Fecal microbiota transplantation in relapsing *Clostridium difficile* infection

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Abstract: *Clostridium difficile* infection rates are Climbing in frequency and severity, and the spectrum of susceptible patients is expanding beyond the traditional scope of hospitalized patients receiving antibiotics. Fecal microbiota transplantation is becoming increasingly accepted as an effective and safe intervention in patients with recurrent disease, likely due to the restoration of a disrupted microbiome. Cure rates of > 90% are being consistently reported from multiple centers. Transplantation can be provided through a variety of methodologies, either to the lower proximal, lower distal, or upper gastrointestinal tract. This review summarizes reported results, factors in donor selection, appropriate patient criteria, and the various preparations and mechanisms of fecal microbiota transplant delivery available to clinicians and patients.

Keywords: *Clostridium difficile*, fecal bacteriotherapy, fecal flora, fecal flora reconstitution, fecal microbiota transplantation, fecal transplant, recurrent *Clostridium difficile* infection, stool transplantation

Introduction

Fecal microbiota transplantation (FMT), often referred to as 'fecal transplant,' is rapidly becoming accepted as a viable, safe, and effective treatment for recurrent *Clostridium difficile* infection (CDI). CDI is a frequent nosocomial illness, and identified as the pathological agent in 10–20% of cases of antibiotic-associated diarrhea [Bartlett, 2002], and as high as 50% in epidemic outbreaks [McFarland, 1998]. CDI infection rates have also been rising: from 1996 to 2003 CDI prevalence doubled in the USA, reaching 61/100,000 [McDonald *et al.* 2006], and in 2010, incidence was estimated at 500,000/year, with mortality rates up to 20,000 cases a year [Heinlen and Ballard, 2010; Rupnik *et al.* 2009]. This growing epidemic is also of global concern with increased CDI being reported in Europe [Bauer *et al.* 2011; Warny *et al.* 2005], Taiwan [Lee *et al.* 2011], Korea [Shin *et al.* 2008], and Canada [Eggertson, 2004]. A survey analysis of European hospitals in 34 countries revealed a weighted mean incidence of *C. difficile* cases per hospital to be 4.1/10,000 hospital patient-days, with a large variance among hospitals in actual incidence rates (range: 0.0–36.3 cases) [Bauer *et al.* 2011].

Treating the increasing volume of CDI patients has simultaneously become increasingly challenging as novel strains of the bacteria have been appearing, particularly BI/NAP1, notable for its increased virulence [Loo *et al.* 2005; McDonald *et al.* 2005; Warny *et al.* 2005]. Hospitalization for more than a week quintuples the risk of acquiring CDI [Ananthakrishnan, 2011; Pepin *et al.* 2005b], and further, CDI is no longer only a concern for hospitalized patients. The greatest risk still remains with antibiotic use in the elderly during in-patient circumstances [Rupnik *et al.* 2009], but recent trends reveal susceptibility in healthy individuals without prior exposure to antibiotics [Hookman and Barkin, 2009]. Increased-risk populations include patients with inflammatory bowel disease (IBD) [Ananthakrishnan *et al.* 2009], peripartum patients [CDC, 2005; Hookman and Barkin, 2009], those older than 65 years of age [Pepin *et al.* 2005a], those that have a severe comorbid illness [Aslam *et al.* 2005; Kyne *et al.* 2002], or are immune compromised [Hookman and Barkin, 2009].

Increased disease prevalence and morbidity has expanded research efforts aimed at improved

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treatment [McFarland, 2009; van Nood *et al.* 2009]. Currently, standard recommendations for treatment of mild CDI include metronidazole or vancomycin, with data suggesting that vancomycin is more efficacious than metronidazole in severe CDI [Pepin *et al.* 2005a; Zar *et al.* 2007]. Conventional therapy for recurring CDI is in flux, but generally includes tapered/pulsed dosing of vancomycin [Cohen *et al.* 2010]. However, current literature is suggesting increasingly that for patients with infections that fail to resolve with traditional antibiotic regimens, FMT's average cure rate of > 90% [Borody *et al.* 2001; Bowden *et al.* 1981; Garborg *et al.* 2010; Girotra *et al.* 2011; Gough *et al.* 2011; Hellemans *et al.* 2009; Khoruts *et al.* 2010; Nieuwdorp *et al.* 2008; Paterson *et al.* 1994; Persky and Brandt, 2000; Rohlke *et al.* 2010; Schwan *et al.* 1983; Silverman *et al.* 2010; Yoon and Brandt, 2010; You *et al.* 2008], low cost, apparent safety, and readily available materials makes microbiota replacement through fecal transplantation an increasingly accepted option [Aas *et al.* 2003; Brandt *et al.* 2012; Rohlke *et al.* 2010].

FMT is of particular utility in recurrent or refractory CDI, which historically is difficult to cure [Ho and Prasad, 2011; van Nood *et al.* 2009]. A long-term, follow-up, multicenter study of interventional colonoscopic FMT for recurrent CDI has demonstrated a primary cure rate of 91% (defined as the resolution of symptoms without recurrence within 90 days of FMT), and a secondary cure rate of 98% (defined as the resolution of symptoms after one further course of vancomycin with or without repeat FMT) [Brandt *et al.* 2012], whereas traditional methods of antibiotic retreatment without FMT have less efficacy [Musgrave *et al.* 2011]. In conventional treatment, once the initial antibiotic course has been completed, recurrence occurs in 6–50% of the time [Aslam *et al.* 2005; Cohen *et al.* 2010; Pepin *et al.* 2005a, 2005b], and after one recurrence incident, patients have up to 65% risk of a subsequent episode of CDI [McFarland, 1998; McFarland *et al.* 2002]. Potential alternatives and adjuvant options include probiotics, resin binders, intravenous immunoglobulins, and monoclonal antibody therapy [Johnson, 2009], however none have been as well studied or demonstrably effective as FMT.

FMT CDI therapy theoretically works by replacing, or buttressing, the protective microbiome of natural colonic flora that has been disrupted by antibiotics and/or other environmental or iatrogenic factors [Grehan *et al.* 2010]. Once the balanced system of commensal gastrointestinal (GI) bacteria is eradicated by antibiotics *Clostridium difficile* has the opportunity to dominate due to the loss of the repressive force of the normal bacterial population. FMT recreates the equilibrated fecal microbiota, allowing the suppression of *C. difficile*, and rebuilding 'colonization resistance' [Brandt and Reddy, 2011]. An additional premise is the possibility that the transplantation of donated flora results in an immunological response, facilitating the eradication of *C. difficile*. Further research will continue to elucidate the mechanisms behind FMT's success in eliminating CDI.

The expanding body of data demonstrating FMT's success includes a variety of methodologies for the delivery of FMT, either to the lower distal, lower proximal, or upper GI tract. For this review, available English language peer-reviewed literature, and several abstracts, through Pubmed, Embase, Web of Science, and general internet searches, were utilized to summarize progress in recurrent CDI treatment using FMT. With a focus on methodology, the aim here is to present a summary of options to implement this effective treatment.

FMT methodologies

There is no clear (or evidence-based) consensus regarding the most appropriate form of delivery for the fecal microbiota transplant. There have been successful results, defined as clearance of diarrhea, or negative *C. difficile* toxin assays, with FMT administered to the proximal colon *via* colonoscope (see Table 1) [Arkkila *et al.* 2010; Brandt *et al.* 2012; Garborg *et al.* 2010; Girotra *et al.* 2011; Hamilton *et al.* 2012; Hellemans *et al.* 2009; Kelly *et al.* 2012; Khoruts *et al.* 2010; Lund-Tonnesen *et al.* 1998; Mattila *et al.* 2011; Mellow and Kanatzar, 2011; Paterson *et al.* 1994; Persky and Brandt, 2000; Rohlke *et al.* 2010; Wettstein *et al.* 2007; Yoon and Brandt, 2010], the distal lower GI tract *via* enema/rectal tube (see Table 2) [Borody *et al.* 2001; Bowden *et al.* 1981; Gustafsson *et al.* 1999; Jorup-Ronstrom *et al.* 2006; Kassam *et al.* 2012; Louie, 2008; Paterson *et al.* 1994; Schwan *et al.* 1983; Silverman *et al.* 2010; Tvede and Rask-Madsen, 1989; You *et al.* 2008], and the upper GI tract *via* nasogastric (NG) tube/gastroscope (see Table 3) [Aas *et al.* 2003; Duplessis *et al.* 2011; Lund-Tonnesen *et al.* 1998; MacConnachie *et al.* 2009; Nieuwdorp *et al.* 2008; Rubin *et al.* 2009; Russell *et al.* 2010].

Regardless of the delivery method chosen the initial steps in the procedure are similar: evaluating patient eligibility, patient consent, determining and screening donors, and in most cases, discontinuing the recipient's antibiotics prior to the procedure. The exact preparation and volume of the donated sample, and location of delivery, can be altered depending on the methodology selected.

Patient indications

FMT for recurrent CDI is not yet a regulatory body 'approved' or 'recognized' modality, however with its consistently effective cures rates of >90% [Gough *et al.* 2011], it stands out as an increasingly viable and appropriate option for patients who have failed to eliminate the infection despite traditional management. Proposed FMT guidelines, submitted by the Fecal Microbiota Transplantation Workgroup [Bakken *et al.* 2011], suggest primary indications for FMT, and outline the specifics that potentially make patients and donors appropriate candidates for FMT. The authors recommend that FMT be considered in the multiply recurrent CDI patient, who has had at least three episodes of mild to moderate CDI, and failure with a tapered course of vancomycin, or at least two episodes of severe CDI that resulted in hospitalization. It was additionally suggested that FMT could be used earlier in the progression of illness if moderate CDI was not responding to vancomycin for at least 1 week, or severe CDI presenting with no response to standard therapy after 48 h [Bakken *et al.* 2011]. In cases of nonresponsive, severe, or fulminant disease it should be considered whether earlier use of FMT would prevent further deterioration [Bakken *et al.* 2011].

FMT is generally considered to be relatively contraindicated in patients with severe comorbid conditions, or those taking immunosuppressants, although anecdotally, such patients have been successfully treated. Duplessis and colleagues reported rapid resolution of refractory CDI complicated by severe Crohn's disease when treated with FMT *via* NG tube [Duplessis *et al.* 2011]. With the increased comorbidity of CDI and IBD [Ananthakrishnan *et al.* 2009], it is not unrealistic to assume that the frequency of patients with recurrent CDI and active IBD being treated with FMT will increase in order to provide swift and effective elimination of CDI. In the absence of CDI, FMT has been reported to provide sustained relief of symptoms due to ulcerative colitis in a small number of series [Bennet and Brinkman, 1989; Borody *et al.* 2003].

Most of the published literature highlighting FMT interventions is limited to the adult population. One case study reports successful FMT *via* NG tube in a 2-year-old pediatric patient, and suggested a potential protocol for use in the pediatric population [Russell *et al.* 2010]. Gough and colleagues' systematic FMT review reported that in 317 patients, 61% were female, the average age was 53 years, with actual ages spanning 2–95 years [Gough *et al.* 2011].

Donor determination

The choice of donors varies among studies, most frequently the donor has been an intimate partner, housemate, or family member [Borody *et al.* 2004; Gough *et al.* 2011; Rohlke *et al.* 2010], however several studies used volunteer donors [Aas *et al.* 2003; Borody *et al.* 2004; Bowden *et al.* 1981; Eiseman *et al.* 1958; Garborg *et al.* 2010; Hamilton *et al.* 2012; Kassam *et al.* 2012; Lund-Tonnesen *et al.* 1998]. Lund-Tonnesen and colleagues used homologous feces from 1 healthy donor in 18 patients (17 colonoscope, 1 gastroscope) [Lund-Tonnesen *et al.* 1998]. A total of 15 patients were considered cured, however 3 patients with the most severe colitis were reported as nonresponsive. Kassam and colleagues treated 27 patients with FMT *via* retention enema, using two prescreened donors for all patients, and reported resolution of symptoms in 88% (22/27) [Kassam *et al.* 2012]. The remaining five patients (5/27) received a second enema FMT and three of those five experienced resolution of symptoms, bringing the secondary cure rate to 93% (25/27).

The University of Minnesota Fairview Medical Center has moved away from the approach of using directly identified individualized donors by creating a standardized laboratory process of banking frozen fecal material. When 12 patients treated for CDI with fresh donor material (10 patient-identified donors, two standardized donors) were compared with 33 patients treated with the standardized frozen material there were no significant differences in infection clearance for fresh *versus* frozen samples, or in patient-identified donors *versus* standardized donors, and no adverse events were reported for either group [Hamilton *et al.* 2012]. The Centre for Digestive Diseases in Sydney, Australia, performs the majority of their FMT

procedures with standardized donor fecal samples. Borody and Khoruts hold the perspective that the burden of rigorous screening should be entrusted to the clinical facility and not to the patient, noting decreased costs of screening, and simplified coordination efforts at their centers [Borody and Khoruts, 2011].

Taking a different approach, clinicians often elect to utilize donations from individuals living in the same household, hypothesizing that in close living arrangements, and particularly with intimate partners, potential pathogens would likely already have been widely shared by both parties [Mellow *et al.* 2011; Rohlke *et al.* 2010; Yoon and Brandt, 2010]. Donation from an intimate partner diminishes the risk of transferring an additional infectious agent (to which the recipient has not been previously exposed) into their GI tract. Regardless of the relationship of the recipient and donor, rigorous screening is recommended. Considering the virulence of *C. difficile*, and the spore's ability to survive in the environment, utilizing a donor from the same household as the infected patient might theoretically be an adverse risk factor. However, the data thus far has demonstrated that transmitting donated stool containing *Clostridium difficile* is not necessarily correlated with treatment success or failure [Bakken *et al.* 2011], presumably because the entire balanced microbiome is transferred it retains the ability to repress the present *C. difficile's* pathogenicity by disallowing it to become an amplified proportion of the flora. Slightly higher rates of CDI resolution have been reported with donation from a partner or relative (93%), in comparison with fecal donations from unrelated sources (84%) [Gough *et al.* 2011]. Controlled studies with balanced treatment groups need to be conducted before reliable recommendations can be made regarding the most effective donator/recipient paradigm.

Donor screening

There have not yet been any adverse events reported that can be conclusively or directly attributed to FMT, and proper donor screening is essential to avoid transmitting communicable diseases from donor to recipient (baseline screening recommendations are listed in Box 1). An oral interview is the clinician's initial tool enlisted in the screening process; it is the primary avenue for identifying potential risk factors that would increase the odds of exposures to pathogens undetectable in the laboratory. The clinician must estimate the risk that the donor had recently contracted a transmissible disease, such as HIV or hepatitis, as well as rule out potential exposure to pathogenic agents that are not identified by laboratory methods to a high degree of sensitivity. This can be facilitated by eliminating donors with a history of engaging in high-risk behaviors, such as illicit drug use, sexual encounters with multiple partners, or unprotected sexual activity.

Box 1. General recommendation for baseline donor screening.

For nonstandardized donors

Donor stool screening

- Ova and parasites
- Stool culture and sensitivity test
  - generally includes: *Salmonella*, *Shigella*, *Escherichia coli*, O157:H7, *Yersinia enterocolitica*, and *Campylobacter*
- *Clostridium difficile* toxins A and B
- Some practitioners additionally screen for *Cryptosporidium* antigen and *Giardia* antigen

Donor serum screening

- HIV-1 and HIV-2
- Hepatitis A, B, and C
- Some practitioners additionally screen for: rapid plasma reagin and fluorescent treponemal antibody-absorbed *Treponema pallidum*

Analysis beyond this baseline should be determined by the physician's interview and risk assessment, in parallel with an evaluation of the donor/recipient relationship, and any clinical factors supporting/opposing an abbreviated screening.

Additional potential exclusions should include donors with a history of incarceration, tattoo or body piercing in the past 6 months, current or known exposure to a communicable disease, use of immunosuppressant agents, or antibiotics within the last 3 months. Travel within the past 6 months to an area known to be a risk factor for diarrheal illness or other infectious diseases should also be considered in the analysis of donors. Current research now suggests that intestinal microbiota plays a role in many chronic diseases, such as IBDs (Crohn's disease and ulcerative colitis), metabolic syndrome, cancer, obesity [Festi *et al.* 2011], and irritable bowel syndrome [Ghoshal *et al.* 2012]. It may be pertinent to exclude donors with any evidence of auto-immune or other chronic conditions until the exact role that microbiota play in the pathophysiology of these conditions is known. Owing to the importance and sensitive nature of identifying behavioral risk factors, it may be most advantageous to interview potential donors separately from the recipient, allowing maximal respect of confidentiality.

When the donation is from an intimate partner, the recipient may opt out of testing or prefer a limited version of the testing [Mellow *et al.* 2011; Rohlke *et al.* 2010], which could both expedite the process and reduce costs. In the rare cases when expedited FMT is the patient's best chance of survival, such as in severe fulminant CDI, it is the physician's obligation to calculate the benefit *versus* harm, and should not be obligated to abide by the abbreviated screening [Bakken *et al.* 2011], if it is not in the best interest of the patient. The best route of avoiding iatrogenic complications and exposures is to complete a comprehensive screening whenever possible.

Donation preparation

Transplantation of fresh donated feces is recommended to take place within 24 h [Bakken *et al.* 2011; Landy *et al.* 2011], and ideally within 6 h [Aas *et al.* 2003; Bakken *et al.* 2011; Kelly *et al.* 2012; Landy *et al.* 2011; Mattila *et al.* 2011; Mellow and Kanatzar, 2011; Rohlke *et al.* 2010; Russell *et al.* 2010]. Exact volumes and preparations/dilutions deviate depending on the avenue of transplantation. In all cases, a large volume of donation suspension should be attempted since resolutions seem to be greatest (97%) when more than 500 ml is transferred (*versus* 80% resolution with less than 200 ml), and relapse rates up to four times higher have been reported when less than 50 g of stool is donated [Gough *et al.* 2011]. It may be helpful to ensure the donor can reliably produce stool on the day of donation by providing a mild laxative the night before, such as citrate of magnesium [Rohlke *et al.* 2010], or milk of magnesia [Kelly *et al.* 2012; Mellow and Kanatzar, 2011; Yoon and Brandt, 2010].

In order to amalgamate the selected fluid (predominantly normal saline [Borody, 2000; Gough *et al.* 2011], or water [Arkkila *et al.* 2010; Kelly *et al.* 2012; Mattila *et al.* 2011]) with the donated fecal matter into a heterogeneous mixture clinicians generally use a blender (standard kitchen or commercial, allocated for the purpose of FMT only), or vigorously hand shake the suspension in a tightly covered container. The resulting viscous liquid can then be filtered into a new container, or into the equipment that will be used for instilling the donation during the FMT process, such as large syringes. The filtration allows for extraction of any larger components of the excrement that will not reduce into a thick liquid form, such as undigested food particles, which could clog the tubal systems of a colonoscope, syringe, endoscope, or NG tube. Various filtration systems have been constructed, and depending on the resources at hand, will vary in cost from a few cents for disposable supplies to more extensive costs associated with reusable equipment. Some clinicians have crafted filtration devices with 4 × 4 sheets of gauze [Brandt *et al.* 2012; Garborg *et al.* 2010; Kelly *et al.* 2012; Nieuwdorp *et al.* 2008; Rohlke *et al.* 2010; Yoon and Brandt, 2010] or coffee filters [Aas *et al.* 2003; Russell *et al.* 2010], which are then secured over the top of the suspension container. The suspension can then be poured from the original container, through the filter, into the second container. A more refined system can be implemented by using a stainless steel strainer [Hamilton *et al.* 2012; Khoruts *et al.* 2010], or urinary calculi strainer [Mellow and Kanatzar, 2011]. When using reusable filtration systems, and canisters, extensive sterilization procedures should always be followed.

Some clinicians have modified the general protocol by including additives in the suspension mixtures. In a multimethods study (colonoscopy followed by enemas) Wettstein and colleagues added psyllium to the 200–300 ml of saline used to mix the donated flora to a liquid consistency [Wettstein *et al.* 2007]. Another clinician prepared the donated stool suspension with pasteurized

cow's milk before transplanting through an enema, based on findings that, compared with controls, patients with recurrent CDI excrete fewer fecal short-chain fatty acids. A total of 7 out of the 9 patients were considered cured at 18 months after transplantation [Gustafsson *et al.* 1998, 1999]. One of the earlier clinicians to publish a case-study account of FMT prepared enemas from fresh feces in an anaerobic cabinet. The protocol called for two enemas directly after their preparation and 3 days apart, and immediately post, and at 9 months, the patient exhibited no signs of CDI [Schwan *et al.* 1983, 1984]. Many of these alternative options were conducted *via* enema, however it is logical to assume they would be viable for any of the available routes of delivery.

Patient (recipient) pre/postpreparation

The decision to eliminate antibiotics prior to FMT was almost universal, however there were variances in the timeframe prior to the procedure, most commonly 1–3 days. The use of bowel lavage was not included in all of the reviewed protocols. The presumed reasoning behind lavage is to enhance FMT success by flushing out residual feces, antibiotics, and *C. difficile* bacteria, toxins, and spores, prior to the administration of the donated flora. Most commonly, polyethylene glycol (PEG) electrolyte lavage is standard protocol prior to colonoscopy and was used the evening prior to colonoscopic FMT administration in almost all of the included studies. Bowel lavage is not frequently utilized with FMT administered *via* NG tube, however one group reported utilizing bowel lavage prior to upper GI FMT in four patients [Nieuwdorp *et al.* 2008]. Lavage may also have a benefit with enema preparations [Borody *et al.* 2004, 2012]. Clone library sequencing has shown that colonic mucosa-associated microbiota composition is altered by standard bowel preparation lavage [Harrell *et al.* 2012], and therefore it could be surmised that it enhances the potential for FMT to provide a 'fresh start' in repopulating the colonic habitat of the recipient. Further investigation is needed before routinely recommending bowel lavage in the varying combinations of FMT procedures, in light of a recent analysis noting that patients who received both bowel lavage and an antibiotic before the fecal transplant had the greatest rate of relapse (12%) [Gough *et al.* 2011].

Slight variances were also present in the reported post-transplant protocol. Some centers instructed patients to take two tablets of over-the-counter loperamide immediately after colonoscopy-induced transplantation and again approximately 6 h later to maximize retention time of the donated microbiota [Brandt *et al.* 2012; Rohlke *et al.* 2010]. Silverman and colleagues continued *Saccharomyces boulardii* in patients receiving the probiotics prior to FMT for 60 days post-enema FMT [Silverman *et al.* 2010]. Many of the patients in Hamilton and colleagues' study were taking probiotics pre-FMT, but all were counseled to discontinue any probiotic treatment post-FMT [Hamilton *et al.* 2012]. One of the authors of this review (NS) continues *Saccharomyces boulardii* indefinitely in almost all patients treated with FMT. Future randomized controlled trials (RCTs) are needed to determine the exact pre/post-transplant protocol that will ensure the greatest ratio of 'clearance' of CDI, with the lowest risk of relapse.

FMT delivery

The first reported FMT in humans, in 1958, was *via* enema [Eiseman *et al.* 1958], and at the time of Gough and colleagues' systematic review, 35% of FMTs had been provided by enema, the largest fraction of FMTs [Gough *et al.* 2011]. Since then at least 192 cases of FMT *via* colonoscopy have been reported, bringing the total FMT *via* colonoscopy to approximately 254 patients [Arkkila *et al.* 2010; Brandt *et al.* 2012; Girotra *et al.* 2011; Hamilton *et al.* 2012; Kelly *et al.* 2012; Mattila *et al.* 2011], and approximately 156 cases of FMT *via* enema/rectal catheter [Gough *et al.* 2011]. Fecal transplantation delivery procedures varied in dispersal location of donated microbiota, volume limits, and method of mixture suspension. There were expected differences in pre/post-patient instructions, in parallel with the typical protocol of the modality used. Some clinicians used a combination of different methodologies, such as first providing a single FMT by colonoscope, and following up with a series of enemas [Wettstein *et al.* 2007]. The available literature is summarized below according to the location of GI tract delivery and equipment category (proximal lower GI – colonoscopy; distal lower GI – enema and rectal tubes; upper GI tract – NG tubes, duodenal tubes, and endoscopy/gastroscopy). The methodology can be replicated according to the clinician's evaluation of the most appropriate avenue, considering the patient's circumstances, the available physician/staff skill sets, and equipment accessibility at the transplantation site.

Proximal lower GI FMT

Procedural details of colonoscopic administration of FMT for CDI varied slightly among the 17 reports included in this category (see Table 1) [Arkkila *et al.* 2010; Brandt *et al.* 2012; Garborg *et al.* 2010; Girotra *et al.* 2011; Hamilton *et al.* 2012; Hellemans *et al.* 2009; Kelly *et al.* 2012; Khoruts *et al.* 2010; Lund-Tonnesen *et al.* 1998; Mattila *et al.* 2011; Mellow and Kanatzar, 2011; Paterson *et al.* 1994; Persky and Brandt, 2000; Rohlke *et al.* 2010; Wettstein *et al.* 2007; Yoon and Brandt, 2010]. The core fundamental features were generally preserved across studies, with each manipulating the sample to produce a thick slurry of liquefied material that could be injected through the working channel of a standard colonoscope. The suspension is then filtered to ensure the larger particulates that could clog the scope are removed. The volume should be limited to increase the likelihood that the sample will be retained, while at the same time aiming to maximize the bacterial flora concentration [Rohlke *et al.* 2010].

Alterations have been developed in the secondary components implemented in colonoscopic FMT. The available literature demonstrated differences in the type and volume of fluid combined with the donation sample, grams of donated fecal matter included, final suspension volume, dispersal location, incorporation of lavage, and pre/post-instructions given to the patients. The variances specific to the colonoscopic route of FMT are discussed below, and to facilitate physician ease of providing FMT, a reasonable suggested basic protocol for colonoscopic FMT is summarized in Box 2.

Not all studies reported the exact measurements of fecal donation or suspension fluid used, or whether the entire suspension was infused into the colon. Owing to the inherent nature of retrospective case studies, rigorous control could not be maintained in the exact methodology used for each patient within a sample, which resulted in a range of treatment volumes within datasets reported for some studies. The volume of fluid mixed with the donated fecal matter ranged on average from 200 ml to 300 ml, and the amount of donated stool ranged from 5 g to 300 g, however some authors reported inclusion of all the fecal matter provided [Brandt *et al.* 2012; Rohlke *et al.* 2010]. The majority preference for anatomical endpoint during colonoscopy was towards a goal of reaching the terminal ileum or cecum whenever possible. Some chose to disperse the entire suspension at the most proximal aspect of the colon reached [Arkkila *et al.* 2010; Garborg *et al.* 2010; Hamilton *et al.* 2012; Khoruts *et al.* 2010; Mattila *et al.* 2011; Wettstein *et al.* 2007], whereas others released the suspension gradually during withdrawal of the scope [Brandt *et al.* 2012; Mellow and Kanatzar, 2011; Persky and Brandt, 2000; Sage *et al.* 2011; Yoon and Brandt, 2010]. One study initially infused the donated stool in a gradual withdrawal process, but later began delivering the entire suspension at the ileum or cecum [Rohlke *et al.* 2010]. A larger portion of the suspension was sometimes delivered to areas of the colon that had the greatest presentation of pathology or diverticulosis [Hamilton *et al.* 2012].

There may be significant potential advantages to utilizing the colonoscopic method of reconstituting the colon of CDI patients with natural flora. The scope allows the clinician to visualize areas of mucosa that have been particularly damaged by the CDI infection, and also identify any complications or comorbid conditions. Proximal colonic installation may also be of advantage since the entire length of colonic mucosa is being exposed to, and repopulated with the donated flora amalgam. The risks of colonoscopy are minimal, and other than the donor-screening costs, which are incurred by all methodologies, the cost of transplantation does not exceed the general cost of colonoscopy. Across all methodologies evaluated, relapse was four times higher when less than 50 g of stool was infused, and independently from the stool content, larger volume suspensions have been shown to be more effective in reducing the risk of treatment failure post-transplantation (97% resolution *versus* 80% with ≥ 500 ml *versus* ≥ 200 ml) [Gough *et al.* 2011]. Colonoscopic FMT is well suited to transfusing these larger volume suspensions.

The combined secondary cure rate of the included cases highlighting FMT *via* colonoscope was 96.3%. This impressive cure rate is consistent with the 98% secondary cure rate reported in the recent long-term, multicenter follow-up study utilizing colonoscopic FMT for CDI treatment [Brandt *et al.* 2012].

Distal lower GI FMT

Historically, the distal lower GI tract has been a popular location selected for FMT instilment. A total of 12 reports of lower GI tract FMT treating

Box 2. Proximal gastrointestinal fecal microbiota transplantation.

FMT procedure outline: colonoscopic method

Pre-FMT process

- Patient and donor consent
- Complete FMT ≤ 2 weeks after donor screening
- **Patient**
  - Terminate antibiotics 1–3 days prior to transplantation
  - Lavage: standard 4.0 L PEG bowel preparation
- **Donor**
  - Mild laxative the night prior to FMT (milk of magnesia [Kelly *et al*. 2012; Mellow and Kanatzar, 2011; Yoon and Brandt, 2010] or citrate of magnesium [Rohlke *et al*. 2010]) if needed
  - Donor may provide fresh stool at the site of transplant, the morning of FMT or be provided with an at-home stool sample kit with lid [Mellow and Kanatzar, 2011]
    - donation must still be provided and transplanted the same day of FMT
- FMT should begin ≤ 6 h after provision of donor stool specimen
- **Stool processing**
  - Follow universal safety precautions for level 2 biohazard
    - appropriate gloves, gown, mask, goggles/eye protection
  - Combine > 50 g of donated stool and 200–800 ml of nonbacteriostatic saline in large suction canister
  - Manually shake the canister, or use a conventional kitchen blender, until reaches thick liquid consistency
  - Filter suspension 1–2 times with multiple 4 × 4 gauze pads draped over the canister and secured in place by rubber bands (or equivalent filtering system)
  - Transfer suspension to large syringes (60 cc)

Colonoscopy

- Moderate sedation used at provider/patient discretion
- Conduct standard colonoscopy to the right colon (adult or pediatric scope), reaching the terminal ileum or cecum whenever possible [Rohlke *et al*. 2010]
- Deliver donated suspension (from prefilled large syringes) through the working channel of the colonoscope
  - deliver entire suspension at most proximal extent reached (goal: terminal ileum)

  or
  - infuse gradually [Persky and Brandt, 2000], every 5–10 cm, during withdrawal (> fraction infused at most proximal point, and at sites with prominent diverticular disease [Hamilton *et al*. 2012] and/or mucosal disruption)

Post-FMT process

- Patients
  - Over-the-counter loperamide [Rohlke *et al*. 2010] or other OTC antidiarrheal, immediately post-FMT
  - Avoid excretion of the FMT donation for > 4 h
  - Bedrest as long as possible post-FMT, until the next day
  - Standard post-procedure dietary instructions
  - Instruct to call provider upon any signs of *Clostridium difficile* infection return

FMT, fecal microbiota transplantation.

CDI were included in this review (see Table 2) [Borody *et al.* 2001; Bowden *et al.* 1981; Duplessis *et al.* 2011; Gustafsson *et al.* 1999; Jorup-Ronstrom *et al.* 2006; Kassam *et al.* 2012; Louie, 2008; Paterson *et al.* 1994; Schwan *et al.* 1983; Silverman *et al.* 2010; Tvede and Rask-Madsen, 1989; You *et al.* 2008]. The main points of variation that were specific to enema, retention enema, or rectal tube methodology pertained to the number of FMTs used per patient (over varying durations of time), volume of mixture fluid, grams of feces, and total infused suspension volume. Two studies are included in Table 2 that were not centrally part of this review due to their

Table 1. Proximal Lower GI.

#	Year	Study	Method	Notes	Secondary Cure Ratio	Stool	Fluid vol, ml	Fluid	Infused *, ml
1	1998	(Lund-Tonnesen *et al.*, 1998)	17- colonoscope, 1- gastroscope		15/18	5–10 g	IDR	IDR	IDR
2	2000	(Persky and Brandt, 2000)	Colonoscope		1/1	ID	IDR	Saline	500
3	2007	(Wettstein *et al.*, 2007)	colonoscope/ enema	Colonoscope, then Enema days 5, 10, up to 24	15/18	200–300 g	200–300	Saline and Psyllium	IDR
5	2009	(Hellemans *et al.*, 2009)	Colonoscope		1/1	IDR	IDR	IDR	IDR
6	2010	(Arkkila *et al.*, 2010)	Colonoscope		34/37	20–30 ml	100–200	Water	IDR
7	2010	(Khoruts *et al.*, 2010)	Colonoscope	Pre/Post Microbiota Analysis	1/1	25 g	300	Saline	250
8	2010	(Garborg *et al.*, 2010)	2- Colonoscopy 38- Gastroscope		2/2	50–100 g	250	Saline	200
9	2010	(Yoon and Brandt, 2010)	Colonoscope		12/12	10–20 ml every 5–10cm	1000	Saline	250–400
10	2010	(Rohlke *et al.*, 2010)	Colonoscope		19/19	Total Provided	200–300	Saline	200–350
11	2011	(Girotra *et al.*, 2011)	Colonoscope / Enteroscope (unspecified)		3/3	IDR	IDR	IDR	IDR
12	2011	(Brandt *et al.*, 2012)	Colonoscope	Long-Term, Multi-Site Follow-Up	76/77	Few Tbls to Total Provided	Varied	Saline	300–700
13	2011	(Mellow and Kanatzar, 2011)	Colonoscope		12/13	Total Provided	Enough to Liquify	Saline	300–600
14	2011	(Mattila *et al.*, 2011)	Colonoscope		66/70	20–30 ml	100–200	Water	100
15	2012	(Hamilton *et al.*, 2012)	Colonoscope	Frozen, standardized Donor Stool	41/43	50 g	250	Saline	220–240
16	2012	(Kelly *et al.*, 2012)	Colonoscope		24/26	6–8 Tbls	100–1000	Water or Saline	500–960

*: If stated to be different from suspension total
IDR: Insufficient Data Reported/or otherwise unavailable.

classification as treatment for pseudomembranous colitis [Eiseman *et al.* 1958; Fenton *et al.* 1974], as opposed to specifically CDI. One of the earlier reports discussed FMT in 20 patients, however only 1 case explicitly treated CDI, and only that patient was included in this review [Bowden *et al.* 1981].

FMT via enema/rectal tube and colonoscopic FMT administration require only minimal alterations to the protocol, largely associated with the location/module of delivery, and use of anesthesia (proximal lower GI FMT). The central process of donation preparation is similar with each method. Distal lower GI methodology requires smaller volumes to be transplanted during a single procedure, but is most feasible as a series treatment, arranged as multiple FMT infusions over a specified duration of time. Borody and colleagues recommend using 200–300 g of donated fecal material, and 200–300 ml sterile saline, homogenized in a blender to liquid consistency, and administered by enema within 10 min of preparation, once daily for 5 days [Borody *et al.* 2012]. As in colonoscopic FMT, clinicians are encouraged to recommend loperamide pretreatment to maximize retention time. Combination treatment with the initial infusion delivered by colonoscope,

Table 2. Distal Lower GI FMT.

#	Year	Study	Method	Notes	Secondary Cure Ratio	Stool, (g)	Fluid vol (ml)	Fluid
PMC	1958	(EISEMAN *et al.*, 1958)	Enema	PMC 1-3 infusions	4/4	IDR	IDR	IDR
PMC	1974	(Fenton *et al.*, 1974)	Enema	PMC	1/1	IDR	IDR	IDR
1	1981	(Bowden *et al.*, 1981)	Enema	1 Patient Included (1/16 patients: Confirmed CDI) 2 Infusions x 5 days	1/1	IDR	IDR	Saline
2	1983	(Schwan *et al.*, 1983)	Enema	2 Infusions, Prepared in Anaerobic Cabinet	1/1	IDR	450	IDR
3	1989	(Tvede and Rask-Madsen, 1989)	Enema	1 Patient: Enema 5 Patients: Mix of Flora	1/1	50	500	Saline
4	1994	(Paterson *et al.*, 1994)	Rectal Tube		7/7	200	200	Saline
5	1999	(Gustafsson *et al.*, 1999)	Enema	5 Confirmed CDI, Failed FMT: 2 Infusions Frozen Fecal Donation	5/6	5–10	IDR	Homogenized Cow Milk
6	2001	(Borody *et al.*, 2001)	Enema	IBD and CDI	6/6	200–300	200–300	IDR
7	2006	(Jorup-Ronstrom *et al.*, 2006)	Enema	Swedish, Abstract 3 lavage, 1 enema, 1 NR	4/5[2]	IDR	30	IDR
8	2008	(You *et al.*, 2008)	Enema	Fulminant CDI	1/1	45	300	Saline
9	2008	(Louie, 2008)	Rectal Catheter	Home-based enemas 1-3 infusions	43/45	300–500	1000–1500	IDR
10	2010	(Silverman *et al.*, 2010)	Enema	Home-Based, Low Volume Enemas	7/7	50	250	IDR
11	2012	(Kassam *et al.*, 2012)	Enema	1 Enema Infusion, 2nd Enema Provided if Symptoms present at 7d post FMT	25/27	150	300	Sterile Water

*: If stated to be different from suspension total
IDR: Insufficient Data Reported/or otherwise unavailable
[1]: Data reported for enema infusion
[2]: Cure ratio reported as combined ratio for all methodologies used

and followed by at least 5 days of rectal enema FMT has been reported [Borody *et al.* 2012].

The range of stool used for the FMT amalgams was from 5–10 g [Gustafsson *et al.* 1999] to 300 g [Borody *et al.* 2001]. There was heterogeneity in the selection of fluids used to mix the suspension, including homogenized cow's milk [Gustafsson *et al.* 1999], psyllium, and saline [Wettstein *et al.* 2007], normal/sterile saline preparations [Bowden *et al.* 1981; Paterson *et al.* 1994; Schwan *et al.* 1983; You *et al.* 2008], or sterile water [Kassam *et al.* 2012]. Many studies reported the use of multiple administrations of FMT [Borody *et al.* 2001; Bowden *et al.* 1981; Kassam *et al.* 2012; Paterson *et al.* 1994; Schwan *et al.* 1983]. Kassam and colleagues' study started with 1 enema, and if diarrhea recurred within 7 days a second enema FMT was administered (4/27 patients, with 2/4 classified as failures after the second FMT) [Kassam *et al.* 2012]. Wettstein and colleagues' study is included under the upper GI FMT category, due to their treatment of CDI initially with colonoscopic FMT, followed by a varying number of enemas [Wettstein *et al.* 2007]. Most authors opted to transplant freshly donated fecal flora, however Gustafsson and colleagues used donated fecal material from a healthy adult volunteer and prepackaged syringes with the composite of filtered suspension donation and pasteurized cow's milk [Gustafsson *et al.* 1999]. The 20 ml syringes were then stored at 20°C and later thawed in 37°C water 30–60 min prior to enema FMT treatment.

Lower GI administration of FMT has a high cure rate of 95.4% and a 4.8% relapse rate [Gough *et al.* 2011], and is generally well accepted by patients. Enema administration has the advantage of being less invasive, lower cost, and a reasonable option for both hospitalized and ambulatory

Table 3. Upper GI FMT.

#	Year	Study	Method	Notes	Secondary Cure Ratio	Stool, g	Fluid vol, ml	Fluid	Infused*, ml
1	1998	(Lund-Tonnesen *et al.*, 1998)	17- Colonoscope 1- Gastroscope	18 Patients, Homologous Feces from 1 Donor	15/18	5–10	IDR	IDR	IDR
2	2003	(Aas *et al.*, 2003)	NG	2 Patients Died, Unrelated, Before Evaluation of Tx Fail/Success	15/16	30	50–70	Saline	25
3	2008	(Nieuwdorp *et al.*, 2008)	Jejunal Infusion via Duodenal Catheter,	Pre-Lavage	7/7	60–120	300–400	Saline	IDR
4	2009	(MacConnachie *et al.*, 2009)	NG		11/15	30	150	Saline	30
5	2009	(Rubin *et al.*, 2009)	NG	NG in Elders	14/16	30	50–70	Saline	30–60
6	2010	(Garborg *et al.*, 2010)	2- Colonoscopy 38- Gastroscope		31/38	50–100	250	Saline	200
8	2010	(Russell *et al.*, 2010)	NG	IBD, Pediatric Patient	1/1	30	50–70	Saline	25
9	2011	(Duplessis *et al.*, 2011)	NG	IBD (Crohn's Disease) Complicated by CDI	1/1	70	200	Saline	Total

*: If stated to be different from suspension total
NG: Nasogastro
IDR: Insufficient Data Reported/or otherwise unavailable.

patients. The risks of perforation, associated with endoscopic methods are avoided by enema delivery, which may be advantageous in the fulminate patient. A case study detailing retention-enema FMT reported a successful outcome in a single patient with fulminant *C. difficile* infection. The 69-year-old male postoperatively developed ileus and oliguric renal failure, hospital-acquired pneumonia, and was febrile, hypotensive, and presented with symptoms consistent with fulminant CDI. Upon receipt of FMT via enema, the patient's blood pressure stabilized, leukocyte count normalized, and oliguria resolved. The patient's bowel function returned, and he was taken off the vasopressors and venovenous hemofiltration [You *et al.* 2008].

Enema administration does not require the specialized skills of endoscopic procedures, and therefore could be applicable in a diverse range of settings. Using low-volume fecal enema preparations, the treatment can potentially be provided at home [Louie, 2008; Silverman *et al.* 2010], by the patient, family member, or caretaker, and potentially would allow a larger number of patients to be treated by FMT in rural or underdeveloped environments.

Upper GI tract FMT

The third mechanism of FMT available today, upper GI tract administration, includes NG tubes, duodenal tubes, and endoscopy/gastroscopy (see Table 3). In 2011, 23% of all FMT procedures were provided by means of NG tube or gastroscope. CDI treatment has been successful, defined as a resolution of symptoms, in 76% of these cases [Gough *et al.* 2011]. In most cases only one infusion was provided, but one study instilled a second treatment in 4 of the 10 patients for which the first NG FMT failed, 3 of the 4 were successful [Garborg *et al.* 2010]. Reports of a combined jejunal and colonic FMT were reported in a recent abstract discussing successful resolution in three patients, however the exact methodology was not reported [Girotra *et al.* 2011]. As with the methods for lower GI FMT, preparation of the donated flora sample was the same, with differences in volumes, and pre/post-treatment strategies. Only one clinician provided bowel lavage prior to FMT [Nieuwdorp *et al.* 2008], however most cases included a proton-pump inhibitor, omeprazole, taken in the evening prior [Aas *et al.* 2003; MacConnachie *et al.* 2009; Rubin *et al.* 2009; Russell *et al.* 2010]. Upper GI FMT requires smaller volumes

of suspension to be transplanted owing to concerns about aspiration. The reported data delineating the NG approach, generally prepared the suspensions with 50–400 ml (majority 50–70 ml) of saline, and 30–100 g (majority 30 g) of stool, with a total of 25–60 ml (majority 25–30 ml) of suspension actually instilled [Aas *et al.* 2003; Duplessis *et al.* 2011; MacConnachie *et al.* 2009; Rubin *et al.* 2009; Russell *et al.* 2010]. Immediately before transfusing the suspension, the NG tube can be placed, and radiography used to verify that the terminal end of the tube has entered the gastric antrum. A syringe can be used to flush the suspension through the tubal system. After the suspension is delivered the tube can be flushed with 0.9 N NaCl saline, and removed. The patient can be released from the clinic and resume all normal dietary patterns immediately afterwards [Aas *et al.* 2003]. The gastroscope FMT approach reported larger volumes (50–100 g stool, 250 ml saline, and 200 ml infused), due to insertion into the distal duodenum, as opposed to the gastric antrum. Instead of blending the suspension, the donated stool was spread on a gauze pad placed in a strainer, and the 250 ml of sterile saline poured through the gauze to filter the donation and create the suspension [Garborg *et al.* 2010].

The case report and proposed protocol of FMT in a single pediatric patient (2 years old) with IBD and CDI followed the protocol outlined by Aas and colleagues [Aas *et al.* 2003], with slight modifications [Russell *et al.* 2010]. Both sets of authors created the suspensions with 50–70 ml saline, and approximately 30 g of donated feces, filtered through a coffee filter, however Russell and colleagues specified only diffusing 25 ml of the fecal flora treatment through the NG tube [Russell *et al.* 2010]. Both Aas and colleagues and Russell and colleagues prescribed vancomycin for 4 days prior to the transplantation, terminating the night prior, with a dose of omeprazole the night prior and the morning of the FMT [Aas *et al.* 2003; Russell *et al.* 2010]. The adult dosages (vancomycin: 250 mg every 8 h; omeprazole: 20 mg) were reduced to pediatric appropriate levels (vancomycin: 10 mg/kg, every 6 h; omeprazole: 1 mg/kg, up to 20 mg maximum), and *Lactobacillus rhamnosus* GG or other probiotic was recommended for 3–6 months post-treatment. Controlled studies need to be performed before this protocol or FMT in general can be recommended as a first-line treatment for pediatric CDI, however early clinical accounts give reason to suspect that this is a viable option that would allow children to avoid the adverse events and complications associated with indefinite use of metronidazole or vancomycin.

Discussion

FMT's high cure rates of multiply recurrent CDI, 83% [Garborg *et al.* 2010] to nearing or at 100%, and reported safety supports the viability of FMT as an acceptable treatment method. In a recent systematic review, based on seven studies that represent the best available clinical research evidence on FMT for CDI, analysis concluded that most patients (83%) experience resolution of diarrhea immediately following the first FMT procedure [Guo *et al.* 2012]. Prior to the recent outbreaks of *C. difficile* with increased virulence, successful treatment of CDI episodes with traditional antibiotics results in an average of 265 additional days/patient of vancomycin and 19.7 days/patient of metronidazole [McFarland *et al.* 1999, 2002]. Continued research, particularly RCTs, will be important in determining which method of delivery is the most efficacious in repopulating the protective micro-ecology of fecal flora, while also maintaining the minimal risk of adverse events, and minimizing costs.

From our current perspective, there are advantages and disadvantages for each modality of administration, and there is no clear consensus on which implementation methodology offers the greatest benefit. Lower proximal GI FMT's advantages of being able to reach the terminal ileum or cecum (*versus* splenic flexure with enema), clinician visibility of relevant pathology, and the capacity to infuse larger volume suspensions, suggests that this approach may be the most anatomically reasonable and advantageous route of FMT in most patients. In univariate analysis, a shorter duration of symptoms before FMT, and naso-duodenal route of administration were associated with treatment failure [Sofi *et al.* 2011]. However, each patient should be evaluated individually to determine their best mode of care. Endoscope procedures carry a small risk of perforation, and this risk is likely enhanced in patients suffering with fulminant toxic megacolon, due to the inflamed mucosa of the affected colon. These patients may endure less risk if treated with enema FMT regardless of the endoscope's ability to deliver the flora transplant at the proximal end of the colon.

Enema utilization may also have advantages in its accessibility, as it does not require an endoscopist, procedure center, or anesthesia, and may carry less cost for the patient, depending on the number of infusions provided. There is greater ease in performing a series of transplants over a compact duration of time, without the patient having to tolerate the longer and more invasive endoscopic procedures. In contrast, since multiple instilments are frequently necessary with enema FMT, this methodology could also potentially be more costly, with lost work time, travel, and procedures factored into the equation [Bakken, 2009]. Enema FMT infusions have also been associated with a considerable amount of retrograde leakage, which leads to additional biohazard potential, and a potentially unpleasant experience for the patient [Bakken, 2009]. In the case series currently available, colonoscopic FMT has demonstrated efficacy without multiple infusions, and has been successful in providing secondary elimination of CDI if the first treatment fails or the patient is reinfected.

Upper GI tract FMT administration has been less favored generally, presumably due to the location of insertion of the fecal flora sample at the gastric antrum or duodenum, instead of directly at the affected sites in the colon. Potential degradation of the sample by gastric and pancreatico-biliary secretions is a concern, as is aspiration. There have also been slightly lower cure rates, although they are still high at 76% [Gough *et al.* 2011]. Despite these concerns, there are scenarios where this method may be the one able to provide the highest quality of care out of the three available modules of delivery. One paper summarized previous results with NG FMT, and noted that this avenue of treatment was well suited to seniors with CDI, a growing population at risk for infection [Rubin *et al.* 2009]. This option may also be particularly appropriate for the pediatric population [Russell *et al.* 2010], as well as for those with severe comorbidities that result in contraindication of lower GI infusion, such as in severe Crohn's disease [Duplessis *et al.* 2011]. Authors have reported that their experiences with a naso-duodenal tube were time efficient, whereas they found infusion through colonoscopy to be a slow process [Nieuwdorp *et al.* 2008; van Nood *et al.* 2009]. However, lower GI delivery may be preferred if the patient has signs of diminished ability to pass fluids through the intestinal tract [van Nood *et al.* 2009], such as ileus.

In avoiding adverse events, the vital step in all FMT procedures is the donor screening, encompassing laboratory tests and the oral interview. Clinical research has started to unravel more information about the intestinal microbiota's role in chronic diseases such as IBDs (Crohn's disease and ulcerative colitis), metabolic syndrome, cancer, and obesity [Festi *et al.* 2011]. It is particularly important to ensure that FMT donations come from healthy, well-screened individuals, without evidence of auto-immune or other chronic conditions. There have been no adverse events reported that can be directly confirmed as a result of FMT treatment. Brandt and colleagues noted that in the long-term follow up of 77 patients no new infections occurred postcolonoscopic FMT, however four patients later presented with new disorders, including peripheral neuropathy, Sjögren's disease, idiopathic thrombocytopenic purpura, and rheumatoid arthritis [Brandt *et al.* 2012]. Other patients in the study reported improvements in pre-existing medical conditions, including allergic sinusitis and arthritis. These new conditions, or improvements, cannot be attributed directly to FMT, but spark interest in further RCTs examining the interplay of microbiota and auto-immune disease, and its role in FMT [Brandt *et al.* 2012].

Most of the reports used fresh donations, which can be defined as unfrozen stool used within 24 h, but preferably within 6 h [Aas *et al.* 2003; Bakken *et al.* 2011; Kelly *et al.* 2012; Landy *et al.* 2011; Mattila *et al.* 2011; Mellow and Kanatzar, 2011; Rohlke *et al.* 2010; Russell *et al.* 2010]. Colonoscopic FMT with frozen standardized donor samples have been successfully implemented at the Centre for Digestive Diseases in Australia [Borody and Khoruts, 2011], and at the Minnesota Fairview Medical Center [Hamilton *et al.* 2012]. The use of frozen standardized donations is not theoretically a methodology restricted to colonoscopic delivery, however Hamilton and colleagues' paper described their methodology in relation to proximal GI FMT [Hamilton *et al.* 2012]. The clinical results were positive, yielding no significant difference in outcome between 10 patient-identified donor FMTs and 33 frozen standardized donor FMTs. The prospective case study was designed as an outcome data collection study, rather than a clinical trial, and was not intended to examine the efficacy of this methodology against other treatment options. Standardizing both the treatment protocol and the donated fecal

flora suspension may offer additional reproducibility benefits in designing a RCT with a high level of reliability and validity. At this time, given the limited information available on the key beneficial factors, many experts recommend the use of fresh donation provided on the day of treatment [Bakken *et al.* 2011].

Another potential benefit of standardized donor material would be in alleviating the burden of screening and acquiring the donation of fecal material from the patient, and might perhaps lead us to a centralized system where specialized facilities process donor material and ship to providers in the requested form. This could allow the development of a standardized interview questionnaire for potential donors and a laboratory screening process for the fecal donations, much like the process developed for blood donation, marrow donation, and tissue harvesting. As the field progresses, FMT may become available in a concentrated form, delivering the exact microbiota constituents needed to eradicate CDI infection and restore homeostasis. Currently, there are still extensive information gaps in our understanding of the human distal GI tract microbiota ecology, and it is not yet known exactly which organisms of the FMT are responsible for its high cure rates.

In one of the earlier reports aimed at identifying the protective constituents of intestinal microbtiota, Tvede and Rask-Madsen treated 5 CDI patients with a mixture of 10 facultative aerobic and anaerobic flora cultured from a donor, and diluted in sterile saline [Tvede and Rask-Madsen 1989]. Following treatment through rectal infusion *C. difficile* and its associated toxin was no longer detectable. Furthermore, *Bacteriodes* sp., which was not present prior to the infusion, was present after, signaling that *Bacteroides* sp. may have application in preventing and eliminating CDI.

In a more recent gene-sequencing study, terminal-restriction fragment length polymorphism and 16sRNA sequencing was used to conduct a case-study analysis of the pre/post-colonoscopic FMT bacterial composition of a patient with multiply recurrent CDI. The recipient's flora prior to therapy was deficient in Firmicutes and Bacteriodetes, but 2 weeks post-transplantation, the patient's symptoms had fully resolved, and the fecal flora of the donor and recipient were significantly similar. The post-transplant flora was predominantly made up of *Bacteriodes* spp., providing further support to its importance in maintaining colonic homeostasis, and an uncharacterized butyrate-producing bacterium. Although research is beginning to unravel the genetic characteristics of the intestinal microbiome, the bacterial concentration in the GI tract reaches 100–200 billion cells/g of feces (dry weight), with the number of bacterial organisms within the lumen approximated to near 10^{14} [Maccaferri *et al.* 2011]. It will be arduous to identify and evaluate each of the represented organisms, and manufacture a concentrated supplement with those deemed to maintain normal functioning. Until it is defined which enteric organisms are responsible for symbiotically restoring the colon to a healthy state, FMT is a relatively easy, and with appropriate screening, safe methodology for the treatment of recurrent CDI, in essence, instilling 'all' the bugs until we understand how best to proceed with a more targeted intervention.

Considering each methodology has its own pros and cons, determining the best method of delivery should be a patient-centered decision. For example, for a patient with less than optimal sphincter tone or lack of assistance at home, an enema might not be the most practical choice, whereas an NG tube would potentially allow easier delivery of the FMT with maximal retention. For anxious patients, a colonoscopy administered under moderate anesthesia may be the most tolerable, and therefore successful. In communities with limited access to endoscopic facilities, or for patients who cannot afford the significant costs of medical care, provider, or self-administered, enemas might be the most convenient and economical approach, although this method must be utilized with caution if prescreening is limited. Another avenue to consider would be to utilize a combination of methods that would give the patient the best chance of complete CDI eradication and relief of symptoms. The initial transplant could be delivered *via* colonoscopy, and follow-up in-office or home enemas could be administered subsequently to maximize establishment of the nascent microflora habitat.

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