Fecal microbiota transplantation: facts and controversies

Els van Nood[a], *Peter Speelman*[a], *Max Nieuwdorp*[a], *and Josbert Keller*[b]

Purpose of review
To review the current evidence on fecal microbiota transplantations (FMTs) for recurrent *Clostridium difficile* infections (CDIs), metabolic syndrome and inflammatory bowel disease.

Recent findings
Recently, a randomized trial confirmed the efficacy of this treatment strategy in patients with recurrent CDI. For other disorders, evidence is still limited. To date, studies have been performed to try and influence the course of metabolic syndrome and inflammatory bowel disease.

Summary
There is increasing interest in the role of altered microbiota in the development of a myriad of diseases. Together with new insights comes an interest in influencing this altered microbiota as a potential target for therapy. FMTs are effective against recurrent CDI, a disorder caused by disruption of the normal microbiota. Restoration of intestinal flora and thereby restoration of colonization resistance is thought to be the mechanism responsible for cure. With the developments in FMT and the extension of this treatment modality to both intestinal and extra-intestinal diseases, a new field of targeted therapy awaits. The ultimate goal is the development of powerful probiotic regimens that can replace FMT. Currently, FMT should only be given in a strict experimental setting for other conditions than CDI.

Keywords
Clostridium difficile, donor feces infusion, fecal microbiota transplantation, inflammatory bowel syndrome, metabolic syndrome

INTRODUCTION AND PURPOSE OF THE REVIEW

The first description of the use of human feces as a remedy for disease derives from ancient Chinese history, when feces were prescribed for a variety of conditions according to the *Handbook of Emergency Medicine*, written by Ge Hong, approximately 1700 years ago [1]. Later, consumption of fresh, warm camel feces was recommended by Bedouins as a remedy for bacterial dysentery; its efficacy was confirmed by German soldiers in Africa during World War II [2]. In modern medical literature, infusion of feces from healthy donors was first reported in 1958, when the surgeon Eiseman described a heroic response in patients with antibiotic-associated diarrhea treated with enemas containing donor feces [3]. Nowadays, infusion of human feces is commonly called fecal microbiota transplantation (FMT), although the terms 'donor feces infusion', 'fecal transplantation' or 'human intestinal microbiota transfer' are also used. In the past decade, FMT has gained increasing interest as an effective treatment strategy against recurrent *Clostridium difficile* infection (CDI) [4–6]. This can be partly explained by the steep increase in CDIs on one hand, and by the increased interest in the composition of the bowel flora and its influence on health and disease on the other [7,8]. FMT could possibly alter the course of other diseases in which an altered microbiota is involved in the pathogenesis. This insight has given birth to a promising area of research.

In general practice, however, experience with this procedure is limited by a lack of randomized trials supporting its efficacy and the unappealing nature of the treatment. This review will address the

[a]Department of Internal Medicine, Academic Medical Center, Amsterdam and [b]Department of Gastroenterology, Haga Teaching Hospital, The Hague, the Netherlands

Correspondence to Els van Nood, AMC-UvA, Department of Internal Medicine, Meibergdreef 9, Room F4-222, 1105 AZ Amsterdam, the Netherlands. Tel: +31 20 5669111; fax: +31 20 5669343; e-mail: e.vannood@amc.nl

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KEY POINTS

- Intestinal microbiota are increasingly recognized as partakers in human diseases, but the true causative role of intestinal microbiota in the development of (autoimmune mediated) diseases needs to be defined for many conditions.
- FMT has been shown to consistently alter intestinal microbiota composition in patients with recurrent CDI.
- FMT temporarily ameliorates insulin resistance and intestinal microbiota composition in obese individuals, when given feces from lean donors.
- Applying a proper fecal donor-screening protocol (minimizing risk of transmissible disease) together with executing randomized controlled double-blind trials are pivotal for determining the therapeutic role of fecal transplantation in clinical practice.
- The optimal protocol of infusing donor feces has to be defined.

use of donor feces for CDI, the metabolic syndrome and inflammatory bowel disease (IBD). In addition, the optimal protocol and future implications will be discussed.

THE COLONIC MICROBIOTA AND COLONIZATION RESISTANCE

The human bowel consists of trillions of bacteria, 1 g of feces containing 10e[11] bacteria [8]. With 16sRNA sequencing, the genetic composition of the microbiota can now be charted in great detail. There are four major bacterial phyla identified: *Bacteroidetes, Firmicutes, Actinobacteria*, and *Proteobacteria* [9]. In early childhood, the gut microbiota finds a unique equilibrium and stays relatively stable throughout life [10]. This is remarkable as the intestinal mucosa and thereby the microbiota is continuously confronted with antigens, food, possible toxic substances, and organisms. In a joint effort with the immune system, the microbiota needs to balance between protective reactions against pathogens and tolerance against commensal bacteria to preserve intestinal homeostasis [11]. This process is called colonization resistance. Even short courses of antibiotic use negatively influence colonization resistance, which is characterized by a lack of diversity and lack of abundance, which gives potential pathogenic bacteria the opportunity to grow and differentiate [12].

CLOSTRIDIUM DIFFICILE INFECTION AND FECAL MICROBIOTA TRANSPLANTATION

Clostridium difficile is one of those potential pathogenic bacteria. Being abundantly present in nature, *C. difficile* is demonstrated in the bowel of approximately 4–13% of asymptomatic people [13–15]. In healthy bowel, it lacks the potential to differentiate and produce toxins, which can cause disruption of colonic epithelium and diarrhea. However, change in colonization resistance due to antibiotics, surgery, chemotherapy, and several other factors create the opportunity for overt disease. CDIs are an increasing cause of infectious diarrhea in hospitals in the western world [7,16]. Metronidazole and vancomycin are the antibiotics routinely prescribed for treatment of CDI. Following initial treatment, a recurrence occurs in approximately 25% of patients [16], who are at increased risk to develop subsequent recurrences. FMTs are used as last resort for patients with recurrent disease, with reported success rates as high as 94% [4]. From 1958 onwards, there have been more than 500 patients described in case series and case reports reporting successful treatment with FMTs for antibiotic-associated diarrhea or microbiologically proven CDIs.

Restoration of the disturbed microbiota seems responsible for restoration of colonization resistance, thereby preventing new recurrences. Recent studies have addressed in detail the changes in microbiota following FMT for recurrent CDI. The residual colonic microbiota of patients suffering from CDI is deficient in members of the bacterial divisions *Firmicutes* and *Bacteroidetes* [17,18,19▪]. FMT has a dramatic and persistent impact on the composition of the patient's microbiota. The fecal bacterial composition of the recipient is strikingly similar to that of the donor, with a restoration of levels of *Bacteroidetes* species and *Clostridium* clusters IV and XIVa (*Firmicutes*), whereas the opportunistic *Proteobacteria* species decrease [19▪,20]. Whether reconstitution of bacteria is solely responsible for the effect against *C. difficile* remains unknown. The disappointing outcome of treatment with probiotics compared to donor feces suggests that feces contain a superior combination of intestinal bacterial strains. However, feces also contain additional substances (bile acids, proteins, bacteriophages) that may contribute to the higher success rate of FMT against recurrent *C. difficile*.

Recently, the first randomized trial of donor feces for recurrent CDI was published, in which patients were randomized to either a standard treatment with vancomycin (with or without a whole bowel lavage) or to a treatment with FMT [19▪]. The study was stopped prematurely after an interim analysis because of a large difference in effect between the standard treatment arm and the experimental arm. Of 16 patients in the infusion group, 15 (94%) showed resolution of CDI after one or two infusions, compared to 23–31% in the

control groups. Currently, there are several other randomized controlled studies recruiting patients with recurrent CDI (clinical trials.gov). In conclusion, evidence increases that donor feces infusion is indeed superior to antibiotics in patients with recurrent CDI. Importantly, severely ill patients, children, and immunocompromised patients were excluded from the randomized trial. Anecdotal reports suggest that FMT is well tolerated in those patients, but further research is needed to confirm these observations [21].

DONOR FECES FOR METABOLIC SYNDROME

There is increasing interest in the (potential causative) role of microbiota in obesity [22]. The colonic microbiota in obese mice shows a lower microbial diversity and is enriched in carbohydrate and lipid users [23,24]. Furthermore, colonization of obese mice with a 'lean microbiota' results in a significantly greater decrease in total body fat (−30%) than colonization with an 'obese microbiota' (+5%) [24]. In humans, a trial in male patients with metabolic syndrome was published in 2012, in which the therapeutic effect of FMT on insulin resistance was studied [25▪]. Patients were given either allogenic feces from lean donors, or an autologous FMT. A temporary reduction in peripheral insulin resistance was found in patients treated with 'lean' feces, implicating substantial effects on whole body glucose metabolism. Moreover, reductions in fasting lipid profiles after allogenic fecal therapy were found. The effect of fecal therapy on metabolism is possibly explained by enhanced production of specific short-chain free fatty acids like butyrate, produced by bacteria in the infused lean donor feces, which restores normal fecal physiology. Although promising, the results of new trials have to be awaited.

FECAL MICROBIOTA TRANSPLANTATION FOR INFLAMMATORY BOWEL DISEASE

The first reports of fecal microbiota transplantation in the colon for ulcerative colitis in literature date from 1989 [26]. Only a handful of cases have been published in case reports [27]. With the interest in the role of the microbiota in the pathogenesis of IBD, FMT is now considered as one of the potential 'novel therapies under investigation' for ulcerative colitis. A recent study of 10 children with ulcerative colitis treated with FMT showed 78% clinical response within 1 week; clinical remission in 33% of patients; and only mild and self-limiting adverse events [28]. Caution is required, as in a small group of four patients with Crohn's disease, adverse effects (transient fever, abdominal pains, bloating, and no clinical improvement with only transient effects on the host's fecal microbial composition) were reported 2 months after treatment with FMTs [29].

There are no results of randomized trials available supporting the efficacy of FMT for ulcerative colitis or Crohn's disease, but several studies are recruiting patients (clinical trials.gov). A blinded randomized trial will end this year in the Netherlands (Dutch Trial Registry, NTR2862, the TURN trial). Until the results of those trials become available, use of FMT in IBD patients should be considered strictly experimental.

OTHER IMPLICATIONS OF FECAL MICROBIOTA TRANSPLANTATION

An altered microbiota might play a causative role in a myriad of diseases. Irritable bowel syndrome, nonalcoholic steatohepatitis, celiac disease, allergies, neurodevelopmental disorders, endocrinopathies, and other autoimmune disorders are associated with a change in bowel flora [11]. Whether a FMT can positively influence these conditions remains to be seen, as there is no literature available at this point [30].

OPTIMAL PROTOCOL OF FECAL MICROBIOTA TRANSPLANTATION

There is no consensus on the optimal protocol for FMT. Feces can be infused both in the upper or lower gastrointestinal tract [4]. Approximately one-third of patients have received feces through a gastric or nasoduodenal tube, two-thirds through an enema or colonoscope [6,31]. The overall reported success rate is around 90%. As it is difficult to compare the different protocols and strategies in different case series and case reports, there is currently no consensus on the preferred route of infusion.

Historically, the majority of successfully treated patients received only one treatment with donor feces, although small early series reported multiple infusions.

If given for CDI, approximately 80% of patients received antibiotic treatment for CDI prior to the FMT. For other indications [metabolic syndrome, irritable bowel syndrome (IBS)] there are no reports in the literature of antibiotic pretreatment. A minority of patients receive an oral whole bowel lavage prior to infusion of feces. Although it certifies normal passage of fluids prior to infusing feces, there is no study that compared donor feces with or without a whole bowel lavage. With regard to

donor preference, most patients receive donor feces from partners or relatives, whereas healthy volunteers are used in a minority of cases. In our experience, there is no relation between sex-matched or relative-matched feces and success rates.

PREPARATION OF FECES

Freshly produced donor stool (200–300 g) is processed and infused preferably as quickly as possible. In case series, this is mostly done within 6 h of passage. Feces mostly are dissolved in sterile saline. Water and other diluents (e.g. yogurt or milk) have also been described as vehicle. Feces have to be liquefied, to allow infusing the solution in the bowel. Unfolded gauzes, paper funnels, and cloths all have been used to strain debris. In case series, the amount of feces used varies from 15 to 300 g, with a trend towards improved outcome using larger volumes of prepared solution [4]. Whether, feces should be prepared in an anaerobic environment to preserve as much obligate anaerobic bacteria as possible is unknown. Most laboratories lack anaerobic chambers, and previous success rates are obtained with aerobic preparation. Nevertheless it seems advisable to cover feces as soon as possible with saline.

More data become available about the use of standardized frozen stool samples, and outcomes do not seem to be affected by this procedure in case series [20]. The logistic advantage of frozen stool stored in advance is obvious, as it can be used directly when necessary.

PROTOCOL FOR SCREENING OF DONORS

With regard to screening of potential donors, it is important that individuals with an increased risk of any (sexually) contractible diseases are excluded in order to reduce transmission of (otherwise unknown) pathogens. Any risk of a recently contracted infectious disease that is still in its window phase (HIV, hepatitis) warrants exclusion of the potential donor. Questions regarding travel history, sexual behavior, previous operations, blood transfusions, piercings, and all other interventions that might contribute to carriage of an infectious disease should be asked [19▪]. Although the potential risk of transferring an infectious disease is probably limited when using a partner or spouse as donor, screening is always preferred. Moreover, keeping in line with current blood transfusion protocols, the use of thoroughly screened standardized frozen stool batches in fecal transplantation might have several logistical advantages over the use of fresh donor stool, with regard to the aforementioned window phase.

An overview of donor screening is given in Table 1.

The risk of transmitting a noninfectious disease has also to be taken into account;

with the increased interest for the interaction between microbiota and its influence on a large

Table 1. Screening of donors

1. Initial screening	Questionnaire addressing risk factors for potentially transmittable disorders
2. Donor screening	Blood
	Cytomegalovirus (IgG and IgM)
	Epstein–Barr virus (VCA IgM, VCA IgG, VCA, anti-EBNA)
	Hepatitis A (total antibodies, if positive and not vaccinated also hepatitis A IgM)
	Hepatitis B (HbsAg, anti-HbsAg, anti-HBcore)
	Hepatitis C (anti-HCV)
	HIV types 1 and 2
	Human T-lymphotropic virus type I and II
	Treponema pallidum (TPHA)
	Entamoeba histolytica (agglutination and dipstick test)
	Strongyloides stercoralis (ELISA)
	Feces
	Bacteriological evaluation by local standards
	Parasitological evaluation by local standards (triple feces test or PCR)
	Test for *Clostridium difficile* (toxin ELISA and culture or PCR)
3. One day before donation of feces	Questionnaire addressing current stool frequency and pattern, general health, use of antibiotics, travel history and (recent) sexual behavior since initial screening

anti-EBNA, anti-Epstein-Barr virus nuclear antigen; VCA, viral capsid antigen.

number of diseases (Crohn's disease, ulcerative colitis, autoimmune diseases, irritable bowel syndrome, and celiac disease) and the possible causative role of microbiota, patients with any of the above mentioned diseases should be excluded as potential donor.

POTENTIAL ADVERSE EVENTS

The potential adverse events from a FMT can be related to the procedure itself or related to the infusion of another human's feces. Procedurally, both routes have their specific (mostly theoretical) risks. Colonoscopy can only be performed by a trained physician, and has a small risk of perforation or bleeding. Although our experience is that infusing feces through a duodenal tube is less invasive and less strenuous than through colonoscopy, there is a potential risk of vomiting if bowel passage is hampered. Donor feces should therefore be infused slowly. Most case series lack reporting adverse effects [4]. In the randomized trial for CDI, no significant differences in adverse events among the three study groups were observed, except for mild diarrhea and abdominal cramping in the infusion group on the day of infusion [19▪]. Transfer of infectious diseases has not been reported.

CONCLUSION

With increasing recognition of the role of disturbed microbiota in development of various diseases, influencing and restoring the microbiota may provide proof of the concept that the microbiota is a causative factor in the pathogenesis. To date, FMT is an established treatment modality for recurrent CDIs. Persisting normalization of the disturbed bowel microbiota associated with CDI seems responsible for the therapeutic effect of FMT. New research has to focus on the optimal composition of microbiota given to patients with recurrent CDI [20,32]. This will lead to better probiotic mixtures, which ultimately eliminate the need for donor screening and donor feces infusion in the future.

In early 2013, the US Food and Drug Administration (FDA) required an investigational new drug (IND) application for each FMT. This greatly restricted the implementation of routine FMTs. This policy has since been changed, and the FDA now exercises discretion if they are provided with an extended informed consent. In Europe, the EMA has not regulated FMTs for CDI thus far. For other diseases, FMT should be considered strictly experimental, only offered to patients in an investigational setting. Although changes have been demonstrated for patients with metabolic syndrome, further studies have to demonstrate signature groups that can establish long-term effects on insulin resistance while taking confounding factors (diet, weight) into account. The results of randomized trials for IBD are eagerly awaited. Although patients with ulcerative colitis have repeatedly demonstrated a positive attitude towards FMT [27,33], caution of physicians is required, as evidence is lacking.

With the increased interest for microbiota and its influence on health and disease, future insights may point to other diseases for which FMT may be beneficial, such as (antibiotic induced) IBS, bacterial overgrowth, autoimmune diseases, multiple sclerosis, allergies, and depression. Although the concept of FMT is promising, current expectations should be dampened until future evidence arises.

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Conflicts of interest

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