Fecal microbiota transplantation in relapsing *Clostridium difficile* infection

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Abstract

Clostridium difficile infection rates are increasing in severity and frequency, and the range of patient populations susceptible/at risk is quickly widening beyond the scope of elderly hospitalized patients. Fecal Microbiota Transplantation is becoming accepted as an effective, safe, and easily accessible avenue of infection elimination in multiply recurrent patients. Secondary cure rates are reported at greater than 90%, much higher than reported for traditional avenues of care. Once antibiotics or other factors have disrupted the protective microbiome of natural colonic flora, Clostridium difficile may become dominant due to the lack of the normal repressive forces provided by the commensal GI flora. Balance can be restored by transplantation of donor stool, harboring a non-disrupted sample of a full GI bacterial microbiome. Transplantation can be provided through a variety of methodologies, either to the lower proximal, lower distal, or upper GI tract. This review summarizes 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 Reconstitution, stool transplantation, fecal transplant, Fecal Microbiota Transplantation, fecal flora, recurrent CDI

Background

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 United States, 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 to 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 one 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 (Ananthakrishnan et al., 2009b), peripartum patients (Centers for Disease Control and Prevention (CDC), 2005; Hookman and Barkin, 2009), those greater than 65 years of age (Pepin et al., 2005a), 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 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 increasingly suggesting that for patients with infections that fail to resolve with traditional antibiotic regimens, Fecal Microbiota Transplant (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; Helleman 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., 2003a; Brandt et al., 2012; Rohlke et al., 2010).

FMT is of particular utility in recurrent or refractory CDI, which are historically difficult to cure (Ho and Prasad, 2011; van Nood et al., 2009). A long-term follow-up, multi-center, study of interventional colonoscopic FMT for recurrent CDI has demonstrated a primary cure rate of 91% (defined as resolution of symptoms without recurrence within 90 days of FMT) and a secondary cure rate of 98% (defined as resolution of symptoms after one further course of vancomycin with or without repeat FMT) (Brandt et al., 2012), whereas traditional methods of antibiotic re-treatment without FMT have less efficacy (Musgrave et al., 2011). In conventional treatment, once the initial antibiotic course has been completed, recurrence occurs 6-50% of the time (Aslam et al., 2005; Cohen et al., 2010; Pepin et al., 2005a; Pepin et al., 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 (IVIG), 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 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).

The expanding body of data demonstrating FMT’s success includes a variety of methodologies for 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, via Pubmed, Embase, Web of Science, and general internet searches, were utilized to summarize progress in recurrent CDI treatment via FMT. With a focus on methodology, the intent 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 (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; 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 (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 (Aas et al., 2003b; 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, having 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 one week, or severe CDI presenting with no response to standard therapy after 48 hours (Bakken et al., 2011). In cases of non-responsive, severe, or fulminant, disease it should be taken into account whether earlier use of FMT would prevent further deterioration (Bakken et al., 2011).

FMT is generally considered relatively contraindicated in patients with severe comorbid conditions or those taking immunosuppressants, although anecdotally, such patients have been successfully treated. Duplessis et al. (2011) reported rapid resolution of refractory CDI complicated by severe Crohn’s disease when treated with FMT via NG tube. With the increased comorbidity of CDI and IBD (Ananthakrishnan et al., 2009a) 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).
The majority of the published literature highlighting FMT interventions is limited to the adult population. One case study reports successful FMT via nasogastric tube in a two-year-old pediatric patient, and suggested a potential protocol for use in the pediatric population (Russell et al., 2010). Gough et al.’s (2011) systematic FMT review reported that in 317 patients, 61% were female, the average age was 53 years, with actual ages spanning 2-95 years.

**Donor Determination**

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., 2003a; 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 et al. (1998) used homologous feces from one healthy donor in 18 patients (17 colonoscope, 1 gastroscope). Fifteen of the patients were considered cured, however three patients with the most severe colitis were reported as non-responsive. Kassam et al. (2012) treated 27 patients with FMT via retention enema, using two pre-screened donors for all patients, reporting resolution of symptoms in 88% (22/27). 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 further moved away from the approach of using directly identified individualized donors by creating a standardized laboratory process of banking frozen fecal material. In comparing 12 patients treated for CDI with fresh donor material (10 patient identified donors, two standardized donors) to 33 patients treated with the standardized frozen material there were no significant differences in infection clearance for fresh vs. frozen samples, or in patient identified donors vs. standardized donors, and no adverse events were reported for either group (Hamilton et al., 2012). The Center for Digestive Diseases in Sydney, Australia, performs the majority of their FMT procedures with standardized donor fecal samples. Borody and Khoruts (2011) 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.

From 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 (that the recipient has been previously unexposed to) 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 to fecal donations from unrelated sources (84%) (Gough et al.,
Controlled studies with balanced treatments groups need to be conducted before reliable recommendations could 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 listed in Box 1). An oral interview will be the clinician’s initial tool enlisted in the screening process, it is the primary avenue of identifying potential risk factors that would increase the odds of exposures to pathogens undetectable in the lab. The clinician must estimate the risk that the donor 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. Additional potential exclusions should include donors with a history of incarceration, tattoo or body piercing in the past six months, current or known exposure to a communicable disease, use of immunosuppressant agents, or antibiotics within the last three months. Travel within the past six months to an area known to be a risk-factor for diarrheal illness or other infectious diseases should also be considered in analysis of donors. Due 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 at survival, such as in severe fulminant CDI, it is the physician’s obligation is to calculate the benefit vs. 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**

Transplant of fresh donated feces is recommended to take place within 24 hours (Bakken et al., 2011; Landy et al., 2011), and ideally within six hours (Aas et al., 2003b; 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 based on avenue of transplantation. In all cases, a large volume of donation suspension should be attempted since resolutions seem to be greatest (97%) when greater than 500 mL is transferred (vs. 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 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 X 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., 2003b; 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 multi-methods study (colonoscopy followed by enemas) Wettstein et al. (2007) added psyllium to the 200-300 mL saline used to mix the donated flora to a liquid consistency. Another clinician prepared the donated stool suspension with pasteurized cow milk before transplanting through an enema, based on findings that compared to controls, patients with recurrent CDI excrete fewer fecal short chain fatty acids. Seven of the nine patients were considered cured at 18 months post transplant (Gustafsson et al., 1998; Gustafsson et al., 1999). One of the earlier clinicians to publish a case study account of FMT prepared enemas in an anaerobic cabinet from fresh feces. The protocol called for two enemas directly after their preparation and three days apart, immediately post, and at nine months, the patient was exhibiting no signs of CDI (Schwan et al., 1983; Schwan et al., 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/Post Preparation

The decision to eliminate antibiotics prior to FMT was almost universal, however there were variances in the time frame prior to the procedure, most commonly 1-3 days prior. The use of bowel lavage was not included in all 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 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 included studies. Bowel lavage is not frequently utilized with FMT administered via nasogastric tube, however one group reported utilizing bowel lavage prior to upper GI FMT in four patients (Nieuwdorp et al., 2008). Lavage may also have benefit with enema preparations (Borody et al., 2012; Borody et al., 2004). Clone library sequencing has shown that colonic mucosa-associated microbiota composition is altered by standard bowel prep 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 to routinely recommend bowel lavage in the varying combinations of FMT procedures, in light of 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 diphenoxylate and atropine (Immodium, McNeil PPC Inc) immediately after colonoscopy induced transplant and again approximately six hours later to maximize retention time of the donated microbiota (Brandt et al., 2012; Rohlke et al., 2010). Silverman et al. (2010) continued *Saccharomyces boulardii* in patients receiving the probiotics prior to FMT for 60 days post enema FMT. Many of the patients in Hamilton et al.’s study (2012) were taking probiotics pre-FMT, but all were counseled to discontinue any probiotic treatment post-FMT. One of the authors of this review (NS) continues *Saccharomyces boulardii* indefinitely in almost all patients treated with FMT. Future RCT trials 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 et al.’s (2011) systematic review, 35% of FMTs had been provided by enema, the largest fraction of FMTs. 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 the 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 via colonoscope, and following up with a series of enemas (Wettstein et al., 2007). The available literature is summarized below according to location of GI tract delivery and equipment category (proximal lower GI – colonoscopy; distal lower GI- enema, rectal tubes; and 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 (Arkkila et al., 2010; Brandt et al., 2012; Garborg et al., 2010; Girotra et al., 2011; Hamilton et al., 2012; Helleman et al., 2009; Kelly et al., 2012; Khoruts et al., 2010; Lund-Tonnesen et al., 1998; 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
colonoscopy. The suspension is then filtered to ensure larger particulates that could clog the scope are removed. The volume should be limited to increase the likelihood 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 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. Due 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 data sets reported for some studies. The volume of fluid mixed with the donated fecal matter ranged on average from 200-300 ml, and the amount of donated stool ranged from 5 – 300 g, however some authors reported inclusion of all 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), where as 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 risk of treatment failure post transplant (97% resolution vs. 80% with ≥ 500 ml vs. ≥ 200 ml) (Gough et al., 2011). Colonoscopic FMT is well suited for transfusing these larger volume suspensions.

The combined secondary cure rate of the included cases highlighting FMT via colonoscopy was 96.3%. This impressive cure rate is consistent with the 98% secondary cure rate reported in the recent long-term multi-center 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 instillation. Twelve reports of lower GI tract FMT treating CDI were included in this review (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 classification as treatment for PMC (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 one case was explicitly treating 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 the most feasible as a series treatment, arranged as multiple FMT infusions over a specified duration of time. Borody et al. (2012) recommends using 200–300 g of donated fecal material, and 200–300 mL sterile saline, homogenized in a blender to liquid consistency and administered via enema within 10 minutes of preparation, once daily for five days. As in colonoscopic FMT, clinicians are encouraged to recommend loperamide pretreatment to maximize retention time. Combination treatment with the initial infusion delivered via colonoscope, 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 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 et al.’s (2012) study started with one enema, and if diarrhea recurred within seven days a second enema FMT was administered (4/27 patients, with 2/4 classified as failures after the second FMT). Wettstein et al. (2007) 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. Most authors opted to transplant freshly donated fecal flora, however Gustafsson et al. (1999) used donated fecal material from a healthy adult volunteer and pre-packaged syringes with the composite of filtered suspension donation and pasteurized cow milk. The 20 mL syringes were then stored at 20 degrees Celsius and later thawed in 37 degree Celsius water 30-60 min prior to enema FMT treatment.

Lower GI administration of FMT has a high cure rate of 95.4% and 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 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 post-operatively 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 care-taker, and potentially would allow a larger number of patients to be treated via FMT in rural or underdeveloped environments.

**Upper GI Tract FMT**

The third mechanism of FMT available today, upper GI tract administration, includes nasogastro tubes, duodenal tubes and endoscopy/gastroscopy. In 2011 23% of all FMT procedures had been provided by means of NG tube or gastroscope. CDI treatment has been successful, defined as 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 four of the 10 patients for which the first NG FMT failed, three of the four were successful (Garborg et al., 2010). Reports of combined jejunal and colonic FMT was reported in a recent abstract discussing successful resolution in three patients, however exact methodology was not reported (Girotra et al., 2011). As with the methods of lower GI FMT, preparation of the donated flora sample was the same, with differences in volumes, and pre/post strategies. Only one clinician provided bowel lavage prior to FMT (Nieuwdorp et al., 2008), however most cases included a proton pump inhibitor, omeprazole, the evening prior (Aas et al., 2003b; MacConnachie et al., 2009; Rubin et al., 2009; Russell et al., 2010). Upper GI FMT requires smaller volumes of suspension to be transplanted due 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., 2003b; Duplessis et al., 2011; MacConnachie et al., 2009; Rubin et al., 2009; Russell et al., 2010). Immediately before transfusing the suspension, the nasogastric tube can be placed, and radiography used to verify 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 NaCl saline, and removed. The patient can be released from the clinic and resume all normal dietary patterns immediately following (Aas et al., 2003b). The gastroscope FMT approach reported larger volumes (50-100 g stool, 250 mL saline, and 200 ml infused), due to the insertion into the distal duodenum, as opposed to 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 (two year-old) with IBD and CDI followed the protocol outlined by 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 Russel et al. (2010) specified only diffusing 25 mL of the fecal flora treatment through the nasogastric tube. Both
Aas (2003) and Russel (2010) prescribed vancomycin for 4 days prior to the transplant, terminating the night prior, with a dose of omeprazole the night prior and the morning of FMT. The adult dosages (vancomycin: 250 mg q8h, omeprazole: 20 mg) were reduced to pediatric appropriate levels (vancomycin:10 mg/kg, every 6 hrs, omeprazole: 1 mg/kg, up to 20 mg max), and *Lactobacillus rhamnosus* GG or other probiotic was recommended for three to six 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**

Fecal Microbiota Transplant’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; McFarland et al., 2002). Continued research, particularly RCTs, will be important in determining which method of delivery is the most efficacious in repopulating the protective microecology 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 of which implementation methodology offers the greatest benefit. Lower proximal GI FMT’s advantages of being able to reach the terminal ileum or cecum (vs 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, shorter duration of symptoms before FMT, and naso-duodenal route of administration were associated with treatment failure (Sofi et al.,) 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 instillments are frequently necessary via 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
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...the case series’ currently available, colonoscopic FMT has demonstrated efficacy without multiple infusions, and has been successful for 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, presumable 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 still high at 76% (Gough et al., 2011). Despite these concerns, there are scenarios where this method may be the able to provide the highest quality of care out of the three available modules of delivery. One paper summarized previous results with nasogastric FMT, and noted that this avenue of treatment was well suited for 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 naoduodenal tube were time efficient, where as 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 intestinal microbiota’s role in chronic diseases such as inflammatory bowel diseases (Crohn’s disease and UC), metabolic syndrome, cancer, and obesity (Festi et al., 2011). It is particularly important to ensure 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 et al. (2012) noted that in long-term follow up of 77 patients no new infections diseases occurred post-colonoscopic FMT, however four patients later presented with new disorders, including peripheral neuropathy, Sjogren’s disease, idiopathic thrombocytopenic purpura, and rheumatoid arthritis. Other patients in the study reported improvements in preexisting medical conditions, including allergic sinusitis and arthritis. These new conditions, or improvements, cannot be attributed directly to FMT, but sparks interest in further RCTs examining the interplay of microbiota and autoimmune 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 hours, but preferably within six hours (Aas et al., 2003b; 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 of 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 et al.’s (2012) paper described their methodology in relation to proximal GI FMT. 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 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 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 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 microbiota, Tvede et al. (1989), treated five CDI patients with a mixture of 10 facultative aerobic and anaerobic flora, cultured from a donor, and diluted in sterile saline. Following treatment through rectal infusion of C. difficile and its associated toxin was no longer detectable. Furthermore, bacteroides sp., which was not present prior to the infusion, was then 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 two weeks post-transplant, 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 bacteria. 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/gram of feces (dry weight), with the number of bacterial organisms within the lumen approximated 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 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, a patient with less than optimal sphincter tone or lack of assistance at home, an enema might not be the most practical choice, whereas a nagogastric 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 being 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.


