Fecal Microbiota Transplantation for the Management of *Clostridium difficile* Infection

Raghavendra Paknikar and Joel Pekow

Abstract

The clinical burden of *Clostridium difficile* infection (CDI) continues to grow. Despite the multitude of treatment options that have been developed and tested to combat the morbidity and death associated with CDI, recurrence remains common. As such, treatment modalities such as fecal microbiota transplantation (FMT) have become studied increasingly; FMT serves to transplant stool from carefully selected healthy subjects into *C. difficile* positive patients through a variety of delivery routes. In doing so, FMT is hypothesized to correct dysbiosis of the recipient gut microbiome addressing the root cause of the pathogenesis of *C. difficile* infection. A growing body of evidence shows FMT to be efficacious in this setting, and the study of FMT accordingly continues to evolve to identify novel indications for its utilization.

Keywords: *Clostridium difficile*; *Clostridium difficile* colitis; fecal microbiota transplantation

CLOSTRIDIUM DIFFICILE is a gram-positive bacillus obligate-anaerobe that is responsible for *C. difficile* infection (CDI). Its pathogenesis comes from the production of cytotoxins, toxin A (TcdA) and toxin B (TcdB), which cause mucosal injury in the gastrointestinal tract [1]. Because *C. difficile* was first identified in 1978 as the etiology of pseudomembranous colitis, its impact on the medical landscape has become recognized increasingly [2]. Specifically, CDI is now the most common health-care-associated infection, with an estimated annual cost to the U.S. healthcare system of approximately \$5 billion [3–5]. Further, CDI contributes to substantial morbidity and death in the United States; in 2011 alone, 29,300 deaths were attributed to CDI [6–8]. In the United States, hospitalizations for CDI among non-pregnant adults doubled from 2000 through 2010 and were projected to continue to increase, lending credence to its ongoing clinical significance [9].

The human gut microbiota, which is an exceptionally diverse ecosystem consisting of thousands of bacterial species, protects against invasive pathogens such as *C. difficile* [10,11]. The disruption of the gut microbiota in the setting of *C. difficile* colonization leads to the onset of symptomatic disease [12]. There are a variety of clinical precipitants for this change in microbiota, although the most notable is exposure to antibiotic agents [13]. Other commonly implicated sources of dysbiosis include immunosuppression, advanced age, abdominal surgery, or other medications such as proton

pump inhibitors. Once this disruption has occurred, CDI presentation may vary from a self-limited diarrheal illness to a fulminant, life-threatening colitis [1].

Pharmaceutical Options in *C. difficile* Infection

The management of CDI has evolved over the years as the medical community continues to gain insight on its pathogenesis. Initially, metronidazole was utilized primarily as first line management. Data before 2000 indicated similarities in the percentage of patients who achieved clinical resolution while taking metronidazole compared with vancomycin. More recent data, however, have demonstrated a significant benefit in symptom resolution with lower recurrences after management with vancomycin [14–17].

This effect may be driven by resistance patterns. In one study of 288 *C. difficile* positive patient stool samples, antimicrobial resistance testing revealed 134/282 (47.5%) patients resistant to imidazole (a class of agents that includes metronidazole), 17 (6.1%) resistant to vancomycin, and 9 (3.2%) resistant to imidazole and vancomycin [18]. As such, the most recent Infectious Disease Society of America (IDSA) guidelines no longer recommend using metronidazole as a first line agent [19]. Fidaxomicin is also approved currently by the Food and Drug Administration (FDA) for an initial episode or first recurrence of CDI, but its use is limited in this setting in many persons given its significant cost [19].

In a randomized, placebo controlled trial, fidaxomicin was non-inferior to oral vancomycin for clinical cure rate for patients who have recurrence of CDI [20].

In cases of primary recurrence, an oral vancomycin taper or pulse dosing schedules are recommended, often with advisement from infectious disease or gastroenterology specialists [19,21]. As an alternative, patients who have recurrence of CDI can be treated with fidaxomicin because randomized placebo controlled trial data demonstrate lower rates of recurrences with fidaxomicin compared with those treated with vancomycin [20]. In addition to vancomycin and fidaxomicin, bezlotoxumab has shown efficacy in the prevention of recurrent CDI. Bezlotoxumab, a monoclonal antibody against *C. difficile* toxin B and actoxumab, a monoclonal antibody against *C. difficile* toxin A, were studied against placebo (all patients were also receiving standard of care oral antibiotic agents) in the MODIFY I/II double-blinded randomized controlled study of 2655 patients with either primary or recurrent CDI. This study showed a reduced rate of CDI recurrence in the treatment group of bezlotoxumab alone and bezlotoxumab + actoxumab, 17% and 16%, respectively, versus 28% in the placebo group [22]. The latest IDSA/Society for Healthcare Epidemiology of America (SHEA) guidelines, however, do not comment on the use of bezlotoxumab for prevention of recurrent CDI [19].

Although not routinely used in clinical practice, other treatment regimens have demonstrated efficacy in managing CDI. One small study demonstrated lower rates of CDI recurrence in patients treated with a 20 day course of rifaximin after standard anti-CDI antibiotic agents compared with placebo, although the difference between groups was not statistically significant [23]. The use of a rifaximin after traditional vancomycin, however, has raised concerns regarding the potential for the development of high level resistance with this treatment regimen [24]. Nitazoxanide, which is a broad-spectrum anti-helminthic agent, has also been investigated as a treatment for patients with CDI. In a randomized, double-blind study, nitazoxanide was compared with vancomycin in 50 patients. The results of this study demonstrated that nitazoxanide may be as effective as vancomycin in managing CDI, but the small sample size precluded conclusions about non-inferiority [25].

Individuals with an impaired anti-toxin response may be more susceptible to CDI. As such, several investigators have hypothesized that use of intravenous immunoglobulin (IVIG) may be an effective treatment strategy in patients with CDI unresponsive to antibiotic therapy. A retrospective study of 14 patients with severe, refractory, recurrent CDI undergoing treatment with IVIG (Flebogamma, 150–400 mg/kg) showed that nine patients achieved normalization of bowel movements at a median of 10 days [26]. Another small, retrospective study of 21 patients receiving IVIG, however, demonstrated only nine patients with colitis resolution [27].

In addition to antibiotic treatment, there has been significant interest in the role of probiotics for preventing recurrence of CDI. A 2017 Cochrane systematic review and meta-analysis of 31 randomized controlled trials (RCTs) including 8672 patients suggested probiotics are effective overall for preventing *C. difficile*-associated diarrhea with a number needed to treat to benefit (NNTB) of 42. Interestingly, a post hoc subgroup analyses suggested that probiotic treatment was most effective in trials with a *C. difficile* baseline risk >5%

(NNTB=12), and ineffective in trials with a *C. difficile* baseline risk ≤5% [28]. Together, these treatments encapsulate the diverse mechanisms through which recurrent CDI has been targeted with variable success.

Increasing Burden and Resistance of *C. difficile* Infection

Despite the treatment options available, CDI remains a clinically challenging condition in large part because of increasing resistance patterns. Even among those that recover, recurrent disease is common. A first recurrence will occur in 15%–20% of successfully treated patients, and a second recurrence will occur in 45% of those patients. In total, multiple recurrences will develop in up to 5% of all patients [29–31]. There are several possible explanations for the high recurrence rate in the population. One is the emergence of new strains such as the North American pulsed-field gel electrophoresis type 1 (NAP1) strain, which has been responsible for geographically dispersed hospital-associated outbreaks [32–34]. Further, laboratories are increasingly switching to polymerase chain reaction-based detection assays with higher sensitivity for CDI diagnosis, leading to increased rates of positive tests in carriers of *C. difficile* in the setting of an aging and thus higher risk population [35,36].

The most common proposed explanation for the high recurrence rate of CDI, however, is that conventional antibiotic treatments for CDI not only work on CDI but also against other gut bacteria, which in turn produces further dysbiosis of the microbiota predisposing patients to recurrence. It follows then that return of the gut microbiome to its original state (pre-CDI) may serve to reduce the possibility of recurrence in patients with CDI. In this context, fecal microbiota transplantation (FMT) has gained considerable attention as a means to combat increasingly difficult to manage CDI [37].

Role of FMT in *C. difficile* Infection

Fecal microbiota transplantation consists of the infusion of feces from a healthy donor to the gastrointestinal tract of a recipient patient to treat a specific disease associated with alteration of gut microbiota. The mechanism of FMT is somewhat debatable but appears to be because of successful retention of donor stool content in the recipient in addition to augmentation of species present in the recipient at low levels before FMT [38]. The FMT remains without comprehensive FDA approval, but its clinical use has been buttressed by a growing amount of literature supporting its efficacy. Presently, the FDA classifies FMT as an investigational new drug, a classification that typically is required for physicians who intend to utilize the treatment for clinical or research purposes. In the case of FMT, however, the FDA has noted a special guidance whereby FMT can be utilized in cases when CDI is not responsive to standard therapies outside of the context of a clinical trial as long as physicians obtain informed consent detailing the risks surrounding the procedure and its designation as an investigational therapy [39].

The FMT has demonstrated the greatest efficacy in recurrent CDI (rCDI), most commonly defined as more than two episodes of CDI [40–46]. Multiple societies including the IDSA, SHEA, European Society of Clinical Microbiology and Infectious Diseases (ESCMID), and the American College of

Gastroenterology recommend FMT as a treatment for those with rCDI [19,21,47].

Two unblinded randomized controlled studies have demonstrated the superiority of FMT compared with recurrent antibiotic treatment for patients with rCDI. In the first unblinded RCT involving 43 patients, van Nood et al. [42] studied a 14-day oral vancomycin treatment course in 13 patients versus vancomycin with bowel lavage in 13 patients versus a four-day vancomycin course followed by FMT delivered by nasoduodenal tube in 17 patients. The study was terminated early after an interim analysis of patients achieving the primary end-point of initial response without relapse for 10 weeks showed a sizable difference between FMT (81%) and non-FMT groups (27%) ($p < 0.001$).

A second unblinded RCT involving 39 patients with rCDI compared 20 patients who received a three-day vancomycin course, followed by bowel cleanse and FMT delivered by colonoscopy, which was repeated until resolution of colitis was noted, to a second group of 19 patients who received vancomycin 125 mg four times daily treatment for 10 days, followed by pulse vancomycin dosing every two to three days for at least three weeks. The study was stopped when interim analysis at one year revealed a 90% (18/20) resolution of CDI in the FMT group compared with 26% (5/19) resolution in the vancomycin group ($p < 0.0001$) [44].

In addition to randomized controlled data, several observational and retrospective studies have also supported the efficacy of FMT for refractory CDI. A systematic review that included two RCTs and 21 case-series studies involving 516 patients reported the overall rate of symptom resolution when FMT was used for rCDI was 85% [45].

In addition to literature examining the benefit of FMT in refractory CDI, several studies have sought to determine the efficacy of FMT in patients with severe CDI unresponsive to antibiotic therapy. In a recent open-label RCT, 56 patients with severe CDI as defined by the ESCMID guidelines that was also refractory to antibiotic agents were studied as follows: Group FMT-S (28 patients) had a single FMT followed by a 14-day vancomycin course and Group FMT-M (28 patients) had multiple (at least two until disappearance of pseudomembranous colitis) FMT plus a 14-day vancomycin course. In this study, both FMT-S (75%) and FMT-M (100%) achieved the primary end-point of cure of refractory severe CDI ($p = 0.01$) [48]. Additional studies have also shown good efficacy of FMT for severe CDI using a variety of FMT protocols, but often requiring re-treatment with FMT [49–51].

Given the effectiveness of FMT in recurrent and refractory CDI, there has also been an interest in the use of FMT as primary management for CDI. A recent small RCT in patients with a primary infection demonstrated that seven of nine patients treated with FMT had a response compared with five of 11 treated with metronidazole ($p = 0.20$). Because of the small sample size in this study, future larger investigations are necessary to draw conclusions in this cohort. In addition, this study used metronidazole as opposed to vancomycin as the first line antibiotic agent of choice, which may be less applicable to institutions where vancomycin is given as a primary therapy [52]. Another study evaluating FMT after the first episode of CDI found a decrease in death in patients infected with the 027 strain who were treated initially compared with those who were treated after recurrence [53]. Further analyses are required

to determine the cost-effectiveness and feasibility of this approach to CDI treatment.

The expanding number of clinical studies examining the efficacy of FMT for *C. difficile* have led to improved understanding of patient populations that are more likely to benefit from FMT. Fischer et al. [54] investigated 328 patients in their developmental cohort and found severe and severe-complicated infection, inpatient status during FMT, and the number of previous CDI-related hospitalizations to be strongly associated with early failure of a single FMT for CDI. Another study of 201 patients demonstrated female gender, previous hospitalization or surgery as significant predictors of FMT failure [55]. A third analysis of 64 patients found severe CDI and inadequate bowel preparation to be independent predictors of FMT failure [56]. Together, these studies will aid in the understanding of patients who more likely need close monitoring after FMT and potentially require multiple infusions of donor stool for clinical cure.

Challenges of FMT

As more is learned regarding FMT, new challenges have arisen. Several factors unique to FMT have been identified by providers as the clinical reach of FMT has broadened including logistics of donor selection, inoculum processing, and route of delivery of the sample to the patient.

FMT donor selection

A variety of donor selection procedures exist. Initial FDA and early joint professional society consensus recommendations had suggested that fecal donors should be known to the recipient or treating physician [39]. Reasons for this are multi-factorial, but known donors are thought to share environmental risk factors and be more likely to have greater overlap in microbiota, reducing the chance for intolerance to the FMT. Given the rapid rise in FMT utilization, however, FMT stool banks, which collect and store stool from anonymous, screened donors, are becoming increasingly popular.

A systematic review and meta-analysis including 273 patients with CDI showed no difference in clinical outcomes between anonymous and patient selected donors [41]. Large third-party donor groups, such as Openbiome, employ several protections to minimize potential harms of banked stool. These include screening donors for occult pathogens in both feces and blood, as well as excluding individual donors who have taken antibiotic agents or other microbiome altering medications, have specific pre-existing medical conditions (including inflammatory bowel disease [IBD], irritable bowel syndrome [IBS], malignancy, chronic infections, metabolic disorders, active autoimmune disease, or receiving active immunosuppression), are older in age, and have high-risk behaviors [57]. For this purpose, some groups utilize negative screening questionnaires similar to those given to potential blood donors [58]. Further study is needed to ascertain whether current screening protocols are sufficient, particularly for recipient groups such as the immunocompromised.

Supply of FMT samples

Given the increasing demand of FMT, attention is being paid to ways to streamline administration of FMT including utilization of previously collected frozen or freeze-dried stool

samples. One double-blind RCT involving 219 patients with recurrent or refractory CDI showed clinical non-inferiority of the use of frozen FMT samples compared with freshly collected stool delivered via enemas [46]. A similar comparative RCT by Satokari et al. [59] displayed similar efficiency of FMT performed with fresh and frozen fecal samples for the management of rCDI.

Given the efficacy of frozen samples, previously collected banked FMT samples are often utilized clinically. The implications of frozen FMT are multi-faceted including reducing the number of donor screenings, increasing the availability of frozen banked samples, delivering FMT samples to locations without on-site facilities, and potentially improving safety because frozen samples can be tested with greater scrutiny while they are preserved [60]. Staley et al. [61] found that after a single administration of encapsulated freeze-dried FMT in 49 patients, 43 patients achieved a clinical success, defined as no recurrence of CDI over two months. Further, the fecal microbiome demonstrated near normalization of the fecal microbial community by one month following this FMT treatment portending both physiologic and clinical implications of treatment.

In a similar study, 72 patients with rCDI received FMT delivery by colonoscopy with either fresh, lyophilized, and frozen samples. This analysis demonstrated that all three delivery methods were efficacious and concluded that cure rates were highest for the group receiving fresh product seen in 25/25 (100%), lowest for the lyophilized product 16/23 (78%; $p=0.022$ vs. fresh and 0.255 vs. frozen), and intermediate for frozen product 20/24 ($p=0.233$ vs. fresh) [62].

FMT routes of administration

A variety of routes of administration are used for FMT. The most common modes to deliver an FMT into the upper gastrointestinal tract involve either a nasogastric tube or endoscopic delivery into the stomach or proximal small bowel. Success rates for FMT through upper gastrointestinal tract administration into the proximal small bowel have ranged between 77% and 94% in previous investigations [63,64]. Delivery of an FMT into the colon by enema or colonoscopy has also demonstrated a high rate of efficacy, with greater success rates when stool is delivered to the proximal colon.

Clinical cure in studies examining colonoscopy-guided administration have ranged from 80% to 100% [40,65–68]. A systematic review and meta-analysis that included 273 patients with rCDI found that lower gastrointestinal delivery (that predominantly was composed of colonoscopic delivery with only one study involving enema delivery) has higher rates of clinical resolution compared with upper gastrointestinal delivery. Specifically, 91.4% of lower gastrointestinal delivery versus 82.3% of upper gastrointestinal delivery patients displayed clinical cure ($p<0.05$) [41].

Both forms of administration are not without clinical risks. One theoretic concern with upper gastrointestinal delivery that arises is the potential for aspiration, which may in turn also lead to a reduction in volume of fecal transplantation and therefore less efficacious treatment. With respect to lower gastrointestinal delivery, risks associated with colonoscopy exist that may be exacerbated in persons with *C. difficile* associated colitis. Colonoscopic delivery, however, does possess clinical utility given the ability to visualize the area

of disease and evaluate for pathology unrelated to CDI. Broadly, however, both categories of endoscopic delivery confer additional expense and healthcare utilization.

In addition to delivery during endoscopy, several recent studies have assessed the efficacy of oral FMT capsules. Hirsch et al. [69] conducted a small study of 19 patients with rCDI who were given oral capsule FMT, of which ultimately 17/19 (89%) patients showed a clinical cure rate in line with other FMT delivery methods described above. Another investigation by Kao et al. [70] of 116 patients concluded in per-protocol analysis that the use of capsule-based FMT was non-inferior to colonoscopy delivered FMT. In this study, the rate of prevention of rCDI after a single treatment was 96.2% in both groups ($p<0.001$). Interestingly, a significantly greater proportion of participants receiving capsules rated their experience as “not at all unpleasant” at 66% versus 44% ($p=0.01$), which may portend an additional benefit of capsule therapy in maximizing the therapeutic reach of FMT.

Safety of FMT

Because FMT involves the transfer of large number of micro-organisms and metabolites from one individual to another, there has been considerable attention paid to the safety profile of FMT. Although the majority of studies evaluating the safety of FMT predominantly include short-term outcomes and are collected retrospectively, there have not been any systemic, reproducible serious adverse events. Kassam et al. [41] reported no adverse events directly associated with FMT in a systematic meta-analysis involving variable follow-up from weeks to years in 273 patients. Another systematic review of 317 patients who received FMT for rCDI concluded that there were similarly rare adverse events with FMT. In this analysis, there was an upper gastrointestinal bleed in one patient, IBS symptoms in four patients, infectious diarrhea symptoms in one patient, constipation in one patient, and signs of irritable colon in one patient, although none of these events could be attributed directly to FMT [65].

The most common adverse events after FMT reported in other studies have been constipation and IBS symptoms (5%–10%) [66,71]. It is unclear whether symptoms consistent with IBS can be attributed solely to FMT, however, because the literature suggests that patients after CDI alone are at risk for new onset IBS symptoms. Specifically, those with long CDI courses, current anxiety, and higher body mass index are at increased risk for development of IBS symptoms after CDI [72].

A recent retrospective multi-center case series report of 80 heterogeneous immunocompromised patients concluded that FMT was safe and well tolerated among this population as well. In this study, two deaths occurred within 12 weeks of FMT, one of which was the result of aspiration during colonoscopy and the other was unrelated to the FMT. No immunocompromised patients had infections develop that were related definitively to FMT; however, two patients had unrelated infections develop and five had self-limited diarrheal illness without identification of a causal organism [73]. A small case review study of 31 patients aged 65 years or older showed CDI revolved in 27/31 patients. In this analysis, five deaths occurred in follow-up, although the FMT was not thought to be a causative factor in these cases, and four patients reported subjective worsening of their arthritis post-FMT leading the

study to conclude that FMT is generally a safe and effective treatment even in older adults with CDI [74].

Because CDI is common in patients with IBD, there are several studies examining the efficacy of FMT for management of rCDI in patients with IBD. Similar to studies involving non-IBD populations, there has been a low rate of adverse events reported [75,76]. Previous work, however, demonstrates a modest percentage of patients report worsening of clinical IBD symptoms after FMT necessitating treatment escalation [73,76–78]. Another related study concluded that patients with ulcerative colitis are particularly susceptible to IBD flares after FMT with greater than 50% requiring additional immunosuppression [79]. It remains unclear, however, whether patients who report worsening of their IBD have changes in mucosal inflammation. In fact, a recent meta-analysis suggested that just 4.6% of patients had worsening of disease activity when analysis was limited to high quality studies [80].

Given the limited number of patients and short-term follow-up in previous studies examining the safety of FMT, larger prospective studies with long-term follow-up are needed. Of significance, the largest planned FMT study in history began enrolling patients this year via the American Gastrointestinal Association FMT National Registry that plans to track 4000 patients for 10 years after their FMT procedure providing a wealth of efficacy and safety data. Special attention will need to be paid to the theoretical risk that the process of FMT may induce chronic diseases over time such as obesity based on what has been described in animal models [81].

Conclusion

To manage recurrent *C. difficile* infection, FMT has become an increasingly utilized and highly efficacious modality. Studies thus far have reinforced its safety profile across multiple methods of administration, and future studies are likely to better characterize the long-term impact of FMT. In addition, novel methods of delivery and storage are likely to further expand use of FMT, potentially to include new indications such as early utilization in CDI, use as a prophylactic measure for CDI, or as a treatment for other disease processes entirely. Accordingly, ongoing clinical trials are studying FMT in several disease states including IBD, metabolic syndrome, decolonization of gastrointestinal multi-drug resistant organisms, and non-alcoholic steatohepatitis based largely on the template developed for FMT in patients with CDI.

Author Disclosure Statement

Dr. Pekow served as a consultant (advisory board) for Pfizer for inflammatory bowel disease, although the company manufactures two of the products that are discussed in the review article. For Dr. Paknikar, no competing financial interests exist.

The authors received support from NIH R03DK109218.

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Address correspondence to:

Dr. Joel Pekow

Section of Gastroenterology, Hepatology, and Nutrition

University of Chicago

900 East 57th Street, MB#9

Chicago, IL 60637

E-mail: jpekow@medicine.bsd.uchicago.edu