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The Efficacy and Safety of Fecal Microbiota Transplant for Recurrent *Clostridium difficile* Infection: Current Understanding and Gap Analysis

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The leading risk factor for *Clostridioides* (*Clostridium*) *difficile* infection (CDI) is broad-spectrum antibiotics, which lead to low microbial diversity, or dysbiosis. Current therapeutic strategies for CDI are insufficient, as they do not address the key role of the microbiome in preventing *C. difficile* spore germination into toxin-producing vegetative bacteria, which leads to symptomatic disease. Fecal microbiota transplant (FMT) appears to reduce the risk of recurrent CDI through microbiome restoration. However, a wide range of efficacy rates have been reported, and few placebo-controlled trials have been conducted, limiting our understanding of FMT efficacy and safety. We discuss the current knowledge gaps driven by questions around the quality and consistency of clinical trial results, patient selection, diagnostic methodologies, use of suppressive antibiotic therapy, and methods for adverse event reporting. We provide specific recommendations for future trial designs of FMT to provide improved quality of the clinical evidence to better inform treatment guidelines.

Keywords. fecal microbiota transplant; *Clostridioides difficile* infection; microbiome; SARS-CoV-2; Shiga toxin–producing *Escherichia coli*.

In this review, we address the current understanding of FMT efficacy and safety to stimulate critical thinking about the gaps in clinical evidence needed to better inform *Clostridioides difficile* infection (CDI) treatment. This discussion also readily applies to investigational microbiome drugs, as we seek to improve therapeutic options for patients to mitigate risk.

Why Do Antibiotics Often Fail in CDI Treatment?

Exposure to broad-spectrum antibiotics is the main risk factor for primary CDI, the leading cause of nosocomial diarrhea [1]. CDI recurrence is common, with rates of up to 40%–60% among those with a history of ≥2 episodes [2]. Recurrences follow treatment discontinuation within 8 weeks, although the majority occur within 1–3 weeks, highlighting the paradox of treating an antibiotic-associated infection with more antibiotics [3].

High rates of treatment failure can only be fully understood within the context of the pathogen's life cycle and the role of the healthy microbiome in reducing recurrence [4–6]. *C. difficile* has a 2-phase life cycle with dormant spores germinating into toxin-producing vegetative bacteria, which leads to colitis [7]. Although the factors that initiate germination are incompletely understood, bile acids play an important role [6]. Primary bile acids, which are synthesized in the liver and secreted into the gastrointestinal tract, set up favorable conditions for germination. By contrast, secondary bile acids, the metabolic product of key commensal bacteria, inhibit vegetative bacterial growth [5]. These and other key microbe-derived metabolites are reduced when antibiotics kill innocent bystander bacteria, which are critical to these metabolic processes. For example, a relative increase of primary to secondary bile acid concentrations facilitates spore germination and vegetative outgrowth, leading to recurrence. Furthermore, CDI-targeted antibiotics maintain and exacerbate this low-diversity microbiome (ie, dysbiosis) [8]. Thus, antibiotics are a main driver of compositional and functional microbiome changes and are insufficient as a sole therapeutic strategy, as microbiome recovery is essential for durable clinical resolution. Other potential mechanisms that inhibit *C. difficile* include niche exclusion, competition for nutrients, and bacteriocins [6].

Recurrent CDI Requires a 2-Pronged Treatment Approach

These foundational principles suggest the need for a 2-pronged therapeutic approach. However, there are currently no approved microbiome therapeutics, although several investigational

agents are in clinical trials [9, 10]. A burgeoning literature has emerged on fecal microbiota transplant (FMT), the transfer of minimally processed stool from a donor to a recipient. Clinical resolution is associated with increased microbial diversity and secondary bile acids, providing proof of concept of the critical role of microbiome restoration [11–13].

The Infectious Diseases Society of America/Society for Healthcare Epidemiology of America (IDSA/SHEA) guidelines recently recommended FMT for multiply recurrent CDI, despite "moderate quality" evidence, reflecting the great unmet need [14]. Why are the data only ranked as "moderate quality" evidence? The IDSA/SHEA guidance suggests that FMT response rates may be influenced by patient selection, proximity of the last CDI episode, and clinical trial design. Herein, we describe the methodologic flaws that limit estimates of FMT efficacy and safety for recurrent CDI:

- Quality of study designs evaluating FMT efficacy
- Selection of appropriate diagnostic assays for CDI
- Importance of a placebo arm when evaluating any investigational agent, particularly in a disease without any pathognomonic symptoms or signs
- Use of prolonged suppressive antibiotic regimens before FMT
- Appropriate comparator treatments

In addition, we address concerns about emerging infections, such as SARS-CoV-2 and transmission of undetected pathogens [15, 16]. Finally, we propose recommendations for future FMT study designs, also applicable to investigational microbiome drugs, to improve the quality of the clinical evidence needed for well-supported treatment guidelines.

What Is the Quality of Data Supporting FMT Efficacy in Reducing Risk of CDI Recurrence?

Although FMT appears to reduce risk of CDI recurrence, accurate estimates of efficacy and safety are limited by the design of published trials [17]. Case series or individual case reports have supported efficacy estimates up to 93%, but small uncontrolled trials are prone to bias [18]. Notably, a 2019 meta-analysis found that FMT was associated with lower cure rates in randomized trials (67.7%) compared with open-label studies (82.7%; $P < .001$) [19].

A 2017 systematic review highlighted that 87% of FMT reports were nonrandomized trials missing details on key methodology, including (a) donor eligibility criteria, (b) stool processing methods, (c) dosage, dosing frequency, route, and types of stool used (eg, fresh or frozen), and (d) methods of preparing the FMT recipient (eg, antibiotics, lavage) [20]. The authors concluded that incomplete reporting "raises multiple problems for patients, clinical researchers and health regulators." The authors of another meta-analysis described the evidence as "low-strength," limiting firm conclusions regarding efficacy rates [21].

Selecting the Appropriate *C. difficile* Diagnostic Assay

Chronic diarrhea is a common complaint in the outpatient clinic attributable to a multitude of noninfectious pathophysiologic etiologies, including osmotic, secretory, and inflammatory mechanisms and altered physiology [22]. Diarrhea is also the chief symptom of CDI, often in isolation of any other clinical finding, underscoring the importance of a rigorous diagnostic test with high specificity and positive predictive value [23].

In primary CDI, proponents of nucleic acid amplification testing (NAAT) affirm its high sensitivity, whereas detractors highlight its inability to distinguish colonization from infection [24]. Supporters of assays for toxin production highlight its greater specificity and positive predictive value compared with NAAT for this toxin-mediated infection, while detractors cite risk of false-negative results. However, studies that have correlated testing with clinical outcomes have supported higher predictive values for toxin testing compared with NAAT for true CDI; thus, NAAT use can lead to overdiagnosis [23]. However, in clinical practice, toxin testing may not always be readily available and may not have optimal sensitivity, and so the "art of medicine" and clinical judgment must guide clinical decision-making.

In recurrent CDI, overdiagnosis with NAAT is of even greater concern. In fact, the recent CDI guidelines made a firm case for always using toxin testing for suspected recurrent infection in clinical practice [14]. Why is toxin testing so critical in this clinical scenario? After treatment, NAAT will likely be positive due to spore persistence, whether or not the patient's symptoms are related to *C. difficile*. In 1 epidemiologic study of patients with CDI resolution, fecal shedding of spores was detected in 56% of patients 1–4 weeks after treatment discontinuation [25]. Up to 25% of CDI patients can develop postinfectious irritable bowel syndrome (IBS), which can be misdiagnosed as active infection if only NAAT is utilized [26].

Although these caveats are important to patient management, they have even greater implications for clinical trial design and subject selection. In the context of a clinical trial, it is essential that all study candidates have the disease of interest and that toxin testing has the best predictive value for true infection. If there is uncertainty about this fundamental point, then the study is essentially flawed; analyses of efficacy (and safety) will be inaccurate, leading to under- or overestimates of effect. In contrast, in diseases with clear-cut surrogate markers, like HIV RNA and CD4 cell counts, enrollment criteria and end points are straight-forward and objective. But in recurrent CDI, toxin testing is a critical element for trial design, particularly in light of the prolonged carrier state [25]. Toxin testing ensures (a) enrollment of subjects with true disease and (b) confirmation that symptomatic recurrence is due to CDI rather than an alternate cause of diarrhea (eg, postinfectious IBS) [26].

Despite the concerns raised above, as the pendulum has swayed in clinical practice toward NAAT, so have clinical studies. Trials performed before NAAT availability (eg, fidaxomicin) relied exclusively on toxin testing [27]. More recent trials have allowed detection of either toxin production (enzyme immunoassay) or toxin genes (polymerase chain reaction) according to investigator discretion, which may have led to inappropriate subject selection [28]. Crucially, to date, no randomized, placebo-controlled FMT trials for recurrent CDI have required toxin testing as a criterion for patient selection. The veracity of the CDI diagnosis in subjects recruited to these relatively more robust trials remains uncertain.

What Insights Does a Placebo Arm Provide in Clinical Trials of Recurrent CDI?

A critical issue in assessing the quality of evidence supporting FMT is the relative lack of high-quality placebo-controlled trials [21]. A placebo arm provides valuable information about the study population that cannot reliably be determined in any other way. For example, if there are low event rates among subjects in the placebo arm, then the efficacy of the treatment intervention is no longer evident, as most subjects are at low risk for the outcome of interest [29].

This concept is well illustrated by a National Institutes of Health (NIH)–funded FMT trial that compared donor FMT with autologous FMT to determine their relative therapeutic efficacy [30]. At 1 of the 2 study sites, rates of clinical resolution were 90% in both the intervention and placebo arms, raising concerns that some subjects did not have true recurrent CDI on study entry. Indeed, the authors acknowledged that many of study participants may have had simple colonization rather than true CDI, due to the use of NAAT for study eligibility. These observations support the importance of a placebo comparator (and toxin-based testing), particularly when interpreting studies suggesting high FMT efficacy rates [31].

What Are the Implications of Using Suppressive Antibiotics Before FMT?

In many trials, FMT is offered to subjects with a "history of recurrence," which may have occurred long before study entry. Many of these study participants are continued on prolonged suppressive antibiotics, such as vancomycin, to "control" their CDI, which confounds the evaluation of FMT and the true performance of a placebo arm. In the NIH-funded trial mentioned above, some patients were on long-term suppressive antibiotics for up to 148 weeks before study entry [30]. This is a critical issue, as 1 retrospective study identified the absence of a vancomycin taper before FMT as the leading risk factor for CDI recurrence [32].

The practice of allowing suppressive antibiotics before entry into an FMT trial raises a key question: Was the subject at risk for recurrence? A Canadian study by Hota and colleagues is very informative in this regard. In this uniquely designed trial, subjects with a history of multiply recurrent CDI were randomized to FMT or a vancomycin taper, only after they presented with another clinical recurrence [33]. Remarkably, 58% of the screened subjects never had a subsequent recurrence, so they were not randomized. CDI experts have hailed this trial as arguably the most rigorous in the field to examine the efficacy of a therapeutic intervention for acute CDI [34, 35]. This study design assesses the true efficacy of FMT to prevent CDI recurrence, because patients are treated during the acute phase immediately after recurrence rather than following a distant episode, where the risk of contemporaneous recurrence is unknown.

What Is the Efficacy of FMT vs a Vancomycin Taper?

Although the IDSA/SHEA guidelines recommend a vancomycin pulse/taper for treatment of recurrent CDI, there are few data examining the efficacy of such regimens or their efficacy compared with FMT. The Canadian study noted above is the only trial that directly compared 2 weeks of vancomycin followed by either FMT or a vancomycin pulse/taper given over an additional 4 weeks [33]. The working hypothesis was that FMT would be superior to vancomycin taper. However, after randomization of 28 subjects, the study was stopped early for futility when an interim analysis showed comparative efficacy of 44% for FMT vs 58% for vancomycin.

The only other trial that compared FMT with vancomycin used a 2-week antibiotic course, shorter than the recommended 6-week course [11, 14]. Thus, the comparator antibiotic arm was suboptimal, as reflected by a clinical resolution rate of 31%.

What Is the Impact of an Open-Label Trial on Estimates of Efficacy?

A major challenge to placebo-controlled trials is the wide availability of FMT. Stool banks distribute FMT under "enforcement discretion," which permits use of this investigational agent without an IND for recurrent CDI treatment, as long as patients give informed consent [36]. The public perception of FMT as a highly effective and safe product represents a challenge for enrolling subjects in placebo-controlled investigational drug trials.

To attract study participants, some company sponsors have adopted a strategy of offering active drug to all nonresponders through an open-label trial, following participation in a placebo-controlled trial. However, the availability of an open-label trial may negatively impact the primary trial if patients seek active drug before meeting protocol-defined criteria. An example of this phenomenon was observed in a double-blind, placebo-controlled trial of RBX2660, a stool-derived microbiota suspension [10]. In this phase 2b study, 4 criteria were required to meet the definition of treatment failure (ie, recurrence of diarrhea, a positive *C. difficile* test, need for retreatment as determined by the investigator, and no alternative cause identified for symptom recurrence). Several subjects were deemed a "treatment failure" and rolled over into the open-label trial,

despite not fulfilling the recurrence criteria. In fact, some subjects had negative *C. difficile* test results. These subjects were strictly counted as treatment failures in this controlled trial, which may have led to an underestimate of efficacy.

Notably, the blinded and open-label arms of this RBX2660 study had remarkably different efficacy estimates; in the open-label study, clinical resolution was achieved in 88%, whereas in the main placebo-controlled study clinical resolution rates were lower, at 61%–67% [10]. The 88% RBX2660 efficacy rates are consistent with those observed in open-label FMT trials. These observations reinforce the importance of a placebo-controlled trial as the preferred high-quality standard, particularly in a disease without any pathognomonic signs or symptoms or a definitive "gold standard" diagnostic test.

What Are the Gaps in Understanding FMT Safety Risk?

Many experts state that FMT is "safe" based on a multitude of uncontrolled trials without a placebo control. Closer examination of adverse event (AE) reporting, however, suggests a need for caution on several grounds.

Absence of Adverse Event Reporting in Published Studies

In 1 systematic review of AEs following FMT, the authors cited concerns about potential underreporting, as many studies did not report any AEs at all [37].

Open-Label Trials

In the largest FMT trial to date, 219 subjects (mean age, 73 years) were randomized to fresh or frozen FMT via enema [38]. Six deaths (5.6%) occurred in the frozen FMT arm, and 11 deaths (11.7%) occurred in the fresh FMT arm; none were attributed by the investigators to FMT. Although we agree with the authors that CDI morbidity and mortality rates have been reported to be as high as 15%, it is difficult to know which AEs may be treatment-related without the benefit of a placebo-controlled arm [39].

A few FMT proponents have argued that some patient subgroups, such as immunocompromised hosts, often do not qualify for placebo-controlled trials and need open access to FMT [40]. However, implicit in this statement is the implication that FMT has been shown to be efficacious and safe in this patient population when there are no controlled trials to support such assumptions.

Prospective Reporting vs Retrospective Reporting of Adverse Events

It is instructive to compare AE rates between 2 FMT products, which used different methods of monitoring and reporting. In a double-blind, placebo-controlled trial of a stool-derived microbiome drug product, RBX2660, 64% of subjects reported an AE; the distribution of these AEs was comparable by treatment arm (2 doses of placebo vs 2 doses RBX2660 vs 1 dose RBX2660/placebo) [10] In contrast, 1 stool bank (OpenBiome, Cambridge, MA, USA) reported 42 AEs in 2050 subjects who received FMT, for an event rate of 2%; furthermore, none of the AEs was judged to be "definitely related to FMT" [41].

It is difficult to attribute the dramatic differences in event rates to major differences in the products themselves, as both are stool-derived. The main differences appear to be the methodologies used in AE collection and reporting. In the placebo-controlled phase 2b trial of RBX2660, AEs were systematically collected on a prospective basis and investigators were mandated to assign causality [10]. In contrast, OpenBiome asks clinicians to retrospectively report, and the stool bank characterizes the relationship of the product to AEs rather than the clinicians [42, 43]. This type of methodology is prone to bias. Retrospective reporting may also miss connections between infections and FMT if the patient is evaluated by a different health care provider who does not recognize the temporal relationship.

Clinical trials can facilitate connections between FMT and AEs. In June 2019, the Food and Drug Administration (FDA) reported 2 cases of invasive infections with extended-spectrum beta-lactamase-producing (ESBL) *Escherichia coli* following FMT linked to a single donor; both patients were immunocompromised, and 1 died [44]. Notably, these 2 serious events were identified during a clinical trial with prospective AE reporting to the FDA [45]. A rigorous lookback investigation using genomic sequencing linked donor stool to both infections and colonization of 5 other asymptomatic recipients. Notably, these 2 serious events, and the more subtle colonization cases, were identified during prospective AE reporting.

In contrast, retrospective reporting can miss FMT-related events. In March 2020, the FDA issued another safety alert regarding 2 patients infected with enteropathogenic *Escherichia coli* (EPEC) and 4 others infected with Shiga toxin–producing *E. coli* (STEC) following FMT provided under Enforcement Discretion [16]. Four patients required hospitalization. All cases were linked to contaminated stool, with a single donor linked to all 4 STEC cases. Another patient died after receiving stool from the STEC-positive donor, although it is unknown if FMT contributed to this person's death as his/her stool was not tested. Recall of this STEC-positive donor did not occur until a second case was reported [46]. The stool bank employed a relatively low-sensitivity test (ie, toxin immunoassay) rather than a more sensitive molecular assay (ie, PCR), which may have enabled these transmissions [47].

Serious transmission events prompt changes in screening algorithms [46]. However, even mandatory guidelines cannot address the issue of emerging infections, including SARS-CoV-2, which is shed in stool for days after clinical resolution of symptoms [48–50]. In response to these new data, the FDA has issued an alert warning for providers and patients of potential transmission via FMT [15]. The rapid emergence of the COVID-19 health care crisis reminds us that donor screening is an imperfect science, particularly if

new infections are unanticipated and only recognized after transmission to the host [51, 52].

Serial FMT Interventions With Invasive Procedures

One trial reported 90% efficacy in 20 subjects treated with FMT, although deeper examination of the paper shows that first-dose efficacy was only 65% [53]. To reach 90% efficacy, multiple infusions (2–4 per patient) were administered. Repeat infusions through invasive means, such as colonoscopy, should also be weighed in the risk/benefit analysis of any procedure, and first-dose efficacy rates should be clearly reported.

Recommendations for Future Clinical Trials

In patients with recurrent CDI, FMT is an effective interrogative tool that provides proof of concept that modulation of the gut microbiome is a promising avenue for drug development and fulfills an unmet need. However, we caution that estimates of efficacy remain unclear and clinical expectations of physicians and patients may be exceeding the quality of the evidence [20, 21].

The safety of any investigational product is best understood in the context of a placebo-controlled trial in a sufficient number of patients with an adequate period of prospective follow-up. A national FMT registry, supported by a grant to the American Gastroenterology Association from the National Institutes of Health, has been initiated in recognition of our limited knowledge of the long-term risks of FMT. The results of this study will not be available for many years to come. A key reason for caution regarding potential long-term consequences of FMT is the ever-increasing list of diseases associated with the microbiome [54]. Thus, manipulation of the gut microbiome, particularly with wholesale replacement via FMT, could have varied and unforeseeable consequences that are only identifiable after the fact [51].

Physicians should be aware of data limitations when counseling patients regarding any investigational therapy. We make the following recommendations for future FMT trials and for reporting of data in publications to improve our fundamental knowledge of FMT safety and efficacy:

- Trials should exclusively employ toxin testing to ensure selection of subjects with true recurrent CDI [14].
- Treatment trials should enroll subjects with acute-onset CDI.
- Long-term suppressive antibiotics for CDI should be considered a key exclusion criterion.
- The number of treatments required to achieve clinical resolution should be reported, as repeated FMT treatments carry procedural risks, depending on route of administration.
- Statistical analyses should take into account loss to follow-up and other AEs that lead to treatment discontinuation, which are considered treatment failures in most clinical trials.

- Large double-blind, placebo-controlled trials are needed for adequate assessment of the efficacy and safety of any investigational intervention, including FMT. Alternatively, future comparator trials with vancomycin pulse-taper regimens should be considered to fully assess if FMT offers additional advantages over other recommended therapeutic approaches [14].

CONCLUSIONS

High-quality trials are needed to improve our understanding of FMT efficacy and safety in order to better inform patients of potential benefit and, more importantly, potential risk. Only a few decades ago, we learned painful lessons regarding the purported safety of plasma-derived factors for hemophiliacs, which unknowingly transmitted HIV [52]. The HIV epidemic was well underway for approximately a decade before it was clinically recognized and identified. Patients deserve risk mitigation, which can be accomplished with thorough vetting and regulation. Mandatory screening guidelines for stool donors are urgently needed, although screening cannot prevent unanticipated emerging infections. Finally, the development of investigational microbiome therapeutics with defined microbial consortia will offer greater confidence in drug purity, identity, and potency, in addition to risk mitigation for improved patient safety [4, 55].

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