: Chemistry, Manufacturing, and Control Information: Guidance for Industry*. Silver Spring, MD: United States Food and Drug Administration (2016).

63. Gonzales-Luna A, Carlson T. Follow your gut: microbiome-based approaches in the developmental pipeline for the prevention and adjunctive treatment of *Clostridioides difficile* infection (CDI). *Curr Infect Dis Rep.* (2020) 22:22. doi: 10.1007/s11908-020-00729-8

64. Khanna S, Pardi D, Kelly C, Kraft C, Dhere T, Henn M, et al. A novel microbiome therapeutic increases gut microbial diversity and prevents recurrent *Clostridium difficile* infection. *J Infect Dis.* (2016) 214:173–81. doi: 10.1093/infdis/jiv766

65. McGovern B, Ford C, Henn M, Pardi D, Khanna S, Hohmann E, et al. SER-109, an investigational microbiome drug to reduce recurrence after *Clostridioides difficile* infection: lessons learned from a phase 2 trial. *Clin Infect Dis.* (2021) 72:2132–40. doi: 10.1093/cid/ciaa387

66. Feuerstadt P, Louie T, Lashner B, Wang E, Diao L, Bryant J, et al. SER-109, an oral microbiome therapy for recurrent *Clostridioides difficile* infection. *N Engl J Med.* (2022) 386:220–9. doi: 10.1056/NEJMoa2106516

67. Orenstein R, Dubberke E, Hardi R, Ray A, Mullane K, Pardi D, et al. Safety and durability of RBX2660 (Microbiota Suspension) for recurrent *Clostridium difficile* infection: results of the PUNCH CD study. *Clin Infect Dis.* (2016) 62:596–602. doi: 10.1093/cid/civ938

68. Khanna S, Assi M, Lee C, Yoho D, Louie T, Knapple W, et al. Efficacy and safety of RBX2660 in PUNCH CD3, a phase III, randomized, double-blind, placebo-controlled trial with a bayesian primary analysis for the prevention of recurrent *Clostridioides difficile* infection. *Drugs.* (2022) 82:1527–38. doi: 10.1007/s40265-022-01797-x

69. Orenstein R, Dubberke E, Khanna S, Lee C, Yoho D, Johnson S, et al. Durable reduction of *Clostridioides difficile* infection recurrence and microbiome restoration after treatment with RBX2660: results from an open-label phase 2 clinical trial. *BMC Infect Dis.* (2022) 22:245. doi: 10.1186/s12879-022-07256-y

70. Orenstein R. The role of microbiome-based therapeutics in *Clostridioides difficile* infection: durable, long-term results of RBX2660. *Infect Dis Ther.* (2022). doi: 10.1007/s40121-022-00714-9 [Online ahead of print].

71. Kwak S, Choi J, Hink T, Reske K, Blount K, Jones C, et al. Impact of investigational microbiota therapeutic RBX2660 on the gut microbiome and resistome revealed by a placebo-controlled clinical trial. *Microbiome.* (2020) 8:125. doi: 10.1186/s40168-020-00907-9

72. Khanna S, Kelly C, Louie T, Fisher M, Hota S, Misra B, et al. CP101, an investigational orally administered microbiome therapeutic, increases intestinal microbiome diversity and prevents recurrent C. difficile infection: results from a randomized, placebo-controlled trial. *Am J Gastroenterol.* (2021) 116:S57. doi: 10.14309/01.ajg.0000772996.83378.7c

73. Lurienne L, Bandinelli P, Galvain T, Coursel C, Oneto C, Feuerstadt P. Perception of quality of life in people experiencing or having experienced a *Clostridioides difficile* infection: a US population survey. *J Patient Rep Outcomes.* (2020) 4:14. doi: 10.1186/s41687-020-0179-1

74. Guthmueller B, Orenstein R, Dubberke E, Khanna S, Gerding D, Mische S, et al. Quality of life increased after treatment for participants in a phase 2b randomized, double-blinded, placebo-controlled clinical trial of RBX2660 for preventing recurrent *Clostridium difficile* infections. *Am J Gastroenterol.* (2016) 111:S1524. doi: 10.14309/00000434-201810001-02738

75. Feuerstadt P, Dubberke E, Guo A, Harvey A, Yang M, García-Horton V, et al. Significant improvement in health-related quality of life (HRQL) with RBX2660: results from a phase 3, randomized, placebo-controlled trial in recurrent *Clostridioides difficile* infection (PUNCH CD3). *Open Forum Infect Dis.* (2022) 9:ofac492.577. doi: 10.1093/ofid/ofac492.577

76. Sonali S, Ray B, Tousif H, Rathipriya A, Sunanda T, Mahalakshmi A, et al. Mechanistic insights into the link between gut dysbiosis and major depression: an extensive review. *Cells.* (2022) 11:1362. doi: 10.3390/cells11081362

frontiers | Frontiers in Medicine

TYPE Clinical Trial
PUBLISHED 12 January 2023
DOI 10.3389/fmed.2022.1049849

OPEN ACCESS

EDITED BY
Angel Lanas,
University of Zaragoza, Spain

REVIEWED BY
Stéphane Isnard,
McGill University Health Center, Canada
Rosa Del Campo,
Ramón y Cajal Institute for Health Research, Spain

*CORRESPONDENCE
Luigi Boccuto
✉ lboccut@clemson.edu

SPECIALTY SECTION
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

RECEIVED 21 September 2022
ACCEPTED 26 December 2022
PUBLISHED 12 January 2023

CITATION
Tkach S, Dorofeyev A, Kuzenko I, Falalyeyeva T, Tsyryuk O, Kovalchuk O, Kobyliak N, Abenavoli L and Boccuto L (2023) Efficacy and safety of fecal microbiota transplantation *via* colonoscopy as add-on therapy in patients with mild-to-moderate ulcerative colitis: A randomized clinical trial.
Front. Med. 9:1049849.
doi: 10.3389/fmed.2022.1049849

Efficacy and safety of fecal microbiota transplantation *via* colonoscopy as add-on therapy in patients with mild-to-moderate ulcerative colitis: A randomized clinical trial

Sergii Tkach[1], Andrii Dorofeyev[2], Iurii Kuzenko[1], Tetyana Falalyeyeva[3,4], Olena Tsyryuk[4], Oleksandr Kovalchuk[4,5], Nazarii Kobyliak[3,5], Ludovico Abenavoli[6] and Luigi Boccuto[7,8]*

[1]Ukrainian Research and Practical Centre of Endocrine Surgery, Transplantation of Endocrine Organs and Tissues of the Ministry of Health of Ukraine, Kyiv, Ukraine, [2]Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine, [3]Medical Laboratory CSD, Kyiv, Ukraine, [4]Educational-Scientific Center, Institute of Biology and Medicine, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine, [5]Endocrinology Department, Bogomolets National Medical University, Kyiv, Ukraine, [6]Department of Health Sciences, University "Magna Graecia", Catanzaro, Italy, [7]Healthcare Genetics Program, School of Nursing, Clemson University, Clemson, SC, United States, [8]Clemson University School of Health Research, Clemson, SC, United States

Introduction: Growing evidence supports the effectiveness of fecal microbiota transplantation (FMT) in treating ulcerative colitis (UC), although its effects seem to depend on the method of introduction, the number of procedures, the donor material, and the severity of UC.

Aim: This study aimed to assess FMT's clinical and microbiological efficacy, tolerability, and safety in patients with mild-to-moderate UC.

Material and methods: Patients with mild-to-moderate UC were randomized into two groups. The first group (standard-care, $n = 27$) was treated with basic therapy–mesalazine–at a daily dose of 3 g (2 g orally + 1 g rectally). In the second group (FMT group, $n = 26$), while taking mesalazine at the indicated dose, each patient with UC as add-on therapy underwent a single FMT procedure with fresh material delivered by colonoscopy from a healthy donor. The clinical efficacy of treatment in both groups was evaluated after 4 and 8 weeks. The primary outcome was remission of UC, defined as a partial Mayo score ≤ 2, and decreased fecal calprotectin. All patients underwent bacteriological examination of feces for quantitative microbiota composition changes.

Results: Clinical response in the form of a significant decrease in stool frequency and a tendency to normalize its consistency after 4 weeks was detected in 14 (51.9%) patients of the standard care group and 16 patients (61.5%) of the FMT group ($p = 0.583$). The Mayo score in the standard care group was 3.59 ± 1.21 and in the FMT group -3.15 ± 1.04 ($p = 0.166$). After 8 weeks, the main primary endpoint was achieved in 70.4% of the standard-care group patients as compared to 84.6% of participants who received FMT as add-on therapy ($p = 0.215$). A more pronounced decrease in Mayo score was observed in the FMT group compared to the standard-care group (1.34 ± 1.44 vs. 2.14 ± 1.4; $p = 0.045$). All patients also showed a significant decrease in fecal calprotectin levels, which correlated with clinical data, stool frequency, and clinical remission. An improvement in gut microbiota composition was noted in both groups, albeit it was significantly more pronounced in the FMT group.

Conclusions: FTM in patients with mild-to-moderate UC is a well-tolerated, effective, and safe method of treatment in comparison to basic therapy.

Clinical trial registration: https://clinicaltrials.gov/ct2/show/NCT05538026?term=kobyliak&draw=2&rank=4, identifier: NCT05538026.

KEYWORDS

fecal microbiota transplantation, gut microbiota, dysbiosis, ulcerative colitis, inflammatory bowel disease

Introduction

Ulcerative colitis (UC) is a chronic immune-mediated inflammatory bowel disease (IBD) that almost always affects the rectum and often extends to the more proximal colon. UC usually begins at a young age (15–30 years) and most patients (∼85%) present with mild or moderate activity, characterized by periods of exacerbation and remission (1).

The severity of UC can be mild, moderate, or severe, with definitions of disease activity varying in clinical practice and the medical literature. Since it is not always possible to clearly distinguish between the severity of the course, in recent years, there has been a tendency to distinguish between mild-to-moderate and moderate-to-severe forms of UC (1). Mild-to-moderate UC is significantly more common, typically characterized by < 4–6 bowel movements per day, mild/moderate stool bleeding, no significant symptoms, low overall inflammatory response, and no evidence of high inflammatory activity based on both the Truelove and Witt criteria and the Mayo clinics ones (1–3).

More than 90% of patients with UC after diagnosis begin treatment with 5-aminosalicylic acid (5-ASA), achieve clinical remission in a fairly short time, and then continue to take these medications to maintain remission (4). Fewer patients with more severe diseases require the use of immunomodulators or biological therapy.

The etiology of UC is not exactly known, although it is multifactorial, and both genetic and environmental factors contribute to its development (5). In recent years, special attention in the study of the mechanisms of development of UC has been paid to the study of the gut microbiome (GM) (6, 7). The data available to date suggest that certain changes in GM can induce disturbances in key links in the pathogenesis of UC: local and systemic immune response, the state of the intestinal mucosal barrier, features of its permeability, and changes in the morphological structure (8–11). It is possible that the severity of gut dysbiosis in patients with UC largely determines the clinical picture of the disease, the severity of exacerbation, and the stability of remission (9). Therefore, the assessment of the impact of various types of UC treatment on changes in GM is of particular interest.

Considering the important pathogenetic role of gut dysbiosis, additional strategies for treating UC have recently been focused on the modification of altered GM using various drug and non-drug methods (12). One such method is fecal microbiota transplantation (FMT), consisting of the simultaneous replacement of the GM of a sick recipient with fecal material from a healthy donor (13). Even though so far the only officially approved indication for FMT is recurrent *Clostridium (C) difficile* infection, the effectiveness of FMT is currently being studied in treating other gastrointestinal and non-gastrointestinal disorders (14–17), and several studies have been conducted to specifically study the effectiveness of FMT in UC, showing encouraging results (18–24).

This study aimed to assess FMT's clinical and microbiological efficacy, tolerability, and safety in patients with mild-to-moderate UC.

Materials and methods

Study design

This open-label, single-center, randomized clinical study was conducted to examine the effectiveness of FMT as add-on therapy in patients with a confirmed clinical diagnosis of mild-to-moderate UC. The study protocol was designed in compliance with principles of the Declaration of Helsinki 1975. The study was approved by the Ethics Committee at Ukrainian Research and Practical Center of Endocrine Surgery, Transplantation of Endocrine Organs and Tissues of the Ministry of Health of Ukraine (protocol number: 6/2020) and was registered in the ClinicalTrial.gov database under entry number NCT05538026. Before RCT was initiated, its purpose and methods were discussed with participants and all patients voluntarily signed the informed consent.

Depending on the treatment, all patients with mild-to-moderate UC were randomized into 2 groups using a computer random number method in a ratio of 1:1. Randomization was carried out by an expert in statistics with blocks of four using a computer-generated list at www.randomization.com. The groups were homogeneous in terms of age, gender, and diagnosis. The patients in the first group (standard-care, $n = 27$) were prescribed basic therapy, mesalazine (Pentasa), at a daily dose of 3 g (2 g orally + 1 g rectally). In the second group (FMT group, $n = 26$), while taking mesalazine at the indicated dose, each patient with UC as add-on therapy underwent a single FMT procedure with fresh material from a healthy donor.

Participants selection

Participants were eligible for inclusion in the trial if they had a verified endoscopically and histologically UC. The severity and degree of activity for UC were assessed based on the Mayo score, which is one of the most commonly used disease activity indices in placebo-controlled trials in UC (25). In its complete form, it is composed of four parts: rectal bleeding, stool frequency, physician assessment, and endoscopy appearance. Each part is rated from 0 to 3, giving a total score of 0–12. A partial Mayo score (eliminates endoscopy) of 2–4 points indicates mildly active disease, a score of 5–6 points indicates moderately active disease, and a score of 7–9 points indicates severely active disease (26). Eligible patients were

with active mild-to-moderate UC (defined as a partial Mayo score of 4–6, and a Mayo endoscopic subscore ≥1). Other inclusion criteria were as follows: adult patients (age: 18–60 years); negative results of stool culture for the presence of pathogenic bacteria (*Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp.) and toxin-producing *C. difficile*; treatment with mesalazine at a daily dose of 3 g during the last 4 weeks; fecal calprotectin over 150 μg/g and a signed informed consent form.

Patients were excluded if pregnant or breastfeeding; with previous surgery on the abdominal cavity; with severe current disease (hepatic, renal, respiratory, or cardiovascular); with corticosteroids, biological agents, probiotic, or antibiotic use within 8 weeks prior to study initiation; or any condition or circumstance that would, in the opinion of the investigator, prevent completion of the study or interfere with the analysis of study results.

Procedure

For our FMT procedures, we used fecal material from one donor tested in accordance with the European Consensus on FMT that was published in the form of clinical guidelines for physicians in 2017 (19). A healthy 39-year-old Caucasian male was recruited as a donor, since he had no harmful habits, adhered to a healthy lifestyle, and had a BMI of 24.5 kg/m^2. His fecal material has already been utilized in other FMTs that have proven effective in the treatment of recurrent *C. difficile* infection. The donor underwent a physical examination, as well as studies and blood tests to exclude pathology of the gastrointestinal tract, metabolic or neurological disorders (complete blood count, blood glucose, electrolytes, and inflammatory markers), liver tests, and thyroid function tests, as well as serological screening tests for HIV, syphilis, and viral hepatitis A, B, and C. The results of his stool culture for the presence of pathogenic bacteria (*Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp. and toxin-producing *C. difficile*), rotaviruses, helminth eggs, and parasites were also negative. His stool culture indicated the absence of gut dysbiosis. Donor fecal samples were tested every 2 months and remained normobiotic with minor variations in the quantitative composition of gut bacteria.

FMTs were prepared as follows: 50–80 g of freshly delivered feces were mixed with 200 mL of isotonic saline and 50 mL of 85% glycerol, homogenized in a blender for 60 s, filtered through a 0.5 mm mesh steel strainer, drawn on 50 mL sterile Luerlock syringes, and sealed.

An appropriately prepared fresh stool suspension from a healthy donor was administered to all patients a single time during a colonoscopy (through a probe inserted into the working channel of the endoscope) while patients were under the effects of intravenous anesthesia.

Outcomes assessment

All patients underwent a comprehensive laboratory and instrumental examination, including general clinical and biochemical blood tests (liver function tests, thyroid hormones, serological examination for celiac disease, electrolytes), fecal examination for calprotectin, helminth eggs and parasites, abdominal ultrasonography, gastroduodenoscopy and colonoscopy with segmental biopsy.

Evaluation of the clinical efficacy of treatment in both groups was carried out after 4 weeks and 8 weeks. The primary outcome was remission of UC, defined as a partial Mayo score ≤2, and decreased fecal calprotectin.

All patients underwent bacteriological examination of feces for quantitative microbiota composition changes in terms of secondary outcome. The gut microbiota of all patients was studied before and 1 month after FMT at the level of the main microbial phylotypes by determining the DNA *Firmicutes, Bacteroidetes,* and *Actinobacteria* in stool samples using a quantitative real-time polymerase chain reaction (PCR) (qRT-PCR). For this, samples of fresh feces were placed in a special container by each patient. An aliquot of feces was taken within 10 min after defecation, immediately frozen, and stored at −20°C until DNA isolation using the phenol-chloroform method according to protocol. DNA was eluted in 200 μl of buffer, and the amount and quality of DNA were measured using a NanoDrop ND-8000 (Thermo Scientific, USA). Samples with a DNA concentration of fewer than 20 ng or with a 260:280 fluorescence ratio of <1.8 were either subjected to ethanol precipitation to become concentrated or further purified according to quality standards. Various taxa were quantified by qPCR using primers targeting the 16S rRNA gene specific for *Firmicutes, Actinobacteria, Bacteroidetes,* and *Faecalibacterium (F) prausnitzi,* as well as universal primers. Genotyping was performed *via* qRT-PCR using the primer structure and temperature cycle parameters.

One of the problems of the PCR approach is related to normalization. To address this issue, the set could be extended by adding a universal pair of primers (and a probe) targeting total prokaryotic content that can be used for normalization purposes (for example, by dividing signals from other taxa by it). Although this would reflect the microbial concentration in the analyzed DNA sample, this concentration could not directly correspond to the concentration in the subject's stool, as it can change considerably during the extraction (27).

Adverse reactions due to FMT were assessed daily over a period of 3 days, and then weekly over the trial.

Sample size calculation

The sample size was calculated using WINPEPI 11.65 (Brixton Health, Israel) software based on the previously published study (21). We calculated that to allow for dropouts at 10% we would need 60 participants in a balanced two-group design ($\alpha = 0.05$; $1\text{-}\beta = 0.80$).

Statistical analysis

Statistical analysis was performed using the standard software SPSS version 20.0 (SPSS, Inc., Chicago, Illinois) and GraphPad Prism, version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Analyses were done according to the intention-to-treat principle, excluding participants without data from the analyses of all clinical endpoints, who did not undergo treatment, and participants diagnosed with any other disease at 8 weeks. Quantitative changes were presented as the mean and standard deviation (M ± SD), and qualitative changes were presented as %. In order to prove the normal distribution hypothesis, Kolmogorov-Smirnov one-sample test was used. To estimate the

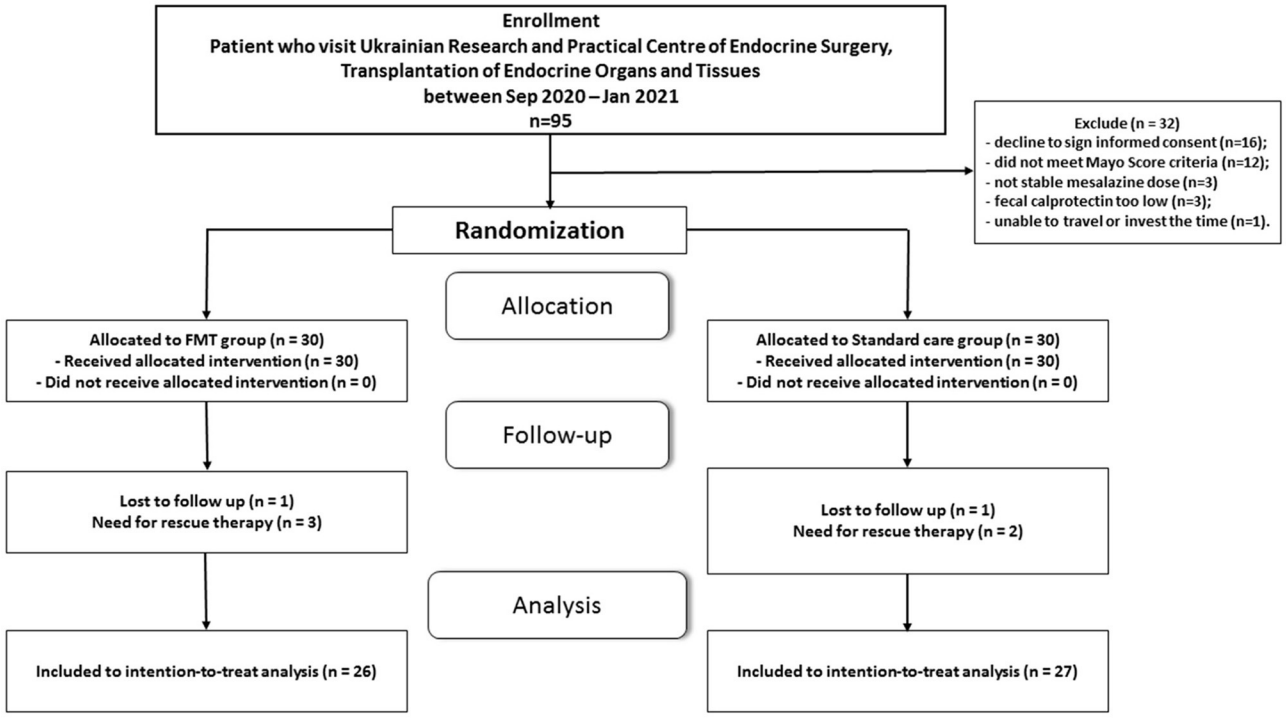


FIGURE 1
Consolidated standards of reporting trials (CONSORT) flow chart-trial protocol.

TABLE 1 Baseline clinical parameters in examined patients (M ± SD or %).

Baseline characteristics	Standard care group ($n = 27$)	FMT group ($n = 26$)	p
Gender (male/female)	10/17	11/15	0.456
Age, years	40.1 ± 12.1	42.4 ± 11.4	0.360
UC duration, years	5.11 ± 2.39	5.81 ± 2.2	0.276
Smoking status, *n* (%)	9 (33.3%)	11 (42.3%)	0.500
Body mass index (BMI), kg/m^2	25.67 ± 2.68	25.26 ± 3.19	0.619
Mayo index, points	5.03 ± 0.80	5.00 ± 0.80	0.868
Fecal calprotectin, μg/g	265.12 ± 47.63	256.36 ± 47.68	0.507
Endoscopic severity index, points	6.78 ± 0.75	6.69 ± 0.89	0.903
Localization			
Proctitis	2	1	
Proctosigmoiditis	12	14	
Left-sided colitis	13	11	

difference in the incoming quantitative data χ^2 criterion was used. A paired *t*-test and a repeated measure analysis of variance (RM-ANOVA) were used to determine, within each group, the difference between the initiation of therapy and the 4 weeks and end of the trial. The changes in outcomes of the participants after the initiation of therapy and the end of the trial were compared by paired sample *t*-tests. Analysis of covariance (ANCOVA) was used to identify any differences between the two groups after the intervention, adjusting for baseline values. Differences between groups were considered significant at a value of $p < 0.05$.

Results

Recruitment for a single-center open comparative randomized clinical trial was started in September 2020 and continued until January 2022 at the Ukrainian Research and Practical Center of Endocrine Surgery, Transplantation of Endocrine Organs and Tissues of the Ministry of Health of Ukraine. For the primary analysis, 95 patients were selected. After carefully considering compliance with the inclusion/exclusion criteria, 18 patients were not eligible. The main reasons were low fecal calprotectin ($n = 3$), not stable

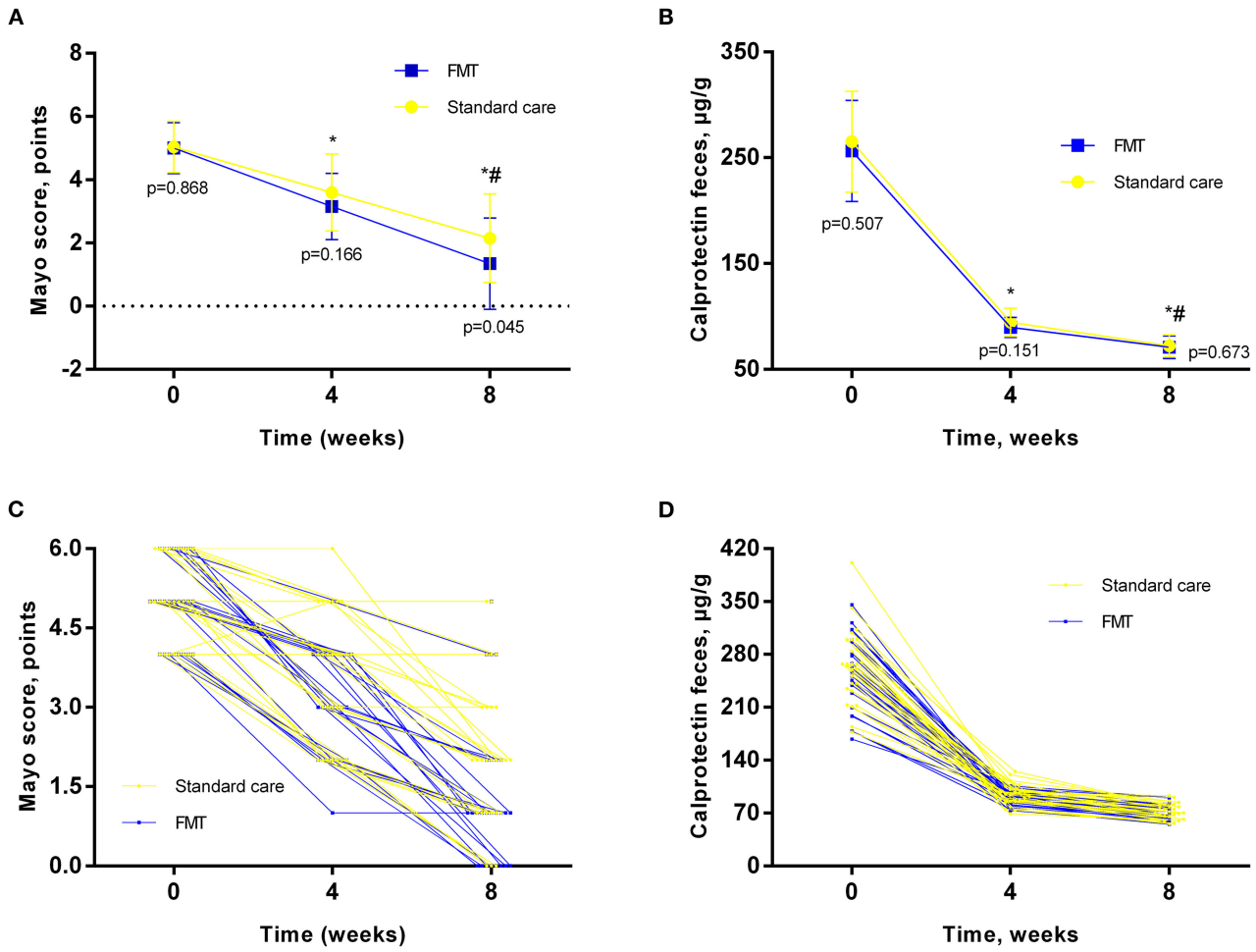


FIGURE 2
Main outcomes analysis in different timepoints. **(A, C)**–Mayo Score; **(B, D)**–fecal calprotectin; Data expressed as mean ± SD **(A, B)** and individual values **(C, D)**. RM-ANOVA was used to identify any differences within groups. *p* – indicates the difference between groups at the same timepoint. *- as compared to baseline; # - compared to 4 weeks.

mesalazine dosage ($n = 3$), and 12 patients who did not meet Mayo score criteria (Figure 1). An in-person consult with all other potential participants allowed us to explain the study criteria, purpose, and methodology of the study. After consideration of the proposal, 16 patients refused to give their informed consent, and 1 was unable to travel or invest the time. At the end of the enrolment period, with possible bias adjustment, 60 patients with mild-to-moderate UC were chosen to be included in the study. All patients were equally distributed in a random order to FMT or standard care group. One randomly assigned participant in both groups withdrew their informed consent without explanation. Moreover, 5 patients (3 in FMT and 2 in standard care group) needed rescue therapy with steroids after initiation of intervention. This left 53 participants for the final modified intention-to-treat analysis. A CONSORT flow chart with a general protocol schedule is shown in Figure 1.

The enrolled patients' baseline demographic and clinical characteristics did not significantly differ between groups (Table 1). A total of 53 patients (32 women, 21 men) with active mild/moderate UC were examined. The severity of UC was assessed based on the Mayo score and fecal calprotectin level. The partial Mayo score at baseline in patients of the standard care group was 5.03±0.8, which does not differ from FMT−5.00±0.8 points ($p = 0.868$) (Table 1). The level of fecal calprotectin in patients with UC before treatment was 265.12 ± 47.63 in standard care and 256.36 ± 47.68 µg/g in the FMT group ($p = 0.507$).

Primary outcomes analysis

The clinical efficacy of the treatment in both groups of patients is presented in Figure 2. The results of the study showed that in both groups of patients with UC, the treatment was effective in most patients. Clinical response in the form of a significant decrease in stool frequency and a tendency to normalize its consistency after 4 weeks was detected in 14 (51.9%) patients in the standard care group and 16 patients (61.5%) of the FMT group ($p = 0.583$). However, in 5 (18.9%) patients of the standard care group, to achieve this intermediate effect, a slight escalation of treatment was required (increasing the dose of mesalazine to 4 g/day), which was significantly higher as compared to FMT, which were only 1 (3.5%) patient required escalation ($p = 0.049$). After 8 weeks, the main primary endpoint was achieved in 70.4 % of patients in the

standard care group as compared to 84.6% of participants who received FMT as add-on therapy ($p = 0.215$). After 4 weeks, the Mayo score in the standard care group was 3.59 ± 1.21, and in the FMT group−3.15 ± 1.04 ($p = 0.166$) (Figures 2A, C). After 8 weeks of therapy, we observed a more pronounced decrease in Mayo score in the FMT group as compared to the standard care group (1.34 ± 1.44 vs. 2.14 ± 1.4; $p = 0.045$) (Figures 2A, C). The same findings for the current endpoint were confirmed in between group ANCOVA analysis (Table 2). All patients also showed a significant decrease in the level of fecal calprotectin (Figures 2B, D) compared to baseline, which correlated with clinical data, stool frequency, and clinical remission. At the same time, even in patients who reached clinical remission after 8 weeks, the level of fecal calprotectin remained elevated (72.15 + 10.45 in the standard care group and 70.92 + 10.68 μg/g in the FMT group). In between group analysis, fecal calprotectin changed insignificantly ($p = 0.575$, Table 2).

Secondary outcomes analysis

We also analyzed the effect of basic therapy and FMT on the gut microbiota composition in patients with UC in terms of secondary outcomes analysis (Table 3). Changes in the qualitative and quantitative composition of the gut microbiota were recorded in most patients with UC before the start of treatment In patients with left-sided UC with moderate disease activity, there was a decrease in the number of *Bacteroidetes* and *Firmicutes* with the growth of *Actinobacteria* and other opportunistic bacteria namely *Proteobacteria*. Accordingly, the Firmicutes/Bacteroidetes (F/B) ratio was 0.64. Four weeks after the start of treatment, a change in the ratio of the main microbial phenotypes was recorded. In all patients, an increase in the number of *Bacteroidetes* and *Firmicutes* was noted. The level of *Bacteroidetes* in the FMT group returned to normal, and the abundance of *Firmicutes* almost reached the normal value and was significantly higher as compared to baseline only in the FMT group (31.5 vs. 23.0%, $p < 0.05$). Normal value was obtained from analysis of microbiota composition in Ukrainian population, fecal concentrations of *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Firmicutes/Bacteroidetes* (F/B) ratio were analyzed in 61 adult individuals (28). It should be noted that the increase in the *Bacteroidetes* and *Firmicutes* and the decrease in *Actinobacteria* and other representatives of opportunistic bacterias in patients after FMT were significantly higher as compared to the standard care group. In addition, after FMT we observed a significant increase in the abundance of butyrate-producing *F. prausnitzii*, which may also indicate an improvement in gut microbiota composition (Table 3).

Thus, the clinical efficacy of treatment in both groups of patients was accompanied by an improvement in gut microbiota composition, which was significantly more pronounced in the group of patients with UC who additionally underwent FMT. We believe that the microbiological efficacy of FMT in patients with mild/moderate UC is associated with a modification of the metabolic activity of the gut microbiome due to the high content of the donor of regulatory molecules and metabolites in the feces, which led to a significant increase in the level *Firmicutes* and *Bacteroidetes* and thereby increasing the synthesis of short-chain fatty acids, in particular butyrate.

Adverse events

Adverse events (AEs) likely related to FMT were stated in patients with UC. No serious AEs were noted. In the FMT group, 6 patients experienced AE. Most often, there was a short-term increase in abdominal pain and bloating (3 patients), 2 patients has complaints of diarrhea, and 1 of constipation. In the standard care group, 1 patient exhibited constipation, and another one had headaches. All AEs reported by patients were estimated as mild in their intensity and disappeared spontaneously. The overall incidence of AEs was higher for FMT but was comparable between groups (23.1 vs. 7.4%, $p = 0.113$).

Discussion

Thus, according to the results obtained, a single FMT improved the results of basic UC therapy with mesalazine, which manifested itself in the form of an insignificant larger number of patients with the clinical response after 4 weeks, which was associated with significantly less amount of patients who required treatment escalation. The clinical remission rate was more pronounced in the FMT group and characterized by a greater decrease in the Mayo score after 8 weeks as compared to the standard care group. Unfortunately, fecal calprotectin, despite its pronounced decrease, did not completely normalize within the treatment periods in both groups, which indicates the need for prolongation of basic therapy.

Our data are consistent with the results of several controlled studies indicating the effectiveness of FMT in patients with active UC. Thus, Moayyedi et al. blindly randomized 70 patients with active UC who received either allogeneic FMT in enemas or water enemas (control) (20). Primary endpoints, such as a decrease in total Mayo score of <3 and endoscopic healing (0 on the endoscopic Mayo scale) after 6 weeks were recorded in 24% of patients who received FMT and 5% of patients who received placebo. Interestingly, the majority of patients who had an effect received FMT from one donor (39 vs.

TABLE 2 Outcomes compared within and between groups.

	Standard care group ($n = 27$)	FMT group ($n = 26$)
Mayo score		
Baseline value	5.03 ± 0.80	5.00 ± 0.80
Week 8 value	2.14 ± 1.40	1.34 ± 1.44
p-value for change from baseline	<0.001	<0.001
Between-group *p*-value	0.048	
Calprotectin feces		
Baseline value	265.12 ± 47.63	256.36 ± 47.68
Week 8 value	72.15 ± 10.45	70.92 ± 10.68
p-value for change from baseline	<0.001	<0.001
Between-group *p*-value	0.575	

For within-group analysis paired sample t-tests were used. ANCOVA was used to identify any differences between the two groups after intervention, adjusting for baseline value.

TABLE 3 Contents of the main phylotypes of microorganisms in patients with UC at baseline and 4 weeks after treatment (%).

Microbial phylotype (%)	Standard care group ($n = 27$)		FMT group ($n = 26$)	
	Baseline	After 1 month	Baseline	After 1 month
Bacteroidetes	35.0	38.0	36.0	42.1
Firmicutes	24.0	26.1	23.0	31.5*
Actinobacteria	23.0	25.9	24.0	19.2*
Other	18.0	10.0	17.0	7.2*
F/B Ratio	0.68	0.68	0.64	0.75
Faecalibacterium prausnitzii	3.0	3.2	3.1	4.3*

* $p < 0.05$ as compared to baseline values.

10% from other donors), which confirms the critical role of donor selection (20). Paramsothy et al. studied the effectiveness of FMT performed by colonoscopy, in patients with mild/moderate UC, while most patients received FMT by introducing material from several donors (from 3 to 7) (22). Steroid-free remission and endoscopic response or remission were achieved in 11 of 41 (27%) patients treated with active fecal material and 3 of 40 (8%) patients treated with placebo (saline). The clinical response was associated with an increase in CM diversity, and the lack of effect was associated with a relative increase in *Fusobacterium*. Costello et al.studied the effectiveness of FMT in patients with mild/moderate UC by repeated administration of frozen fecal material from several donors in enemas (21). At the same time, results were obtained compared with the previous study (remission in 32% of patients treated with fecal material vs. 9% in patients treated with placebo). The LOTUS study, the first which used oral FMT as maintenance therapy in UC, assessed donor engraftment's long-term effectiveness with clinical, endoscopic, and histological outcomes (29). The primary outcome was corticosteroid-free clinical remission with endoscopic remission or response at week 8. At week 8, FMT responders were randomly assigned to either continue or withdraw FMT for a further 48 weeks. At week 8, 53% of patients in the FMT group achieved the primary endpoint as compared to 15% in the placebo group ($p = 0.027$; OR 5.0, 95% CI 1.8–14.1) (29). All patients who continued FMT in the open-label phase were in clinical, endoscopic, and histologic remission at week 56 compared with none of the patients who had FMT withdrawn (29).

A systematic meta-analysis was conducted to assess FMT as a treatment for active UC in 277 participants. FMT was connected with better remission between four RCTs than placebo (30). A most recent meta-analysis involving 6 RCT and 324 patients with UC demonstrated that compared with placebo, FMT has a significant benefit in inducing combined clinical and endoscopic remission (OR 4.11; 95% CI 2.19–7.72; $p < 0.0001$). Subgroup analyses of influencing factors showed no differences between fresh or frozen FMT ($p = 0.35$) and different routes or frequencies of delivery ($p = 0.80$ and $p = 0.48$, respectively) (31). In contrast, a recent meta-analysis, with the inclusion of 14 RCT found that fresh (40.9%) as compared to frozen (32.2%) FMT can increase clinical remission rates in IBD patients, with no significant risk of study heterogeneity ($I^2 = 38\%$, $p = 0.03$) (32).

In our study, the clinical efficacy of treatment in both groups of patients was accompanied by an improvement in gut microbiota composition, which was significantly more pronounced in the group of patients with UC who additionally underwent FMT. It should be noted that the *Firmicutes* phylotype includes one of the main representatives of the obligate *Lactobacillus*, which plays a significant role in the formation of colonization resistance and stability of the gut microbiome. In addition, representatives of *Firmicutes* have a significant effect on the metabolic activity of the gut microbiota, taking part in the synthesis of short-chain fatty acids, including butyrate, thereby modifying the state of the intestinal mucosal barrier (33, 34). The number of *F. prausnitzii* belonging to the family Ruminococcaceae, a member of the *Firmicutes* phylotype, is considered a regulatory and plays an important role in maintaining intestinal homeostasis, was also significantly reduced before treatment ($p < 0.05$). It's believed that decreased *Firmicutes* and *Faecalibacterium prausnitzii* in patients with UC can be an unfavorable prognostic sign and a marker of the severity of changes in the gut microbiome (11). The number of *Actinobacteria* in patients with UC was significantly higher than in healthy individuals, this is because the *Actinobacteria* family includes many representatives of opportunistic microbiota, the number of which increases with intestinal dysbiosis associated with UC.

So, FMT is an emerging treatment strategy for UC. Clinical research on FMT in treating gastroenterological diseases has dramatically increased in the last few years and is still ongoing. However, there are many issues to solve before FMT can become standard therapy for UC, including donor selection, administration routes, frequencies, easy-to-administer formulation development, and optimal patient population (35).

Conclusion

Even single transplantation of fecal microbiota (fresh material) bears the potential to be a well-tolerated and safe method of treatment in a large number of patients with mild-to-moderate UC, contributing to an increase in the effectiveness of basic therapy after 4 and 8 weeks, as well as a significant improvement in the abundance of the gut microbiota as early as 4 weeks after FMT. The addition of FMT to the standard therapeutic protocols for UC warrants efficacy at reaching clinical improvement and preservation of gut eubiosis, in line with the goals of precision medicine.

In our opinion, the effectiveness of FMT depends primarily on the microbial composition and quality of the donor material used (from one or several donors; fresh or frozen material), the number of procedures (single or repeated FMT), routes of administration of

the material (colonoscopy, enemas, naso-duodenal probe), previous treatment, the prevalence of the process and severity of UC. Therefore, future studies are recommended to further characterize these parameters and develop the necessary guidelines to routinely add FMT to the treatment options for UC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee at Ukrainian Research and Practical Center of Endocrine Surgery, Transplantation of Endocrine Organs and Tissues of the Ministry of Health of Ukraine (protocol number: 6/2020). The patients/participants provided their written informed consent to participate in this study.

Author contributions

ST, AD, and NK contributed to the conceptualization and the original idea of this manuscript. ST, AD, OT, IK, and NK contributed to methodology and reviewed the literature. ST, AD, LA, OK, and TF involved in validation and revised and validated the literature findings. IK and OT performed formal analysis. ST and AD contributed to investigation. ST and NK involved in data curation, did writing–original draft preparation, and did writing–review and editing. ST, OT, and IK did visualization. ST, LB, LA, and NK involved in supervision. ST and IK contributed to project administration. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Ko CW, Singh S, Feuerstein JD, Falck-Ytter C, Falck-Ytter Y, Cross RK, et al. AGA clinical practice guidelines on the management of mild-to-moderate ulcerative colitis. *Gastroenterology*. (2019) 156:748–64. doi: 10.1053/j.gastro.2018.12.009

2. Mak WY, Zhao M, Ng SC, Burisch J. The epidemiology of inflammatory bowel disease: East meets west. *J Gastroenterol Hepatol*. (2020) 35:380–9. doi: 10.1111/jgh.14872

3. Forss A, Clements M, Bergman D, Roelstraete B, Kaplan GG, Myrelid P, et al. A nationwide cohort study of the incidence of inflammatory bowel disease in Sweden from 1990 to 2014. *Aliment Pharmacol Ther*. (2022) 55:691–9. doi: 10.1111/apt.16735

4. Le Berre C, Roda G, Nedeljkovic Protic M, Danese S, Peyrin-Biroulet L. Modern use of 5-aminosalicylic acid compounds for ulcerative colitis. *Expert Opin Biol Ther*. (2020) 20:363–78. doi: 10.1080/14712598.2019.1666101

5. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. *Lancet*. (2017) 389:1756–70. doi: 10.1016/S0140-6736(16)32126-2

6. Guo XY, Liu XJ, Hao JY. Gut microbiota in ulcerative colitis: insights on pathogenesis and treatment. *J Dig Dis*. (2020) 21:147–59. doi: 10.1111/1751-2980.12849

7. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clin J Gastroenterol*. (2018) 11:1–10. doi: 10.1007/s12328-017-0813-5

8. Carstens A, Dicksved J, Nelson R, Lindqvist M, Andreasson A, Bohr J, et al. The gut microbiota in collagenous colitis shares characteristics with inflammatory bowel disease-associated dysbiosis. *Clin Transl Gastroenterol*. (2019) 10:e00065. doi: 10.14309/ctg.0000000000000065

9. Khan I, Ullah N, Zha L, Bai Y, Khan A, Zhao T, et al. Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? IBD treatment targeting the gut microbiome. *Pathogens*. (2019) 8:126. doi: 10.3390/pathogens8030126

10. Frank DN. St. Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A*. (2007) 104:13780–5. doi: 10.1073/pnas.0706625104

11. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, et al. A decrease of the butyrate-producing species roseburia hominis and faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. *Gut*. (2014) 63:1275–83. doi: 10.1136/gutjnl-2013-304833

12. Glassner KL, Abraham BP, Quigley EMM. The microbiome and inflammatory bowel disease. *J Allergy Clin Immunol*. (2020) 145:16–27. doi: 10.1016/j.jaci.2019.11.003

13. Tkach S, Dorofeyev A, Kuzenko I, Boyko N, Falalyeyeva T, Boccuto L, et al. Current status and future therapeutic options for fecal microbiota transplantation. *Medicina*. (2022) 58:84. doi: 10.3390/medicina58010084

14. D'Haens GR, Jobin C. Fecal microbial transplantation for diseases beyond recurrent clostridium difficile infection. *Gastroenterology*. (2019) 157:624–36. doi: 10.1053/j.gastro.2019.04.053

15. Kobyliak N, Falalyeyeva T, Tsyryuk O, Eslami M, Kyriienko D, Beregova T, et al. New insights on strain-specific impacts of probiotics on insulin resistance: evidence from animal study. *J Diabetes Metab Disord*. (2020) 19:289–96. doi: 10.1007/s40200-020-00506-3

16. Kobyliak N, Falalyeyeva T, Mykhalchyshyn G, Molochek N, Savchuk O, Kyriienko D, et al. Probiotic and omega-3 polyunsaturated fatty acids supplementation reduces insulin resistance, improves glycemia and obesity parameters in individuals with type 2 diabetes: a randomised controlled trial. *Obesity Med*. (2020) 19:100248. doi: 10.1016/j.obmed.2020.100248

17. Kobyliak N, Falalyeyeva T, Beregova T, Spivak M. Probiotics for experimental obesity prevention: focus on strain dependence and viability of composition. *Endokrynol Pol*. (2017) 68:659–67. doi: 10.5603/EP.a2017.0055

18. Suez J, Zmora N, Zilberman-Schapira G, Mor U, Dori-Bachash M, Bashiardes S, et al. Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. *Cell*. (2018) 174:1406–23.e16. doi: 10.1016/j.cell.2018.08.047

19. Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, et al. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut*. (2017) 66:569–80. doi: 10.1136/gutjnl-2016-313017

20. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology*. (2015) 149:102–9.e6. doi: 10.1053/j.gastro.2015.04.001

21. Rossen NG, Fuentes S, Van Der Spek MJ, Tijssen JG, Hartman JHA, Duflou A, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology*. (2015) 149:110–8.e4. doi: 10.1053/j.gastro.2015.03.045

22. Paramsothy S, Kamm MA, Kaakoush NO, Walsh AJ, van den Bogaerde J, Samuel D, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet*. (2017) 389:1218–28. doi: 10.1016/S0140-6736(17)30182-4

23. Costello SP, Waters O, Bryant RV, Katsikeros R, Makanyanga J, Schoeman M, et al. Short duration, low intensity, pooled fecal microbiota transplantation induces remission in patients with mild-moderately active ulcerative colitis: a randomised controlled trial. *Gastroenterology*. (2017) 152:S198–9. doi: 10.1016/S0016-5085(17)30969-1

24. Holvoet T, Joossens M, Vázquez-Castellanos JF, Christiaens E, Heyerick L, Boelens J, et al. Fecal microbiota transplantation reduces symptoms in some patients with irritable bowel syndrome with predominant abdominal bloating: short- and long-term results from a placebo-controlled randomized trial. *Gastroenterology*. (2021) 160:145–57.e8. doi: 10.1053/j.gastro.2020.07.013

25. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med*. (2005) 353:2462–76. doi: 10.1056/NEJMoa050516

26. Lewis JD, Chuai S, Nessel L, Lichtenstein GR, Aberra FN, Ellenberg JH. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. *Inflamm Bowel Dis*. (2008) 14:1660–6. doi: 10.1002/ibd.20520

27. Rivera-Pinto J, Egozcue JJ, Pawlowsky-Glahn V, Paredes R, Noguera-Julian M, Calle ML. Balances: a new perspective for microbiome analysis. *mSystems*. (2018) 3:e00053–18. doi: 10.1128/mSystems.00053-18

28. Koliada A, Syzenko G, Moseiko V, Budovska L, Puchkov K, Perederiy V, et al. Association between body mass index and firmicutes/bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiol*. (2017) 17:120. doi: 10.1186/s12866-017-1027-1

29. Haifer C, Paramsothy S, Kaakoush NO, Saikal A, Ghaly S, Yang T, et al. Lyophilised oral faecal microbiota transplantation for ulcerative colitis (LOTUS): a randomised, double-blind, placebo-controlled trial. *Lancet Gastroenterol Hepatol*. (2022) 7:141–51. doi: 10.1016/S2468-1253(21)00400-3

30. Narula N, Kassam Z, Yuan Y, Colombel JF, Ponsioen C, Reinisch W, et al. Systematic review and meta-analysis: fecal microbiota transplantation for treatment of active ulcerative colitis. *Inflamm Bowel Dis*. (2017) 23:1702–9. doi: 10.1097/MIB.0000000000001228

31. El Hage Chehade N, Ghoneim S, Shah S, Chahine A, Mourad FH, Francis FF, et al. Efficacy of fecal microbiota transplantation in the treatment of active ulcerative colitis: a systematic review and meta-analysis of double-blind randomized controlled trials. *Inflamm Bowel Dis*. (2022) doi: 10.1093/ibd/izac135

32. Tan X-Y, Xie Y-J, Liu X-L, Li X-Y, Jia B. A systematic review and meta-analysis of randomized controlled trials of fecal microbiota transplantation for the treatment of inflammatory bowel disease. Evidence-based complementary and alternative medicine. *eCAM*. (2022) 2022:8266793. doi: 10.1155/2022/8266793

33. Bahman Y, Maryam M, Aisa B, Falalyeyeva T, Kobyliak N, Majid E. Immunomodulatory role of *Faecalibacterium prausnitzii* in obesity and metabolic disorders. *Minerva Biotechnol Biomol Res*. (2021) 33:76–85. doi: 10.23736/S2724-542X.21.02759-2

34. Kobyliak N, Falalyeyeva T, Kyriachenko Y, Tseyslyer Y, Kovalchuk O, Hadiliia O, et al. Akkermansia muciniphila as a novel powerful bacterial player in the treatment of metabolic disorders. *Minerva Endocrinol*. (2022) 47:242–52. doi: 10.23736/S2724-6507.22.03752-6

35. Matsuoka K. Fecal microbiota transplantation for ulcerative colitis. *Immunol Med*. (2021) 44:1–5. doi: 10.1080/25785826.2020.1792040

Frontiers in Medicine

Translating medical research and innovation into improved patient care

A multidisciplinary journal which advances our medical knowledge. It supports the translation of scientific advances into new therapies and diagnostic tools that will improve patient care.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact