The use of fecal microbiota transplantation in gastrointestinal diseases

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ACRONYMS AND ABBRIVATIONS

et al.	and others
AE	Adverse events
CD	Crohn's disease
CDI	*Clostridioides difficile* infection
FDA	Food and Drug Administration
FFT	Fecal filtrate transfer
FGID	Functional gastrointestinal disorders
FMT	Fecal microbiota transplantation
GI	Gastrointestinal
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
NDT	Nasoduodenal tube
NJT	Nasojejunal tube
PBA	Primary bile acid
PPI	Proton-pump inhibitors
rCDI	Recurrent *Clostridioides difficile* infection
RCT	Randomized controlled trial
SAE	Serious adverse events
SBA	Secondary bile acid
SCFA	Short-chain fatty acid
UC	Ulcerative colitis
USA	United States of America

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ABSTRACT

The use of fecal microbiota transplantation in gastrointestinal diseases

Jannat Monosi Ali

Fecal microbiota transplantation (FMT) is a treatment modality that involves the process of transplanting fecal matter obtained from a selected donor to a recipient in order to restore the microbial homeostasis in the intestine. Although the utilization of fecal matter to treat diseased individuals can be traced to the 4th century, China, it was not until the end of the 20th century that Western countries began to study the potential role of FMT. Since then, there has been a growing interest in the scientific community to understand the composition and the function of the human microbiota. In addition, there has also been a great deal of interest in how to manipulate the microbiota and to understand the relationship between various disease conditions and the perturbated microbial community.

In contrast to antibiotics and immunomodulators, FMT seems to be an excellent therapy option for several gastrointestinal diseases, as it is a restorative treatment modality and not destructive or suppressive. The mechanism of action of FMT is not fully understood, however, its effectiveness is thought to be attributable to the recovery of the microbiota from dysbiosis to eubiosis. To date, the only formally approved indication for FMT is *Clostridioides difficile* infection (CDI), in addition, the reported efficacy rates have been very high. The findings on employing FMT for treating inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are not as straightforward, possibly due to the limited large-scale RCTs, the heterogenous nature of the disease, the various phenotypic presentations and the still unknown factors associated to the transplanted microbiota. Nevertheless, FMT seems to have a role in both IBD and IBS management and could be incorporated as an adjunctive therapy to combat not only dysbiosis and related pathology but also the additional pathogenetic mechanisms that usually underlie these multifactorial-related conditions.

Key words: *Clostridioides difficile* infection, Crohn's disease, dysbiosis, fecal microbiota transplantation, inflammatory bowel disease, irritable bowel syndrome, microbiota, ulcerative colitis

SAŽETAK

Primjena transplantacije fekalne mikrobiote u gastrointestinalnim bolestima

Jannat Monosi Ali

Transplantacija fekalne mikrobiote (FMT) je modalitet liječenja koji uključuje proces transplantacije fekalne tvari dobivene od odabranog darivatelja kako bi se povratila homeostaza crijevne mikrobiote primatelja. Iako se korištenje fekalne tvari za liječenje oboljelih osoba može pratiti od 4. stoljeća u Kini, zapadne zemlje su tek krajem 20. stoljeća počele proučavati potencijalnu ulogu FMT-a. Od tada, u znanstvenoj zajednici raste interes za razumijevanje sastava i funkcije ljudske mikrobiote, kao i poremećaja u ljudskim mikrobnim zajednicama povezanih s raznim bolestima.

Za razliku od antibiotika i imunomodulatora, čini se da je FMT izvrsna terapijska opcija za nekoliko gastrointestinalnih bolesti, budući da predstavlja obnavljajući modalitet liječenja, a ne destruktivan ili supresivan. Mehanizam djelovanja FMT-a nije u potpunosti razjašnjen, međutim, njegova se učinkovitost pripisuje obnavljanju mikrobiote iz stanja disbioze u stanje eubioze. Do danas, jedina službeno odobrena indikacija za FMT je infekcija bakterijom *Clostridioides difficile* (CDI) s vrlo visokim stopama učinkovitosti. Nalazi o korištenju FMT-a za liječenje upalne bolesti crijeva (IBD) i sindroma iritabilnog crijeva (IBS) nisu tako jednostavni, vjerojatno zbog ograničenih randomiziranih i kontroliranih ispitivanja velikih razmjera, heterogene prirode bolesti, njenih različitih fenotipskih prikaza kao i još uvijek nepoznatih čimbenika povezanih s transplantiranom mikrobiotom. Ipak, čini se da bi FMT mogla imati ulogu i u liječenju IBD-a i IBS-a te bi se mogala koristiti i kao pomoćna terapija u borbi ne samo s disbiozom i srodnom patologijom nego i s dodatnim patogenetskim mehanizmima koji obično leže u osnovi ovih multifaktorskih stanja.

Ključne riječi: Infekcija Clostridioides difficile, Crohnova bolest, disbioza, transplantacija fekalne mikrobiotedisbioza, upalna bolest crijeva, sindrom iritabilnog crijeva, mikrobiota, ulcerozni kolitis,

1 | INTRODUCTION

The importance of the intestinal microbiota for human health emerged as early as 2500 years ago, when the ancient Greek physician Hippocrates said, “All disease begins in the gut”. The human gastrointestinal (GI) tract harbors a rich and diverse community of microorganisms that differ from individual to individual, making it unique as a fingerprint. Since no two intestinal microbiota profiles are the same, the definition remains unclear in what a healthy microbial community is, however, it is thought that a stable and diverse microbiota correlates with a healthy state. The microbiota is vastly diverse and its density changes along the GI tract being the densest in the large intestine. The composition, diversity and functionality of the microbiota are highly dynamic and change throughout life. The quantity and quality of the microbiota is easily altered by both endogenous and exogenous factors such as diet, hormonal cycles, health status, drugs, pre- and probiotics, and the surrounding environment (1). The microbiota has a vital part in human health and plays a fundamental role in the modulation of several local functions including, but not limited to, defending the host against pathogens, energy production and metabolism, aiding in nutritional provision, fine-tuning the immune system, and detoxifying xenobiotics (2,3).

Dysbiosis is the condition of a disrupted microbial homeostasis resulting in alterations in the symbiotic relationship between the microbiota, the enteric microenvironment, and the host. Intestinal dysbiosis has been shown to contribute to the pathogenesis of several common GI-related conditions, including infectious diarrhea, chronic inflammatory bowel disease and functional GI conditions. Current evidence has demonstrated that intestinal dysbiosis also has role in several extraintestinal conditions such as oncological conditions (4), obesity and metabolic syndrome (5), autism spectrum disorder (6), multiple sclerosis (7), graft-versus-host disease (8), Parkinson’s disease (9), hepatic encephalopathy (10), nonalcoholic fatty liver disease (11), and atherosclerosis and hypertension (12).

There has been a great interest in manipulating the intestinal microbiota in order to amend the dysbiotic condition. Several strategies have been used to target the disrupted microbiota including dietary interventions, prebiotics, probiotics, antibiotics, phage therapy, and fecal microbiota transplantation (FMT). Among these, FMT seems to be the most powerful intervention with the most satisfactory results. FMT is the procedure that involves the administration of minimally manipulated fecal material obtained from a healthy donor to a patient (recipient), with the intention of improving and restoring the balance of the microbial community in the intestine (13). In this thesis, the history of FMT, microbiota and related dysbiosis, procedural aspects of FMT, safety profile and clinical applications in selected gastrointestinal diseases will be discussed.

2 | HISTORY OF FMT: THE NEW OLD THERAPY

The use of fecal material as a medical therapy is nothing new and has been used for almost two millennia (Figure 1) (14). The first record of FMT can be traced back to the 4th century, performed by a well-recognized traditional Chinese medicine practitioner named Ge Hong (15). Hong used fresh and fermented fecal suspension orally and coined the term “yellow soup” to avoid patients’ repugnance. It was used as a rescue treatment for serious food poisoning, febrile illness, typhoid fever, and diarrhea (16). It is the first literary record of fecal transplantation, described in the Chinese emergency medicine handbook “Hand Therapy for Emergencies”, which at the time was considered a medical miracle that brought back patients from the brink of death (17).

In the 16th century, another practitioner of traditional Chinese medicine, Li Shizhen, recorded the use of transplanted fecal material to treat abdominal diseases in the most famous book of traditional Chinese medicine called “Ben Cao Gang Mu” or Compendium of Materia Medica (15,18). It was the most complete record of fecal material transplantation in traditional human medicine (16).

In the West in the 17th century, an Italian surgeon Acquapendente described the use of enteric flora transplantation in veterinary medicine and coined the term “transfaunation”. The term implied the transfer of GI contents from a healthy to a diseased animal and was widely used in the field of veterinary medicine (3,15,19).

During World War II, an interesting report revealed that cross-species FMT might work as well (18,20). German soldiers residing in North Africa suffered from episodes of recurrent diarrhea and were treated with fresh, warm camel dung (19). The treatment was inspired by the native Bedouins as a remedy for bacterial dysentery, the efficacy of which was anecdotally confirmed by the German soldiers.

In modern medicine, it was not until the second half of the 20th century that Western countries began to study the potential beneficial role of FMT. In 1958, the first report on FMT in English literature was made by the Chief of Surgery, Dr. Ben Eiseman, and his colleagues at Denver General Hospital (18). They utilized their combined expertise to treat four patients that suffered from pseudomembranous colitis with fecal enema (21). Researchers realized 20 years later that the condition they were treating was in fact pseudomembranous colitis caused by *C. difficile* (22).

The use of FMT beyond infectious diseases was first published in 1989. Borody *et al.* performed “an exchange of bowel flora” on a 45-year-old male with refractory ulcerative colitis (UC), showing full and lasting clinical recovery after treatment (14,19). In Amsterdam 2013, the first randomized controlled trial (RCT) was conducted and demonstrated high efficacy in the treatment of recurrent *C*. *difficile* infection (rCDI) with FMT. The authors reported that one treatment with FMT by nasoduodenal route was superior to standard antibiotic therapy with vancomycin (23).

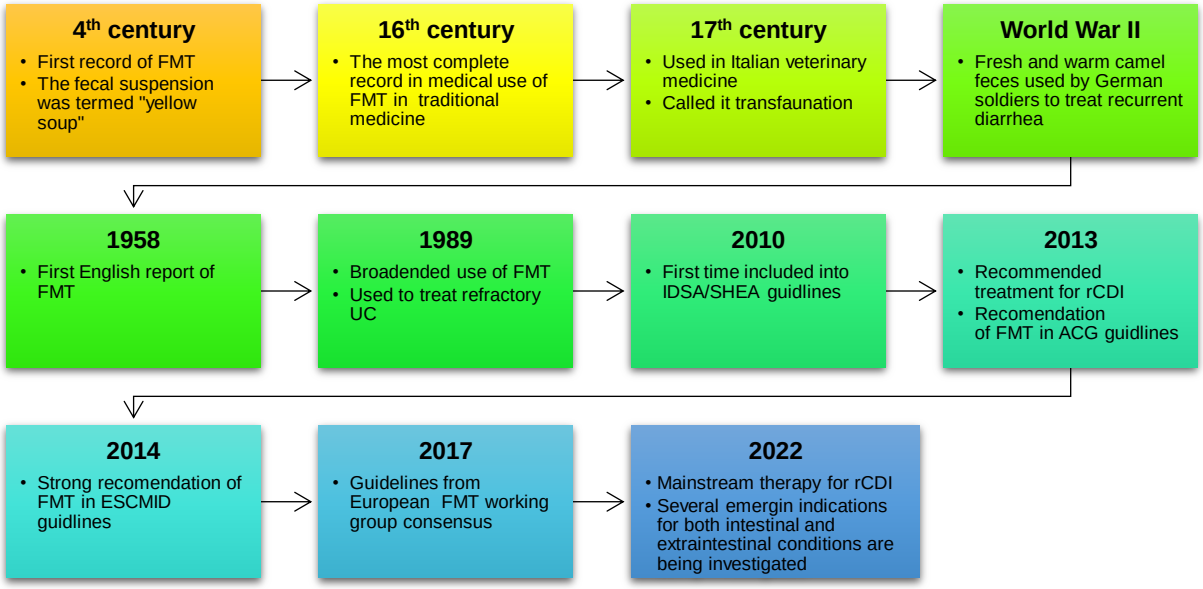

Figure 1. History timeline of major events in FMT therapy

FMT, Fecal microbiota transplantation; UC, Ulcerative colitis; rCDI, Recurrent *Clostridioides difficile* infection; IDSA, Infectious Diseases Society of America; SHEA, Society for Healthcare Epidemiology of America; ACG, American College of Gastroenterology; ESCMID, The European Society of Clinical Microbiology and Infectious Diseases

3 | HUMAN GUT MICROBIOTA

BASIC TERMINOLOGY

The term microbiota describes the community of all microorganisms that are present in a defined environment. Microbiota includes all three known domains of life (archaea, bacteria, eukaryote as well as viruses) and is a preferred term to the older “microflora” which only encompasses bacteria (24,25). The microbiome is the collective composition of genetic material from all organisms present in a given habitat, including both symbiotic and pathogenic microbes. Metagenomics is the study of the microbiome, which is the study of the genomes from a community of mixed organism. In the normal healthy state, the microbial community is in a state of balance called eubiosis, where the symbiotic relationships are maintained. On the other hand, dysbiosis is the condition of perturbation in the ecosystem so that the microbial homeostasis becomes disrupted, which in turn leads to a loss of diversity and a reduced quality of microbial functions.

In the last decade, the role that microbiota plays in human health has become a topic of growing interest. The scientific understanding has increased remarkably during this time, but there is yet a lot to discover, such as the fundamental functions of majority of the microorganisms that still remain to be determined.

The intestinal microbiota is a complex of microorganisms that inhabits the GI tract and forms a multifaceted ecosystem consisting of both known and unknown species. This ecosystem is dominated by bacterial species compared to other microbes found in the gut, such as fungi, viruses, archaea, and protists (26).

This complex ecosystem is diverse and dynamic, and its density varies depending on the location, being the densest in the colon. The dominant bacterial species found in the intestine consist of four main phyla: *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria* (18,26,27). The composition of the gut microbiota can be altered by a variety of factors including diet, the environment, physical activity, hormones, and medication such as antibiotics and proton-pump inhibitors (PPI) (28). Each individual possesses their own unique constitution of microbiota, just like a fingerprint, and therefore an ideal healthy microbiota composition has not yet been defined. It has been proposed that the overall function of the microbiota with the resulting host interactions may be of a greater value than the specific microbial composition itself (27).

The microbiota influences several bodily functions such as GI motility, the regulation of mucosal barrier function and epithelial turnover, it affects immune response, and suppresses pathogen overgrowth. It also plays an important role in host metabolism, converting dietary fiber to short-chain fatty acids, which act as energy substrate for colonocytes (29). The intestinal microbiota as an integral part of the digestive tract influences its functional development and overall physiological functions. It is important to appreciate that the human gut microbiota is not just an assemblage of microorganisms, but a well-organized integrated network of microorganisms that interacts intensely with each other as well as with its host (30).

The dysbiosis of the gut microbiota has been implicated in several intestinal disease states, with the prototypical example being *C. difficile* infection (CDI). The disturbances in the microbiota can be attributed to an increase in pathogenic microbes, reduced diversity and/or loss of beneficial microbes which in turn can lead to the production of harmful metabolites and induce host damage (28). Dysbiosis has also been linked to several non-communicable extraintestinal conditions such as metabolic, neurological, and behavioral disorders.

4 | FECAL MICROBIOTA TRANSPLANTATION

FMT, also known as stool transplantation, is the process of transferring fecal material from a healthy donor into the recipient's GI tract in order to correct the dysbiosis-associated conditions. It involves rigorous screening of donor, preparation of fecal material as well as the recipient, and close monitoring of the procedure as well as the patient even thereafter (Figure 2).

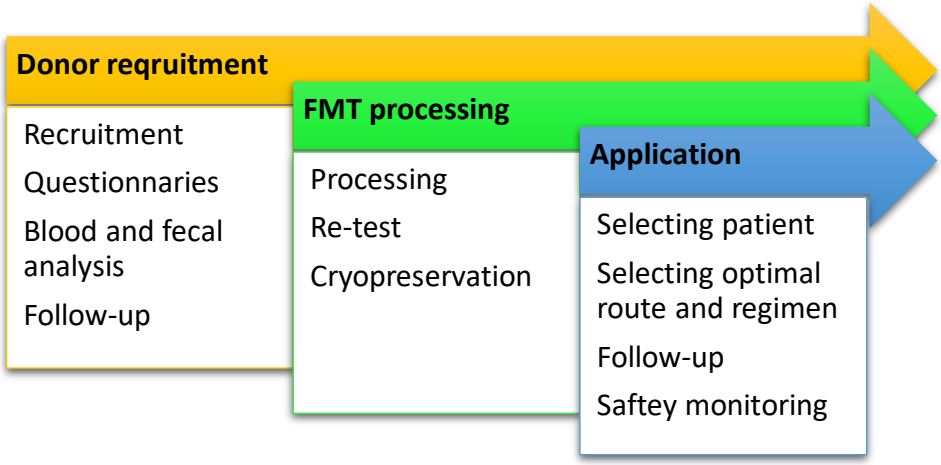

Figure 2. The outline of the process for FMT

4.1 | MECHANISM OF ACTION

Dysbiosis, the perturbation in the function and composition of the microbiota, causes interference in metabolic pathways that affect immunological and mechanical processes both locally (intestinal) and systematically (extraintestinal). In this regard, the dysbiotic condition of the intestinal microbiota leads to various disease states.

The disturbance of the microbiota has been studied the most in *Clostridioides difficile* infection (CDI) patients, with established pathogenesis of dysbiosis as the predominant culprit. There is a general acceptance in that the microbiota is a factor in the pathogenesis of several GI conditions including some subgroups of IBS and IBD. However, it is not known whether dysbiosis constitutes the cause or the outcome of the latter. Hence, the relative importance of the microbiota in the pathogenesis is different for different diseases. In addition, it is worth mentioning that apart from CDI, there is a huge lack of studies focusing on the mechanism of action of FMT.

One might assume that the "active compounds" of the FMT's efficacy would only be related to the microorganisms and their restoration, but there are several additional factors that may contribute to its efficacy.

For instance, a recent pilot study (31) investigated whether sterile fecal filtrates containing proteins, oligonucleotides/DNA, metabolic products, antimicrobial compounds, and bacterial debris were effective in CDI patients. The researchers prepared the so-called fecal filtrate transfer (FFT) by passing the fecal suspension through progressively finer pore filters, cumulating in a 0.2 µm pore filter (0.2 µm < size of a bacteria). In all the subjects treated with FFT the symptoms were eliminated and their stool habits resorted to normal 6 months post-FFT. The authors concluded that FFT alone, rather than conventional FMT may be sufficient in treating CDI patients. In addition, these findings suggests that some soluble factors rather than the intact bacteria *per se* could potentially be the key mediator in the mechanism of action. In addition, to minimize the risk of transmitting unknown infectious agents to immunocompromised patients, FFT might be of great value in this subset of patient population.

Although the exact mechanism of FMT is not yet defined, based on currently available evidence, the benefits of FMT are thought to work through various processes, including but not limited to the following: colonization resistance, restoration of metabolites, and restoration of immune function (32).

COLONIZATION RESISTANCE

Colonization resistance is a well-known phenomenon that implies the mechanism by which the commensal bacteria protect themselves by competing for the ecological niches and thereby preventing colonization and overgrowth of pathogenic and opportunistic (pathobiont) microorganisms. To maintain a healthy microbial composition, the commensal bacteria regulate the activity of pathogenic bacteria by microbe-microbe (direct) and microbe-host (indirect) interactions.

The microbiota mediates colonization resistance through several direct and indirect mechanisms (Figure 3) (33). Some of the direct microbiota-mediated mechanisms include competition for nutrients, production of toxins and other compounds (bacteriocin, secondary bile acids, SCFAs), and niche exclusion (space competition). The indirect mechanisms include stimulation of the mucosal immune system to enforce the intestinal barrier defenses, activate the host's immune response, promote immune cell recruitment, promote activation and differentiation of key immune cells, and enhance both innate and adaptive mucosal immunity. With this regard, FMT may through direct ecological competition increase host resistance to pathogens and restore the microbial diversity and its functions.

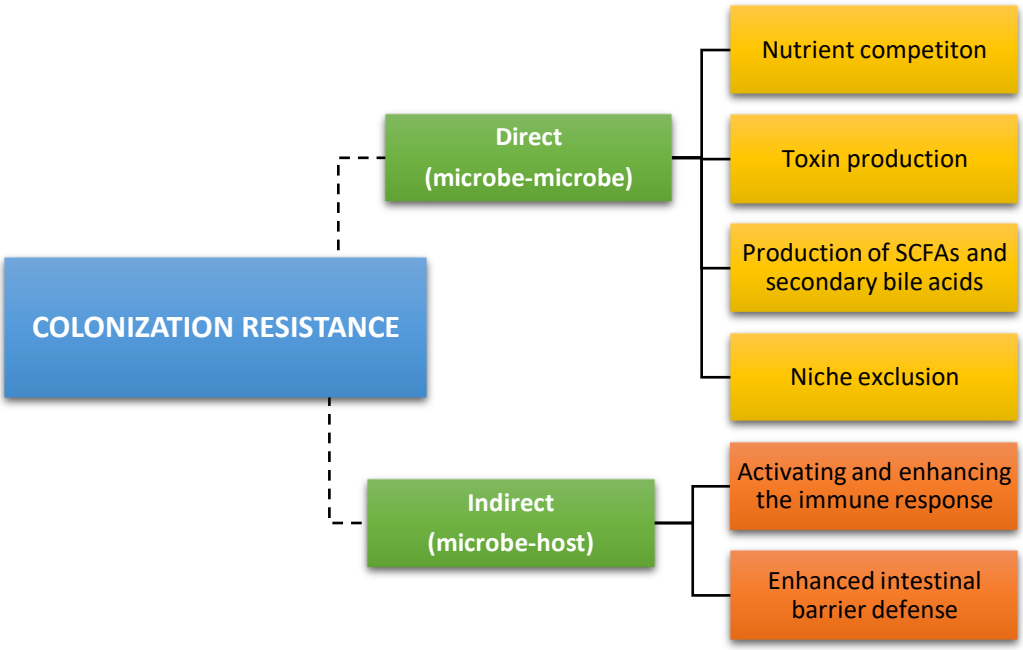

Figure 3. Colonization resistance - One of the proposed mechanisms of action that may underlie the efficacy of FMT

RESTORATION OF METABOLITES

One of the main proposed theories in FMT mechanism of action is the restoration of essential microbial metabolites and/or co-metabolites, which are usually produced by interactions between the host and the microbes. The intestinal microbiota facilitates the synthesis of metabolites that have important properties for host metabolism including short-chain fatty acids (SCFAs) and bile acids (32).

One of the key metabolites in the colon are SCFAs, which are produced by the microbiota through fermentation of dietary fibers and indigestible starch. The two dominant bacterial phyla in the human intestine are *Bacteroidetes* and *Firmicutes* and they are also the main producers of the common SCFAs like acetate, propionate, and butyrate. Some of the immunomodulatory properties of SCFA include stimulation of T regulatory cells, oppose translocation of lipopolysaccharides (LPS) and bacteria, enhance barrier function and proliferation of intestinal epithelial cells, and inhibit production of proinflammatory cytokines. All these effects protect the intestinal cells from inflammation and promote mucosal homeostasis (32,34). The effects of FMT may be from either resolving the dysbiosis *i.e.,* restoring the SCFA-producing bacteria or from directly transferring SCFAs thereby restoring the essential metabolites.

Bile acids facilitate digestion of dietary fats and oils but also serve as signaling molecules with important roles such as immune regulation and modulation, induction of epithelial integrity, protection against pathogenic overgrowth, and energy metabolism (34). The liver synthesizes and conjugates primary bile acids (PBAs) to be stored in the gallbladder between meals.

During a meal the bile acids are secreted into the gut, however, 95% of the PBA are reabsorbed to enter the enterohepatic circulation. The remaining are converted to secondary bile acids (SBAs) through enzymatic processes, which are provided by the intestinal bacteria. The use of antibiotics may lead to the destruction of secondary bile acid-producing bacteria, resulting in decreased levels of secondary bile acids and increased levels of primary bile acids (25).

Both PBAs and SBAs have important roles in suppressing bacterial overgrowth, as well as inducing and maintaining epithelial integrity. Furthermore, it has been demonstrated that SBAs promote the generation of peripheral regulatory T cells which in turn enhance the colonic immunity (35). In addition, they regulate cholesterol, glucose and energy homeostasis, and inhibit proinflammatory transcription genes (36). PBAs generally promote *C. difficile* spore germination, while SBAs are inhibitors of *C. difficile* spore germination (25,34,37). FMT is thought to restore bile acid metabolism by directly transferring both primary- and secondary bile acids into the donor or indirectly by correcting the dysbiotic condition.

RESTORATION OF IMMUNE FUNCTION

In a healthy physiological state, the microbiota signals to the immune cells to support their development and to fine-tune the immune responses. In return, the immune system helps to maintain a stable microbial community and promotes the growth of beneficial commensal microbes (38). It has been estimated that about 75% of the immune cells are located in the intestine, in this regard it can be said that a well-developed microbial community is associated with a sophisticated immune system, which in turn leads to a stable and good health (32). Healthy crosstalk between the immune system and the intestinal microbiota is essential to support the protective responses to pathogenic microbes, to promote tolerance to beneficial microbes and their products, and to maintain self-tolerance (39).

Disturbance in the function and composition of the microbiota, activates an aggressive immune response in the intestinal mucosa that may in turn cause chronic inflammation and subsequently lead to mucosal lesions (40). Correction of the dysbiotic condition by FMT normalizes and maintains the function of immune cells (32)

FMT is known to increase the diversity of the microbiota, maintain the ecological balance, and to rebuild the immune function. The related mechanism may involve the introduced (donor stool) microbiota’s capacity to maintain mucosal integrity, limit permeability, and inhibit apoptosis to reestablish intestinal barrier. The introduced microbiota may also better combat proinflammatory cytokines by synthesizing antagonizing factors, thereby reducing local and systemic inflammatory responses. FMT also restores the metabolism of secondary bile acids, competes with pathogenic bacteria, and improves insulin resistance, as a result, the immune function becomes improved (41).

4.2 | FMT PROCEDURE

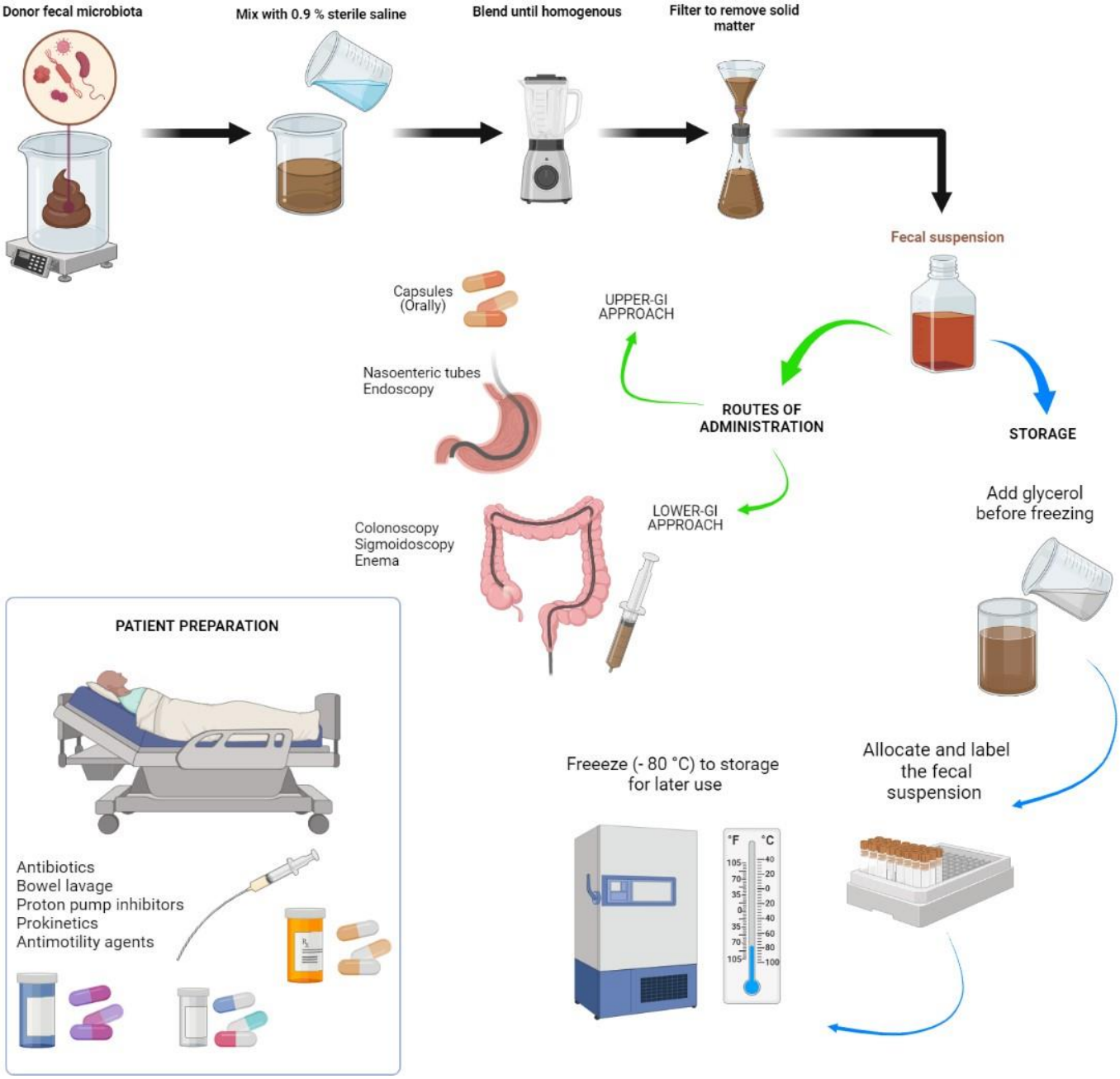

Figure 4. An overview of FMT's procedural part and the handling of donor fecal matter

4.2.1 | DONOR SELECTION

The different recommendations between medical societies, the lack of strong evidence-based guidelines, and the lack of standardization make the selection process for a suitable donor challenging. It is worth mentioning that there are currently no regulatory guidelines for fecal or donor screening. Despite the inconsistency by different working groups, there are some general criteria that form the basis for most of the recommendation/guidelines provided by experts within the field.

The European FMT Working Group recommends that potential donors go through four consecutive steps to be selected, as follows:

- Preliminary interview
- General clinical examination
- Blood and stool examination
- Further questionnaire at the day of donation

Donor selection for FMT has two main purposes, the first is to prevent potential adverse events associated with infusion of fecal material, and the second is to avoid the transmission of pathogenic microbes (42,43). For this reason, the European FMT Working Group recommends general exclusion criteria for the selection of potential donors, regardless of the indication (43). The exclusion criteria in the preliminary interview are summarized in Table 1.

The selection process begins with a medical interview focusing on potential donors' medical history and lifestyle habits to identify risk factors. This first step in the donor evaluation process and the use of exclusion criteria is of great value in excluding issues that cannot be detected by laboratories. To take a step further to reduce the risk of transmitting donor comorbidities, subjects aged 18–60 years are preferred. However, the age limit used for the inclusion criteria for eligibility should be optional to enable the inclusion of suitable healthy relatives or acquaintances (42,43).

Potential candidates selected based on the results of the preliminary questionnaire will undergo both blood and stool tests (Table 2), which should be done no longer than 4 weeks before donation. Under certain circumstances and when there are no changes in the donor's health, this testing can be done for up to 8 weeks before donation. However, after the first stool donation, repeated tests are required before further donation can be made. The main purpose of this step is to identify potentially communicable diseases that can be transmitted via FMT. Some of the tests are mandatory and some should be directed according to geographical location, the clinical condition of the recipient (e.g., immunosuppressed), and the medical history of the donor (42,43).

When all tests are negative, the potential candidate becomes an accepted stool donor. Finally, on the day of the donation the selected donors will have an additional interview to ensure the safest possible procedure. This interview (Table 3) is done to check if any new issues have emerged (43).

Table 1. Exclusion criteria for potential donors addressed in the preliminary interview (43)

INFECTIOUS DISEASES
▫ History or known exposure to: HIV, HBV, HCV, HTLV-1, HTLV-2, Malaria, Syphilis, Trypanosomiasis, Tuberculosis
▫ Known systemic infection not controlled at the time of donation
▫ Use of illegal drugs
▫ Risky sexual behavior
▫ Previous reception of tissue/organ transplant
▫ Previous (<12 months) reception of blood products
▫ Recent (<6 months) needle stick accident
▫ Recent (<6 months) body tattoo, piercing, earring, acupuncture
▫ Recent medical treatment in poorly hygienic conditions
▫ Risk of transmission of diseases caused by prions
▫ Recent parasitosis or infection from *Rotavirus*, *Giardia lamblia* and other microbes with GI involvement
▫ Recent (<6 months) travel in tropical countries, or at high risk of communicable diseases or traveler's diarrhea
▫ Recent (<6 months) history of vaccination with a live attenuated virus, if there is a possible risk of transmission
▫ Healthcare workers (to exclude the risk of transmission of multidrug-resistant organisms)
▫ Individual working with animals (to exclude the risk of transmission of zoonotic infections)
GI, METABOLIC AND NEUROLOGICAL DISORDERS
▫ History of IBS, IBD, functional chronic constipation, coeliac disease, other chronic GI disorders
▫ History of chronic, systemic autoimmune disorders with GI involvement
▫ History of, or high risk for, GI cancer or polyposis
▫ Recent appearance of diarrhea, hematochezia
▫ History of neurological/neurodegenerative disorders
▫ History of psychiatric conditions
▫ Overweight and obesity (body mass index >25)
DRUGS THAT CAN IMPAIR GUT MICROBIOTA COMPOSITION
▫ Recent (<3 months) exposure to antibiotics, immunosuppressants, chemotherapy
▫ Chronic therapy with proton pump inhibitors

GI, Gastrointestinal; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; HTLV-1, -2, Human T-cell lymphotropic virus type 1, type 2; IBD, Inflammatory bowel disease; IBS, Irritable bowel syndrome

Table 2. Tests to check if there are any infectious diseases that can be potentially transmitted (43)

BLOOD TESTS — GENERAL	**STOOL TESTS — GENERAL**
▫ CBC with differential ▫ CRP and ESR ▫ Albumin ▫ Creatinine and electrolytes ▫ Aminotransferases, bilirubin, GGT, ALP ▫ Syphilis ▫ Hepatitis viruses (HAV, HBV, HCV, HEV) ▫ HIV-1 and HIV-2 ▫ *Entamoeba histolytica* ▫ *Cytomegalovirus, Epstein-Barr virus*	▫ Fecal occult blood testing ▫ Detection of *Clostridioides difficile* ▫ Detection of enteric pathogens, including *Salmonella, Shigella* ▫ *Campylobacter, E. coli* O157 H7, *Yersinia*, VRE, MRSA ▫ Gram-negative multidrug-resistant bacteria ▫ *Norovirus* ▫ Antigens ± acid-fast staining for *Giardia lamblia* and *Cryptosporidium parvum* ▫ Protozoa (including *Blastocystis hominis*) and helminths
BLOOD TEST — SPECIFIC SITUATIONS	**STOOL TEST — SPECIFIC SITUATIONS**
▫ Human T-lymphotropic virus types I and II antibodies ▫ *Strongyloides stercoralis*	▫ Calprotectin ▫ Detection of *V. cholera* and *L. monocytogenes* ▫ Antigens ± acid-fast staining for *Isospora*, *Microsporidia* ▫ *Helicobacter pylori* fecal antigen ▫ *Rotavirus*

HAV, Hepatitis A virus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HEV, Hepatitis E virus; MRSA, Methicillin-resistant *Staphylococcus aureus*; VRE, Vancomycin-resistant *Enterococci*; CBC, Complete blood count; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; GGT, Gamma-glutamyl-transferase; ALP, Alkaline phosphatase

Table 3. Issues to be addressed on the day of donation (43)
▫ Newly appeared GI signs and symptoms (e.g., diarrhea, nausea, vomiting, abdominal pain, jaundice)
▫ Newly appeared illness or general signs as fever, throat pain, swollen lymph nodes
▫ Use of antibiotics or other drugs that may impair gut microbiota, new sexual partners or travels abroad since the last screening
▫ Recent ingestion of a substance that may result harmful for the recipients
▫ Travel in tropical areas
▫ Contact with human blood (sting, wound, showing, piercings, tattoos)
▫ Sexual high-risk behavior
▫ Diarrhea (more than three loose or liquid stools per day) among members of the entourage (including children) within 4 weeks of donation

4.2.2 | PREPARATION OF FMT MATERIAL

Although FMT is becoming the standard of care in both Europe and the USA for the treatment of rCDI, there is still a lack of standardization regarding FMT product preparation. In terms of stool volume, single or pooled donor, anaerobic or aerobic conditions, and reconstitution method, different medical centers have their own preferred processes (25).

There is a minimum of general steps that need be followed for the preparation of fresh and frozen fecal material according to the guidelines from the European FMT working group. The steps are summarized in Table 4 (43).

Table 4. Minimum general steps to follow for the preparation of fresh and frozen fecal material (43)
FRESH FECAL MATERIAL
▫ Fresh stool should be used within 6 hours after defecation
▫ To protect anaerobic bacteria, the storage and preparation should be as brief as possible
▫ Until further processing, the stool sample can be stored at ambient temperature (20°C–30°C)
▫ Anaerobic storage and processing should be applied if possible
▫ A minimum amount of 30 g of feces should be used
▫ Fecal material should be suspended in saline using a blender or manual effort and sieved to avoid the clogging of infusion syringes and tubes
▫ A dedicated space, disinfected using measures that are effective against sporulating bacteria, should be used
▫ Protective gloves and facial masks should be used during preparation
FROZEN FECAL MATERIAL
▫ At least 30 g of donor feces and 150 mL of saline solution should be used
▫ Before freezing glycerol should be added up to a final concentration of 10%
▫ The final suspension should be clearly labelled and traceable, and stored at –80°C
▫ On the day of fecal infusion, fecal suspension should be thawed in a warm (37°C) water bath and infused within 6 hours from thawing
▫ After thawing, saline solution can be added to obtain a desired suspension volume
▫ Repetitive thawing and freezing should be avoided

The amount of fecal matter used varies from study to study, with most studies using 30–50 g of fecal material. After thorough testing and collection of the fecal sample, it is then diluted with normal sterile saline solution (0.9 %) with a ratio of solute to solvent of 3-5:1. The solution is then mixed in a blender to get it homogenized and filtered through a gauze or the like to remove larger particles. Lastly, the final suspension is poured into a sterile syringe, ready to be infused into the patient. When preparing frozen fecal material, glycerol should be added before freezing up to a final concentration of 10 % and stored at –80 °C.

When the frozen fecal material is ready to use, it should be thawed in a warm water bath (37 °C) and infused within 6 hours from thawing. The fresh fecal material should be used within 6 hours after donation. The sensitivity of the microbial cells increases after defrosting, to avoid wastage of material, repeated freezing and thawing should be avoided, and samples should be prepared in doses that will be needed (3,28,43,44).

4.2.3 | PREPARATION OF THE RECIPIENT

Patients with CDI should be pre-treated with antibiotics such as vancomycin or fidaxomicin at least 3 days prior to FMT to eradicate/suppress the abundance of vegetative *C. difficile.* The antibiotic regimen should be discontinued 12–48 hours before fecal transfusion (43). Antibiotics should be avoided within 8 weeks after FMT, as it could increase the risk of FMT failure (25).

When performing colonoscopy, bowel lavage should be performed in order to purge bowel toxins and flush out any residual pathogenic microbiota as much as possible. It is reasonable to do so even when using the upper GI route in the treatment of rCDI as it is able to decrease the abundance of *C. difficile* in the intestine (43,45).

In case of FMT delivery through the upper GI route, the use of proton-pump inhibitors may increase the survival of the transplanted microbiota from gastric acid. Some studies have recommended the use of antimotility agents such as loperamide in order to maximize retention for at least four hours of the transplanted fecal microbiota (23,28,46).

The preparation of CDI patients described above also applies to other indications treated with FMT.

4.2.4 | ROUTES OF ADMINISTRATION

Several routes have been studied for the administration of fecal material, with the intention of having three main targets in the GI tract including the upper, middle, and lower intestines. Oral ingestion of FMT capsules is a means of delivery through the upper gut (16,47). Some other methods of targeting the upper GI tract above the second part of the duodenum include the use of a nasogastric tube (NGT) and a nasoduodenal tube (NDT). Mid-gut delivery, beyond the second part of the duodenum is done through endoscopy, nasojejunal tube (NJT), mid-gut transendoscopic enteral tubing (TET), small intestine stoma or percutaneous endoscopic gastro-jejunostomy.

Fecal microbiota can also be delivered to the lower (large) intestines by colonoscopy, enema, distal ileostomy, colostomy, and colonic TET (16,48). For each mode of administration, fecal infusions may be repeated if a single course fails to cure the condition being treated (42).

There is no single best universal delivery method that matches all patients, instead the decision on the optimal route should be individualized, based on the clinical condition, and on the currently available evidence-based resources. The main advantages and disadvantages of the different administration routes are summarized in Table 5.

Table 5. Advantages and disadvantages of different modes in FMT administration

DELIVERY METHODS	ADVANTAGES	DISADVANTAGES
Oral Capsules	▫ Non-invasive ▫ High patient acceptability ▫ No sedation risks	▫ Large capsule burden ▫ Risk of vomiting ▫ Expensive, but eliminates cost of endoscopic procedures
Naso-gastric/ -duodenal/-jejunal tube	▫ Avoids sedation ▫ Alternative to lower route (e.g., due to inflamed colon)	▫ Discomfort of tube placement ▫ Risk of vomiting and aspiration
Enema	▫ Less expensive ▫ Less invasive ▫ No sedation risks ▫ Can be performed in outpatient setting ▫ Can more easily be repeated	▫ Limited to distal colon ▫ May require multiple treatments
Colonoscopy	▫ Directly evaluates colon mucosa ▫ Sampling tissue ▫ Can reach proximal colon	▫ Invasive ▫ Expensive ▫ Sedation risks ▫ Standard risk of colonoscopy

4.3 | SAFTEY OF FMT

In general, the current evidence implies that FMT overall is a safe and well-tolerated procedure and treatment modality with few adverse events (AE). The most reported AEs have been immediate, mild and self-limiting. However, there are concerns related to both the procedural part and the transplanted fecal matter, which are summarized in Table 6. One of the most feared AE related to FMT is the transmission of life-threatening infections. There is a lack of data on long-term complications and there may therefore be unidentified risks of FMT that need to be considered in the screening protocol and discussed with the recipients. To reinforce the latter, the potential AEs should be clearly discussed through a documented process of informed consent with the patient (recipient). Some of the considerations are summarized in Table 7 (49).

Table 6. Summary of adverse event of fecal microbiota transplantation

MINOR AE (common)	Serious AE (rare)	Potential AE
▫ Abdominal pain/discomfort ▫ Altered bowel habits ▫ Bloating ▫ Borborygmus ▫ Constipation ▫ Diarrhea ▫ Flatulence ▫ Nausea/Vomiting (mostly with upper routes) ▫ Transient low-grade fever	▫ Endoscopic complication (perforation, bleeding) ▫ Sedation related (aspiration) ▫ High-grade fever ▫ Infection, sepsis ▫ Transmission of enteric pathogens ▫ IBD flares ▫ CMV reactivation ▫ Death ▫ Pneumonia	▫ Induction of chronic diseases ‣ Metabolic syndrome ‣ Diabetes ‣ Cancer ‣ IBD ‣ NAFLD ‣ IBS ‣ Other ▫ Transmission of unrecognized infectious agents

AE, Adverse event; IBD, Inflammatory bowel disease; CMV, Cytomegalovirus; IBS, irritable bowel syndrome; NAFLD, Non-alcoholic fatty liver disease

Table 7. Discussing considerations for informed consent (49)

CATEGORY	CONSIDERATIONS	
Procedure-related risk	▫ Aspiration ▫ Perforation of bowel	
Symptoms post-FMT	▫ Diarrhea ▫ Constipation ▫ Cramping ▫ Discomfort	▫ Belching/bloating/flatulence ▫ Nausea/vomiting ▫ Borborygmus ▫ Fever of unknown origin
Infection risks	▫ Life-threatening sepsis ▫ Antibiotic-resistant infections ▫ STEC ▫ EPEC ▫ *Blastocystis* spp.	▫ CMV/EBV infection in immunocompromised ▫ COVID-19/SARS-CoV-2 ▫ Future novel pathogens
Theoretical risks	▫ Allergy or anaphylaxis ▫ Small-intestinal bacterial overgrowth ▫ IBD flare ▫ Autoimmune disease	▫ Transmission of noninfectious disease / phenotype ▫ Limited evidence on long-term safety outcomes ▫ Unknown risks
Patient factors	▫ Post-infectious IBS ▫ Treatment failure and appropriate follow-up	

Although FMT is considered a safe procedure with minor AEs, it is still noteworthy to mention that there have been cases of serious adverse events (SAE) and even a few cases of death. Some of the cases but not all might be attributed to FMT directly, but with the rest there is still some uncertainty. In June 2019, the FDA issued a warning about two cases of bacteremia from extended-spectrum beta lactamase (ESBL)-producing *Escherichia coli* (*E. coli*). Both subjects were immunocompromised and received investigational FMT from the same donor, unfortunately this resulted in the death of one of the patients. Consequently, the FDA released a list of minimum screening requirements including improved screening for donor feces and colonization of multidrug-resistant organisms (MDRO) (50,51). Screening for such organisms is already recommended by the European FMT Working Group and has been standard practice at a universal stool bank (OpenBiome, Cambridge, MA) (43,52).

In March 2020, the FDA issued another safety alert regarding the potential risk of life-threating infection caused by enteropathogenic *E. coli* (EPEC) and Shiga toxin-producing *E.coli* (STEC). Six patients developed infections caused by EPEC (n = 2) and STEC (n = 4), four of the six patients required hospitalization and two patients infected with STEC died (53,54). All four patient who developed STEC infection received FMT from a single donor who had previously been screened negative for Shiga-like toxin by enzyme immunoassay (EIA). Subsequently, the stool was tested positive with the more sensitive nucleic acid amplification testing (NAAT) (49).

The two patients who died did not have their stool tested but retained stool samples that were used and administered to the patients were tested with NAAT and found to be positive for one patient and negative for the other patient. In this regard, there is uncertainty as to whether the cause of death of the positive STEC patient was really the infection which was the main contributing factor.

For the negative STEC patient, the FDA did not suspect that STEC was transmitted by this FMT product (49,53,54). There have also been cases of SAEs related to the procedural risks. Two deaths of fecal aspiration pneumonia administered to the mid-gut via upper endoscopy or NDT have been reported. Consequently, it is now advised to avoid sedation and the use of antiemetic agents in upper GI delivery (55).

The COVID-19 pandemic has raised many concerns, including emerging concerns about future pathogens that may have an impact on FMT. Therefore, it is essential to continuously update the screenings and test accordingly, so that they are as up to date as possible in order to reduce the risk of transmission of unwanted pathogen, especially in situations where new ones emerge.

5 | CLINICAL APPLICATION OF FMT IN GI DISEASES

5.1 | CLOSTRIDIOIDES DIFFICILE INFECTION

C. difficile is a Gram-positive, obligatory anaerobe, toxin-producing, and spore-forming bacillus. It is the causative agent of *C. difficile* infection (CDI) that can lead to pseudomembranous colitis which could be a life-threatening disease.

The perturbation of the intestinal microbiota in CDI has been well documented and it is the prototype disease for treatment with FMT (56). Key events in the disease pathogenesis are characterized by both quantitative and qualitative loss of the microbiota, leading to dysbiosis and predisposing the individual to colonization and sporulation of *C. difficile*. This in turn leads to reduced microbial competition for available space and vital substrates, resulting in *C. difficile* outgrowth and toxin production.

The clinical presentation of CDI ranges from mild diarrhea to pseudomembranous colitis. CDI represents the leading cause of nosocomial infectious diarrhea and is associated with significant morbidity and mortality (57). The disease incidence has increased radically over the past three decades along with increased hospitalization of affected patients (58). The incidence of community-acquired CDI is rising in both Europe and USA (25). It is noteworthy that a significant portion of the infected individuals did not carry the typical risk factors for CDI such as antibiotic use and hospitalization (59). However, 31% of community-acquired *C. difficile*-infected patients who were not exposed to antibiotics received PPI (60). PPIs are known to alter the intestinal microbiota with a significant reduction in microbial diversity, which in turn leads to an increased risk of acquiring CDI (61).

A recent systematic review identified independent risk factors for acquiring CDI including antibiotic use, recent hospitalization, increasing age, female sex, and the use of PPIs (62). In fact, CDI only develops in individuals with a disrupted intestinal microbiota (63).

Antibiotics remain the primary therapy strategy in the management of the first CDI episode. However, it is important to remember to use narrow-spectrum antibiotics and to consider immune-enhancing strategies as a preventive measure. The main principles of managing rCDI are as follows: use of narrow-spectrum antibiotics for the first episodes, treat recurrent CDI with preventive measures, try to eliminate risk factors which may contribute to the recurrences. With regard to the treatment of the first, second and subsequent rCDIs with antibiotics, the strength of recommendation and the quality of evidence are weak and low, respectively (64,65).Vancomycin or fidaxomicin are the drugs of choice, alternatively metronidazole may be used when the others are not available. In the first CDI episode, a 10-day course of either vancomycin or fidaxomicin may be used. For recurrent CDI, the same antibiotics are used but with an extended course with a gradually reduced and pulsatile regimen.

Since the use of antibiotics is one of the major risk factors for acquiring CDI, its current management seems rather counterintuitive as the first line of treatment consists of antibiotics. Antibiotics lack the ability to discriminate between pathogenic and beneficial bacteria. Hence, when used to treat infections, they destroy not only the targeted pathogen but also certain unintentional species leading to a perturbed microbial community.

The global recurrence rate of rCDI in patients after their first incidence of CDI is about 10–20% (62). Repeated antibiotic regimens cause further disturbances and an inadequate recovery of the intestinal microbiota, enabling regrowth of antibiotic-resistant *C. difficile* spores (25,66). This explains the extremely high recurrence rate in subsequent episodes, along with the fact that it also becomes more difficult to treat. To highlight the problem of recurrence, it has been shown that there is an emergence of multiresistant and novel hypervirulent strains of *C. difficile*. Compounding this with the current antibiotic crisis, there is an increased threat of severe CDIs with increased morbidity and mortality as the clinical success rate tends to decrease with each antibiotic treatment. Consequently, more effective therapies have been proposed with the main goal of restoring or balancing the intestinal microbiota.

As the main goal of treating CDI is to restore the composition and function of the intestinal microbiota to a pre-disease (pre-antibiotic) state, FMT seems to be the ideal treatment modality as it is a restorative treatment as opposed to antibiotics which is a disruptive treatment. Since 2013, FMT has evolved dramatically from a little explored and unknown to the West, to almost a mainstream therapy of global interest.

To date, based on evidence-based research, the only formally recognized indication for FMT is recurrent C. difficile infection. However, different medical societies differ in when FMT is to be introduced in the management of rCDI (Table 8).

Table 8. Indications for FMT in the management of CDI from medical societies worldwide

Guideline (Year)	Initiating FMT	Strength of recommendation & Quality of evidence
FGFT (2016) (67)	FMT is indicated in multiple rCDI (defined as > 1 recurrence), performed only after failure of standard treatment with antibiotics	NS
European FMT Working Group (2017) (43)	FMT is recommended as treatment option for both mild and severe rCDI	Recommendation: Strong Evidence: High
	FMT can be considered as a treatment option for refractory CDI	Recommendation: Strong Evidence: Low
Joint BSG/HIS FMT Working Group (2018) (68)	FMT is recommended after at least two rCDI, or after one rCDI with high risk for further episodes including severe and complicated CDI	Recommendation: Strong Evidence: High
	FMT should be considered in refractory CDI	Recommendation: Strong Evidence: Moderate
	Repeated FMT is recommended after initial FMT failure	Recommendation: Strong Evidence: High
IDSA/SHEA (2021) (69)	FMT is recommended after the 3rd CDI episode who have failed appropriate antibiotic treatment	Recommendation: Strong Evidence: Moderate
ESCMID *Clostridioides difficile* Study Group (2021) (70)	FMT as an option should be included after second or subsequent rCDI	Recommendation: Weak Evidence: Moderate
	FMT may be a rescue therapy for patients with severe complicated CDI who failed antibiotic treatment and for whom surgery is not feasible	Recommendation: Weak Evidence: Very low
American College of Gastroenterology (2021) (71)	FMT should be considered for patients with severe and fulminant CDI refractory to antibiotic therapy	Recommendation: Strong Evidence: Low
	FMT is recommended in second or subsequent rCDI	Recommendation: Strong Evidence: Moderate
	Repeated FMT is recommended in rCDI within 8 weeks of an initial FMT	Recommendation: Conditional Evidence: Very low
Australian Working Group (2020) (72)	FMT is recommended for patients with recurrent CDI	Recommendation: Strong Evidence: High
	FMT should be considered in patients with refractory or severe CDI	Recommendation: Strong Evidence: High
	For the treatment of CDI, repeated FMT should be considered for patients who do not respond to initial therapy	Recommendation: Strong Evidence: Moderate
Korean Working Group (2022) (73)	FMT is recommended in second or subsequent rCDI	Recommendation: Strong Evidence: High
	FMT should be considered for refractory or severe CDI	Recommendation: Strong Evidence: High

FMT, Fecal microbiota transplantation; NS, not specified; rCDI, recurrent *C. difficile* infection; CDI, *Clostridioides difficile* infection; FGFT, French Group of FMT; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; BSG, British Society of Gastroenterology; HIS, Healthcare Infection Society; IDSA, Infectious Diseases Society of America; SHEA, Society for Healthcare Epidemiology of America

A recent systematic review with meta-analysis evaluated the overall effectiveness and safety of FMT (74). It included a total of 15 studies with 12 clinical trials (Table 9) and three cohorts. The primary outcome of the meta-analysis was to demonstrate the effectiveness of FMT defined as the absence of diarrhea between weeks 8 and 13 post-FMT. Secondary outcomes included the most effective route of administration and the proportion of subjects who experienced any AEs. The authors also assessed the quality assessment of the included articles, which demonstrated a remarkable homogeneity with a low overall bias. In addition, all the studies had “low concern” with regards to applicability.

All the included studies demonstrated effectiveness in treating CDI. The mean treatment effectiveness was 82% and it was demonstrated that its efficacy increased with the number of doses received, implying a highly effective treatment of CDI. Overall, the analysis demonstrated that FMT is equivalent to or superior to the first-line antibiotic regimens. AEs were reported in 14 of the 15 articles. The most common organ system affected was GI tract along with the most common symptom being diarrhea. Majority of the AEs were mild and self-limiting within days. The proportion of patients experiencing at least one AE in association with the route of administration is in descending order as follows: colonoscopy (71.6%), enema (37.1%), oral capsule (23.1%), and upper endoscopy (3.4%). Although endoscopy showed a low number of AEs, it is noteworthy to mention that these patients experienced clinically more severe AEs (e.g., fecal aspiration) comparing to other routes of administration. Colonoscopy is considered as the choice of route of administration for FMT delivery, as it allows the physician to directly observe the colon and collect samples as needed. When comparing colonoscopy to enema and upper endoscopy, it was shown to be superior to both (74). However, colonoscopy versus upper endoscopy had conflicting results, as another study demonstrated no statistically significant difference between these routes in the efficacy of FMT treating CDI (75). Finally, comparing FMT capsules to colonoscopy was shown to be statistically significant and demonstrated that capsules are more effective than colonoscopy (74).

In conclusion, FMT is a well-established treatment modality for the management of rCDI. Although antibiotics continue to provide the mainstream therapy to treat the first episode of CDI, due to its high efficacy and superior safety profile, FMT should be considered earlier in the management of rCDI instead of using it as a last resort. In addition, there is a need to standardize and uniform FMT protocols and to open more universal stool banks in order to make it more accessible.

Table 9. Summarized findings from RCTs investigating FMT's effectiveness and safety profile in CDI

Author (year)	*N*	Route	Recovery	Adverse events
Garza-González *et al.* (2019) (76)	Total: 21 FMT: 13 FMT-L: 8	Capsules	Overall efficacy 95.2%	Minor AEs Burping: 14.2% (3/21) Constipation: 19% (4/21) Vomiting: 9% (2/21)
Dubberke *et al.* (2018) (77)	Total: 127 FMT-A: 41 Placebo-B: 44 FMT-C: 42	Enema	Overall efficacy: 89% FMT-A: 61% Placebo-B: 45% FTM-C: 67%	Total AEs: 64.1% Total SAEs: 20.3% - FMT-A: 31.7% - Placebo-B: 13.6% - FMT-C: 16.3 %
Ianiro *et al.* (2018) (78)	Total: 56 FMT-S: 28 FMT-M: 28	Colonoscopy	FMT-S: 75% FMT-M: 100%	FMT-S - Mild diarrhea: 64% (18/28) - Constipation: 61% (17/28) FMT-M - Mild diarrhea: 71% (20/28) - Constipation : 82% (23/28)
Juul *et al.* (2018) (79)	Total: 20 FMT: 9 Metronidazole: 11	Enema	Overall efficacy: 78% FMT: 56% Metronidazole: 45%	FMT: 11.11% (Foul stool smell) Metronidazole: N/S
Jiang *et al.* (2018) (80)	Total: 65 Lyophilized capsule: 31 Frozen enema: 34	Enema	Overall efficacy: 86% Lyophilized capsules: 84% Frozen enema: 88%	Total SAEs: 14% Lyophilized capsules: 13% Frozen enema: 15%
Jiang *et al.* (2017) (81)	Total: 72 Fresh: 25 Lyophilized: 23 Frozen: 24	Colonoscopy	Overall efficacy: 87% Fresh: 100% Lyophilized: 78% Frozen: 83%	Mild diarrhea: 86% (62/72) Fatigue: 8% (6/72) Headache: 6% (4/72) Weight gain: 3% (2/72)
Kao *et al.* (2017) (82)	Total: 105 (116) Capsule: 57 Colonoscopy: 59	Colonoscopy Capsule	Overall efficacy: 96% Capsule: 96% Colonoscopy: 96%	Minor AEs Capsule: 5.4% Colonoscopy: 12.5%
Hota *et al.* (2017) (83)	Total: 28 (30) FMT: 16 Vancomycin: 12	Enema	FMT: 43.7% Vancomycin: 58.3%	Early AEs: 57% Late AEs: 48%
Kelly *et al.* (2016) (84)	Total: 46 FMT: 22 Autologous FMT: 24	Colonoscopy	Donor FMT: 90.9% Autologous FMT: 62.5%	Most common reported AEs in both groups: abdominal pain, fatigue, flatulence, and diarrhea SAEs - FMT: 4 (none related to FMT) - Autologous FMT: 3
Lee *et al.* (2016) (85)	mITT - Total: 219 Fresh: 111 Frozen: 108 Protocol - Total: 178 Fresh: 87 Frozen: 91	Enema	mITT Fresh: 70.3% Frozen: 75% Protocol Fresh: 85.1% Frozen: 83.5%	Most common minor-moderate AEs: - Diarrhea: 70% - Flatulence: 25% - Constipation: 20% - Abdominal cramps: 10% - Nausea: < 5% SAEs: - Fresh: 3% - Frozen: 5 %
Orenstein *et al.* (2016) (86)	Total: 31	Enema	Overall efficacy: 87.1%	Total 188 AEs in 28 (90%) patients Total of 20 SAEs in 7 (23%) patients
Cammarota *et al.* (2015) (87)	Total: 39 FMT: 20 Vancomycin: 19	Colonoscopy	FMT: 90% Vancomycin: 26%	Mild, self-limiting AEs (FMT) - Diarrhea: 94% - Bloating and cramps: 60%

mITT, modified intention-to-treat; AEs, Adverse events; SAEs, Serious adverse events
FMT-L = L, *Lactobacillus* ; FMT-A, Placebo-B, FMT-C = A,B,C designated group; FMT-S, FMT-M = S, single, M, multiple

5.2 | INFLAMMATORY BOWEL DISEASE

In addition to the well-established indication for rCDI, there are several emerging clinical conditions for which FMT could be a promising alternative to the current standard regimen or even as an adjunct therapy. Many recent studies have focused on using FMT to manage inflammatory bowel disease (IBD).

IBD is the umbrella term for disorders that cause chronic inflammation of the mucosal lining of the GI tract from mouth to anus. It is an incurable chronic inflammatory, destructive disease with a relapsing-remitting pattern characterized by inflammation of the mucosa of the GI tract. There are mainly two subtypes of IBD, namely ulcerative colitis (UC) and Crohn's disease (CD) which are primarily differentiated by the location and depth of the mucosal inflammation.

The exact etiology of IBD is still unknown, but its pathophysiology is multifactorial and includes both genetic and environmental factors. It is postulated that in genetically predisposed individuals, the disease arises from an overly reactive immune response to the dysbiotic gut microbiota (88). There is supporting evidence that the microbiota plays a role in the pathogenesis of IBD (89), in addition, it has been proposed that the dysbiotic gut is a contributing factor to the development of the hyperreactive immune system in the GI tract (40). However, it is not clear whether the presence of a dysbiotic state in IBD patients, which usually manifests itself in an overall diminished diversity and loss of anaerobic bacterial species (90) is the consequence or the cause of the mucosal inflammation seen in IBD patients (91).

5.2.1 | ULCERATIVE COLITIS

There are currently five randomized controlled trials (RCTs) available with results that investigated the efficacy of FMT in UC (Table 10) (92–96). All five RCTs varied considerably in their study design, hence the interpretation is limited by their heterogeneity. However, there are certain interesting findings that have provided a deeper insight into donor selection and FMT processing for UC compared to CDI.

Four of the five studies showed superiority of FMT compared to placebo in achieving remission. The only study that showed insignificant results used an upper GI approach with NDT, which may imply that the method and route of administration may have an impact on the efficacy of FMT. It is noteworthy to point out that it is not the route itself but the method of administration that may have a greater impact, which has been demonstrated in the Haifer *et al.* study using an upper GI route with lyophilized oral capsules.

Costello *et al.* demonstrated high efficacy (clinical remission and steroid free response) of FMT (96), despite it being the only study that utilized aerobically prepared fecal material and had a lower number of treatments compared to the other RCTs that used lower GI-route. Donor characteristics appear to be a relevant factor, Moayyedi *et al.* reported higher treatment success with one particular donor compared to other donors (93). Selection of a suitable donor seems to have a greater value in UC than is the case for CDI.

A systematic review with meta-analysis that included four RCTs (93–96) demonstrated a pooled rate of achieving combined outcome (clinical remission with endoscopic remission/ response) were 27.9 % in the intervention arm and 9.5 % in the control arm (97).

Despite its moderate to high remission rates, FMT has still not entered clinical practice in the management of UC. This may be due to the heterogeneity of the study design, including but not limited to fecal material preparation, patient preparation, donor characteristics, single or pooled donor, delivery method, treatment regimen, and the definition of remission. The variability in the studies makes it a disadvantage to draw definitive conclusions. Therefore, future studies should be more consistent in the study design to ensure better replicability and reproducibility as well as to avoid systematic errors.

Another systematic review with a meta-analysis compared the efficacy and safety of FMT, tofacitinib, and biological agents (infliximab, adalimumab, golimumab, vedolizumab) in patients with UC (98). It included sixteen RCTs for efficacy analysis in which seven therapies (including placebo) were analyzed for their effectiveness by network meta-analysis. The findings demonstrated that all interventions were superior to placebo. Furthermore, the three best interventions with the greatest efficacy (clinical remission and clinical response) were FMT, vedolizumab, and infliximab. In terms of absolute effects and relative ranking, infliximab and vedolizumab showed better efficacy. Tofacitinib and FMT also showed high efficacy, but due to the limited RCTs for these interventions, an absolute conclusion cannot yet be drawn.

Based on the results of the above-mentioned studies and the meta-analysis, FMT appears to be beneficial and may play a role in the induction of remission in active mild-to-moderate UC. However, the lack of long-term follow-up data on safety profile together with the heterogeneity in the studies and small study groups contributes to an overall uncertainty with regard to its safety and efficacy.

Table 10. Summary of RCTs investigating the efficacy of FMT in UC

First author (year)	Eligibility for inclusion	No. Patients FMT/Placebo	Administration	Intervention	Placebo	Treatment regimen	Follow-up	Combined remission*	
								FMT	Placebo
Moayyedi (2015) (93)	Mild-to-moderate UC (Mayo score ≥ 4, with endoscopic subscore ≥1)	75 (38/37)	Enema	Aerobically prepared, fresh/frozen, single/pooled donor, allogenic stool	Water	6 (weekly)	Week 7	24 % (9/38)	5 % (2/37)
Rossen (2015) (94)	Mild-to-moderate UC (SCCAI 4–11, with endoscopic subscore ≥1)	48 (23/25)	NDT	Aerobically prepared, fresh, single donor, allogenic stool	Autologous stool	2 (wk. 0 and 3)	Week 12	30 % (7/23)	20 % (5/25)
Paramsothy (2017) (95)	Mild-to-moderate UC (Mayo score 4–10, with endoscopic subscore ≥1)	81 (41/40)	Colonoscopy + Enema	Aerobically prepared, frozen, pooled donor, allogenic stool	Saline + odorant + coloring	41 (Colonoscopy) wk. 0; Enemas 5/wk. for 8 weeks)	Week 8	27 % (11/41)	8 % (3/40)
Costello (2017) (96)	Mild-to-moderate UC (Mayo score 3–10, with endoscopic subscore ≥2)	73 (38/35)	Colonoscopy + Enema	Anaerobically prepared, frozen, pooled donor, allogenic stool	Aerobically prepared, autologous stool	3 (Colonoscopy) day 0; 2 (Enemas) over 7 days)	Week 8	32 % (12/38)	9 % (3/35)
Haifer (2021) (92)	Mild-to-moderate UC (Mayo score 4–10, with endoscopic subscore ≥1)	35 (15/20)	Orally	Lyophilized FMT capsules	Placebo capsule	Loading with 24 capsules daily for 1 week, then 12 capsules daily for 1 week, followed by a maintenance dose of 6 capsules daily thereafter	Week 8	53 % (8/15)	15 % (3/20)
							Week 56	100 % (4/4)	

*Combined remission implies to the response group that had both clinical and endoscopic remission
SCCAI, simple clinical colitis activity index; wk., Week; UC, Ulcerative colitis; FMT, Fecal microbiota transplantation; RCT, Randomized controlled trials; NDT, Nasoduodenal tube

5.2.2 | CROHN'S DISEASE

While a number of prospective cohorts, few case studies and some systematic reviews with meta-analysis have been presented, there is still a lack of adequate RCTs investigating FMT in CD patients. To date, only two completed RCTs (99,100) have been performed that explore the value of FMT in CD patients. On the other hand, there are currently 29 registered RCTs investigating the use of FMT in CD with or without other IBD subtypes on ClinicalTrials.gov.

Yang *et al.* compared the efficacy and safety of different routes of administration for FMT, demonstrating that there was no significant difference in the efficacy of FMT between the different administration routes. Overall, the clinical response rate was 77.8 % (21/27) and clinical remission 2 weeks post-FMT was achieved by 66.7% (18/27). Separating the clinical response and clinical remission for each route demonstrated 78.6% and 64.3% respectively in the colonoscopy group and 76.9% and 69.2%, respectively in the gastroscopy group (99). The RCT done by Sokol *et al.* investigated the efficacy of FMT on maintaining remission in CD and defined their primary endpoint as successful colonization of the donor microbiota at 6 weeks which none of the participants achieved (100). Although RCTs are considered to be in the higher level of evidence hierarchy, these trials are insufficient to draw any conclusions due to their small sample size (n = 21-31), in addition to the fact that one of the RCTs actually lacks a control group.

A recent systematic review with meta-analysis was done to evaluate the efficacy of FMT in CD, consisting of 15 studies published between 2014–2020 that included two RCTs and 13 cohorts (101). There were 10 studies that investigated patients with only CD and the remaining five studies included other IBD subtypes as well. However, the results on CD in the studies investigating several IBD subtypes were separated to include only the patients with CD in the final analysis. Six of the 13 cohorts obtained data from the same clinical trial issued in China, but only the most recently published one with the largest database were included in the analysis. Three studies were performed in the pediatric population and the remainder performed in adults. The final analysis included 10 studies with a total of 293 patients. The studies that were included in the final analysis are summarized in Table 11 and Table 12.

Given the positive findings from several studies on FMT to induce remission in UC patients, it is compelling to believe that similar optimistic results would be revealed in patients with CD. Unfortunately, the evidence to date is not as appealing as it is for UC for several reasons, including the heterogeneity of the study design, the characteristics, and the different FMT administration protocols. The marked variation between the studies limits the possibility of interpreting the findings. One thing that is certain is that larger, high-quality RCTs are needed to see if FMT has a place in CD management.

Table 11. Characteristics of selected studies investigating the efficacy of FMT in CD

First author (year)	Country	Study type	Sample size	IBD type	Population	Eligibility for inclusion	Concomitant therapy	
COHORTS								**Total quality score**
Suskind (2015) (102)	USA	Prospective non-comparative cohort	9	CD	Pediatric	Mild to moderate CD; PCDAI 10–29	Stable doses of CD drug therapy continued Exclusion criteria: prior use of biologic agent	Medium
Karolewska-Bochenek (2018) (103)	Poland	Prospective non-comparative cohort	2	CD/UC	Pediatric	CD or UC; Refractory to standard therapy; Colonic disease	Stable doses of CD drug therapy continued	Medium
Goyal (2018) (104)	USA	Prospective non-comparative cohort	7	CD/UC/IC	Pediatric	Mild to moderate IBD (CD, UC, IC); PCDAI 10–40; Biomarkers > × 2 upper limit	Stable doses of CD drug therapy continued	Medium
Wei (2015) (105)	China	Prospective non-comparative cohort	3	CD/UC	Adult	Mild to moderate CD; CDAI score of >150 to <400; C-reactive protein >10 mg/L	Stable doses of 5-ASA or corticosteroid continued Exclusion criteria: anti-TNF agent within 2 months	Medium
Vermeire (2016) (106)	Belgium	Prospective non-comparative cohort	6	CD/UC	Adult	IBD; Refractory to immunomodulators	CD drug therapy continued	Medium
Vaughn (2016) (107)	USA	Prospective non-comparative cohort	19	CD	Adult	Active CD; HBI ≥ 5; >3 years duration; Refractory to standard therapy	Stable doses of 5-ASA or thiopurines continued. Corticosteroids tapered to 20 mg of prednisone. 12-week washout for cyclosporine, tacrolimus, and biologic agents	Medium
Gutin (2019) (108)	USA	Prospective non-comparative cohort	10	CD	Adult	Active CD; HBI ≥ 3	Stable doses of CD drug therapy continued	Medium
Xiang (2019) (109)	China	Prospective non-comparative cohort	174	CD	Adult	CD with any therapeutic target	All CD drug therapy was ceased. Commenced corticosteroids ± azathioprine / thalidomide / exclusive enteral nutrition as part of 3rd stage of step up FMT	High
RCTs								**Risk of bias**
Yang (2019) (99)	China	Randomized, double blinded, parallel two-arm trial	31	CD	Adult	Mild to moderate CD; CDAI > 150; Colonic disease	Stable doses of 5-ASA, thiopurines or corticosteroid continued	High
Sokol (2020) (100)	France	Randomized, single-blind, placebo-controlled trial	21	CD	Adult	Active CD at screening (HBI > 4); Clinical response to corticosteroid induction (HBI < 5)	Corticosteroids were tapered Exclusion: anti-TNF agent within 1 month, immunosuppressants within 3 months.	High

CD, Crohn's disease; HBI, Harvey-Bradshaw index; FMT, fecal microbiota transplantation; UC, ulcerative colitis; IBD, inflammatory bowel disease; CDAI, Crohn's disease activity index; PCDAI, pediatric Crohn's disease activity index; IC, indeterminate colitis; RCT, randomized controlled trial

Table 12. Protocols of selected studies investigating the efficacy of FMT in CD

COHORTS

Study	Pre-FMT preparation	Pre-antibiotics	Route of administration	FMT regimen	Donor	Follow-up	Definitions		
							Clinical response	Clinical remission	Endoscopic endpoints
Suskind *et al.*(102)	PPI day before + morning of FMT. PEG 3 × day for 2 days prior to FMT	Yes	Nasogastric tube	Fresh, 30 g, single infusion	Relative; Single donor	2, 6 and 12 weeks	Engraftment	↓ PCDAI ≥ 10; ↓ CRP, ↓ Calprotectin	None
Karolewska-Bochenek *et al.* (103)	PPI + Antiemetic morning of FMT	No	NDT/ Gastroscopy	Frozen, 50 g, 8 doses (in 12 days)	Unrelated; Single donor	18–33 days	↓ PCDAI ≥ 15	↓ PCDAI ≥ 10; ↓ Calprotectin	None
Goyal *et al.* (104)	PPI 5 days prior to FMT for 7 days; Antidiarrheal 2 hrs. prior to FMT	Yes	Endoscope then colonoscopy	Fresh, 150 g, Single infusion	Relative/ acquaintance; Single donor	1 week, 4 weeks, 24 weeks	↓ PCDAI > 12.5	PCDAI 0; Normalization of biomarkers	None
Wei *et al.* (105)	Bowel lavage, PEG	Yes	NJT/ Colonoscopy	Fresh, 60 g, single infusion	Unrelated; Single donor	4 weeks	↓ CDAI > 70	CDAI < 150; CRP < 10 mg/L	None
Vermeire *et al.* (106)	Bowel lavage, PEG	No	NDT/ Ileocolonoscopy	Fresh, 200 g, 2 doses (1 day apparat)	Relative/ acquaintance; Single donor	8 weeks, 24 weeks	Bacterial richness as the No. of different OUT	Changes in CDAI, CRP	SES-CD < 3
Vaughn *et al.* (107)	Bowel lavage, Magnesium citrate	No	Colonoscopy	Froze, 50 g, single infusion	Unrelated men; Single donor	12 weeks, 26 weeks	↓ HBI > 3 w/o medication	HBI < 5	None
Gutin *et al.* (108)	Bowel lavage	Yes	Colonoscopy	Frozen, 250 cc single infusion	USB; Single donor	1, 3, 6, 9, and 12 months	↓ HBI ≥ 3	HBI < 3	SES-CD
Xiang *et al.* (109)	Antiemetic + PPi prior to FMT (except in NJT)	No	Endoscopy/ NJT/ Mid-gut or colonic TET	Fresh/Frozen/ Both, 60 cm^3, single/multiple (2-5) infusions	Relative/ acquaintance/ USB; Single donor	Median follow up 43 months	↓ HBI > 3	HBI ≤ 4	None

RCTs

Study	Intervention	Comparison	Pre-FMT preparations	FMT regimen	Donor	Follow-up	Definitions		
							Clinical response	Clinical remission	Endoscopic endpoints
Yang *et al.* (99)	Arm 1: Gastroscopy Arm 2: Colonoscopy	N/A	Arm 1: PPI Arm 2: Bowel lavage + Antidiarrheal	Fresh, 200 g, 2 doses (1 week apart)	Relative/ acquaintance/ volunteers; Single donor	1, 2, 4, 6, and 8 weeks	↓ CDAI > 100	CDAI < 150	Remission: SES-CD 0-2 Response: ↓ SES-CD > 50 %
Sokol *et al.* (100)	Colonoscopic FMT	Colonoscopic, Physiological serum	Bowel lavage, PEG	Fresh,50–100 g, single infusion	Volunteers; Single donor	2, 6, 10, 14, 18, and 24 weeks	N/A	N/A	CDEIS

PPI, Proton pump inhibitor; NS, Not specified; PEG, polyethylene glycol; N/A, Not applicable; OUT, Operational taxonomic units; USB, Universal stool bank; NJT, Nasojejunal tube; NDT, Nasoduodenal tube; CDAI, Crohn's disease activity index; CDEIS, Crohn's disease endoscopic index of severity; CRP, C-reactive protein; FMT, fecal microbiota transplantation; HBI, Harvey-Bradshaw index; PCDAI, pediatric Crohn's disease activity index; PEG, polyethylene glycol; SES-CD, simple endoscopic score for Crohn's disease; TET, transendoscopic enteral tubing; CD, Crohn's disease; CDAI, Crohn's disease activity index; CDEIS, Crohn's disease endoscopic index of severity; FMT: fecal microbiota transplantation; PEG: polyethylene glycol; SES-CD: simple endoscopic score for Crohn's disease

5.3 | IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome (IBS) falls under the umbrella term disorders of gut-brain interaction (DGBI), formerly known as functional gastrointestinal disorders (FGID). Since most IBS research studies investigating the effectiveness of FMT still use the older term FGID instead of DGBI, the term FGID will be used in this section for the sake of simplicity.

These conditions are diagnosed and classified according to the ROME IV criteria and are characterized by chronic GI symptoms in the absence of a detectable organic cause with conventional diagnostics. The current system (ROME IV) divides FGID into 20 pediatric and 33 adult conditions, of which the most common subtype is IBS. FGID is a widespread condition, with a worldwide prevalence of 40 % and is one of the most common conditions encountered by gastroenterologist (110).

IBS is the most prevalent subtype of FGID, and it is a common condition with a world prevalence of 5–10 % (111). Its symptoms are sometimes difficult to differentiate from other common GI conditions as it often overlaps with other FGIDs and sometimes even with IBD. Its course is typically a relapsing and remitting pattern with common symptoms such as abdominal discomfort, bloating, and altered bowel habits. Although the condition does not have a malignant course, it has an immense impact on the quality of life (QoL) and constitutes a high socioeconomic burden (112).

Some authors still like to subdivide IBS according to the older ROME III criteria, which classified IBS according to the predominant symptom in bowel habits including, constipation (IBS-C), diarrhea (IBS-D), IBS with mixed bowel habits (IBS-M), and unclassified type (IBS-U). In Rome IV criteria, functional bowel disorders including functional diarrhea, functional constipation, and IBS with all its subtypes are now considered to be in a continuum rather than as independent entities (113). However, for treatment purposes, dividing IBS based on the predominant symptom makes the therapeutic approach more feasible.

Current clinical management of IBS is often based on the prevailing symptomatology and includes both pharmacological and non-pharmacological approaches. Despite a high prevalence, the diagnosis and treatment are still a challenge and the integral to break this pattern is a high-quality consultation with a strong doctor-patient relationship.

The pathogenesis of IBS is thought to be multifactorial, although its precise mechanism remains unclear. It has been postulated that the most crucial abnormality is in the communication between the brain and the gut (and vice versa), as is suggested by the current valid term DGBI. The brain-gut axis is affected by emotions, which can influence the intestinal barrier, mucosal secretion, and intestinal motility (114).

Abnormal secretion of dopamine and serotonin has been reported in IBS patients, these alternations may have a role in the type of clinical presentation they will have (115). An increased permeability of the intestinal mucosa has been found to be associated with hypersensitivity to somatic and visceral stimuli. This leads to a change in the perception of pain, which have been demonstrated in IBS patients, particularly those with IBS-D subgroup (116).

Intestinal microbiota and its role in the IBS pathogenesis are not well defined. Studies confirm that there are certain alterations in the intestinal microbiota of IBS patients compared to healthy individuals (117–119) and that the microbiota is likely involved in the alteration of mucosal inflammation and in barrier function (120,121). There is a higher prevalence of bacterial overgrowth in the small intestine in IBS patients, although the causal and temporal relationship between these conditions remains unclear (122). Some of the environmental factors thought to be involved in triggering IBS include diet, food intolerance, use of antibiotics, infections, and psychosocial distress (112).

Lately, there has been an increased interest in utilizing FMT in the management of IBS. However, the findings have been inconsistent, which may be due to the heterogeneity of the study design together with the heterogenous nature of the pathogenesis and the clinical phenotype of IBS patients.

A recent systematic review conducted a meta-analysis of RCTs to assess the short- and long-term effects of FMT in IBS (112). The analysis included seven RCTs with a total of 472 IBS patients. Six were conducted in Europe and one in USA. Four of the seven studies were performed in a single center, the rest were performed in multiple centers ranging from two to three locations. Six studies used Rome III criteria to diagnose IBS and one used Rome IV criteria. Characteristics of included studies are summarized in Table 13.

Five of the seven RCTs demonstrated positive results for FMT, while the remaining two favored the placebo group. The mode of administration seems to play a role in the efficacy, as two out of three RCTs reported that FMT was inferior to placebo used capsule based FMT, and the third used colonoscopy. The analysis (112) demonstrated that fresh donor stool was superior to frozen fecal material, but the heterogeneity was high among the studies using frozen fecal material, which made it more difficult to confirm increased effectiveness of fresh fecal material.

The long-term efficacy of FMT demonstrated that it was not associated with improvement in global symptoms at 1-year follow-up. This may be explained by the chronic course of relapsing and remitting pattern, which is why a single treatment did not achieve the desired long-term effect. One of the RCTs found that IBS patients who responded to their first FMT treatment, showed an efficacy of 67% on the second treatment (123). This specifies the importance of repeated FMT regimen as an option for first responders to achieve long-term remission.

Donor selection was again shown to play a crucial role in the efficacy of FMT, as demonstrated by one of the RCTs (124). In the absence of clear criteria for selecting a super-donor, the study used both fecal bacterial profiling and clinical criteria in an attempt to select the donor who had positive factors and lacked negative factors known to affect the intestinal microbiota. The study showed a high response rate of over 76% when using the super-donor.

Some general conclusions that can be made are that FMT seems to have a role in certain IBS subtypes, and it seems to be donor-dependent much like IBD. The optimal route, together with doses and treatment regimens, should be further investigated to increase the efficacy of FMT in IBS management. In addition, because IBS is heterogenous in its presentation, a microbial mapping should be considered for both the donor and the recipient to have a better match between them *i.e.,* so that the donor complements the missing factors in the recipient. Finally, due to its chronic course, repeated FMT should be considered especially in those who responded to their first FMT treatment.

There has been a challenge to compare these RCTs because of the variations in the FMT protocols, donor selection criteria, and the treatment regimen along with doses used for the transplant. In addition, different measurements were used to assess the efficacy of these studies, and different IBS subtypes were included without separating the findings by their subtype. Regardless of their outcomes, all RCTs provide some essential information that can be used in future investigations to further improve the quality of the studies.

Table 13. Characteristics of RCTs investigating the efficacy of FMT in IBS

Author (Year)	Study	IBS criteria and subtypes	No.	Intervention	Control	Primary endpoint	Secondary endpoint	Follow-up and responders %
Halkjaer et al. (2018) (125)	Randomized with 1:1 allocation, in blocks, double-blinded, placebo-controlled	Rome III IBS-SSS ≥ 175 IBS-C 33.3 %, IBS-D 29.4 %, IBS-M 37.3 %	52	25 capsules, frozen, 50 g, 25 capsules/day for 12 days, pooled donor	25 placebo capsules daily for 12 d.	↓ IBS-SSS ≥ 50 points at 3 months	Change in IBS-QoL, microbiota profile	6 months
Johnsen et al. (2018) (126)	Randomized with 2:1 allocation, in blocks, double-blinded, placebo-controlled, parallel group study	Rome III IBS-SSS ≥ 175 IBS-D 53 %, IBS-M 47 %	83	Colonoscopy, fresh/frozen (1:1), 50–80 g single infusion, pooled donor	Colonoscopy,50–80 g, single infusion autologous stool	↓ IBS-SSS ≥ 75 points at 3 months	↓ IBS-SSS ≥ 75 points at 12 months	12 months FMT: 56.3 % Control: 35.7 %
Holster et al. (2019) (127)	Randomized with 1:1 allocation, in double-blinded study	Rome III IBS-C 25 %, IBS-D 56.2 %, IBS-M 18.8 %	16	Colonoscopy, fresh 30 g, single infusion, single donor	Colonoscopy,30 g, single infusion autologous stool	↓ GSRS-IBS ≥ 30 %	Change in IBS-QoL, IBS-SSS, anxiety, depression, barostat test, microbiota profile	6 months
Aroniadis et al. (2019) (128)	Randomized with 1:1 allocation, in blocks, double-blinded cross-over study	Rome III IBS-SSS ≥ 175 IBS-D 100 %	48	25 capsules, frozen, 28 g 25 capsules/day for 3 days, single donor	25 placebo capsules daily for 3 d.	↓ IBS-SSS ≥ 50 points at 3 months	Change in IBS-QoL, depression, anxiety, stool consistency, microbiota profile	6 months
Lahtinen et al. (2020) (129)	Randomized with 1:1 allocation, in blocks, double-blinded study	Rome III IBS-C 6.1 %, IBS-D 51 %, IBS-M 14.3 %, IBS-U 28.6 %	49	Colonoscopy, frozen 30 g, single infusion, single donor	Colonoscopy,30 g, single infusion, autologous stool	↓ IBS-SSS ≥ 50 points at 3 months	Change in IBS-QoL, depression, anxiety, stool consistency, microbiota profile	13 months FMT: 21.7 % Control: 30.8 %
El-Salhy et al. (2020) (124)	Randomized with 1:1 allocation, double-blinded study	Rome IV IBS-SSS ≥ 175 IBS-C 37.8 %, IBS-D 38.4 %, IBS-M 23.8 %	164	Gastroscopy (duodenum), frozen 30/60 g, single infusion, single (super) donor	Gastroscopy, single infusion, autologous stool	↓ IBS-SSS ≥ 50 points at 3 months	Change in IBS-QoL, dysbiosis index, microbiota profile	12 months FMT 30g: 58.2 % FMT 60g: 63.6 %
Holvoet et al. (2021) (123)	Randomized with 2:1 allocation, in double-blinded cross-over study	Rome III IBS-D, IBS-M, Refractory IBS with severe bloating	62	NJT (duodenum), fresh, single infusion, single donor	NJT, single infusion, autologous stool	Adequate relief of symptoms at 3 months	Change in IBS-QoL, IBS symptom, stool consistency, microbiota profile	12 months FMT: 11.6 % Control: 0 %

IBS-SSS, IBS-severity scoring system; IBS-QoL, IBS-Quality of Life; IBS, irritable bowel syndrome; GSRS, Gastrointestinal Symptom Rating Scale; NJT, Nasojejunal tube; IBS-D, Diarrhea-predominant; IBS-C, Constipation-predominant; IBS-M, Mixed type; IBS-U, Unclassified type; QoL, Quality of life;

CONCLUSION

In the field of chronic GI conditions, the outcomes of the currently used therapies are far from ideal and the promise of a new efficacious, readily available, and potentially curative treatment has been eagerly expected. One such emerging and promising therapeutic modality is FMT.

The current knowledge about the mechanistic effects that underpin the efficacy of FMT stems from results in studies investigating CDI. The high efficacy rate of FMT in CDI patients suggest that it is most likely driven by disease-specific factors rather than stool-specific factors. However, this has not been the case for IBD and IBS as it has been demonstrated that donor microbiota characteristics play a significant role in a successful FMT treatment. To overcome this, the donor microbiota profile should be characterized in order to find the most appropriate donor for each recipient and condition. To take it a step further, the microbiome should be characterized for both the recipient and the donor so that the donor can supplement the missing elements in the recipient. This will make it possible to refine the FMT to selectively obtain the missing components of the recipient from the donor sample, and at the same time also reduce the risk for transmitting unknown possible pathogenic microbes.

As FMT is becoming more relevant in the management of several non-communicable chronic diseases like IBD and IBS, more research is needed to examine the mechanistic insights for each disease indication to underpin and thereby to understand the factors behind a successful FMT. When these unknown factors are specified, only then will it be possible to refine the current crude FMT to a more individually adapted, personalized FMT. In some IBD and IBS cases, the achieved eubiosis through FMT may bring resolution of some symptoms but may not cure the disease itself. Therefore, the role of FMT in the management of these conditions should not be absolute, rather it should be incorporated as an adjunct in combination with other specifically selected therapy modalities targeting the different pathological mechanisms and thereby achieving maximal clinical outcome.

The safety profile of FMT seems to be very good as the most reported AEs was found to be mild, self-limiting, and local (GI-related). Nevertheless, SAEs have been reported although in many cases it has been found to be related to other causes/factors than FMT *per se* such as the physical state of the patients and their comorbidities. However, there are a small but real risk inherited to the procedure of administrating FMT which always should be considered when selecting the optimal route, so it is best suited for the recipient.

As demonstrated by numerous studies using advanced sequencing technologies, the highly diverse microbiota differs from individual to individual, so it is only reasonable to assume that each individual would need their own personalized FMT to achieve an optimal effect. Therefore, it is a necessity to take the step into the new era of personalized medicine in order to achieve this, and thereafter to fine-tune FMT accordingly. Hence, the dogma of “one stool fits all” will need to be reconsidered.

To conclude, future research should focus on mechanistic insights that underpin a successful treatment, identify and characterize the microbiota and their functions, and to match the donor to specific case. Furthermore, standardized and uniform FMT protocols needs to be established for each indication, and longer-term follow-up investigations with long-term safety monitoring are also required. Next phase of FMT will most likely be highly personalized thereby being able to match the selective FMT for different patients and diseases accordingly.

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REFERENCES

1. Anwar H, Iftikhar A, Muzaffar H, Almatroudi A, Allemailem KS, Navaid S, et al. Biodiversity of Gut Microbiota: Impact of Various Host and Environmental Factors. BioMed Research International. 2021;2021.

2. Spanogiannopoulos P, Bess EN, Carmody RN, Turnbaugh PJ. The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. Nat Rev Microbiol [Internet]. 2016 May 1 [cited 2022 Jun 4];14(5):273. Available from: /pmc/articles/PMC5243131/

3. Wang JW, Kuo CH, Kuo FC, Wang YK, Hsu WH, Yu FJ, et al. Fecal microbiota transplantation: Review and update. Vol. 118, Journal of the Formosan Medical Association. Elsevier B.V.; 2019. p. S23–31.

4. Routy B, le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science [Internet]. 2018 Jan 5 [cited 2022 Jun 5];359(6371):91–7. Available from: https://pubmed.ncbi.nlm.nih.gov/29097494/

5. Zhang Z, Mocanu V, Cai C, Dang J, Slater L, Deehan EC, et al. Impact of Fecal Microbiota Transplantation on Obesity and Metabolic Syndrome-A Systematic Review. Nutrients [Internet]. 2019 Oct 1 [cited 2022 Jun 5];11(10). Available from: https://pubmed.ncbi.nlm.nih.gov/31557953/

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 [Internet]. 2017 [cited 2022 Jun 5];5(1). Available from: https://pubmed.ncbi.nlm.nih.gov/28122648/

7. Makkawi S, Camara-Lemarroy C, Metz L. Fecal microbiota transplantation associated with 10 years of stability in a patient with SPMS. Neurology® Neuroimmunology & Neuroinflammation [Internet]. 2018 Jul 1 [cited 2022 Jun 5];5(4):459. Available from: /pmc/articles/PMC5882466/

8. 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. Frontiers in Immunology [Internet]. 2018 Sep 25 [cited 2022 Jun 5];9(SEP):2195. Available from: /pmc/articles/PMC6167440/

9. Minato T, Maeda T, Fujisawa Y, Tsuji H, Nomoto K, Ohno K, et al. Progression of Parkinson's disease is associated with gut dysbiosis: Two-year follow-up study. PLoS One [Internet]. 2017 Nov 1 [cited 2022 Jun 5];12(11). Available from: https://pubmed.ncbi.nlm.nih.gov/29091972/

10. 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 [Internet]. 2017 Dec 1 [cited 2022 Jun 5];66(6):1727–38. Available from: https://pubmed.ncbi.nlm.nih.gov/28586116/

11. 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 [Internet]. 2017 Dec 1 [cited 2022 Jun 5];7(1). Available from: https://pubmed.ncbi.nlm.nih.gov/28484247/

12. Peng J, Xiao X, Hu M, Zhang X. Interaction between gut microbiome and cardiovascular disease. Life Sci [Internet]. 2018 Dec 1 [cited 2022 Jun 5];214:153–7. Available from: https://pubmed.ncbi.nlm.nih.gov/30385177/

13. 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 Jul 1;149(1):223–37.

14. Bibbò S, Settanni CR, Porcari S, Bocchino E, Ianiro G, Cammarota G, et al. Fecal microbiota transplantation: Screening and selection to choose the optimal donor. Vol. 9, Journal of Clinical Medicine. MDPI; 2020. p. 1–14.

15. Brandt LJ. Response to Zhang et al. Vol. 107, American Journal of Gastroenterology. 2012. p. 1755–6.

16. Zhang F, Cui B, He X, Nie Y, Wu K, Fan D, et al. Microbiota transplantation: concept, methodology and strategy for its modernization. Vol. 9, Protein and Cell. Higher Education Press; 2018. p. 462–73.

17. Sbahi H, di Palma JA. Faecal microbiota transplantation: applications and limitations in treating gastrointestinal disorders. BMJ Open Gastroenterology. 2016 Dec 31;3(1).

18. Ser HL, Letchumanan V, Goh BH, Wong SH, Lee LH. The Use of Fecal Microbiome Transplant in Treating Human Diseases: Too Early for Poop? Vol. 12, Frontiers in Microbiology. Frontiers Media S.A.; 2021.

19. Borody TJ, Warren EF, Leis SM, Surace R, Ashman O, Siarakas S. Bacteriotherapy Using Fecal Flora Toying With Human Motions. 2004.

20. Gasbarrini G, Bonvicini F, Gramenzi A. Probiotics History. J Clin Gastroenterol [Internet]. 2016 Dec 1 [cited 2022 May 9];50:S116–9. Available from: https://pubmed.ncbi.nlm.nih.gov/27741152/

21. EISEMAN B, SILEN W, BASCOM GS, KAUVAR AJ. Fecal enema as an adjunct in the treatment of pseudomembranous. Surgery. 1958;44(5):854–9.

22. Masucci L, Quaranta G. Fecal microbiota transplantation: What's new? Vol. 10, Microorganisms. MDPI; 2022.

23. 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 [Internet]. 2013 Jan 31 [cited 2022 Apr 15];368(5):407–15. Available from: https://pubmed.ncbi.nlm.nih.gov/23323867/

24. Quigley EMM. Basic Definitions and Concepts: Organization of the Gut Microbiome. Gastroenterol Clin North Am [Internet]. 2017 Mar 1 [cited 2022 Apr 10];46(1):1–8. Available from: https://pubmed.ncbi.nlm.nih.gov/28164844/

25. Fadda HM. The Route to Palatable Fecal Microbiota Transplantation. Vol. 21, AAPS PharmSciTech. Springer; 2020.

26. Antushevich H. Fecal microbiota transplantation in disease therapy. Vol. 503, Clinica Chimica Acta. Elsevier B.V.; 2020. p. 90–8.

27. Malikowski T, Khanna S, Pardi DS. Fecal microbiota transplantation for gastrointestinal disorders. Vol. 33, Current Opinion in Gastroenterology. Lippincott Williams and Wilkins; 2017. p. 8–13.

28. Hsu WH, Wang JY, Kuo CH. Current applications of fecal microbiota transplantation in intestinal disorders. Vol. 35, Kaohsiung Journal of Medical Sciences. John Wiley and Sons Inc.; 2019. p. 327–31.

29. Cibulková I, Řehořová V, Hajer J, Duška F. Fecal microbial transplantation in critically ill patients—Structured review and perspectives. Vol. 11, Biomolecules. MDPI; 2021.

30. Vaughn BP, Rank KM, Khoruts A. Fecal Microbiota Transplantation: Current Status in Treatment of GI and Liver Disease. Vol. 17, Clinical Gastroenterology and Hepatology. W.B. Saunders; 2019. p. 353–61.

31. 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 [Internet]. 2017 Mar 1 [cited 2022 Jun 3];152(4):799-811.e7. Available from: https://pubmed.ncbi.nlm.nih.gov/27866880/

32. Ademe M. Benefits of fecal microbiota transplantation: A comprehensive review. Vol. 14, Journal of Infection in Developing Countries. Journal of Infection in Developing Countries; 2020. p. 1074–80.

33. Khan I, Bai Y, Zha L, Ullah N, Ullah H, Shah SRH, et al. Mechanism of the Gut Microbiota Colonization Resistance and Enteric Pathogen Infection. Frontiers in Cellular and Infection Microbiology. 2021 Dec 23;11:1273.

34. Ooijevaar RE, Terveer EM, Verspaget HW, Kuijper EJ, Keller JJ. Annual Review of Medicine Clinical Application and Potential of Fecal Microbiota Transplantation. Annu Rev Med [Internet]. 2018; Available from: https://doi.org/10.1146/annurev-med-111717-

35. Campbell C, McKenney PT, Konstantinovsky D, Isaeva OI, Schizas M, Verter J, et al. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. Nature 2020 581:7809 [Internet]. 2020 Apr 15 [cited 2022 Jun 4];581(7809):475–9. Available from: https://www.nature.com/articles/s41586-020-2193-0

36. Feng W, Ao H, Peng C. Gut Microbiota, Short-Chain Fatty Acids, and Herbal Medicines. Front Pharmacol [Internet]. 2018 Nov 23 [cited 2022 Jun 4];9(NOV). Available from: https://pubmed.ncbi.nlm.nih.gov/30532706/

37. Khoruts A, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. Vol. 13, Nature Reviews Gastroenterology and Hepatology. Nature Publishing Group; 2016. p. 508–16.

38. Levy M, Thaiss CA, Elinav E. Metagenomic cross-talk: the regulatory interplay between immunogenomics and the microbiome. Genome Medicine 2015 7:1 [Internet]. 2015 Nov 20 [cited 2022 Apr 16];7(1):1–13. Available from: https://genomemedicine.biomedcentral.com/articles/10.1186/s13073-015-0249-9

39. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. Nature Reviews Immunology 2016 16:6 [Internet]. 2016 May 27 [cited 2022 Apr 16];16(6):341–52. Available from: https://www.nature.com/articles/nri.2016.42

40. Cammarota G, Ianiro G, Cianci R, Bibbò S, Gasbarrini A, Currò D. The involvement of gut microbiota in inflammatory bowel disease pathogenesis: potential for therapy. Pharmacol Ther [Internet]. 2015 [cited 2022 Apr 16];149:191–212. Available from: https://pubmed.ncbi.nlm.nih.gov/25561343/

41. Zeng W, Shen J, Bo T, Peng L, Xu H, Ide Nasser M, et al. Cutting Edge: Probiotics and Fecal Microbiota Transplantation in Immunomodulation. J Immunol Res [Internet]. 2019 [cited 2022 Jun 3];2019. Available from: https://pubmed.ncbi.nlm.nih.gov/31143780/

42. Committee on Organ Transplantation E. Guide to the quality and safety of TISSUES AND CELLS for human application European Committee (Partial Agreement) on Organ Transplantation (CD-P-TO).

43. 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. In: Gut. BMJ Publishing Group; 2017. p. 569–80.

44. Thabit AK, Alsolami MH, Baghlaf NA, Alsharekh RM, Almazmumi HA, Alselami AS, et al. Comparison of three current Clostridioides difficile infection guidelines: IDSA/SHEA, ESCMID, and ACG guidelines. Infection [Internet]. 2019 Dec 1 [cited 2022 Apr 10];47(6):899–909. Available from: https://pubmed.ncbi.nlm.nih.gov/31428991/

45. Ianiro G, Segal JP, Mullish BH, Quraishi MN, Porcari S, Fabiani G, et al. Fecal microbiota transplantation in gastrointestinal and extraintestinal disorders. Vol. 15, Future Microbiology. Future Medicine Ltd.; 2020. p. 1173–86.

46. Allegretti JR, Kao D, Sitko J, Fischer M, Kassam Z. Early Antibiotic Use After Fecal Microbiota Transplantation Increases Risk of Treatment Failure. Clin Infect Dis [Internet]. 2018 Jan 1 [cited 2022 Apr 15];66(1):134–5. Available from: https://pubmed.ncbi.nlm.nih.gov/29020157/

47. 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 [Internet]. 2014 Nov 5 [cited 2022 Apr 11];312(17):1772–8. Available from: https://pubmed.ncbi.nlm.nih.gov/25322359/

48. Zhang F, Zhang T, Zhu H, Borody TJ. Evolution of fecal microbiota transplantation in methodology and ethical issues. Vol. 49, Current Opinion in Pharmacology. Elsevier Ltd; 2019. p. 11–6.

49. Gupta S, Mullish BH, Allegretti JR. Fecal Microbiota Transplantation: The Evolving Risk Landscape. Vol. 116, The American journal of gastroenterology. NLM (Medline); 2021. p. 647–56.

50. Important Safety Alert Regarding Use of Fecal Microbiota for Transplantation and Risk of Serious Adverse Reactions Due to Transmission of Multi-Drug Resistant Organisms | FDA [Internet]. [cited 2022 Apr 17]. Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/important-safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse

51. 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 . New England Journal of Medicine [Internet]. 2019 Nov 21 [cited 2022 Apr 17];381(21):2043–50. Available from: https://www.nejm.org/doi/full/10.1056/NEJMoa1910437

52. Kassam Z, Dubois N, Ramakrishna B, Ling K, Qazi T, Smith M, et al. Donor Screening for Fecal Microbiota Transplantation. N Engl J Med [Internet]. 2019 Nov 21 [cited 2022 Apr 17];381(21):2070–2. Available from: https://pubmed.ncbi.nlm.nih.gov/31665572/

53. Update to March 12, 2020 Safety Alert Regarding Use of Fecal Microbiota for Transplantation and Risk of Serious Adverse Events Likely Due to Transmission of Pathogenic Organisms | FDA [Internet]. [cited 2022 Apr 17]. Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/update-march-12-2020-safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious

54. Safety Alert Regarding Use of Fecal Microbiota for Transplantation and Risk of Serious Adverse Events Likely Due to Transmission of Pathogenic Organisms | FDA [Internet]. [cited 2022 Apr 17]. Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse-events-likely

55. Baxter M, Ahmad T, Colville A, Sheridan R. Fatal Aspiration Pneumonia as a Complication of Fecal Microbiota Transplant. Clin Infect Dis [Internet]. 2015 Jul 1 [cited 2022 Apr 18];61(1):136–7. Available from: https://pubmed.ncbi.nlm.nih.gov/25805303/

56. Waller KMJ, Leong RW, Paramsothy S. An update on fecal microbiota transplantation for the treatment of gastrointestinal diseases. Journal of Gastroenterology and Hepatology [Internet]. 2022 Feb 1 [cited 2022 Jun 21];37(2):246–55. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/jgh.15731

57. Lofgren ET, Cole SR, Weber DJ, Anderson DJ, Moehring RW. Hospital-acquired Clostridium difficile infections: estimating all-cause mortality and length of stay. Epidemiology [Internet]. 2014 [cited 2022 May 1];25(4):570–5. Available from: https://pubmed.ncbi.nlm.nih.gov/24815305/

58. Voth E, Khanna S. Fecal microbiota transplantation for treatment of patients with recurrent Clostridioides difficile infection. https://doi.org/101080/1478721020201752192 [Internet]. 2020 Jul 2 [cited 2022 May 1];18(7):669–76. Available from: https://www.tandfonline.com/doi/abs/10.1080/14787210.2020.1752192

59. Crobach MJT, Planche T, Eckert C, Barbut F, Terveer EM, Dekkers OM, et al. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for Clostridium difficile infection. Clinical Microbiology and Infection [Internet]. 2016 Aug 1 [cited 2022 May 2];22:S63–81. Available from: http://www.clinicalmicrobiologyandinfection.com/article/S1198743X16300258/fulltext

60. Chitnis AS, Holzbauer SM, Belflower RM, Winston LG, Bamberg WM, Lyons C, et al. Epidemiology of community-associated Clostridium difficile infection, 2009 through 2011. JAMA Intern Med [Internet]. 2013 Jul 22 [cited 2022 May 2];173(14):1359–67. Available from: https://pubmed.ncbi.nlm.nih.gov/23780507/

61. Imhann F, Bonder MJ, Vila AV, Fu J, Mujagic Z, Vork L, et al. Proton pump inhibitors affect the gut microbiome. Gut [Internet]. 2016 May 1 [cited 2022 May 2];65(5):740–8. Available from: https://gut.bmj.com/content/65/5/740

62. Finn E, Andersson FL, Madin-Warburton M. Burden of Clostridioides difficile infection (CDI) - a systematic review of the epidemiology of primary and recurrent CDI. BMC Infectious Diseases [Internet]. 2021 Dec 1 [cited 2022 May 2];21(1):1–11. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-021-06147-y

63. Bibbò S, Lopetuso LR, Ianiro G, di Rienzo T, Gasbarrini A, Cammarota G. Role of microbiota and innate immunity in recurrent Clostridium difficile infection. J Immunol Res [Internet]. 2014 [cited 2022 May 1];2014. Available from: https://pubmed.ncbi.nlm.nih.gov/24995345/

64. Kukla M, Adrych K, Dobrowolska A, Mach T, Reguła J, Rydzewska G. Guidelines for Clostridium difficile infection in adults. Przegląd Gastroenterologiczny [Internet]. 2020 [cited 2022 May 11];15(1):1. Available from: /pmc/articles/PMC7089862/

65. 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). Clinical Infectious Diseases [Internet]. 2018 Mar 19 [cited 2022 May 3];66(7):987–94. Available from: https://academic.oup.com/cid/article/66/7/987/4942452

66. Adamu BO, Lawley TD. Bacteriotherapy for the treatment of intestinal dysbiosis caused by Clostridium difficile infection. Current Opinion in Microbiology [Internet]. 2013 Oct [cited 2022 May 2];16(5):596. Available from: /pmc/articles/PMC3840269/

67. Sokol H, Galperine T, Kapel N, Bourlioux P, Seksik P, Barbut F, et al. Faecal microbiota transplantation in recurrent Clostridium difficile infection: Recommendations from the French Group of Faecal microbiota Transplantation. Dig Liver Dis [Internet]. 2016 Mar 1 [cited 2022 May 29];48(3):242–7. Available from: https://pubmed.ncbi.nlm.nih.gov/26433619/

68. 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 [Internet]. 2018 Nov 1 [cited 2022 May 3];67(11):1920–41. Available from: https://pubmed.ncbi.nlm.nih.gov/30154172/

69. Johnson S, Lavergne V, Skinner AM, Gonzales-Luna AJ, Garey KW, Kelly CP, 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. Clinical Infectious Diseases [Internet]. 2021 Sep 7 [cited 2022 May 14];73(5):e1029–44. Available from: https://academic.oup.com/cid/article/73/5/e1029/6298219

70. van Prehn J, Reigadas E, Vogelzang EH, 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 [Internet]. 2021 Dec 1 [cited 2022 May 3];27 Suppl 2:S1–21. Available from: https://pubmed.ncbi.nlm.nih.gov/34678515/

71. Kelly CR, Fischer M, Allegretti JR, LaPlante K, Stewart DB, Limketkai BN, et al. ACG Clinical Guidelines: Prevention, Diagnosis, and Treatment of Clostridioides difficile Infections. Am J Gastroenterol [Internet]. 2021 Jun 1 [cited 2022 May 13];116(6):1124–47. Available from: https://journals.lww.com/ajg/Fulltext/2021/06000/ACG_Clinical_Guidelines__Prevention,_Diagnosis,.12.aspx

72. Haifer C, Kelly CR, Paramsothy S, Andresen D, Papanicolas LE, McKew GL, et al. Australian consensus statements for the regulation, production and use of faecal microbiota transplantation in clinical practice. Gut [Internet]. 2020 May 1 [cited 2022 May 1];69(5):801–10. Available from: https://gut.bmj.com/content/69/5/801

73. Gweon TG, Lee YJ, Kim KO, Yim SK, Soh JS, Kim SY, et al. Clinical Practice Guidelines for Fecal Microbiota Transplantation in Korea. Journal of Neurogastroenterology and Motility [Internet]. 2022 Jan 30 [cited 2022 May 1];28(1):28–42. Available from: https://www.jnmjournal.org/journal/view.html?doi=10.5056/jnm21221

74. Pomares Bascuñana R, 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 [Internet]. 2021 Aug 1 [cited 2022 May 1];73(2):149–58. Available from: https://pubmed.ncbi.nlm.nih.gov/33864273/

75. Ianiro G, Masucci L, Quaranta G, Simonelli C, Lopetuso LR, Sanguinetti M, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy plus vancomycin for the treatment of severe refractory Clostridium difficile infection-single versus multiple infusions. Aliment Pharmacol Ther [Internet]. 2018 Jul 1 [cited 2022 May 4];48(2):152–9. Available from: https://pubmed.ncbi.nlm.nih.gov/29851107/

76. Garza-González E, Mendoza-Olazarán S, Morfin-Otero R, Ramírez-Fontes A, Rodríguez-Zulueta P, Flores-Treviño S, et al. Intestinal Microbiome Changes in Fecal Microbiota Transplant (FMT) vs. FMT Enriched with Lactobacillus in the Treatment of Recurrent Clostridioides difficile Infection. Can J Gastroenterol Hepatol [Internet]. 2019 [cited 2022 Jun 5];2019. Available from: https://pubmed.ncbi.nlm.nih.gov/31976311/

77. Dubberke ER, Lee CH, Orenstein R, Khanna S, Hecht G, Gerding DN. Results From a Randomized, Placebo-Controlled Clinical Trial of a RBX2660-A Microbiota-Based Drug for the Prevention of Recurrent Clostridium difficile Infection. Clin Infect Dis [Internet]. 2018 Sep 28 [cited 2022 Jun 5];67(8):1198–204. Available from: https://pubmed.ncbi.nlm.nih.gov/29617739/

78. Ianiro G, Masucci L, Quaranta G, Simonelli C, Lopetuso LR, Sanguinetti M, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy plus vancomycin for the treatment of severe refractory Clostridium difficile infection—single versus multiple infusions. Alimentary Pharmacology & Therapeutics [Internet]. 2018 Jul 1 [cited 2022 Jun 5];48(2):152–9. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/apt.14816

79. Juul FE, Garborg K, Bretthauer M, Skudal H, Øines MN, Wiig H, et al. Fecal Microbiota Transplantation for Primary Clostridium difficile Infection . New England Journal of Medicine [Internet]. 2018 Jun 28 [cited 2022 Jun 5];378(26):2535–6. Available from: https://www.nejm.org/doi/full/10.1056/NEJMc1803103

80. Jiang ZD, Jenq RR, Ajami NJ, Petrosino JF, Alexander AA, Ke S, et al. Safety and preliminary efficacy of orally administered lyophilized fecal microbiota product compared with frozen product given by enema for recurrent Clostridium difficile infection: A randomized clinical trial. PLoS ONE [Internet]. 2018 Nov 1 [cited 2022 Jun 5];13(11). Available from: /pmc/articles/PMC6214502/

81. 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 [Internet]. 2017 Apr 1 [cited 2022 Jun 5];45(7):899–908. Available from: https://pubmed.ncbi.nlm.nih.gov/28220514/

82. 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 [Internet]. 2017 Nov 28 [cited 2022 Jun 5];318(20):1985–93. Available from: https://pubmed.ncbi.nlm.nih.gov/29183074/

83. Hota SS, Sales V, Tomlinson G, Salpeter MJ, McGeer A, Coburn B, et al. Oral Vancomycin Followed by Fecal Transplantation Versus Tapering Oral Vancomycin Treatment for Recurrent Clostridium difficile Infection: An Open-Label, Randomized Controlled Trial. Clin Infect Dis [Internet]. 2017 Feb 1 [cited 2022 Jun 5];64(3):265–71. Available from: https://pubmed.ncbi.nlm.nih.gov/28011612/

84. Kelly CR, Khoruts A, Staley C, Sadowsky MJ, Abd M, Alani M, et al. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. Ann Intern Med [Internet]. 2016 Nov 1 [cited 2022 Jun 5];165(9):609–16. Available from: https://pubmed.ncbi.nlm.nih.gov/27547925/

85. 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 [Internet]. 2016 Jan 12 [cited 2022 Jun 5];315(2):142–9. Available from: https://pubmed.ncbi.nlm.nih.gov/26757463/

86. Orenstein R, Dubberke E, Hardi R, Ray A, Mullane K, Pardi DS, et al. Safety and Durability of RBX2660 (Microbiota Suspension) for Recurrent Clostridium difficile Infection: Results of the PUNCH CD Study. Clin Infect Dis [Internet]. 2016 Mar 1 [cited 2022 Jun 5];62(5):596–602. Available from: https://pubmed.ncbi.nlm.nih.gov/26565008/

87. Cammarota G, Masucci L, Ianiro G, Bibbò S, Dinoi G, Costamagna G, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. Aliment Pharmacol Ther [Internet]. 2015 May 1 [cited 2022 Jun 5];41(9):835–43. Available from: https://pubmed.ncbi.nlm.nih.gov/25728808/

88. Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology [Internet]. 2014 [cited 2022 Apr 23];146(6):1489–99. Available from: https://pubmed.ncbi.nlm.nih.gov/24560869/

89. McIlroy J, Ianiro G, Mukhopadhya I, Hansen R, Hold GL. Review article: the gut microbiome in inflammatory bowel disease-avenues for microbial management. Aliment Pharmacol Ther [Internet]. 2018 Jan 1 [cited 2022 Apr 23];47(1):26–42. Available from: https://pubmed.ncbi.nlm.nih.gov/29034981/

90. de Cruz P, Prideaux L, Wagner J, Ng SC, McSweeney C, Kirkwood C, et al. Characterization of the gastrointestinal microbiota in health and inflammatory bowel disease. Inflamm Bowel Dis [Internet]. 2012 Feb [cited 2022 Apr 23];18(2):372–90. Available from: https://pubmed.ncbi.nlm.nih.gov/21604329/

91. 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 [Internet]. 2019 Sep 1 [cited 2022 Apr 23];8(3). Available from: https://pubmed.ncbi.nlm.nih.gov/31412603/

92. 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. The Lancet Gastroenterology and Hepatology [Internet]. 2022 Feb 1 [cited 2022 Apr 23];7(2):141–51. Available from: https://researchers.mq.edu.au/en/publications/lyophilised-oral-faecal-microbiota-transplantation-for-ulcerative

93. 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 Jul 1;149(1):102-109.e6.

94. 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 Jul 1;149(1):110-118.e4.

95. 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 [Internet]. 2017 Mar 25 [cited 2022 Apr 21];389(10075):1218–28. Available from: https://pubmed.ncbi.nlm.nih.gov/28214091/

96. Costello S, Waters O, Bryant R, Katsikeros R, Makanyanga J, Schoeman MN, et al. Short duration, low intensity pooled faecal microbiota transplantation induces remission in patients with mild-moderately active ulcerative colitis: a randomised controlled trial. Journal of Crohn's and Colitis [Internet]. 2017 Feb 1 [cited 2022 Apr 21];11(S1):S23–S23. Available from: https://research.monash.edu/en/publications/short-duration-low-intensity-pooled-faecal-microbiota-transplanta

97. 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 [Internet]. 2017 Oct 1 [cited 2022 Apr 24];23(10):1702–9. Available from: https://pubmed.ncbi.nlm.nih.gov/28906291/

98. Zhou HY, Guo B, Lufumpa E, Li XM, Chen LH, Meng X, et al. Comparative of the Effectiveness and Safety of Biological Agents, Tofacitinib, and Fecal Microbiota Transplantation in Ulcerative Colitis: Systematic Review and Network Meta-Analysis. Immunol Invest [Internet]. 2021 [cited 2022 Apr 23];50(4):323–37. Available from: https://pubmed.ncbi.nlm.nih.gov/32009472/

99. 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 [Internet]. 2020 Jan 1 [cited 2022 Apr 24];65(1):150–7. Available from: https://pubmed.ncbi.nlm.nih.gov/31367877/

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 [Internet]. 2020 Feb 3 [cited 2022 Apr 24];8(1). Available from: https://pubmed.ncbi.nlm.nih.gov/32014035/

101. Fehily SR, Basnayake C, Wright EK, Kamm MA. Fecal microbiota transplantation therapy in Crohn's disease: Systematic review. J Gastroenterol Hepatol [Internet]. 2021 Oct 1 [cited 2022 Apr 24];36(10):2672–86. Available from: https://pubmed.ncbi.nlm.nih.gov/34169565/

102. 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(3):556–63.

103. 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 [Internet]. 2018 [cited 2022 Apr 26];1047:81–7. Available from: https://pubmed.ncbi.nlm.nih.gov/29151253/

104. 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 [Internet]. 2018 Jan 18 [cited 2022 Apr 26];24(2):410–21. Available from: https://pubmed.ncbi.nlm.nih.gov/29361092/

105. 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 [Internet]. 2015 [cited 2022 Apr 26];2015. Available from: https://pubmed.ncbi.nlm.nih.gov/26146498/

106. 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 [Internet]. 2016 Apr 1 [cited 2022 Apr 26];10(4):387–94. Available from: https://pubmed.ncbi.nlm.nih.gov/26519463/

107. 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 [Internet]. 2016 Aug 10 [cited 2022 Apr 26];22(9):2182–90. Available from: https://pubmed.ncbi.nlm.nih.gov/27542133/

108. 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 European Gastroenterol J [Internet]. 2019 Jul 1 [cited 2022 Apr 26];7(6):807–14. Available from: https://pubmed.ncbi.nlm.nih.gov/31316785/

109. 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 [Internet]. 2020 May 1 [cited 2022 Apr 24];13(3):760–9. Available from: https://pubmed.ncbi.nlm.nih.gov/31958884/

110. Fikree A, Byrne P. Management of functional gastrointestinal disorders. FUNCTIONAL DISORDERS Clinical Medicine [Internet]. 2021 [cited 2022 Apr 29];21(1):44–52. Available from: www.orcha.co.uk

111. Ford AC, Sperber AD, Corsetti M, Camilleri M. Irritable bowel syndrome. The Lancet [Internet]. 2020 Nov 21 [cited 2022 Apr 29];396(10263):1675–88. Available from: http://www.thelancet.com/article/S0140673620315488/fulltext

112. Wu J, Lv L, Wang C. Efficacy of Fecal Microbiota Transplantation in Irritable Bowel Syndrome: A Meta-Analysis of Randomized Controlled Trials. Frontiers in Cellular and Infection Microbiology. 2022 Feb 28;12:179.

113. Schmulson MJ, Drossman DA. What Is New in Rome IV. Journal of Neurogastroenterology and Motility [Internet]. 2017 Apr 31 [cited 2022 Apr 29];23(2):151–63. Available from: https://www.jnmjournal.org/journal/view.html?doi=10.5056/jnm16214

114. Drossman DA. Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features, and Rome IV. Gastroenterology [Internet]. 2016 May 1 [cited 2022 May 1];150(6):1262-1279.e2. Available from: http://www.gastrojournal.org/article/S0016508516002237/fulltext

115. Chojnacki C, Błońska A, Kaczka A, Chojnacki J, Stępień A, Gasiorowska A. Evaluation of serotonin and dopamine secretion and metabolism in patients with irritable bowel syndrome. Polish Archives of Internal Medicine. 2018;128(11):711–3.

116. Zhou QQ, Zhang B, Nicholas Verne G. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. Pain [Internet]. 2009 Nov [cited 2022 May 1];146(1–2):41–6. Available from: https://journals.lww.com/pain/Fulltext/2009/11000/Intestinal_membrane_permeability_and.12.aspx

117. Pittayanon R, Lau JT, Yuan Y, Leontiadis GI, Tse F, Surette M, et al. Gut Microbiota in Patients With Irritable Bowel Syndrome-A Systematic Review. Gastroenterology [Internet]. 2019 Jul 1 [cited 2022 May 1];157(1):97–108. Available from: https://pubmed.ncbi.nlm.nih.gov/30940523/

118. Carroll IM, Ringel-Kulka T, Siddle JP, Ringel Y. Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. Neurogastroenterol Motil [Internet]. 2012 Jun [cited 2022 May 1];24(6). Available from: https://pubmed.ncbi.nlm.nih.gov/22339879/

119. Tap J, Derrien M, Törnblom H, Brazeilles R, Cools-Portier S, Doré J, et al. Identification of an Intestinal Microbiota Signature Associated With Severity of Irritable Bowel Syndrome. Gastroenterology [Internet]. 2017 Jan 1 [cited 2022 May 1];152(1):111-123.e8. Available from: https://pubmed.ncbi.nlm.nih.gov/27725146/

120. Polster A, Öhman L, Tap J, Derrien M, le Nevé B, Sundin J, et al. A novel stepwise integrative analysis pipeline reveals distinct microbiota-host interactions and link to symptoms in irritable bowel syndrome. Scientific Reports 2021 11:1 [Internet]. 2021 Mar 9 [cited 2022 May 1];11(1):1–13. Available from: https://www.nature.com/articles/s41598-021-84686-9

121. Mei L, Zhou J, Su Y, Mao K, Wu J, Zhu C, et al. Gut microbiota composition and functional prediction in diarrhea-predominant irritable bowel syndrome. BMC Gastroenterology [Internet]. 2021 Dec 1 [cited 2022 May 1];21(1):1–12. Available from: https://bmcgastroenterol.biomedcentral.com/articles/10.1186/s12876-021-01693-w

122. Shah A, Talley NJ, Jones M, Kendall BJ, Koloski N, Walker MM, et al. Small Intestinal Bacterial Overgrowth in Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis of Case-Control Studies. Am J Gastroenterol [Internet]. 2020 Feb 1 [cited 2022 May 1];115(2):190–201. Available from: https://pubmed.ncbi.nlm.nih.gov/31913194/

123. 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 [Internet]. 2021 Jan 1 [cited 2022 Apr 30];160(1):145-157.e8. Available from: https://pubmed.ncbi.nlm.nih.gov/32681922/

124. El-Salhy M, Hatlebakk JG, Gilja OH, Bråthen Kristoffersen A, Hausken T. Original research: Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. Gut [Internet]. 2020 May 1 [cited 2022 Apr 30];69(5):859. Available from: /pmc/articles/PMC7229896/

125. Halkjær 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 [Internet]. 2018 Dec 1 [cited 2022 Apr 30];67(12):2107–15. Available from: https://pubmed.ncbi.nlm.nih.gov/29980607/

126. Johnsen PH, Hilpüsch 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 [Internet]. 2018 Jan 1 [cited 2022 Apr 30];3(1):17–24. Available from: https://pubmed.ncbi.nlm.nih.gov/29100842/

127. Holster S, Lindqvist CM, Repsilber D, Salonen A, de Vos WM, Konig J, et al. The Effect of Allogenic Versus Autologous Fecal Microbiota Transfer on Symptoms, Visceral Perception and Fecal and Mucosal Microbiota in Irritable Bowel Syndrome: A Randomized Controlled Study. Clinical and Translational Gastroenterology [Internet]. 2019 Apr 15 [cited 2022 Apr 30];10(4). Available from: /pmc/articles/PMC6602784/

128. 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 [Internet]. 2019 Sep 1 [cited 2022 Apr 30];4(9):675–85. Available from: https://pubmed.ncbi.nlm.nih.gov/31326345/

129. Lahtinen P, Jalanka J, Hartikainen A, Mattila E, Hillilä M, Punkkinen J, et al. Randomised clinical trial: faecal microbiota transplantation versus autologous placebo administered via colonoscopy in irritable bowel syndrome. Aliment Pharmacol Ther [Internet]. 2020 Jun 1 [cited 2022 Apr 30];51(12):1321–31. Available from: https://pubmed.ncbi.nlm.nih.gov/32343000/