Bacteriotherapy Using Fecal Flora

Toying With Human Motions

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Abstract: The intestinal flora may play a key role in the pathogenesis of certain gastrointestinal (GI) diseases. Components of bowel flora such as *Lactobacillus acidophilus* and *Bifidobacterium bifidus* have long been used empirically as therapeutic agents for GI disorders. More complex combinations of probiotics for therapeutic bacteriotherapy have also recently become available, however the most elaborate mix of human-derived probiotic bacteria is, by definition, the entire fecal flora. Fecal bacteriotherapy uses the complete normal human flora as a therapeutic probiotic mixture of living organisms. This type of bacteriotherapy has a longstanding history in animal health and has been used sporadically against chronic infections of the bowel, especially as a treatment of last resort for patients with severe *Clostridium difficile* syndromes including recurrent diarrhea, colitis, and pseudomembranous colitis. Encouraging results have also been observed following infusions of human fecal flora in patients with inflammatory bowel disease, irritable bowel syndrome, and chronic constipation. The therapeutic use of fecal bacteriotherapy is reviewed here and possible mechanisms of action and potential applications explored. Published reports on fecal bacteriotherapy are few in number, and detail the results of small uncontrolled open studies and case reports. Nevertheless, given the promising clinical responses, formal research into fecal bacteriotherapy is now warranted.

Key Words: feces, *Clostridium difficile*, intestinal diseases, probiotics, therapy

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There has been a recent resurgence of interest in the therapeutic use of probiotics for gastrointestinal (GI) diseases where the enteric flora is presumed to play a permissive or even pathogenic role.[1–4] *Clostridium difficile*-related diarrhea and colitis, inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS) have all, to varying degrees, been causally associated with disturbances in the composition of natural flora, although the precise etiology of IBD and IBS remains unclear.[5–10] Probiotics and fecal bacteriotherapy utilizing the entire human fecal flora as the ultimate therapeutic bacterial mixture may have value in the treatment of such disorders, especially when refractory to other treatments.

Historically, the transplantation of viable enteric bacteria was probably first described in animals as "transfaunation" by an Italian anatomist named Fabricius Aquapendente in the 17th century: "I have heard of animals which lose the capacity to ruminate, which, when one puts into their mouths a portion of the materials from the mouth of another ruminant which that animal has already chewed, they immediately start chewing and recover their former health".[11] Further aspects of the history of transfaunation in animals have since been reviewed,[12] and the procedure has also been recommended for the prevention of GI disorders in ruminants.[13,14] For example, transfaunation in ruminants is currently used in severe ruminal acidosis following grain overload. In cattle, this can be achieved by using an instrument specifically developed to facilitate the transfer of gut flora.[15]

We reviewed available articles on the use of fecal bacteriotherapy in the management of various human GI disorders found through MEDLINE, EMBASE and Current Contents medical literature databases. The search also included international gastroenterology conference proceedings and the bibliographies of key reviews. This review is all-inclusive because there is a lack of controlled trials in this area. Publications reviewed here encompass 3 different GI disease groups, not to mention a wide range of therapeutic doses and varying periods of treatment and follow-up. This article has aimed to provide an overview of the existing literature in this narrow field to determine whether further studies into the mechanisms and clinical benefit of fecal bacteriotherapy are indicated.

The use of fecal bacteriotherapy in human subjects has been reported in 17 publications to date (2 of these describe the same series of patients[16,17]). These case reports and prospective studies document the therapeutic effects of infused human fecal flora in a total of 150 patients (84 with relapsing *Clostridium difficile*-associated diarrhea, colitis or pseudomembranous colitis (PMC), 9 with ulcerative colitis, 5 with chronic constipation, 1 with Crohn disease, and 51 patients without

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clear diagnoses but with symptoms including constipation, diarrhea, and abdominal pain). The results are outlined in Table 1. In most cases, fecal flora replacement was used alone following the failure of other standard therapies or due to recurrence of the condition after withdrawal of conventional medications.

Clostridium difficile-associated Diarrhea and Colitis

C. difficile is the cause of at least 25% of all cases of antibiotic-associated diarrhea. This may be an underestimate of the real incidence of *C. difficile* infection because largely employed testing methods of looking for *C. difficile* toxin A alone were unsuccessful at detecting variant strains of *C. difficile* that produced toxin B only. Variant toxin B-producing strains *of C. difficile* have been reported to be as low as 0.1% in the United States and up to 16% in Japan.[18,19] Variant strains were discovered in the early 1990s and thought to be clinically unimportant.[20–22] Studies have since shown that cytotoxin is as lethal as enterotoxin and may be responsible for patient fatality.[23,24]

The administration of antibiotics can alter the balance of normal colonic flora to permit the overgrowth of pathogenic *C. difficile* strains that produce toxins causing diarrhea and associated symptoms. Successful treatment of a primary episode of *C. difficile* diarrhea by withdrawal of the inciting antibiotic or using oral metronidazole or vancomycin is associated with relapse in 10% to 25% of patients.[25,26] There are few effective therapeutic approaches to chronic *C. difficile* infection. Generally, recurrences are treated with a second course of metronidazole or oral vancomycin, however up to 65% of these patients will suffer multiple relapses despite maximal antibiotic therapy.[27] Furthermore, the repeated use of these agents to treat recurring *C. difficile*-associated diarrhea may predispose patients to further relapses through the maintenance of a disturbed intestinal flora and may also contribute to the emerging problem of *C. difficile* resistance. In a recent Spanish report, the rate of resistance of nosocomially acquired *C. difficile* isolates to metronidazole was 6.3% and, whereas no full resistance to vancomycin was observed, the rate of intermediate resistance to vancomycin was 3.1%.[28] More concerning is the finding that the gene responsible for causing vancomycin resistance in enterococci (*van*B) has been detected in the genome of clostridia.[29] It is likely that this gene will migrate to *C. difficile* if has not done so already.

A small proportion of patients suffering recurrent *C. difficile*-associated diarrhea risk progression to colitis, PMC and, albeit rarely, toxic megacolon and death, which appears to be increasing in frequency. The reported incidence of fulminant *C. difficile* colitis in hospitalized patients in Pennsylvania increased from 0% in 1990 to 3.6% in 2000.[30] The mortality rate among in-patients diagnosed with *C. difficile* colitis at Oregon Health and Science University Hospital increased from 3.5% in 1990 to 15.3% in 2000.[31] Standard treatment of life threatening *C. difficile* colitis is colectomy, which, under the circumstances, generally carries a high mortality rate of up to 80%.[32] It is clear that, at times, treatment of relapsing *C. difficile*-associated diarrhea may present a serious problem for the clinician. Thus, there is a need for safe and effective alternative second-line interventions.

The use of probiotics is an appealing approach for the treatment of recurrent *C. difficile*-associated diarrhea because the therapeutic aim is to restore the "colonization resistance" of the bowel and to eradicate *C. difficile*. *Saccharomyces boulardii* and *Lactobacillus* strains as adjuncts to standard anti-*C. difficile* therapy have shown promise in preventing recurrences of *C. difficile*-related diarrhea in clinical trials and anecdotal reports.[2,27,33–35] As an extension of probiotic therapy, fecal bacteriotherapy comprises the entire normal human flora. *In vitro* studies have confirmed the antagonistic activity of the normal fecal flora toward *C. difficile*.[36–39] *In vivo,* clinical manipulation of the bowel flora by infusing homogenized human feces has been consistently effective against recurrent *C. difficile*-associated diarrhea without the need to resort to vancomycin or metronidazole.[40]

The use of fecal bacteriotherapy has been most widely applied to the treatment of chronic relapsing *C. difficile*-associated diarrhea and PMC. Of the 84 patients detailed in the relevant literature with these conditions (36 patients with *C.* difficile-associated diarrhea, 22 with *C. difficile* colitis and 26 with PMC), 72 (86%) experienced immediate and prolonged resolution of severe symptoms following administration of fecal retention enemas. None of the patients relapsed for the duration of follow-up (1 day to 5 years).[16,41–51] Fecal bacteriotherapy cured refractory *C. difficile*-related diarrhea in 33 out of 36 patients (92%) with a single course of treatment.[16,44–49] In addition, of the 22 patients with *C. difficile*-associated colitis, 16 (72%) reported improvement,[43,48,51] albeit 2 patients died of unrelated illnesses.[51]

Most of *C. difficile* infections were associated with the use of broad-spectrum antibiotics. Nine patients were diagnosed with PMC after a chemical bowel preparation for colonoscopy.[42] Prior to bacteriotherapy, symptoms of colitis and diarrhea improved with oral vancomycin in 2 patients[43,44] and "most patients" included in the Aas et al *C. difficile* colitis series.[51] In 15 other patients, *C. difficile* disease recurred despite intense treatment with vancomycin, and/or metronidazole together with bacitracin or cholestyramine.[45–47,49,50] For all reported *C. difficile*-related conditions, *C. difficile* culture and cytotoxin assays were performed on 65 fecal specimens prior to fecal bacteriotherapy and 62 of these were positive for both. Twenty-three patients were re-tested after fecal bacteriotherapy and 20 of these were negative for *C. difficile* and its toxin.[44,46,49–51]

Though the diagnosis of *C difficile*-associated PMC is generally confirmed by detection of *C. difficile* toxin in stools,

TABLE 1. Results of 17 Reports on the Therapeutic Use of Fecal Bacteriotherapy for Gastrointestinal Disorders

Reference	Type of Study	No. of Patients	Disease	Dose and Method of Delivery*	Follow-Up Period	Outcome
Eiseman et al, 1958[41]	Case series	4	PMC	1–3 retention enemas	1–10 days	Severe diarrhea ceased within 48 hours.
Bowden et al, 1981[42]	Case series	16	PMC	Single or twice daily retention enemas (over 1–12 days) (2 patients had fecal infusions via enteric tube to mid-jejunum)	5 days to 3 years	13/16 patients responded dramatically within 1–12 days; 3 patients died—2 had no evidence of disease at autopsy, 1 had evidence of PMC throughout small bowel.
Schwan et al, 1984[43]	Case report	1	Relapsing *C. difficile* enterocolitis	2 retention enemas (over 3 consecutive days)	1 year	Prompt and complete normalization of bowel function with disappearance of IBS symptoms; weight gain 6kg; 1–2 stools/day
Bennet and Brinkman, 1989[58]	Case report	1	Severe UC	Large-volume retention enemas	6 months	Patient symptom-free (no bloody diarrhea, cramping, tenesmus, skin lesions or arthritis) for the first time in 11 years without any medication.
Tvede and Rask-Madsen, 1989[44]	Open	2	Relapsing *C. difficile* diarrhea	1–2 retention enemas (50 g feces suspended in 500 ml saline)	6 months	Patient 1: complete clinical recovery with eradication of *C. difficile* and its toxin after 1 infusion; Patient 2 had 2 infusions but did not respond (but did respond to a cultured bacterial mixture).
Borody et al, 1989[45]	Case series	55	IBS and IBD	Retention enemas	1–12 months	20 patients deemed "cured"; 9 patients experienced a decrease in symptoms; 26 patients failed to respond.
Flotterod et al, 1991[46]	Case report	1	Relapsing *C. difficile* diarrhea	Single enema via duodenal tube (10 g feces suspended in saline)	3 days	Diarrhea cured after fecal infusion.
Andrews et al, 1992[73]	Case report	1	Chronic constipation	2 retention enemas (in 2 days)	18 months	Prompt and long-term resolution of symptoms.
Paterson et al, 1994[47]	Case series	7	Chronic *C. difficile* diarrhea	Single daily retention enemas for 3 days (400 mL consisting of 200 ml stool mixed with 200 ml saline)	Up to 2 years	All patients experienced rapid resolution of disabling persistent *C. difficile* infection without relapse.
Lund-Tonnesen, et al, 1998[48]	Open	18	*C. difficile* diarrhea	Single enema via colonoscope (10 g feces suspended in saline); via gastrostomy tube in one patient	1–5 days	15/18 clinically cured without relapse; 3 patients with severe colitis did not respond.
Gustaffson et al, 1998 and 1999[16,17]	Open	9	Antibiotic-associated diarrhea	Single enema (20 mL containing 5–10 g homogenized feces in ordinary pasteurised cow's milk)	18 months	7/9 patients recovered rapidly with cessation of diarrhea within 6 days without long-term relapse.
Persky and Brandt, 2000[49]	Case report	1	*C. difficile* diarrhea	Single enema via colonoscope (500 mL containing stool mixed in saline)	5 years	Immediate and complete resolution of diarrhea that was maintained long-term. Repeat *C. difficile* toxin assay negative.
Borody et al, 2001[59]	Case series	6	UC and chronic constipation	Single daily retention enemas for 5 days (donor stool suspended in 200 mL saline with one tablespoon of psyllium husks)	8–28 months	Constipation (3 patients): Long-term restoration of normal bowel function to 1–2/day without laxatives. UC (3 patients): disappearance of symptoms within 1 month, maintained long-term without medications.
Faust et al, 2002[50]	Case series	6	Recurrent PMC	Stool transplantation (method unstated)	9–50 months	All patients responded promptly and continued to be asymptomatic; 4/6 patients *C. difficile* toxin negative.
Aas et al, 2003[51]	Case series	18	Recurrent *C. difficile* colitis	Single 25 ml dose of stool suspension (30 mg feces homogenized in 50–70 mL saline) via nasogastric tube	90 days	15/18 patients asymptomatic for duration of follow-up. 13 patients were *C. difficile* negative (2 patients not tested); 2 died of unrelated illnesses; 1 treatment failure (*C. difficile* positive).
Borody et al, 2003[60]	Case series	6	Severe UC	Single daily enemas for 5 consecutive days (consisting of 200–300 g stool and 200–300 mL saline)	1–13 years	All patients showed complete resolution of symptoms by 4 months and were able to cease all UC medications. No clinical, colonoscopic or histological evidence of UC at long-term follow-up.

*Details of dose and method of stool transplantation provided where available.

this test was unavailable prior to the discovery of *C. difficile* in 1978.[52,53] Two publications describing the use of fecal bacteriotherapy for PMC pre-date 1978, thus 20 patients were diagnosed on the presence of classic symptoms alone including abdominal distention, diarrhea, fever or visualization of a pseudomembrane on endoscopy.[41,42] Details of 26 patients suffering PMC are recorded in the literature and 23 of these (88%) showed dramatic resolution of symptoms and disappearance of the pseudomembrane following fecal bacteriotherapy though of the 3 patients who died, 2 had no evidence of PMC at autopsy.[42] Despite the lack of controlled trials, the apparent benefit of fecal bacteriotherapy for relapsing *C. difficile* diarrhea and PMC seems to warrant further attention.

Inflammatory Bowel Disease

The rising incidence of Crohn's disease (CD) and high prevalence of ulcerative colitis (UC) is a burden to the community and the health system. The causes of IBD remain unknown, although accumulating evidence suggests that an excessive mucosal immune response to the enteric flora, or perhaps a specific infectious agent, in combination with genetic predisposition, may be responsible for the chronic inflammatory process. Accepted treatments for CD and UC target inflammation using long-term anti-inflammatory and immunosuppressive agents. Although corticosteroids can be effective in the management of moderate to severe IBD, this is offset by a high potential for dependency, systemic toxicities and lack of efficacy as maintenance therapy. Corticosteroid-sparing medications include azathioprine, 6-mercaptopurine, methotrexate, cyclosporine, infliximab, and other anti-tumor necrosis factor agents. These drugs are also associated with a range of adverse effects and the therapeutic efficacy may vary between patients with long-standing disease. Surgery is usually indicated when IBD is refractory to medical therapy or when the adverse effects of long-term drug use become too restrictive to continue. Surgery may eventually be required in up to 90% of patients with ileo-colonic disease.[54] Probiotics may be useful as an alternative therapeutic approach to IBD with minimal adverse effects.

Several controlled trials have shown that probiotic therapy can influence the course of IBD. By reducing intestinal inflammation, probiotics are thought to stabilize the gut mucosal barrier and consequently reduce the systemic inflammatory response in patients with IBD.[55] The use of single and multiple probiotics such as *Escherichia coli* strain Nissle, *Saccharomyces boulardii* and the lactic acid bacteria for the management of IBD has already been reviewed extensively.[3,56] Results of 13 trials to date (7 in CD and 6 in UC), including 2 randomized controlled trials, outlining the use of various single and combination probiotics for IBD have been encouraging.[3] The optimum bacterial combination and dosage remain to be determined. Intuitively the use of fecal bacteriotherapy should be more applicable to UC than CD given that CD etiology may entail tissue infection with a specific pathogen (eg, *Mycobacterium avium* ssp. *Paratuberculosis*).[57]

Fecal bacteriotherapy as a complete bacterial therapeutic mixture for the management of refractory UC has been described in 4 publications comprising 9 patients to date.[45,58–60] One of these publications reported the use of human fecal flora in an unspecified number of patients with IBD which described 1 example of UC and 1 of CD as individual case reports.[45] Of the 9 patients detailed in the literature with severe, longstanding UC (18 months to 20 years, mean 8.6 years), all were refractory to standard management including corticosteroids, 5-aminosalicylates (salazopyrin, mesalamine, olsalazine), and azathioprine. The presence of active colitis was confirmed by colonoscopy and histology prior to treatment. Stool culture and microscopy were performed in 7 out of 9 patients and were negative for common detectable bacterial pathogens and parasites including *C. difficile* and its toxin. Following administration of fecal retention enemas, all 9 patients responded dramatically within 6 weeks with resolution of bloody diarrhea, abdominal pain, cramping, and urgency. All patients ceased taking their UC medications within this initial period without relapse. Remission was maintained without medication during follow-up ranging from 3 months to 13 years. Follow-up colonoscopy was carried out and biopsies taken for histology in 8 out of 9 patients, revealing no evidence of UC in 6 patients and the presence of mild chronic inflammation in the remaining 2 patients.[45,58,60] The patient with CD refractory to prednisone and salazopyrin responded to the infusion of fecal flora within 3 days with disappearance of symptoms, persisting for 4 months without medication. The disease ultimately relapsed after 18 months (Borody, unpublished data). It is possible that fecal bacteriotherapy has some value in the management of refractory IBD, and this may justify further investigation to elucidate mechanisms of action.

Irritable Bowel Syndrome

Irritable bowel syndrome is a common condition in Western populations. The prevalence of IBS in North America ranges from 3% to 20%[61] with the latest prevalence estimate in Australia reported to be 12% using the Rome criteria.[62] The etiology of IBS is unknown although there is unsubstantiated evidence to suggest that a disturbance in the normal intestinal flora may be responsible for the initiation and persistence of symptoms.[63,64] Standard medical management of IBS is often unsatisfactory. Remodeling of the composition of fecal flora with probiotics seems a logical treatment option considering the tentative causal association.

The administration of probiotics either singly or in combination has been tried in some patients with IBS. The results of 12 studies on the use of probiotics for IBS in 1371 patients have been reviewed in detail.[4] Most reviewed papers, including 5 randomized, double-blind, placebo-controlled trials, showed some benefit with the use of a single strain or combi-

nation of bacteria including lactobacilli, bifidobacteria, and *Enterococcus fecalis* among others. Three trials cited the therapeutic value of *Lactobacillus plantarum* 299v for the alleviation of IBS symptoms including pain and bowel function.[65–67] Results of a similar recent study however failed to reveal any benefit following the administration of *L. plantarum* 299v.[68] *L. casei* strain GG also recently failed to significantly improve symptoms in a subgroup of patients with IBS, though a trend was noted in the reduction of unformed stools.[69] In another publication, a >80% global clinical response was reported when *Clostridium butyricum* strain MIYARI 588 was given to randomized patients suffering diarrhea-predominant IBS.[70] In addition, the composition of intestinal flora was altered in patients with IBS in this Chinese study, increasing lactobacilli and bifidobacteria and decreasing clostridia counts. Significant symptomatic improvement was also noted in 2 reports following the endoscopic administration of a mixture of 16 enteric bacteria (comprising mostly *Bacteroides* and *Bifidobacterium* sp.) in 50 patients with IBS.[71,72]

Fecal bacteriotherapy has shown some efficacy in the treatment of IBS and chronic functional constipation. Three publications reported the treatment of 5 patients with chronic constipation-predominant IBS (duration 3–6 years; mean 3.75 years) with a stool frequency of 1 every 4–7 days despite laxative use.[45,59,73] One patient described by Andrews et al[73] developed constipation and associated symptoms including bloating, daily nausea, and headaches following a hysterectomy. Her 3-year-long symptoms resolved promptly during a 4-week course of oral vancomycin, but returned on withdrawal of antibiotics. Prior to fecal infusion the patient was unable to expel a rectal balloon despite maximal straining pressure to 167 cmH_2O, and melanosis coli was present on colonoscopy and histologic biopsy. Two to 3 days following fecal bacteriotherapy, the patient's stool frequency increased to 1–2 per day without laxative-use and the patient was able to expel a water-filled rectal balloon with ease. Colonoscopy revealed normal mucosa with no evidence of melanosis. This improvement persisted for the 18-month follow-up period. Further, 4 other cases were reported by Borody et al.[45,59] All 4 patients experienced immediate resolution of symptoms of constipation to 1 to 2 stools per day following fecal bacteriotherapy and this improvement persisted for 6 to 28 months of follow-up. Despite the small number of patients studied, the marked clinical improvement following fecal bacteriotherapy warrants further study to elucidate the contribution of the fecal flora in the etiology of constipation-predominant IBS.

METHODOLOGY OF FECAL BACTERIOTHERAPY

Donor Screening

A major fear associated with the therapeutic use of donated human feces is the potential for transmission of viral, bacterial, and parasitic infections and the existing literature does not detail screening methods. Adequate descriptions of the techniques used by clinicians to prevent possible cross-infection are lacking. We have attempted to extract the essential details from the available published material.

Ideally a person could be considered for selection as a donor if investigations similar to those required of organ transplant donors are normal. These would include full blood count, liver function tests,[60] and negative viral screening for human immunodeficiency virus (type 1 and type 2), hepatitis A, B and C,[47,59] cytomegalovirus, Epstein-Barr virus,[16,48] and syphilis.[51,60] Several reports have suggested that donor fecal specimens, preferably 3,[74] should be screened on selective media for common detectable pathogens including *Salmonella, Shigella* and *Campylobacter* sp., *Aeromonas hydrophila, Yersinia* sp., *Vibrio parahaemolyticus, Vibrio cholerae, Candida albicans,* enterohaemorrhagic *E. coli* O157, and *Clostridium difficile* and its toxin.[16,43,47,48,50,51,59,60] It has also been emphasized that stool microscopy should be negative for protozoa (trophozoites and cysts), helminths and ova including *Entamoeba histolytica, Giardia lamblia, Cryptosporidium* sp., *Dientamoeba fragilis, Blastocystis hominis, Ascaris lumbricoides*, trematodes, and tape worms.[60] As many as 7 publications contain no information on donor screening methods prior to treatment. To help establish a standard for the use of fecal bacteriotherapy, future publications should detail techniques used to screen donors and fecal specimens before infusion.

Most studies have used patients' relatives as donors,[43,44,47,49–51,60,73] although medical students, surgical residents,[42] and unrelated healthy individuals have also been chosen successfully.[16,41,45,48,51,58,59] Selecting a patient's long-term partner has the theoretical advantage that any transmissible disease should have emerged well before treatment with fecal bacteriotherapy. Indeed, in 1 report it was deemed unnecessary to screen for any pathogens or viruses due to the monogamous, 30-year relationship of the patient with her healthy donor husband.[49] Other studies have also preferred immediate family members for donors and hence disregarded the need for donor screening.[43,50,73] The selection of relatives or spouses as fecal donors should probably not exclude the necessity for comprehensive screening, just as donations of blood should not be transfused until standard screening has been completed.

Preparation and Administration of Fecal Suspension

Treatment protocol for the use of fecal bacteriotherapy for GI illnesses should be standardized. Publications on this novel therapy have listed various doses of human fecal flora ranging from 5 – 300 g donor stool suspended in 10–300 mL saline (or 10–15 mL milk in 1 case,[16] with the addition of 1 tablespoon of psyllium husks in another report[59]). One recent paper describes filtering the fecal suspension twice through a paper coffee filter prior to transplantation.[51] Seven papers

however, fail to list any information regarding the dose of feces given. The fecal suspensions have generally been administered to the patient within 10 minutes to 2 hours after preparation,[42,43,47,59,60] most commonly via retention enema as a single dose or once or twice daily for up to 14 days.[16,41–45,47,58–60,73] Other successful methods of administration have also included an enteric tube to mid-jejunum,[42] duodenal tube,[46] nasogastric tube[48,51] and a colonoscope to infuse homologous feces every 10 cm throughout the colon ensuring delivery to the proximal colon.[48,49]

Aware of the inadequately detailed methods in the existing literature, we attempted to specify and clarify the particulars of preparation and administration of fecal bacteriotherapy in our most recent report on its use for UC, though these directions are largely empiric.[60] This included a protocol designed to "sterilize" the bowel prior to fecal infusion with antibiotics followed by a single orthostatic lavage with 3L of polyethylene glycol-based oral solution. A similar method has been detailed and performed previously.[58,75] Aas et al also pre-treated patients with oral vancomycin to "reduce the *C. difficile* load".[51] We used donor feces (200–300 g) diluted in 200–300 mL normal saline administered via enema within 10 minutes of preparation, repeated daily for 5 days. One publication advises that no more than 6 hours should elapse between donor sample collection and fecal administration.[51] We have recently suggested that patients should be encouraged to retain the enemas for at least 6–8 hours and then follow a high-fiber diet. Clearly, experimental data are required to develop a logical therapeutic approach to the preparation, dosing, and duration of administration of fecal bacteriotherapy specific to the targeted gastrointestinal disorder. Such an approach has in part been attempted by Lund-Tonnesen et al where 18 patients were treated using a single donor, in an attempt to standardize the protocol, resulting in cure of *C. difficile* diarrhea in 15 patients without relapse.[48]

Use of Concurrent Medication

When assessing effectiveness of fecal bacteriotherapy one needs to take into account the use of prior and concomitant medications to differentiate the effect of medications from the effect of bacteriotherapy. Little work has been reported in this respect save 1 reference to recurrent PMC where it was claimed that to withhold other forms of treatment during fecal bacteriotherapy would be "an unjustifiably stringent experiment in view of the gravity of the illness".[41] Perhaps randomization would be more acceptable in less severe disorders.

Analysis of Stool Composition Pre- and Post-infusion

Analysis of stool composition prior to and post-infusion was reported in 2 publications. Tvede and Rask-Madsen noted the complete absence of luminal *Bacteroides* sp. before treatment with human fecal flora, which was restored to predominance after patients received either a single fecal enema or a mixture of 10 bacterial species.[44] Aerobic gram-negative bacteria dominated the bowel of another patient prior to fecal infusion, after which the major colonic organism was anaerobic *E. coli*.[43] In addition, deficiencies of fecal short-chain fatty acids, thought to possess anti-diarrheal properties, were restored to normal levels following rectal administration of human fecal flora in 7 out of 9 patients with antibiotic-associated diarrhea.[16] To validly assess the restorative function of fecal bacteriotherapy, future focus should perhaps be directed toward quantitatively evaluating the luminal contents of each patient before and after treatment.

Attempts to compare fecal compositions before and after bacteriotherapy with any precision have proven difficult.[16,41,43,44] In a complex bacterial population conventional culture techniques are of limited value as no more than half of the intestinal flora can be identified by conventional culture methods. There are also inherent difficulties in the logistics involved in sampling, transporting, and storing the obligate anaerobes that predominate in the gastrointestinal tract.[76] Focus is therefore shifting toward molecular approaches incorporating polymerase chain reaction-based techniques, fluorescence *in-situ* hybridization and DNA microarray gene expression analysis. Using these newer methods is currently prohibitively expensive, but as they become more widely available, better understanding of fecal compositions may be achieved and lead to a more logical approach to therapy.

Outcome Measures

Symptoms

The treatment value of fecal bacteriotherapy has been appraised by various outcome measures for each illness. Publications have generally cited clinical improvement for extended periods as a criterion of efficacy. Though it could be argued that clinical improvement is the crucial measure, patient reporting is subjective and a more formalized symptom scale would be preferable. None of the articles reviewed here have used a standardized outcome measure.

Markers

In *C. difficile*-associated diseases, outcomes have included the absence of *C. difficile* and its toxin post-infusion and the restoration of short-chain fatty acid profiles and other microflora-associated biochemical characteristics.[16,17] Only 23 of 84 patients diagnosed with *C. difficile* diarrhea or colitis were tested for the presence of *C. difficile* and its toxin after fecal treatment.[44,46,49,50] This is in part due to the fact that in 20 cases *C. difficile* testing had not yet been discovered.[41,42] Currently, testing for *C. difficile* should be performed prior to and post-therapy with a combination of toxin assay and culture, which should be repeated over long-term and equal follow-up to truly evaluate the efficacy of fecal bacteriotherapy.[77] The

restoration of anaerobic microorganisms such as *E. coli* and *Bacteroides* sp. to predominance in the bowel would also be of scientific interest.[41,42] Another outcome measure could include the endoscopic and histologic normalization of colonic mucosa in patients with UC[58–60] and in chronic constipation.[73]

Colonoscopy & Histology

Five publications have used colonoscopy or flexible sigmoidoscopy to view the effect of fecal flora infusions[42,58–60,73] with 3 reports also confirming this through histologic biopsy.[58,60,73] Where relevant, mucosal imaging and histology would constitute an ideal monitoring method, especially in UC.

Duration of Follow-up

Existing papers report varying follow-up periods ranging from 1 day to 13 years. The duration of follow-up for the use of fecal bacteriotherapy for *C. difficile*-associated diarrhea and colitis ranges from 1 day to 3 years; for UC from 3 months to 13 years; and for chronic constipation from 6 to 28 months. It is difficult to determine the effectiveness of fecal bacteriotherapy after short-term follow-up. Ideal duration is unclear but a period of 12 months with interim screening at 6 months would seem reasonable.

Adverse Effects

In the available 17 publications describing the use of fecal bacteriotherapy, there is no mention of any specific adverse effects associated with this treatment. It is unclear whether this is due to good fortune, under-reporting, or indeed fecal bacteriotherapy having caused few side effects. Although probiotics are considered non-pathogenic, even seemingly harmless microorganisms can be infective in severely immunocompromised individuals or if introduced into a sterile body cavity. There have been sporadic reports of adverse effects associated with the therapeutic use of probiotics. For example, *S. boulardii* fungemia has been documented[78] and a liver abscess has been linked to the use of *L. rhamnosus*.[79] *L. reuteri* however has been safely administered to HIV-positive patients without complications.[80] Formalized trials on the use of fecal bacteriotherapy would provide valuable information on the safety of this treatment modality. The use of a nasogastric tube to deliver the fecal suspension in 1 study may have been associated with the death of 1 patient due to peritonitis.[51] Although deemed unlikely, the authors could not discount that possibility. This further highlights the need for vigilance in this area.

DISCUSSION

Human fecal bacteriotherapy has been sporadically used in one form or another by a several physicians since mid 1950s, primarily for antibiotic-associated diarrhea and severe *C. difficile*-related diarrhea. Its often surprising that efficacy as a treatment of last resort seems to have stood the test of time. There has been increased understanding of the human gut flora and a paralleled understanding of why and how bacteriotherapy should work. In addition, there has also been a recent renaissance worldwide in the use of probiotics, generally used singly with often unproven results. More recently, however, mixtures of probiotics such as VSL#3 have been used with some clinical efficacy. Moreover, fecal bacteriotherapy—an even more complex probiotic—has returned to clinical use particularly for unremitting *C. difficile*-related infection. Empirically, after examining the available literature, bacteriotherapy using human fecal flora seems to have its greatest value in the treatment of *C. difficile* infection. Fecal bacteriotherapy has arguable success in up to 92% of chronic *C. difficile*-associated diarrhea cases. Despite the empirical nature of these data, at least for chronic relapsing *C. difficile* infection, there does not seem to be a more reliable treatment than fecal bacteriotherapy.

The precise mechanisms of bacteriotherapy using fecal flora in the treatment of *C. difficile* are unclear but probably involve the recolonization of flora with missing components, (eg, *Bacteroides* sp.), to regenerate colonization resistance.[81] The other major mechanism could be the direct antagonistic activity of the normal flora to *C. difficile*.[36–39] Unlike the transient use of antibiotics (eg. vancomycin) for *C. difficile*, implanted flora provides a prolonged presence of "antagonistic" activity that not only cures the current infection, but also prevents future colonization by *C. difficile*, presumably by restoring flora colonization resistance.

The failure of 6 patients reviewed in this article with *C. difficile* colitis to recover following fecal bacteriotherapy may be the result of the relatively low doses of fecal mass used in treatment. Three of these patients received a single enema comprising 10 g stool suspended in saline,[48] whereas the other 3 were treated with only 25 mL fecal-saline suspension via nasogastric tube where bacteria inactivation by acid may have played a role.[51] Perhaps severe disease requires a higher therapeutic dose to combat established infection in the involved region.

Six studies have reported the concomitant use of oral vancomycin therapy with or without metronidazole in the days prior to fecal bacteriotherapy to reduce the bacterial load or "sterilize" the bowel.[43,44,51,58–60] In these instances one could question whether the symptoms resolved as a result of pretransplantation antibiotics or indeed as the result of fecal treatment itself. In all these cases however, prior to fecal transplantation, symptoms invariably recurred on withdrawal of the same antibiotic medications, whereas fecal bacteriotherapy seemed to induce long-term remission in the majority of patients.

Apart from its use in *C. difficile*, other conditions, including IBS and IBD have been treated with bacteriotherapy using fecal flora. Fecal bacteriotherapy induced long-term remission in 9 patients with severe UC and resolved symptoms in

5 patients with chronic constipation-predominant IBS, with concomitant resolution of colonoscopic and histologic appearance of intestinal mucosa. Because the patients with UC tested negative for *C. difficile*, their improvement could conceivably be explained by bacteriotherapy affecting a related, though occult, infective agent. This may point to an infective etiology for ulcerative colitis.[9,10,60] This observation serves as an interesting point-of-departure from which to investigate the relationship between intestinal flora and these GI conditions.

Although it would be desirable to carry out definitive trials in the above-mentioned conditions, it seems unlikely that fecal bacteriotherapy compared with placebo will be subject to a randomized, controlled trial. For one, it may be difficult to find a placebo resembling fecal matter. Nevertheless, to standardize a therapeutic protocol for fecal bacteriotherapy, future publications should contain: (1) greater detail on the precise techniques used to prepare and administer the fecal suspension; (2) a thorough list of side effects and complications experienced; and (3) methods used to monitor long-term follow-up, including regular stool tests for *C. difficile*, and preferably colonoscopy and biopsy for histologic examination. Quantitative analysis of stool composition prior to and post-treatment should be attempted and described to the best of the researchers' ability governed by available technology.

CONCLUSION

Despite deficiencies in study designs and publication details, the existing literature seems to adequately support the therapeutic benefits of fecal bacteriotherapy for the treatment of *C. difficile*-related disease and suggests potential for this inexpensive and apparently safe treatment modality to undergo further investigations for clinical use.

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