Fecal microbiota transplantation and emerging applications

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Abstract | Fecal microbiota transplantation (FMT) has been utilized sporadically for over 50 years. In the past few years, *Clostridium difficile* infection (CDI) epidemics in the USA and Europe have resulted in the increased use of FMT, given its high efficacy in eradicating CDI and associated symptoms. As more patients request treatment and more clinics incorporate FMT into their treatment repertoire, reports of applications outside of CDI are emerging, paving the way for the use of FMT in several idiopathic conditions. Interest in this therapy has largely been driven by new research into the gut microbiota, which is now beginning to be appreciated as a microbial human organ with important roles in immunity and energy metabolism. This new paradigm raises the possibility that many diseases result, at least partially, from microbiota-related dysfunction. This understanding invites the investigation of FMT for several disorders, including IBD, IBS, the metabolic syndrome, neurodevelopmental disorders, autoimmune diseases and allergic diseases, among others. The field of microbiota-related disorders is currently in its infancy; it certainly is an exciting time in the burgeoning science of FMT and we expect to see new and previously unexpected applications in the near future. Well-designed and well-executed randomized trials are now needed to further define these microbiota-related conditions.

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Introduction

Microbial communities populate all surfaces of the human body, but are present at their greatest density in the distal gut, where they exceed the total number of human cells by an order of magnitude.[1] In fact, the distal gut microbiota could be considered a distinct human organ responsible for multiple physiological functions, including various aspects of energy metabolism and the development and modulation of our immune system.

As in any organ, the gut microbiota is comprised of specialized cells that work symbiotically with each other and the host.[2] However, not all gut microbial species are dependent on host health, and relationships with these microbes can become problematic.[3] In the past six decades, our gut microbes have been under constant antibiotic assault in the form of medical therapies and routine use of antibiotics in farming practices. The concerns over potential unanticipated health consequences are only now beginning to be realized, with multiple diseases associated with Western lifestyles hypothesized as causally linked to alterations in the gut microbiota,[3–5] including constipation, IBS, IBD, neurological diseases, cardiovascular diseases, obesity, the metabolic syndrome, autoimmunity, asthma and allergic diseases, many of which have reached epidemic proportions in the past few years.

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Technological limitations have hampered our attempts to enumerate the various gastrointestinal microbial populations, with the vast majority of dominant anaerobic species largely individually unculturable by traditional microbiological techniques. However, the introduction of high-throughput DNA sequencing technologies, increasing computational capabilities and new analytical techniques have revolutionized this area of science and provided the opportunity to speculate about the existence of a 'phylogenetic core'—a core microbiota persistent and abundant among most members of the global population. Major efforts are now underway, such as the Human Microbiome Project in the USA and the MetaHIT project in Europe, that are aimed at characterizing the microbial communities of the human body to determine their role in both human health and disease.[6]

The notion of the gut microbiota as a regulator of health and disease dates back to Elie Metchnikoff's[7] work more than a century ago, in which he hypothesized that toxins produced by putrefactive microbes in the colon accelerate senescence, and that useful microbes could be used to replace harmful ones. Metchnikoff[7] noted the large consumption of fermented milk in certain Eastern European rural populations famed for their purported longevity; he introduced sour milk into his own diet, and noticed a subsequent improvement in his own health, thus forming the foundation for probiotics.[8]

One obstacle facing probiotic development today is a quantitative one. Oral probiotic doses are typically 3–4 orders of magnitude lower than the 100 trillion native micro-organisms contained within the colon.[1,2] This

number is likely to be reduced further after their passage through the harsh environments of the stomach and small bowel. Furthermore, although most species—for example, various strains of lactobacilli, bifidobacteria and *Escherichia coli* Nissle—used in probiotic formulations have originated in the gut, they have probably lost some adaptation to this environment during *ex vivo* cultivation. These problems might not be insurmountable—even small numbers of certain micro-organisms can exert profound effects on large microbial communities. These bacteria can promote biofilm formation by facilitating microbial co-aggregation and production of biosurfactants; produce bacteriocins, which can selectively kill micro-organisms and are important in maintaining microbiota stability; enhance gut barrier function through their effects on the epithelia; and can signal to the host immune system and elicit immunomodulatory effects.[6] Fecal microbiota transplantation (FMT)—the transfer of gut microbiota from a healthy donor to introduce or re-establish a stable microbial community in the gut—is now being utilized for a number of disorders. In this Review, we will summarize the hypothesis behind FMT, its current clinical use and emerging applications.

Key points

- Fecal microbiota transplantation (FMT) is arguably the most effective method in treating recalcitrant *Clostridium difficile* infection (CDI)
- FMT is the engraftment of microbiota from a healthy donor into a recipient, which results in restoration of the normal gut microbial community structure
- Standardization of FMT protocols should overcome the major practical barriers to its wider clinical implementation
- As multiple major diseases might be linked to dysfunction of gut microbiota, FMT could have potential applications beyond CDI

Gut microbiota disruption

Arguably, one of the best examples of a disease resulting from major disruption of the gut microbiota by antibiotics is *Clostridium difficile* infection (CDI). Generally acquired after antibiotic treatment and ingestion of environmental spores, CDI has become a growing public health problem in the past two decades. In the USA alone, the National Hospital Discharge Survey revealed a twofold increase in CDI between 1996 and 2003 to approximately 0.6 per 1,000 patients.[9] A 2009 survey of 12.5% of all US acute care facilities showed a CDI prevalence rate among inpatients of 13.1 per 1,000 patients.[10] This increase in CDI has been accompanied by increasing rates of colectomy and death, with approximately 100,000 people dying annually in the USA with CDI, whereby the infection is at least one of the contributing factors to death.[10,11] This increase in morbidity is in part driven by the emergence of *C. difficile* strains with increased virulence, such as PCR ribotype 027/North American Pulsed-field type 1 (NAP1), which is characterized by resistance to fluoroquinolones and increased toxin production attributable to a mutation in *tcdC*, as well as binary toxin production.[12,13]

Standard CDI treatment is currently based on antibiotics such as metronidazole and vancomycin, which exhibit broad activity against the dominant colonic microbiota phyla, but can also perpetuate recurrence of CDI after their discontinuation. The risk of CDI relapse after initial treatment is approximately 20–25%.[14,15] This risk is increased further by the use of additional interim antibiotics for treatment of other infections.[16] Thus, a portion of patients can develop chronic, recurrent CDI that can last indefinitely. Chang and colleagues[17] analyzed the fecal microbiota of seven patients with CDI using 16S rDNA sequencing and found a progressive reduction in species diversity in patients with initial CDI compared with healthy controls, and patients with recurrent CDI compared with those who had an initial infection. In fact, in the three patients with recurrent CDI, disruption of the distal gut microbiota was evident at the phylum level with marked reduction in levels of *Bacteroidetes* species and relative increases in numbers of *Proteobacteria* and *Verrucomicrobia* species, both usually only minor constituents of the fecal microbiota. This finding is consistent with the 1989 report by Tvede and Rask-Madsen,[18] which noted an absence of *Bacteroides* species in patients with recurrent CDI and the reversal of deficiencies after successful microbiota transplantation. Interestingly, the new macrocyclic antibiotic fidaxomicin, which spares *Bacteriodes* species, reduced the initial relapse rate of CDI by half compared with vancomycin, but did not differ in recurrence rate for the virulent PCR 027/NPA1 strain.[15] Although the emerging narrow-spectrum antibiotics are hoped to permit restoration of the gut microbiota in patients with the chronic relapsing form of CDI, they are yet to be tested in this population of patients. Similarly, whether the latest antibiotics will reduce the unacceptably high rates of mortality and colectomy currently associated with severe and fulminant forms of CDI is unknown.

Fecal microbiota transplantation

Clinical use

Pseudomembranous colitis (one of the most severe clinical manifestations of CDI) was recognized as a complication of antibiotic therapy shortly after the inception of antibiotics in clinical practice—that restoration of the normal gut microbiota could solve this problem was quickly realized. The earliest and most frequently quoted report of FMT is that by Eiseman and colleagues,[19] a team of surgeons from Colorado, who successfully treated four patients using fecal enemas in the late 1950s. Three of the patients were critically ill with fulminant pseudomembranous colitis, which at the time had a 75% mortality rate. The patients were treated with antibiotics, hydration, vasopressors, hydrocortisone and *Lactobacillus acidophilus* probiotic without success. In desperation, the physicians resorted to fecal retention enemas, which resulted in prompt recovery of all patients, facilitating their hospital discharge within days of treatment, with the study authors expressing their hope that a "more complete evaluation of this simple therapeutic measure can be given further clinical trial by others".[19]

FMT, previously known as 'fecal bacteriotherapy', has been offered in select centers across the world for

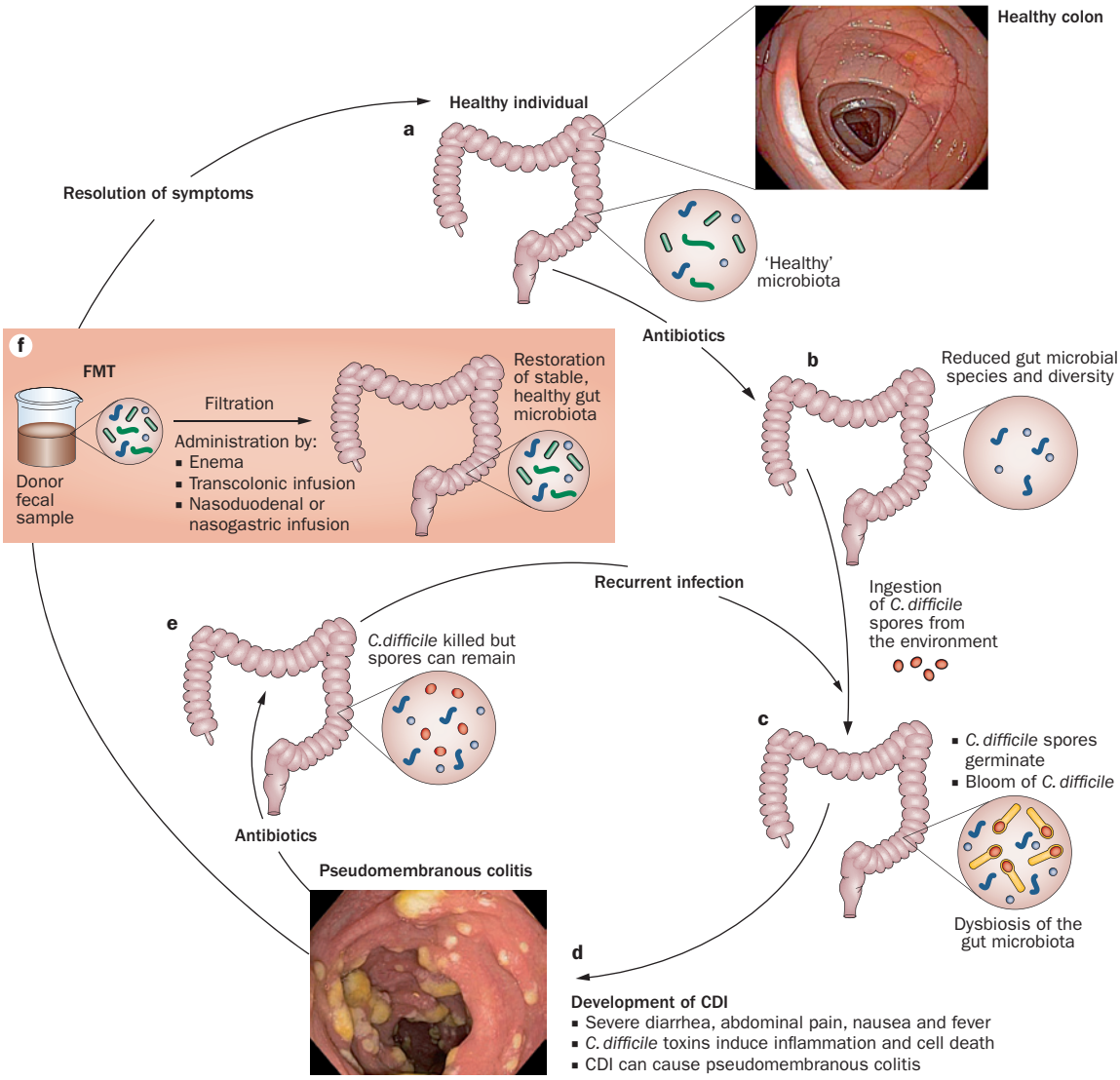

Figure 1 | FMT for patients with recalcitrant CDI. CDI causes severe diarrhea, intestinal inflammation and cell death as a result of toxin-mediated infection with the pathogenic bacteria. Patients with CDI are typically treated with antibiotics, which not only kill the pathogenic *C. difficile* but also exhibit activity against the dominant colonic microbiota phyla. Incomplete antibiotic eradication of *C. difficile* can result in recurrent CDIs. Transplantation of fecal microbiota from a healthy donor into an individual with CDI can restore the healthy gut microbiota in the patient's diseased colon, leading to resolution of symptoms. Abbreviations: CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation.

decades, primarily as a last-ditch resort for recalcitrant CDI, which is characterized by rapid infection recurrence upon antibiotic discontinuation (Figure 1). Infection cycles ultimately become predictable with near certainty and are frequently accompanied by considerable morbidity and mortality. In these most difficult cases, the reported cumulative success rate of FMT in eradicating the infection is ~90%.[20] Published FMT experience encompasses approximately 376 patients thus far (Table 1), consisting of small case series and individual case reports, primarily on patients with recurrent disease. FMT was initially performed using fecal enemas, with nasoduodenal tube[21] and colonoscopy administration[22,23] introduced later. No adverse events have been reported. Two extensive reviews have since summarized our current knowledge on FMT for recurrent CDI. Van Nood *et al.*[24] covered some of the history, screening of donors, pretreatment processes and routes of fecal infusion, while Bakken's review[20] covers similar topics, but also focuses on patient preparation and methodology for instillation of the donor stool slurry. In 2010, Borody *et al.*[25] also dealt with this subject comprehensively and discussed some of the methods for carrying out FMT in recalcitrant CDI. More recently, a group of international infectious disease and gastroenterology specialists have published formal standard practice guidelines for performing FMT in CDI, outlining the rationale, methods and use of FMT, including screening procedures, material preparation, FMT administration and other practical pointers (Box 1).[26] However, the technical aspects of FMT are likely to rapidly evolve over the next few years with its increased use. We expect the specifications for

quantification, preparation and storage of donor material will become stringently standardized.

Selection of an administration route is largely dependent on the clinical situation, although transcolonoscopic infusion is probably favored for the vast majority of patients.[27] In our experience, severely ill patients might require several infusions, given the potentially impaired deep instrumentation of the colon, and the burden of *C. difficile* organisms could be higher in these patients than in those who are less ill. Even in these extreme situations, enema or transcolonoscopic infusion into the distal colon can achieve clinical success.[28] Nasoduodenal or nasogastric infusions might not succeed in such cases as a result of ileus. Data on FMT success in fulminant disease is unavailable at this stage, except for one published case in which this approach was successful.[29] Although CDI occurs in acute, relapsing and fulminant categories, much of the data currently stems from the relapsing category. A great deal more work is needed to reverse the high mortality in fulminant CDI—in excess of 50%[30]—and the high rate of colectomy. With the burgeoning success of FMT we hope that a fundamental and systematic re-evaluation of the standard antibiotic regimens used in CDI treatment will occur, and future therapeutic approaches will be aimed at minimizing further gut microbiota disruption and optimizing their restoration. Grehan *et al.*[31] reported the durable persistence of donor flora at 24 weeks post-transplantation; by comparison, oral probiotics have been shown to persist in the gut microbiota once consumption has ceased; however, they rarely persist beyond 14 days.[32] The prompt reconstitution of normal microbiota in FMT, even with a single infusion, is therefore so complete and durable[31,33,34] that early incorporation of FMT into standard treatment algorithms for CDI is a reasonable consideration. The challenge now is to develop methods, such as stored transplant material, which can be rapidly accessed and deployed for patients with severe CDI and early signs of fulminant disease.

Mechanisms of action

Unlike the concept of probiotics, which at best aims to somehow alter the metabolic or immunological activity of the native gut microbiota, the premise of FMT has always been to introduce a complete, stable community of gut micro-organisms, which are aimed at repairing or replacing the disrupted native microbiota. This scenario has in fact been documented in one case report of FMT for recalcitrant CDI, with the patient's fecal microbiota composition consisting predominantly of the bacteria derived from the healthy donor 2 weeks and 1 month post-FMT.[33] Engraftment of donor microbiota was accompanied by normalization of the patient's bowel function. The exact mechanism that achieved this normalization remains to be elucidated.

FMT seems to be effective in treating infective species such as *C. difficile*, and replacing microbiota deficiencies as described in CDI; although, similar pathological states might drive other gastrointestinal diseases in which in-depth study is still required. In addition, other mechanisms could be involved that might explain how FMT works. The metabolic activities of gut bacterial species can have consequences both locally, on the gut mucosa, and systemically. Disruption of these bacterial species can result in potentially harmful metabolic alterations, leading to the partitioning of toxic substances across the gastrointestinal mucosa where these substances are absorbed into systemic circulation. Gustaffson *et al.*[35] analyzed gut microbiota metabolism pre-FMT and post-FMT in 32 patients with antibiotic-associated diarrhea and found marked disturbances in the majority of microflora-associated characteristics in patients with antibiotic-associated diarrhea. Administration of a human fecal enema corrected these alterations and relieved diarrhea, usually within 4 days. Ultimately, such metabolic changes could one day be used in the diagnosis of specific variations within bacterial species.

Guidance on FMT

Various institutions have devised individual protocols regarding donor and recipient selection, material preparation and route of administration. According to the 2011 formal standard practice guidelines for FMT, Bakken and colleagues[26] suggest that a number of criteria need to be satisfied in universal donor selection. Briefly, at a minimum, the donor is screened for infectious agents, but much more rigorous donor screening is recommended. For example, given the important roles gut microbiota have in the digestive system (including systemic energy metabolism and modulation of the immune system), donors with any gastrointestinal complaints, the metabolic syndrome, autoimmune diseases or allergic diseases should be excluded. At this time, no test exists to determine the microbial composition of the microbiota in such a way as to predict the therapeutic activity and function of the material, although exclusion of pathogens is crucial. Overall donor health is, therefore, an important guide to health of the gut microbiota. Clearly, if donor selection is to be as rigorous as suggested, it would be unreasonable to burden patients who are often quite ill with sourcing potential donors and, in our opinion, the onus of donor selection should fall on the treatment center and not the patient themselves. A possible solution to this problem is the establishment of donor programs in which volunteers are recruited and screened. This approach, for example, is what we have performed at the University of Minnesota, USA, and Centre for Digestive Diseases in Sydney, Australia, whereby the vast majority of FMT is performed using volunteer donor material. This protocol has greatly simplified procedural coordination and markedly decreased laboratory donor screening costs.

As FMT development moves forward, in the foreseeable future, we envision the task being best conducted by a few centralized facilities, capable of processing the donor material and shipping it to individual providers in frozen, lyophilized or encapsulated forms.

Barriers to implementation

Today, FMT still remains at the fringes of medicine for various reasons. Although case series—on which the

Table 1 | FMT treatment in CDI

Study	Indication	Patients (*n*)	Mode of administration	Outcome
Eiseman *et al.*[19] (1958)	Severe PMC	4	Fecal enema	Dramatic resolution of PMC in all patients (100%)
Cutolo *et al.*[62] (1959)	PMC	1	Cantor tube, then fecal enema	Resolution
Fenton *et al.*[63] (1974)	PMC	1	Fecal enema	Symptom resolution within 24 h; sigmoidoscopy at 4 days revealed normal mucosa
Bowden *et al.*[64] (1981)	PMC	16	Fecal enema (*n*=15); enteric tube (*n*=1)	Rapid and dramatic response in 13 of 20 (65%) patients. 3 of 20 (15%) patients died; no evidence of PMC on autopsy in 2 of those patients, the third patient was found to have small-bowel PMC
Schwan *et al.*[65] (1984)	Recurrent CDI	1	Fecal enema	Prompt and complete normalization of bowel function
Tvede & Rask-Madsen[18] (1989)	Recurrent CDI	6	Fecal enema	Prompt *C. difficile* eradication and symptom resolution, including restoration of normal bowel function within 24 h
Flotterod *et al.*[21] (1991)	Refractory CDI	1	Duodenal tube	*C. difficile* eradication
Paterson *et al.*[66] (1994)	Chronic CDI	7	Colonoscope	Rapid symptom relief without relapse in all (100%) patients
Lund-Tonneson *et al.*[22] (1998)	CDAD	18	Colonoscope (*n*=17); gastrostoma (*n*=1)	15 of 18 (83.3%) patients clinically cured postinfusion without relapse
Persky & Brandt[23] (2000)	Recurrent CDAD	1	Colonoscope	Immediate symptom resolution; *C. difficile* eradication, which persisted at 5-year follow-up
Faust *et al.*[67] (2002)	Recurrent PMC	6	Unknown	All patients (100%) clinically cured postinfusion
Aas *et al.*[68] (2003)	Recurrent *C. difficile*-colitis	18	Nasogastric tube	15 of 18 (83.3%) patients cured; 2 (11.1%) patients died of unrelated illnesses; 1 treatment failure (5.5%)
Borody *et al.*[45] (2003)	Chronic CDI	24	Colonoscope and/or rectal enema and/or nasojejunal tube	Eradication of CDI in 20 of 24 (83.3%) patients, confirmed via negative stool culture
Jorup-Ronstrom *et al.*[69] (2006)	Recurrent CDAD	5	Fecal enema	All (100%) patients clinically asymptomatic postinfusion
Wettstein *et al.*[70] (2007)	Recurrent CDI	16	Colonoscope (day 1), then rectal enemas for 5, 10 or up to 24 days	Eradication of CDI in 15 of 16 (93.8%) patients, confirmed via negative culture or toxin assay
Louie *et al.*[71] (2008)	Recurrent CDI	45	Rectal catheter	CDI arrested in 43 of 45 (95.6%) patients
Niewdorp *et al.*[72] (2008)	Recurrent CDAD	7 (2 with PCR ribotype 027 strain)	Jejunal infusion via duodenal catheter	*C. difficile* eradication in all patients (100%), confirmed via culture and/or toxin assay
You *et al.*[29] (2008)	Fulminant CDI	1	Fecal enema	Bowel function, blood pressure and leukocyte count normalized; oliguria resolved, and both vasopressin and venous hemofiltration were discontinued
Hellemans *et al.*[73] (2009)	CDAD	1	Colonoscope	*C. difficile* eradication
MacConnachie *et al.*[74] (2009)	Recurrent CDAD	15	Nasogastric tube	13 of 15 (86.7%) patients asymptomatic post-FMT
Arkkila *et al.*[75] (2010)	Recurrent CDI	37 (11 with PCR ribotype 027 strain)	Colonoscope	*C. difficile* eradication in 34 of 37 (92%) patients
Khoruts *et al.*[33] (2010)	Recurrent CDAD	1	Colonoscope	*C. difficile* eradication, confirmed via negative culture; remained negative at 6-month follow-up
Yoon & Brandt[76] (2010)	Recurrent CDAD PMC	12 (2 with classic PMC on colonoscopy)	Colonoscope	All patients (100%) exhibited durable clinical response
Rohlke *et al.*[28] (2010)	Recurrent CDI	19	Colonoscope	18 of 19 (94.7%) patients clinically asymptomatic between 6 months and 5 years post-FMT
Silverman *et al.*[77] (2010)	Chronic recurrent CDI	7	Low-volume fecal enema	All (100%) patients clinically asymptomatic
Garborg *et al.*[78] (2010)	Recurrent CDAD	40	Colonoscope (*n*=2); duodenal instillation (*n*=38)	Eradication of *C. difficile* in 33 of 40 (82.5%) patients
Russell *et al.*[79] (2010)	Recurrent CDI	1	Nasogastric tube	Resolution of diarrhea within 36 h; repeat *C. difficile* toxin assay negative
Kelly *et al.*[80] (2010)	Chronic, recurrent CDI	12	Colonoscope	All (100%) patients exhibited clinical response
Mellow *et al.*[81] (2010)	Recurrent and refractory CDI	13	Colonoscope	12 of 13 (92.3%) patients were *C. difficile* toxin negative, coinciding with rapid resolution of diarrhea

Table 1 (cont.) | FMT treatment in CDI

Study	Indication	Patients (*n*)	Mode of administration	Outcome
Kassam *et al.*[82] (2010)	CDAD	14	Fecal enema	All (100%) patients had complete clinical resolution
Kelly *et al.*[83] (2011)	Relapsing CDI	26	Colonoscope	24 of 26 patients cured of CDI with resolution of diarrhea

Abbreviations: CDAD, *C. difficile*-associated diarrhea; CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation; PMC, pseudomembranous colitis.

majority of evidence for FMT is based—have obvious limitations given their generally small sample size, lack of a control or comparative group and possibility of selection and adverse event reporting bias, the success of FMT in eradicating infection and rapidly returning critically ill patients to health cannot be denied. Ongoing randomized placebo-controlled studies are currently underway, which should satisfy any remaining doubts about the efficacy of FMT in the near future.[24]

Other factors are likely to have a role in preventing the procedure from becoming a standard therapeutic option. The issue of simple aesthetics—the so-called 'yuck factor' can be a challenge in the medical office or endoscopy suite. However, in our experience this aspect is virtually nonexistent in patients with recalcitrant CDI. This point was reiterated by Kahn *et al.*[36] who conducted a qualitative study assessing patients' readiness for FMT for ulcerative colitis and found that not only did the overwhelming majority of patients welcome this therapy, but expressed their desire that the treatment be readily available. Although this organ (as in, the transplanted microbiota) undoubtedly presents a unique set of challenges and considerations, we feel that this 'yuck factor' should not deny patients a potentially life-saving therapy.

The CDI epidemic has forced the re-evaluation of FMT as a procedure, one which begs further development. Unfortunately, as donor material is both widely available and complex in composition, little interest has been expressed by the pharmaceutical industry with regard to the technological development of FMT-based therapeutics. Development has, therefore, largely been driven by individual clinicians who are facing increasing numbers of patients requiring FMT as an optimal and potentially life-saving treatment.

We recognize FMT to be a form of organ transplantation. The idea of a human microbial organ is a novel paradigm, but one well-supported by modern science. In some aspects, FMT is simpler to perform than other organ transplants, without the need for immunological matching of donor and recipient, or the need for immunosuppression after the procedure; yet, many aspects of this therapy are still unknown.

Emerging FMT applications

Although recalcitrant and severe CDI constitutes the most immediate indication for FMT, which urgently warrants further development for wider dissemination in clinical practice, other potential indications in which this procedure might be beneficial should be considered. Publicity concerning scientific advances in enumerating and understanding the gut microbiota has already convinced some patients that FMT can be curative in their individual conditions; we frequently field inquiries about the possibility of FMT for a variety of clinical problems, including IBD, IBS, obesity, anorexia nervosa, systemic autoimmunity, food allergies, eosinophilic disorders of the gastrointestinal tract, as well as neurodegenerative and neurodevelopmental disorders. However, more preliminary science and clinical work needs to be performed to develop optimal protocols that can be implemented in systematic clinical trials to test the therapeutic potential of FMT in these indications. Unlike recalcitrant CDI, in which the native microbiota have been severely damaged by repeated antibiotic courses, microbial communities in these diseases might be quite resilient to change—whether antibiotic conditioning regimens are needed to suppress or eliminate the native microbiota before FMT is unknown. Moreover, what antibiotics should be used and for how long is also an uncertainty, as is whether one FMT infusion is sufficient for treatment success, or if multiple scheduled infusions should be administered. More work is therefore required to elucidate whether antibiotic pretreatment does provide an improved therapeutic response. It should be noted that antibiotic conditioning regimens have the potential to not only open a niche for microbiota implantation, but also damage the incoming microbiota, which is a key consideration before implementing these treatments.

The central role of the gut microbiota in the pathogenesis of IBD is well established.[37,38] However, the current paradigm places the dominant focus on host factors, such as the immune system and the gut barrier, while the microbiota is regarded more generically as sources of microbe-associated molecules that can stimulate inflammatory responses. Yet, work has demonstrated nonequivalence of different gut microorganisms with respect to their interaction with the

Box 1 | Key concerns of the clinical guidelines for FMT

- Indications: recurrent or relapsing CDI; moderate CDI not responding to therapy; severe CDI, with no response to standard therapy after 48 h
- Donor selection, eligibility (those at risk of harboring an infectious agent should be excluded) and testing
- Recipient exclusion criteria (e.g. patients on major immunosuppressive agents or those with serious comorbidities require close assessment of risk–benefit)
- Protocol for performing FMT: donor and recipient preparation (including laxatives for the donor and large-volume bowel preparation for the recipient); donor sample preparation (e.g. use within 24 h, choice of diluent, homogenization and filtration of stool sample); administration (e.g. enema or nasoduodenal tube)
- Evaluation of success: resolution of symptoms is the primary end point; absence of relapse within 8 weeks of FMT and absence of CDI as secondary end points

Abbreviations: CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation.

host immune system. Some bacterial species (for example, segmented filamentous bacteria) were found to be uniquely capable of inducing T-helper-17 cells,[39] while others (for example, *Bacteroides fragilis* and clusters IV and XIVa of the genus *Clostridium*) augmented responses of regulatory $CD4^+$ T cells.[40,41] Furthermore, some patients with Crohn's disease have a reduction in levels of *Fecalibacterium prausnitzii* in mucosa-associated microbiota.[42] This micro-organism—a member of the dominant phylum *Firmicutes*—secretes metabolites that can reduce the production of proinflammatory cytokines such as IL-12 and IFN-γ, increase production of IL-10 and inhibit development of colitis in a mouse model.[42] Similarly, Qin *et al.*[43] have also reported reduced diversity of the fecal microbiota in patients with IBD, finding that these patients, on average, harbor 25% fewer microbial genes than healthy controls. If these alterations are somehow involved in the pathogenesis of IBD, replacement with a more favorable composition of microbiota might be therapeutic.

The scientific rationale for FMT development beyond CDI is quite compelling, with clinically gratifying outcomes achieved in other suspected microbiota infections. Prolonged remissions of ulcerative colitis after FMT have been reported in the literature.[44,45] In 1989, Bennet *et al.*[44] reported the first published case of FMT in ulcerative colitis, documenting reversal of his own disease after large-volume retention enemas of healthy donor flora; he had continuously active, severe ulcerative colitis of 7-year duration, with disease relapse whenever his prednisone dosage was reduced below 30 mg per day. At 3 months post-FMT, histology revealed no active inflammation, and he remained asymptomatic without therapy for the first time in 11 years. This finding was followed in 2003 by a report documenting the reversal of ulcerative colitis after FMT in all patients ($n = 6$) with previously severe, refractory disease;[45] the patients remained asymptomatic with normal colonoscopic and histological findings after 1–13 years without medication. However, it should be noted that unlike CDI, in which a single infusion of FMT is curative in most patients, recurrent infusions are typically required to induce profound remission in patients with ulcerative colitis. Clearly, the FMT mechanism of action in this disease is quite different to that of CDI.

Similarly, preliminary results have also reported on the resolution of constipation and IBS after FMT. Andrews *et al.*[46] treated 45 patients with chronic, severe constipation with FMT and reported a substantial improvement in 40 (89%) of these patients, with improved defecation and an absence of bloating and abdominal pain. Of 30 patients contacted at long-term follow-up (9–19 months), 18 (60%) continued to report normal defecation without laxative use. Borody and colleagues,[47] in a case series of 55 patients with IBS and IBD treated with FMT, reported that 20 of 55 (36%) patients were deemed cured post-FMT, nine (16%) patients reported a decrease in symptoms and 26 (47%) failed to show long-term response to FMT.[47]

Whilst using FMT to treat ulcerative colitis or constipation-predominant IBS in the Sydney clinic, serendipitous improvements in extraintestinal conditions not previously considered to be microbiota-related have also been observed. These include the virtually complete and prolonged (>15 years) normalization of previously severe multiple sclerosis symptoms in three patients whose constipation was the target of FMT,[48] and progressive normalization of platelet counts in a patient with idiopathic thrombocytopenic purpura whose ulcerative colitis was successfully treated with FMT.[49] In addition, we have also previously reported on the improvement of chronic fatigue syndrome using FMT in a long-term follow-up study.[50] Of the 34 patients who underwent FMT and were available for follow-up, 14 (41.2%) patients obtained persisting relief and seven reported mild or gradual improvements.[50]

The metabolic syndrome epidemic, associated with obesity and numerous other health problems, is arguably the greatest single health-care challenge in the industrialized world, one now rapidly spreading to encompass less developed nations. Energy metabolism is a well-recognized function of gut microbiota. The potential role of the gut microbiota and its influence on body size has long been acknowledged in the usage of low-dose antibiotics in farming practices.[51,52] In fact, similar effects for low-dose antibiotics have been shown in humans in the 1950s in the absence of any effects on rates of clinically significant infection.[53] Interestingly, comparisons between the distal gut microbiota of obese and lean individuals, as well as genetically obese and lean mice, have revealed differences in the distal gut microbiota composition and their metabolites.[54–56] Furthermore, the gut microbiota is involved in multiple elements of energy metabolism, including energy harvest, metabolic rate and energy storage.[57–60] Germ-free mice, which have a naturally low body weight, gain more body fat after colonization with gut microbiota from obese mouse donors compared with lean mouse donors, without increases in food consumption or obvious energy expenditure.[55] In 2010, Vrieze and colleagues[61] reported the results of a double-blind, randomized, controlled trial of FMT in 18 men with the metabolic syndrome. Half of the patients received fecal material from lean male donors and half were implanted with their own feces as controls. After transplantation of fecal flora from lean donors, fasting triglyceride levels in patients with the metabolic syndrome were markedly reduced; no effect was observed in the control group re-instilled with their own feces. In addition, peripheral and hepatic insulin sensitivity markedly improved after 6 weeks in the lean donor group. Again, this finding was not observed in the control group.

Such clinical observations urgently need to be followed with well-designed, randomized trials. The therapeutic action of FMT in some of these disorders is probably similar to that operating in the treatment of CDI. Although such observations are exciting and provocative starting points, they should prompt the systematic study of microbiota composition pre-FMT and post-FMT in sufficiently powered randomized trials.

Conclusions

In summary, the growing CDI epidemic has led to increasing use of FMT. The procedure is now being developed toward more standardized protocols, which should enable large randomized, controlled studies. Nonetheless, the focus of FMT on repairing the most obvious severe damage induced by antibiotic medications in CDI could be only the first chapter in a much larger task. If some of the major diseases, such as the metabolic syndrome, IBD and other autoimmune conditions, are causally linked to microbiota dysfunction, FMT can be anticipated to have a role in therapeutic gut microbiota restoration on a society-wide scale.

Review criteria

A search for original articles published between 1950 and 2011 was performed using MEDLINE and PubMed. The search terms used were "fecal microbiota transplantation", "fecal bacteriotherapy", "fecal enema" and "rectal enema" alone and in combination. References from all identified articles and recent review articles were cross-checked to ensure a thorough search. Any articles not available via MEDLINE and PubMed were obtained from the author's own personal archives. Additional references were identified using the authors expertise.

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Author contributions
Both authors contributed equally to researching data for the article, discussion of content, writing, reviewing and editing the manuscript.