Fecal Transplant: How an Ancient Therapy Is Finding a New Use in Today's Antibiotic-Resistant Era

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ABSTRACT

Fecal transplant refers to any method of delivery of healthy human stool to the colon of a recipient. This therapy is now gaining standard-of-care designation in the United States, Australia, and many parts of Europe for treating resistant *Clostridium difficile* infection). This literature review describes fecal transplant protocols. It highlights the variety of techniques used to screen stool donors; prepare and deliver treatment; and how, despite these variations, safety and efficacy remain high. It highlights the various ways to best mitigate safety while also recommending the direction in which clinical and research communities can move to continue to provide access to fecal microbiota transplant in a cost-effective manner.

Keywords: Fecal microbiota transplant; FMT; *Clostridium difficile*; Microbiome; Antibiotic resistance

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INTRODUCTION

In 1958, a US physician, Dr. Eiseman, successfully treated four patients with pseudomembranous colitis using healthy human stool.[1] The feces from a donor were mixed with buffered saline and then delivered via enema to the patients. The dramatic improvements in their symptoms were published in a case series. This represented the first use in modern medicine of what we now refer to as *fecal microbiota transplant* (FMT). This treatment is much older than midcentury American medicine. Fourth-century Chinese literature references stool-derived "golden soup" being used to treat human diarrheal disease.[2] Seventeenth-century European veterinary medicine used the technique, terming it *transfaunation*.[3] Therapeutic use of stool has been used informally for centuries, but it was largely lost in the toolbox of modern medicine with the integration of sanitation, antibiotics, and germ theory.

After Dr. Eiseman's publication, it was another 55 years before the first randomized controlled trial was done using fecal transplant to treat patients. FMT was used to treat antibiotic-resistant *Clostridium difficile* (rCDI; renamed *Clostridioides difficile* in 2016), a bacterium that causes diarrheal disease and can progress to pseudomembranous colitis. The fecal transplant arm of the clinical trial was so successful, with a 90% cure rate, that the trial was suspended, and all patients were administered FMT treatment instead of the other arm of treatment, which was standard antibiotic therapy.[4] Since then, stool banks and clinics across the United States have been treating patients with rCDI using fecal transplant, with consistent cure rates between 83% and 95% being achieved, regardless of protocols.[5–7] *Fecal transplant* refers to any method of delivery of healthy human stool to the colon of a recipient.

This literature review describes fecal transplant protocols. It highlights the variety of techniques used to screen stool donors; prepare and deliver treatment; and how, despite these variations, safety and efficacy remain high. This therapy is now gaining standard of care designation in the United States, Australia, and many parts of Europe for treating rCDI.[8] As it becomes more integrated into health care, it is important to reflect on which protocols work and why so that cost-effective treatments can be made available.

REGULATION OF FMT

Fecal transplant is currently considered an Investigational New Drug (IND) in the United States under Food and Drug Administration (FDA) guidance published in 2013.[9] This guidance was quickly modified to include a discretionary enforcement that allowed stool banks and physicians to manufacture and use FMT to treat patients with rCDI. Fecal transplant can be used for other medical treatment, but only through an FDA-approved IND study. To adequately track protocols and outcomes, INDs require a clinical trial to be approved by the FDA. The problem with this designation for CDI is that trials take time to enroll patients and therefore are prohibitive for the acute and sometimes deadly nature of CDI. The FDA's guidance for CDI was intentionally vague and provided little to no instruction regarding a legal definition of fecal transplant and how it should be manufactured or delivered. This guidance was not meant to be a permanent solution. In fact, several pharmaceutical trials have worked to create consortium probiotic formulas to match the efficacy of FMT and eventually replace it. So far, they have not had much success. FMT is derived from human stool, which is difficult to standardize in a way that would meet FDA guidelines for manufacturing of drugs. This has left fecal transplant in a limbo of sorts, waiting for a change in designation or a complete ban if a viable alternative drug is approved. There have been numerous agency and policy papers calling for the FDA to change the regulation of fecal transplant to something more similar to how blood is regulated in the United States.[10] However, these calls to action have not seen legislative outcomes yet. This complex policy struggle has also left the clinical use of fecal transplant without a standardized protocol for almost a decade. The lack of a standardized protocol is the major contributor to the variation in protocols reviewed in this paper.

DONOR SCREENING

For patients requiring FMT treatment acutely, it has been proposed that rigorous screening of potential universal donor stool material should be made available to all practitioners.[11–13] FMT maintains similar success rates for both related and unrelated donors.[11,12] Screening costs are high and access is difficult when a patient is in acute need. This problem can be solved by using a stool bank. In order for these banks to exist and be run safely, overall donor health is the primary goal of recruitment and screening. Obtaining a careful history and physical exam and acquiring various screening laboratory tests allow stool banks to minimize the potential for transmitting infection.[11,12,14–16] Medical screening is also imperative for improving safety regarding transmission of other chronic diseases, such as cardiovascular disease or obesity, which have links to the health of the microbiota.[17]

There is consensus that rigorous screening of donor stool and serum is important to minimize infectious disease risk.[18] There is no consensus, however, regarding which screening tests should be used.[19] On June 18, 2019, the FDA issued its first guidance for infectious disease screening after an immunocompromised patient died in a clinical trial as a result of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* that was acquired from FMT.[20] The new recommendations require screening and testing for multidrug-resistant organisms, specifically ESBL-producing *Enterobacteriaceae*, vancomycin-resistant enterococci, carbapenem-resistant *Enterobacteriaceae* (CRE), and methicillin-resistant *Staphylococcus aureus* (MRSA). Prior to this guidance, one review published in 2017 suggested screening for ESBL, but several others did not.[21–23] Owing to discrepancies in recommendations, clinical trials had not adopted ESBL as a standard screening metric for donor exclusion criteria, nor had many stool banks.[24–26] The guidance provided was a step in the right direction. A vast number of patients seek FMT as a therapeutic option; yet, a legal consensus regarding minimum screening requirements is still lacking. More guidance is anticipated and encouraged to maintain safe access to care.

Medical history of donors is a much more complex tool for screening. Donor exclusion criteria are vast, as they should be. There are concerns regarding pathogen transmission risk when considering FMT, with additional concerns regarding preexisting chronic diagnoses that have also been correlated with dysbiosis.[27] Some of the conditions that have been linked to dysbiosis or an imbalance to the intestinal microbial ecosystem are any gastrointestinal disorder, metabolic disease, obesity, autoimmune diseases, allergic disorders, and neuropsychiatric disorders.[11,19,27–29] The literature also recommends that donors should not have had exposure to antibiotics for some time before donation.[30] However, this timeline is rarely supported by evidence and varies widely in the literature from 3 to 6 months, even though research shows that antimicrobial exposure can have impacts lasting longer than 6 months.[22,26,31,32]

Age has also been indicated as an important factor to consider. A suggested age range for donors is often between 18 and 50 years old.[33] Being 18 years of age or older is attributed more to consent than to microbial health. The perceived benefit of using younger donors is reduced exposure to environmental contaminants and the assumption that these individuals are not sexually active, significantly reducing the risk of sexually transmitted illnesses. Younger donors have been used in clinical trials; however, they have not been directly compared with older donors regarding efficacy. In terms of the microbiome, microbial diversity has been shown in cohort studies to positively associate with age but to plateau after the age of 40.[34] Moreover, women were found to have higher species diversity (known as α-diversity) than men.[35] The difficulty is defining what constitutes a healthy donor, or at least what can exclude an unhealthy donor.

Kim and Gluck[36] outlined a suggested list of exclusion criteria consisting of age (<18 or >65 years), BMI >30 kg/m^2; metabolic syndrome; moderate to severe undernutrition; history of antibiotic use in the last 6 months; diarrhea within the last 3–6 months; history of *C. difficile* colitis, immune disorder, or use of immunosuppressive medications; history of drug use or other recent risk factors for human immunodeficiency virus or viral hepatitis; history of travel to a tropical region in the last 3

months; any gastrointestinal illness or complaints; history of autoimmune or atopic illness; history of chronic pain syndromes or neurologic or neurodevelopmental disorders; or history of malignancy.[36] All of these criteria have direct evidence for or theoretical potential to transfer via transplant of the microbiota. They are all important considerations and imperative to reducing risk associated with fecal transplant; however, they leave the question of what defines health unanswered.

A donor's lifestyle should be considered because many factors, such as diet, exercise, stress management, and even mental/emotional health, impact the health of the microbiome.[18,37] Diet especially plays a substantial role.[37] Unfortunately, dietary guidance is grossly missing in most fecal transplant donor screening.[38] The common recommendations seen in multiple studies allow stool donor volunteers to eat as they wish or suggest avoidance of common food allergens 5 days prior to stool donation.[39,40] At the very least, a discussion regarding food sourcing and fiber content should be considered. A paper published in *Nature* in 2018 investigated the effects of specific environmental pollutants on a fecal suspension in a colon medium for 24 h. This study found that four of the five pollutants included in their study (deltamethrin, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, 2,3,7,8-tetraclorodibenzo-*p*-dioxin, and polycyclic aromatic hydrocarbons) significantly altered the "volatolomics," or volatile metabolites produced by the human microbiome.[41] There is also a large body of evidence reflecting the importance of dietary fiber and the short-chain fatty acid secondary metabolites derived from them in microbial and human health.[42]

STOOL PREPARATION

Donor selection criteria have proven to be extremely difficult and ambiguous, and stool preparation is not far behind. Stool preparation varies widely among studies; yet, efficacy seems to remain high. It was previously believed that fresh stool would show greater efficacy, but this has not proven to be the case.[6] One of the first studies to determine efficacy of stored, frozen stool revealed an overall cure rate for rCDI of 90%.[43] Another study conducted in 2014 consisting of 23 recipients of frozen FMT versus 15 receiving fresh FMT revealed an 88% cure rate at 1-year follow-up for both groups.[30] A meta-analysis in 2017 confirmed no difference between fresh or frozen FMT.[44] Additionally, a small study of three participants revealed effective engraftment of previously frozen (at −80°C) FMT thawed on ice for 2 h before colonoscopic delivery. Engraftment was confirmed by microbial shifts in the stool compared before and after treatment. Participants had an increased abundance of both Bacteroidetes and Firmicutes.[45] The dose of microbial content also varies widely from study to study. A single dose, on average, consists of 50–60 g of stool added to 250–300 mL of phosphate-buffered saline.[12,46,47]

The time from donation to processing was assumed to be important for preserving microbial diversity. Nonetheless, studies do not necessarily agree about the ideal time frame. β-Diversity (the differences in microbial composition of samples taken from different environments) has been analyzed to better understand metrics with regard to freezing recommendations. One study compared stool samples that were immediately frozen with samples of 3- and 7-day delayed freezing. Their findings suggest that bacterial diversity can maintain stability for up to 7 days at room temperature.[48] A study in 2015 revealed a significant reduction in bacterial species *Bacteroidetes* and an increase in *Firmicutes* when stool was sampled 30 min after exposure to room temperature conditions.[49] One study suggested processing no later than 2 h after donation, but there is little data to support that metric.[12] Although no head-to-head studies have been conducted to compare efficacy in regard to timeliness of stool processing, a protocol using a 24-h guideline maintained overall clinical efficacy of approximately 90%.[18] With the conflicting data about viability and lack of comparative efficacy data, there is not consensus on timing for stool processing.

One study comparing thawing and refreezing determined that a sample can be freeze-thawed up to four times and also revealed that thawing processes of more than 10 min significantly decreased bacterial taxa abundance.[49] However, another study recommended a 2- to 4-h thaw in an ice bath, which

maintained over 90% efficacy.[12] A randomized controlled trial testing the stability of FMT after prolonged freezing revealed equal product efficacy after being frozen for up to 7 months. Beyond this time frame, the product was determined to be unstable.[50] Another study treating human subjects with frozen fecal matter determined stability of the product when frozen in 10% glycerol for up to 6 months; however, efficacy was maintained in treatment of stool frozen for up to 10 months for rCDI.[51] A study published in 2016 looked at fecal samples that had been freeze-dried and then frozen over a 14-year period in a −20°C freezer. After evaluating DNA quality and comparing the samples with fresh stool obtained from a similar cohort via the American Gut Project, the study concluded that with appropriate storage, microbial profiles were preserved and robust for extended storage periods.[52] It is not recommended to store FMT capsules that have not been freeze-dried in a frost-free freezer that varies from −20°C to −2°C in a 24-h period. Evidence indicates that diversity decreases significantly after only 3 days in this setting.[49] With the contradictory data about viability and lack of comparative efficacy data, there is currently also no consensus for storage of stool.

Because shelf stability and frozen storage have been proved to be limiting factors in access to FMT, future considerations are centered on a freeze-drying process. Preliminary investigations have revealed freeze-dried preparations to be 85% effective after the first dose. This dose consisted of approximately 60 mg of freeze-dried stool.[53] Freeze-dried material delivered in capsules provides multiple benefits, from shelf stability to reduced number of capsules needed. One study reported an 87.8% success rate using freeze-dried, double-encapsulated oral capsules. The same study identified 5% mannitol and 10% trehalose to be superior cryoprotectants, with trehalose maintaining superiority when bacterial viability was assessed.[54]

ORAL FMT CAPSULES

Oral FMT taken via capsules has been suggested as an easier, safer alternative to other delivery methods that require preparation and anesthesia.[55–57] Through the years, various capsule protocols have been described, with slight differences among them.[32,38] Many of the protocols for capsule production differ in regard to blending solutions, preserving agents, and dosing schemes. The data suggest that a variety of preparation methods and time frames maintain efficacy for the treatment of rCDI. No difference in product viability has been shown between aerobic and anaerobic settings.[58] Capsule production under ambient air has been shown to be more than 90% effective.[59] Publications have shown greater resolution of symptoms when the stool is suspended in water (98.5% efficacy) than when it is suspended in normal saline (86%); however, discrepancies in sample size limit any meaningful conclusions when water suspension was n=1 and normal saline was n=20.[47] Other studies blend stool with 1× phosphate-buffered saline or physiological saline, and all have proved to be incredibly efficacious.[18,30] Some theoretical concerns have been posed regarding hypotonicity of distilled water and its potential to lyse bacteria, but this has not been shown to be influential.[46] Few studies have shown head-to-head comparisons regarding efficacy, but it is important to note that all methods maintain high clinical efficacy.

PREPARATION FOR THE PATIENT

Most of the medical literature on FMT has largely focused on colonoscopic delivery until Kao's breakthrough paper in 2017, which showed equivalent efficacy for frozen oral capsules when compared with colonoscopic delivery.[56] Because this delivery method had dominated the field for the decade preceding, colonoscopic bowel preparation was traditionally accepted as a required process for patients to undergo before receiving FMT therapies. However, studies without bowel preparation do not show an appreciable impact on clinical efficacy.[54] In fact, bowel preparation is deemed unnecessary with delivery routes such as the nasoenteric tube.[47,60] Furthermore, more simplified protocols for patients are similar in efficacy to those requiring various preparatory steps, and they also reduce patient stress.

Because FMT is not considered a treatment option until standard therapies have failed, most patients are presented with it as an option while they are pursuing antibiotic therapy. All studies require discontinuation of antibiotic therapy before treatment, but timelines vary. In some studies, participants discontinue antibiotics at least 24 h before treatment. Other studies suggest discontinuing antimicrobial therapy 48 h in advance.[6,30,39,61,62] Still other studies suggest that liquid fasting the evening before therapy, with fluid restriction 2 h prior to administration, is sufficient compared with bowel preparation for colonoscopy.[54,62] A study testing doses of freeze-dried FMT reveal an 88% cure rate (defined as no *C. difficile* after 2 months) with no preliminary measures taken at all.[54]

Antibiotic therapy is required prior to FMT in the United States. This is important when considering transplantation of microbes from one human to another. A required cessation of antibiotics is recommended, and guidance ranges from 24 to 72 h with maintained efficacy of treatment.[3,39,55,63] Some studies use H2 blockers such as ranitidine[61] or proton pump inhibitors (PPIs) such as omeprazole prior to FMT administration. However, PPIs have a known association with CDI recurrence,[64] and the increased failure rates associated with their use in FMT should give clinicians pause regarding this recommendation.[47] Active opiate use during the initial infection of CDI has been shown to decrease efficacy of FMT.[65] Many pharmaceutical medications have negative impacts on the diversity of the human microbiome;[66] all medications should be considered when exploring this therapeutic option for patients.

CAPSULE ADMINISTRATION

Variance in the protocol for the administration of capsules can be added to the complexity already seen in FMT protocols. Various studies have demonstrated efficacy with differing dosing requirements. It is known that repeating FMT increases clinical efficacy.[67–69] It has also been shown to be as efficacious as standard medical treatment, trending toward favoring FMT over standard-of-care treatment (P=0.11).[70] A 2019 systematic review suggested that patients may require up to three treatments, with doses varying from as small as 0.25–0.50 g to as large as 100 g of stool, for clinical efficacy to reach 92.6%.[47,69] One or two administered doses of 30 capsules, divided over 2 days (15 capsules on day 1, 15 capsules on day 2), revealed a 91% cure rate in 180 participants, with cure being defined as resolution of diarrhea or no relapse of diarrhea at 8-week follow-up.[59] In a trial of 13 women and 6 men, an 89% clinical cure rate was achieved with a single dose for 13 subjects and two doses for 4, whereas 2 were determined to have experienced treatment failures.[68] A dosing scheme that included 15 capsules for 2 consecutive days revealed an overall clinical efficacy of 90%.[39] This protocol was repeated in a small 2018 study consisting of 15 participants. In this study, antibiotics were discontinued >48 h before 2 consecutive days of 15 capsules administered each day.[71] This revealed 86.6% and 100% cure rates in one and two FMTs, respectively.[71] These data provide evidence that increased exposure to the newly transplanted microbiome can provide greater treatment efficacy and potentially reduce the number of treatments needed to cure patients with *C. difficile* infection.

SAFETY OF FMT

With adequate donor screening and laboratory testing before administration, FMT is globally accepted as generally safe.[13,72] Long-term data are limited, and further investigation into sustained effects and adverse outcomes is necessary.

Reported short-term adverse events appear to be minor. Mild adverse events, such as abdominal discomfort and bloating, have been reported with administration of frozen oral capsules, but resolution of symptoms usually occurs within 3 days of treatment.[39,69,73,74] The most commonly reported symptom on the day of infusion is diarrhea, generally followed by cramping or belching, depending on administration route.[17] Common mild to moderate symptoms include abdominal pain, abdominal cramping, flatulence, increased stool frequency, constipation, vomiting, belching, fever, and transient increase of C-reactive protein.[74] By contrast, severe adverse events were frequently

associated with the colonoscopic or nasogastric procedure (40%) rather than with the infusion of fecal material.[74] In a study of pediatric patients (average age 9 years), the only reported adverse event after treatment through a nasogastric tube was transient vomiting in 13% of participants (6 of 47).[61]

Severe side effects are extremely rare. All but one death associated with FMT have been related to the delivery procedures (eg, perforation, aspiration during sedation, small bowel involvement of CDI).[75–77] Regarding the one event that led to sepsis and ultimately death, two immunocompromised individuals who received FMT from the same donor became infected with a multidrug-resistant organism, extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli.* Currently, the only information known about the incident was released in a statement by the FDA and is detailed above in Donor Screening. This event highlights the importance of rigorous screening measures, along with understanding the vulnerabilities of patient populations treated. In nonimmunocompromised healthy individuals, multidrug-resistant bacteria might be able to cohabitate safely with normal flora. Two case reports have shown successful decolonization of ESBL, CRE, MRSA, and mass drug administration (MDA) using FMT in healthy populations.[78,79] A retrospective study conducted in a cohort of immunocompromised patients revealed a 78% cure rate after a single dose of FMT, with repeat FMT providing an 89% cure rate. This study reported two deaths, both unrelated to FMT.[80] Regardless of these reports, special caution should be observed when considering FMT for patients who are severely immunocompromised.[13]

Few studies have been conducted on the long-term effect of FMT, but the evidence thus far is promising. Jalanka *et al.*[81] tracked 84 participants in total for 3.8 years. Of these, 45 received FMT therapy and 39 served as control subjects using antibiotic therapy (either metronidazole or vancomycin). After 3.8 years, there was no observed difference in newly developed conditions between groups, and weight gain was not significant between groups. However, the benefits reported in the FMT group were significantly better than in the antibiotic group. Participant reports revealed significant differences in improvement of bowel habits, reduced gastrointestinal disturbance in the upper and lower intestines, and mental health in the FMT arm.[81]

CLINICAL OUTCOMES

C. difficile infection can be debilitating to patients, but with FMT as a treatment option, patient suffering can be drastically reduced and lives saved. An additional benefit of FMT is significant improvement in self-reported health ratings.[39] A study of 19 individuals, mean age 49, in whom at least three methods of standard therapy had failed, including both a pulsed and a tapered vancomycin prescription, found a 100% cure rate of rCDI.[82] In larger-cohort studies, FMT has maintained an efficacy of over 90%.[56] A systematic review in 2017 reported that in 657 participants, FMT was more effective than vancomycin or placebo.[83] A randomized study with three trial arms – FMT via nasoduodenal tube, a standard vancomycin regimen (500 mg orally four times daily for 14 days), and a standard vancomycin regimen with bowel lavage – was stopped early after interim analysis revealed infusion of donor feces to be significantly more effective than the use of vancomycin ($P<0.001$).[4] A study using freeze-dried capsules containing approximately 2.1×10^{11} bacteria in two or three capsules showed an 87.8% cure rate at 8 weeks, with no serious adverse events reported.[54] A prospective study of 167 patients initially had a 16.7% failure rate with 28 primary nonresponders. Among these 28 nonresponders, 20 received repeat FMT, with the result that 15 (75%) of them achieved clinical cure at 8 weeks.[84]

CONCLUSION

Although safety and efficacy data on FMT for the treatment of rCDI are excellent, as with all therapies, FMT can be improved. One way to mitigate poor outcomes is to check with patients frequently to treat any potential recurrence quickly and effectively. Patients with rCDI who receive FMT often experience symptom relief within 24–48 h.[39] Mean failure time has been shown to be approximately 14.5 ± 12.5 days.[84] It is suggested, therefore, that clinicians counsel their patients to be alert to any symptoms of recurrence up to 4 weeks after treatment.[84] Many studies define clinical cure as being asymptomatic for 8 weeks.

Fecal transplant protocols have come a long way since fourth-century China, but access to this treatment option is still limited to a small percentage of patients with rCDI. The variety of successful protocols currently being used reflects the complexity of human stool and how little we understand about its mechanism of action as a therapy. The strong efficacy seen within the variety of protocols provides insight into the incredible power of the therapy. Moreover, it is important to recognize the potential of this therapy for wider application beyond rCDI as further data become available. This review highlights the various ways to best mitigate safety while also recommending the direction in which clinical and research communities can move to continue to provide access to FMT in a cost-effective manner. Our hope is to encourage further research so that this therapy can routinely be provided to patients in need and help mitigate the huge problem of global antibiotic resistance.

COMPETING INTERESTS

The authors co-own and run Flora Medicine, which is a stool bank and clinic in Portland, OR.

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