EXPERT REVIEWS

Alteration of the intestinal microbiome: fecal microbiota transplant and probiotics for *Clostridium difficile* and beyond

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Clostridium difficile infection is increasingly common with a high risk of recurrence despite antibiotic treatment. In cases of recurrent *C. difficile* infection, fecal microbiota transplant (FMT) is a highly effective treatment option promoting the restoration of normal gut microbiota. Furthermore, preliminary uncontrolled evidence demonstrates possible benefit of FMT in the management of some cases of inflammatory bowel disease and chronic constipation. In addition to presenting an overview of FMT, we discuss the role of probiotics, a more common approach to modifying the intestinal microbiome. Probiotics have been utilized broadly for many disease processes, including gastrointestinal, cardiovascular and allergic disease settings, although with limited and inconsistent results. Multiple potential areas for research are also identified.

KEYWORDS: *Clostridium difficile* • fecal microbiota transplant • intestinal microbiome • probiotics

Clostridium difficile infection (CDI), traditionally a nosocomial, antibiotic-associated, toxin-mediated diarrheal disease, has become increasingly common [1]. With the emergence of a hypervirulent strain (BI/NAP1/027), cases have been more severe with longer hospitalizations, increased numbers of colectomies and a significant rise in healthcare costs [2]. A 2008 study showed a CDI prevalence rate of 13.1 out of 1000 inpatients [2,3]. Additionally, there have been community-acquired cases of CDI in overall healthy adults without prior antibiotic exposure. Although in most cases, CDI is adequately treated with antibiotics, up to 30% of patients develop recurrent CDI, which is associated with significant morbidity and mortality [4].

The hypothesis for the underlying etiology of severe CDI is disruption in gut microbiota [4]. The intestinal microbiome makes up a complex interdependent ecosystem responsible for food digestion, immune system activation, vitamin production and protection from invasive nonindigenous bacteria, which is known as colonization resistance [5].

Despite antibiotic regimens incorporating pulsed oral vancomycin, fidaxomicin, rifaximin and probiotics for the treatment of recurrent CDI, it is not uncommon for patients to have CDI recurrence, possibly due to persistent spores despite initial elimination of the *C. difficile* bacteria. Although historically performed without a clear evidence base, multiple studies now demonstrate a role for fecal microbiota transplant (FMT) as a means to restore healthy gut bacteria [6,7].

Fecal microbiota transplant

The earliest reports of FMT come from the Dong-Jin dynasty in 4th century China. Patients with food poisoning or severe diarrhea were given human feces by mouth with report of positive results, although the details of this intervention are unknown [8]. FMT has subsequently been described during the 16th century Ming dynasty and in 17th century veterinary medicine by Fabricius Acquapendente, an Italian anatomist [8,9]. Although first reported in US literature by Eiseman in the 1950s for

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treatment of patients with pseudomembranous colitis, FMT has became more common practice with numerous case reports and case series highlighting its effectiveness [10,11].

Rather than eradicating the pathogen as has traditionally been the focus of antibiotic treatment, the goal of FMT is to re-establish the diverse normal microbiome within the large intestine. Multiple studies analyzing the intestinal microbiota of healthy people, CDI patients and recurrent CDI patients have demonstrated significant differences [12,13]. Although healthy individuals are colonized with many bacteria including a predominance of bacteroidetes and firmicutes, CDI patients harbor less or none of these bacteria and have decreased microbiome bacterial diversity overall [12–14]. Instead, recurrent CDI patients have high levels of proteobacteria and verrucomicrobia [13]. These findings support the hypothesis that CDI results from altered intestinal microbiota, which FMT restores. FMT repopulates bacteria relatively quickly and the effect persists. Khoruts assessed the intestinal microbiome of CDI patients pre- and post-FMT [12]. Two weeks following transplant, and persisting out to 33 days, the recipient's microbiota was similar to that of the donor stool with a dominance of *Bacteroides sp*. A long-term follow-up study describes patients who are disease-free up to 68 months following FMT [11].

Success of FMT

FMT is viewed as a success or 'cure' if the patient does not have a CDI recurrence within 8 weeks [4]. Multiple studies and subsequent systematic reviews have described high levels of success with FMT for the treatment of recurrent CDI [10,11,14–29].

In the only long-term follow-up study to date, 77 patients were treated with colonoscopic fecal transplant for recurrent CDI at five different medical centers [11,16,24]. Although there were variations in transplant protocols between hospitals (e.g., infusate volume, location of infusate delivery and donor exclusion criteria), long-term follow-up (>3 months) demonstrated that 91% of patients had primary cure (diarrhea resolution within 90 days following FMT) and 98% of patients were secondarily cured after additional FMT, probiotics or antibiotics. Qualitative assessment showed improvement in diarrheal symptoms and abdominal pain [11]. A systematic review of seven studies showed an 83% success rate after one infusion, although it included stool delivered via colonoscopy or nasogastric tube (NGT) or retention enema, which may not be equal in terms of efficacy [30]. Another study similarly found cure rates of 92% after a single treatment in patients with refractory CDI [9].

Despite a multitude of case reports and low scale cross-sectional studies, there has been only one randomized, controlled trial with adequate comparison groups to evaluate FMT in recurrent CDI. Van Nood assigned recurring CDI patients (n = 43) to one of the three groups: vancomycin only; vancomycin with colonic lavage; or vancomycin, colonic lavage and stool transplant via nasoduodenal tube [29]. Due to an overall success rate of 93.8% in patients receiving donor feces infusion ($p < 0.001$), the study was terminated early. Of note, two patients required a second infusion and one patient remained symptomatic despite two infusions. The recipients' (n = 9) intestinal microbiome showed a restoration of bacteroidetes post-FMT [29].

Donor selection & FMT process overview

An effective stool donor can be a spouse, close relative or healthy unrelated donor. Studies have shown slightly better resolution of symptoms in FMT recipients who receive transplanted stool from intimately- or genetically related donors (93.3%) compared to healthy unrelated donors (84%) [31]. Donors must also be screened for blood-borne and enteric infectious diseases [26,32].

The procedure for donor stool preparation and transplantation has been extensively described in multiple reviews with varying protocols depending on the route of transplant and volume infused [5,6,9,23,33]. One review suggests a larger volume of infused stool promotes CDI resolution. When patients were administered >500 ml of stool, 97% had resolution, whereas only 80% improved with <200 ml of stool [31]. Although informative, various protocols may have utilized differing amounts of stool in making a stool dilution; thus, these results are more challenging to interpret. Nonetheless, patients who received <50 g of stool had a fourfold greater risk of CDI recurrence [31].

Mode of transplant delivery

Nasogastric tube

NGT or endoscopic administration may be technically easier to perform, requires less patient preparation and is less costly compared to other modalities [15,21]. There are concerns, however, that adequate amounts of viable bacteria may not reach the colon [26].

A retrospective study reviewing the medical records of 18 patients with recurrent CDI who had received stool by NGT demonstrated a 94% cure rate for 16 patients following transplantation; most were treated in the outpatient gastroenterology clinic setting. Two hospitalized patients who were severely debilitated died following transplantation; a third patient had CDI recurrence within 90 days [15]. In another study, 15 patients with recurrent CDI were treated with FMT via NGT and 11 of 15 patients were cured (73%) [21]. The randomized controlled trial mentioned modified this approach by administering FMT via nasoduodenal tube with a success rate of 93.8% as described above [29].

Colonoscopy

Colonoscopic FMT has become increasingly common with multiple studies demonstrating high success rates [16,17,20,23,24,26]. Several studies have included colonic lavage prior to transplant as part of the FMT protocol. It is hypothesized that lavage may reduce colonic biomass, fostering restoration by transplanted bacteria [26].

Infusion via colonoscopy offers the ability to deliver larger volumes of donor stool to the proximal colon, including into

the terminal ileum, and allows the endoscopist to visualize the entire colon with an opportunity to obtain biopsies if needed. Additionally, sedation at the time of colonoscopy increases patient comfort. Risks of colonoscopy include sedation risk, bleeding, infection and colonic perforation, which is increased if the patient exhibits signs of toxic colitis [4].

Enema

In the first English language report, Eiseman and colleagues used fecal retention enemas to treat four patients with pseudomembranous enterocolitis; all four patients had significant clinical improvement [10]. In small series, others have found similarly successful results and dramatic improvements in patient symptoms [14,18,19,25,34,35]. A recent study assessed 27 patients with either refractory or recurrent CDI treated with fecal retention enemas [36]. Following enema, 93% of patients had clinical resolution, most within the first 24 h following transplant. Compared with NGT and colonoscopy, enemas are less invasive, less expensive and pose fewer medical risks.

Treatment with enemas may be difficult in those unable to retain the transplanted stool, particularly in elderly patients who have decreased sphincter tone [36]. Additionally, enemas do not reach proximal to the splenic flexure, limiting contact with the right and transverse colon, and sometimes require multiple infusions.

Barriers & risks of FMT

For many symptomatic patients with recurrent CDI who have exhausted all alternative antibiotics, FMT is embraced. Although FMT does possess a 'yuck factor' or a 'lack of palatability,' patients are overcoming this, likely related to increased public discussions of FMT in mass media [4,16,37]. Studies focused on patient attitudes toward FMT have shown them to be very receptive. In a long-term follow-up study, 53% of patients (n = 77) indicated that they would prefer FMT as first-line therapy if CDI were to recur, and among patients who already had FMT in the past, 97% would be willing to undergo another transplant if CDI recurred [11]. A broader survey demonstrated a strong willingness by patients (179 of 192) to undergo FMT for recurrent CDI, especially if a physician recommended it as treatment (94%). Responders preferred either the hospital setting (48%) or physician's office (39%) and identified the most unappealing aspects as handling stool and receiving FMT via NGT [37].

When considering risk, it is important to remember that FMT involves the infusion of a microbially active suspension. Despite this perceived risk, there has not been documentation of any direct and serious adverse effects of FMT [11,38]. Rare side effects include constipation, diarrhea, abdominal pain, belching and one case report each of gastrointestinal hemorrhage, peritonitis and pneumonia, with none being clearly and directly attributable to FMT [15,29–31]. The authors are aware of at least two cases of norovirus infection with the details to be published [38]. In contrast, a long-term study reported patient improvement in arthritis and allergic sinusitis, although there were new presentations of peripheral neuropathy, Sjogren's disease, idiopathic thrombocytopenic purpura and rheumatoid arthritis in four patients [11]. These newly presenting diseases have not been directly correlated to FMT and may have developed regardless of transplant.

Alteration of the microbiome through FMT in other diseases

Although the most common application for FMT has been in the setting of recurrent CDI, there is ongoing research to assess benefit in other gastrointestinal diseases. These include inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and chronic constipation. There are also isolated reports of FMT effects in nongastrointestinal disease, including multiple sclerosis and Parkinson's disease. The current areas of intestinal microbiome research are presented in Box 1.

Inflammatory bowel disease

IBD is a chronic inflammatory disease commonly composed of Crohn's disease (CD) and ulcerative colitis (UC). Although a clear etiology for IBD remains unknown, hypotheses include exposure to an unidentified infectious agent, genetic predisposition and/or an excessive mucosal immune response contributing to chronic inflammation and inevitable disruption of normal enteric microbiota [9].

FMT via retention enema was initially performed as a self-experiment by Bennet, who was afflicted with UC. He had successful alleviation of symptoms (bloody diarrhea, cramping, tenesmus, skin lesions and arthritis) that persisted for at least 6 months [39]. Also in 1989, Borody documented two patients, one with CD and the other with UC, who were treated with FMT via enemas. Both patients remained symptom free for at least 3 months following donor stool transplant [40]. More recently, Borody retrospectively reviewed the outcomes of FMT for six UC patients with severe disease for >5 years. These patients had previously been treated with steroids and anti-inflammatory agents, and then with donor stool retention enemas for 5 days followed by tapering off of all UC medications. All six patients experienced disease remission ranging from 1 to 13 years post-FMT [41].

With the ongoing work of the Human Microbiome Project, additional characterization of the intestinal microbiota in IBD patients is underway. A 2012 systematic review of existing research acknowledges a limited and weak evidence base, but highlights the potential for FMT to be a safe and effective IBD treatment [42]. Building on these initial studies by Bennet and Borody in UC patients, there are ongoing studies investigating FMT as treatment for this population on a larger scale.

IBS & chronic constipation

Few studies have evaluated the benefit of FMT on IBS and/or chronic constipation. One of the earliest reported case series included 55 patients with IBD and/or IBS treated with fecal retention enemas [40]. In this report, Borody describes

Box 1. Various diseases currently being evaluated through intestinal microbiome research[†].

Fecal microbiota transplant
- Gastrointestinal disease
  - Recurrent *Clostridium difficile* infection
  - Inflammatory bowel disease
  - Irritable bowel syndrome and chronic constipation
- Neurological disease
  - Multiple sclerosis
  - Parkinson's disease
- Hematologic disease
  - Immune thrombocytopenic purpura

Probiotics
- Gastrointestinal disease
  - Prevention and treatment of *C. difficile* infection
  - Prevention of antibiotic-associated diarrhea
  - Inflammatory bowel disease, specifically ulcerative colitis and pouchitis
  - Irritable bowel syndrome
  - Traveler's diarrhea
  - *Helicobacter pylori* infection
  - Acute pancreatitis[‡]
  - Hepatic encephalopathy
- Cardiovascular disease
  - Atherosclerosis
  - Hyperlipidemia
  - Obesity and metabolic syndrome
  - Diabetes mellitus
- Allergic disease
  - Allergic rhinitis
  - Dermatitis/eczema
- Oral disease
  - Dental caries
  - Gingivitis
  - Periodontitis
  - Halitosis
  - Oral candidiasis
- Gynecological disease
  - Bacterial vaginosis
  - Vulvovaginal candidiasis
- Infectious disease
  - Respiratory tract infections
  - Ventilator-associated pneumonia
  - HIV/AIDS
  - Infectious mastitis
- Rheumatologic disease
  - Spondyloarthritis
- Psychiatric disease
  - Anxiety
  - Depression

[†]The effects of microbiome alteration have been studied in the context of the above diseases with varying results. This listing does not condone the use of FMT or probiotics in the prevention or treatment of any individual disease.
[‡]Probiotics are advised against in the setting of acute pancreatitis.

20 patients with 'cure' (as defined by improvement in bowel frequency, less need for laxatives, becoming pain free and resolution of diarrhea), 9 patients with improvement in symptoms (bowel habits and abdominal pain) and 26 patients with no response [40]. In a subsequent study, Andrews and Borody created a mixture of 18 different bacteria paralleling the normal colonic microbiota and infused this 'starter culture' into the cecum of IBS patients [43]. They report that 76% of patients with severe chronic constipation benefitted without any further need for laxatives, with a 4–52 week follow-up period (mean of 21 weeks). Andrews presents a case report of a woman with chronic idiopathic constipation who had long-term resolution (at least 18 months) following retention enema with her husband's stool suggesting that there may be a role for FMT in constipation-predominant IBS [44]. Despite these positive findings, additional studies are required to further evaluate the benefit of FMT in this population, particularly in the setting of a benign disease, whereby patients would be exposed to an invasive therapy with low, but nontrivial risk of complications.

Neurological disease

There is limited information on the relationship between the gut microbiota and neurologic disease. Collins comments on the ability of the gut to influence the brain and behavior. There are weak associations between depression and carbohydrate malabsorption, varying levels of Clostridia in autistic patients and alterations in feeding in patients with chronic *Helicobacter pylori* infection [45].

There is one case series of FMT performed in three patients with 'atypical' multiple sclerosis [46]. One patient underwent five FMT infusions with resolution of constipation and remission of multiple sclerosis symptoms, regaining the ability to walk again after being wheelchair-bound. A second patient underwent 10 FMT infusions and also regained the ability to walk and experienced overall resolution of neurologic symptoms. The third patient was 80-years old and had resolution of both neurologic symptoms and constipation following five FMT infusions. Borody hypothesized that a gastrointestinal infection may be responsible for symptoms of multiple sclerosis in these patients and FMT resulted in restoration and/or alteration of microbiota contributing to resolution of clinical symptoms [46]. Similarly, it has been suggested that Parkinson's disease may be related to an intestinal pathogen that crosses the mucosal barrier of the gastrointestinal system via enteric neurons, thereby entering the central nervous system [47]. Borody has performed similar FMT infusions on patients with Parkinson's disease, also with positive results, although the details have not been published [48].

Alteration of the microbiome through probiotics

In addition to CDI, researchers have postulated associations between alteration of gut microbiota and other diseases ranging

from gastrointestinal (e.g., IBD and IBS) to extraintestinal (e.g., cardiovascular and allergic) [2,49]. Established by Metchnikoff in the early 1900s who reported increased longevity and improvement in personal health when consuming fermented milk, this became the basis for probiotics [2]. Although FMT is the 'ultimate probiotic,' live bacterial cultures consumed orally in the form of yogurt or capsules have been more common.

Clostridium difficile infection

Multiple probiotic regimens have been studied independently and in combination. These have included *Saccharomyces boulardii, Streptococcus thermophilus, Lactobacillus rhamnosus, L. plantarum, L. casei, L. bulgaricus* and *L. acidophilus* [7,50–53].

Of the various probiotics combinations, few have shown significant efficacy in primary or secondary prevention of CDI. A recent review of randomized controlled trials (n = 11) of probiotics for primary CDI or antibiotic-associated diarrhea prevention revealed only two studies with significant results, one using a combination of *L. bulgaricus*, *L. casei* and *S. thermophilus* and the other using a combination of *L. acidophilus* and *L. casei* [51,54,55]. These data suggest that a multistrain probiotic may be required to achieve significant results. The latter study additionally found that higher doses of probiotics resulted in significantly less antibiotic-associated diarrhea compared with lower doses [54]. This finding is not surprising as the amount of bacteria present in oral probiotic is generally four-times less than the total gut bacteria, and it is not known how much of these bacteria survive the acidic gastric environment as they are transported to the colon [56].

For prevention of CDI after one or more recurrence, *S. boulardii* has shown some benefit, particularly in combination with high-dose vancomycin. Only 17% of patients had CDI recurrence, compared with 50% in the placebo group who received high-dose vancomycin alone ($p = 0.05$) [52]. *In vitro S. boulardii* has the ability to produce a protease capable of inactivating *C. difficile* toxin receptors.

Additional studies have suggested that probiotic bacteria rarely survive in the colon beyond 14 days when the patient ceases consumption of the agent [57,58]. This is in comparison to transplanted donor bacteria that appears to persist >24 weeks suggesting a significant change in the baseline gut microbiota [57].

Given the above findings, the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America do not recommend probiotics for secondary prevention of CDI in their 2010 updated practice guidelines for management of CDI [59]. More recent systematic reviews have demonstrated at least moderate benefit with probiotic prophylaxis in preventing CDI, but there remains no clear role for probiotics in the treatment of CDI [53,60].

Inflammatory bowel disease

Probiotics have been studied in IBD patients with variable clinical success. The results have been disappointing in CD, with no significant effects found across multiple studies [61]. Probiotics as treatment for UC, however, have shown promising results, both with induction and maintenance of remission. UC patients with mild-to-moderate disease (n = 90) had significantly decreased symptoms (e.g., reduced bowel frequency) and improved colon endoscopic appearance when administered VSL#3, a mixture of probiotic bacteria, in addition to balsalazide ($p < 0.01$) [62]. When probiotic enemas of *Escherichia coli Nissle 1917* were given, there was a dose-dependent remission of UC [63]. A more recent study reported that VSL#3 added to immunosuppressants (azathioprine, 6-mercaptopurine and methotrexate) and/or 5-aminosalicylic acid significantly (63.1% versus 40.8%, $p = 0.01$) lowered the UC disease activity index (UCDAI), a composite score assessing rectal bleeding, stool frequency, mucosal appearance and a physician rating of disease activity. The study did not find that probiotics significantly induced UC remission, although it may have been underpowered to achieve this [64]. Additional studies similarly found VSL#3-induced remission with decreases in UCDAI and individual symptoms [65]. In pediatric UC patients (n = 29), VSL#3 not only induced remission (92.8% in the intervention group compared with 36.4% in the placebo group, $p < 0.001$), but also promoted maintenance of remission with 21.4% of children treated with probiotics having recurrence within 1 year compared with a recurrence rate of 73.3% in the placebo group ($p = 0.014$). Probiotics were given in addition to steroid induction and mesalamine maintenance [66].

Some of the strongest evidence for probiotic therapy is in both the prevention of a first episode and treatment of pouchitis [61,67]. In patients with severe IBD requiring colectomy, an ileal pouch may be created and attached to the anus, known as an ileal pouch–anal anastomosis (IPAA). This pouch may become inflamed, possibly due to bacterial overgrowth. Treatment with VSL#3 in patients (n = 40) with IPAA demonstrated significantly increased ($p < 0.05$) duration of pouchitis remission compared with placebo groups [68]. The same probiotics were effective in prevention of pouchitis flare-ups ($n = 40$, $p < 0.001$) [69].

Irritable bowel syndrome

Although it is unclear how much microbiota disruption occurs with IBS, multiple studies have demonstrated some benefit with the use of probiotics, although the response appears to be variable depending on the bacterial strain and dose prescribed [9,70–74]. This has been an extensive area of research, yet questions still remain as to the most effective probiotic in the context of a common, but poorly understood disease process. Given that many patients present with IBS symptoms following acute gastrointestinal illness, it is suggested that microbiota disruption plays a role and therefore would be improved by probiotics [57,75]. Despite limitations in performing meta-analyses assessing over 20 randomized controlled trials, each using varying probiotic strains, pediatric versus adult populations and differing outcome measures, there appears to be at least some overall benefit from probiotic supplementation in mitigating IBS symptoms [75].

A meta-analysis assessing 20 clinical trials found 65% of publications reported a decrease in global IBS symptoms, 80% reported a decrease in abdominal pain and fewer commented on bloating (50%), flatulence (40%) and stool frequency (25%) [71]. The magnitude of global IBS symptom improvement was variable depending on the trial and is likely affected by individual study designs and inherent limitations. This meta-analysis concluded that probiotics are significantly protective in alleviating global IBS symptoms compared to placebo with a relative risk of 0.77 and a number needed to treat equal to 7.3 [71]. Moayyedi performed a systematic review of probiotics on decreasing IBS symptoms and reported a relative risk of 0.71 with a number needed to treat equal to four [71,72]. The fact that additional studies have shown no benefit, or in rare cases, worsening of symptoms with probiotics, further suggests a poor understanding of the underlying disease process, which is likely to be multifactorial in etiology.

Other gastrointestinal diseases

Additional studies have evaluated the potential benefit of probiotics in other gastrointestinal diseases, including traveler's diarrhea, *H. pylori* infection, acute pancreatitis and liver disease.

With increased frequency of international travel, researchers have assessed the benefit of prophylactic probiotic therapy in the prevention of traveler's diarrhea. Although some studies have shown mild-to-moderate reductions in diarrhea, others have shown no clear benefit [67,76]. These equivocal findings may be related to specific probiotic strains and doses, but also may be affected by the destination country.

As there are ongoing concerns of increased antibiotic resistance of *H. pylori*, researchers have been seeking alternative treatment options including probiotics. Despite some studies showing a decrease in urea levels on breath testing thought to be related to decreased bacterial load, gastric biopsies have not demonstrated disease eradication [76,77]. There may be some benefit, however, in alleviating symptoms associated with standard triple therapy.

Fewer studies have evaluated the effects of probiotics on pancreatitis. Since many severe acute pancreatitis cases have infectious complications, there is concern for bacterial translocation in the setting of necrosis. A small-scale trial by Olah showed benefit of probiotics added to early enteral nutrition in decreasing pancreatic necrosis and abscesses [78]. Besselink performed the larger PROPATRIA trial, a randomized, multicenter study of 298 first-time acute pancreatitis patients randomly assigned to probiotic or placebo [79]. The results showed no reduction in infectious complications despite probiotic therapy [80]. More concerning, the authors found that subjects in the probiotic group not only had significantly increased bowel ischemia, but also significantly increased mortality rates with 24 deaths (16%) compared with nine deaths (6%) in the placebo group ($p = 0.01$). Although the explanation for these findings remains unclear, probiotics are strongly advised against in this population [80].

There are few studies assessing the potential benefit of probiotics in the setting of liver disease. Bajaj treated patients (n = 25) with minimal hepatic encephalopathy with a 60-day regimen of probiotic-supplemented yogurt alone [81]. Patients in the yogurt group had reversal of their encephalopathy (71%) compared with 0% in the placebo group (no yogurt; $p = 0.003$). Despite this, only two patients in the placebo group went on to develop overt hepatic encephalopathy, so other factors may have contributed to reversal beyond probiotics alone in this small study. A randomized study by Liu assessed the intestinal microbiota of chronic liver disease patients (n = 81) following 14 days of probiotic-enhanced yogurt [82]. Overall, patients had decreased symptoms (i.e., abdominal distention, level of tiredness when performing daily activities) compared to the placebo group.

Cardiovascular disease

Karlsson and colleagues draw a parallel between intestinal microbiota becoming pro-inflammatory resulting in increased mucosal permeability and the oxidative stress-induced subclinical inflammation that leads to chronic inflammation within arteries as seen in atherosclerosis [83]. Although difficult to prove, ongoing research suggests increased translocation of intestinal microbiota into the bloodstream through disruption of epithelial tight junctions. Karlsson performed a randomized controlled trial with 16 male patients diagnosed with carotid wall atherosclerotic plaques. Nine patients were given a 4-week trial of a *L. plantarum*-fermented oat drink and seven patients were in the placebo arm (unfermented oat drink without *L. plantarum*). Study patients demonstrated increased intestinal microbiome diversity and lower concentrations of fecal carboxylic acids, specifically isovaleric ($p = 0.006$) and valeric ($p = 0.029$) acid, surrogate markers for inflammatory disease. There were no significant changes in serum inflammatory markers (e.g., CRP, TNF-α and IL-6). Of note, the study did not assess progression of atherosclerosis or mortality [83].

Naruszewicz similarly gave 36 heavy smokers a *L. plantarum* drink for 6 weeks and found significant decreases in systolic blood pressure ($p < 0.001$), serum leptin ($p < 0.001$) and fibrinogen ($p < 0.001$) [84]. The authors suggest that a combination of lipid peroxidase inhibition, propionic acid-related anti-inflammatory effects and decreased leptin contribute to reduced insulin resistance in tissues [84]. Emerging research additionally suggests intestinal microbiota breakdown of lecithin, a common dietary phospholipid, results in production of trimethylamine-*N*-oxide), a pro-atherosclerotic metabolite which significantly increases cardiovascular risk, including death, nonfatal myocardial infarction and stroke [85].

Several studies have evaluated the benefit of probiotics on serum cholesterol levels. Although prior studies have shown mixed results, Ataie-Jafari found that patients consuming probiotic yogurt (with added *L. acidophilus* and *Bifidobacterium lactis*) compared with regular yogurt demonstrated significantly decreased total cholesterol levels (n = 14; $p < 0.05$) [86–91]. Although this decrease may be a result of probiotics, Fabian found decreased serum cholesterol levels in the placebo group as well, suggesting that consumption of yogurt or milk may be a more significant factor [92]. Guo performed a systematic review

of 13 randomized controlled trials pooling 485 patients and found significantly lower total cholesterol (95% CI: - 9.93 to -2.87) and low-density lipoprotein (95% CI: -7.91 to -1.90) among participants ingesting probiotic, but no significant change in high-density lipoprotein or triglyceride levels [93]. Ejtahed found similar reductions in serum cholesterol in diabetic patients consuming probiotic yogurt [94]. Although the mechanism is unclear, it is hypothesized that lactobacilli prevent the formation of micelles and produce bile salt hydrolases which help to breakdown conjugated bile acid salts [93].

In addition to diabetes and hyperlipidemia, obesity has also been associated with cardiovascular disease. Flint suggests a complex system of weight gain or weight loss depending on the composition of intestinal bacteria present and its role in energy expenditure [95]. Through increased energy recovery from dietary fiber breakdown, modifications in gut transit and production of metabolites promoting lipogenesis, there may be increased fat deposition. Alternatively, he proposes that intestinal bacteria may lead to inflammation, activation of host defense systems and creation of new intestinal tissue, all of which increase energy expenditure and therefore promote weight loss [95]. Although a specific bacteria modulating obesity has not been identified, murine studies show obese mice to have less bacteroidetes and more firmicutes compared to lean mice [96]. Similar results were found in obese humans. Following enrollment in a diet program, the quantity of bacteroidetes increased and Firmicutes decreased with overall maintenance of bacterial diversity [97]. Furthermore, this change was correlated with percentage of weight loss and not a reduction in caloric intake suggesting that firmicutes may play a significant role in energy extraction. Additional studies suggest a potential therapeutic target to *Methanobrevibacter smithii*, which has been linked to energy extraction from polysaccharides contributing to obesity [96,98].

Attempts at modifying obesity through probiotics have not demonstrated successful results thus far. The majority of studies have been in mouse models showing an ability to modify gut microbiota through probiotics with a potential to affect lipid metabolism, although direct effects on obesity in humans have not been demonstrated. Additionally, it is difficult to account for confounders including dietary habits, exercise, antibiotics and nutritional supplements [99]. Furthermore, gut microbiota in the early stages of life may play a role in future obesity. A study by Kalliomäki suggests that alteration in gut microbiota precedes obesity development [100]. Similarly, Luoto suggests that overweight mothers and those gaining excess weight during pregnancy may alter the microbiota composition of the maternal gut and therefore affect the inoculum that serves as the source of infant intestinal microbiota [101]. The administration of probiotics to mothers, however, did not appear to produce a significant effect in a child's weight, even at 10 years of age when the intervention and placebo groups diverged, but not significantly ($p = 0.063$).

Larsen has characterized the intestinal microbiome of diabetic patients [102]. Stool studies indicate higher proportions of bacteroidetes and proteobacteria with reduced levels of firmicutes, although this finding contradicts similar microbiome testing in obese patients, which is often associated with diabetes [97,102]. Ejtahed has focused on the effects of probiotics in diabetic patients. It is proposed that oxidative stress contributes to the pathogenesis of diabetes progression and that probiotics, such as *Lactobacillus*, may possess antioxidant properties, including the ability to scavenge reactive oxygen species. [103]. A double-blinded, randomized controlled trial of probiotic yogurt compared with conventional yogurt ($n = 60$) showed a significant decrease in both fasting blood glucose levels ($p < 0.05$) and hemoglobin A1c ($p < 0.05$) within the intervention group, although the study was of short duration (6 weeks) and did not have a control group without consumption of any yogurt [103].

Considering the risks of diabetes and metabolic syndrome on mortality and with an understanding that the proximal intestine is most responsible for carbohydrate and fat uptake, Vrieze hypothesized that if she replaced the intestinal microbiome in an obese patient with that of a lean donor, the recipient's metabolism may be affected, particularly by increasing insulin sensitivity [104]. Male obese patients were randomized to receive a duodenal infusion of lean donor feces (allogenic group, $n = 9$) or placebo infusion of their own feces (autologous group, $n = 9$). The allogenic group had significantly improved peripheral insulin sensitivities ($p < 0.05$) 6 weeks post-transplant in addition to increased microbiome diversity and increased butyrate-producing bacteria, particularly *Roseburia intestinalis*. The authors hypothesize a role for butyrate in modulating insulin sensitivity [104].

Allergic disease

It has been suggested that as daily living has become more hygienic with decreased microbial contact and an overall decreased stimulation of the immune system, including in the GI tract, there has been a reduction in microbial diversity and a predisposition toward allergy-prone immunity [105,106]. For this reason, it has been hypothesized that by enhancing the gut microbiome, there may be an effect on allergy-induced disease.

Multiple studies have been performed with variable benefit of probiotics on allergic disease, although the results have been positive overall [107]. In a randomized clinical trial, Japanese researchers evaluated the benefit of two probiotics on the treatment of a locally common allergic rhinitis, known as Japanese cedar pollinosis. Forty-four patients were randomized to receive either daily milk fermented with *Lactobacillus* GG and *Lactobacillus gasseri* or placebo yogurt and their clinical symptoms were monitored over 10 weeks. Patients had overall improvement in nasal blockage symptoms after 9 weeks of treatment, but no significant change with regard to sneezing, rhinorrhea or itching, nor was there any change in serum IgE levels [105]. A similar study was performed in 44 children with allergies and/or asthma administered *L. gasseri* and *L. coryniformis*-fermented yogurt, and there was a significant decrease ($p = 0.03$) in plasma IgE [106]. Additional studies have shown similar results with significant decreases in total IgE and increases in

regulatory T cells that are suspected to ultimately blunt allergic inflammation [106,108,109]. Unfortunately, long-term benefits of probiotics have not been demonstrated [110,111].

Similar studies have been performed in patients with atopic dermatitis (or eczema), an inflammatory dermatological disease. Probiotics are believed to reduce intestinal permeability, improve the gut immune system and limit inflammatory response through downregulation of cytokines [112]. Much of this research has been in children. Preschool children treated with an 8-week course of probiotics had their SCORAD index, a measure of rash spread and intensity, decrease by 33.7% [113]. On subgroup analysis, the authors found that children with higher serum IgE and eosinophil levels had increased benefit from treatment with probiotics. Similarly, a group of adult patients (n = 38) with atopic dermatitis treated with *L. salivarius* also had significant reduction in their SCORAD index ($p < 0.001$) [114]. Iemoli also finds significant decreases in the SCORAD index ($p < 0.0001$), in addition to reduced microbial translocation ($p = 0.050$) and immune activation ($p < 0.001$) in the probiotic group of adults with atopic dermatitis [115]. Probiotics have been shown to affect the immune system by decreasing the percentage of $CD4^+$ and $CD25^+$ T cells and increasing the percentage of $CD8^+$ T cells, which supports an immunoregulatory role for probiotics in atopic dermatitis [113]. Although research is ongoing, some studies suggest that there may be a role for prophylactic pre- and post-natal probiotics in preventing eczema development in infants [116,117].

Other diseases

Probiotics have also been utilized in other nongastrointestinal applications with varying benefit. For example, there is strong evidence supporting the use of probiotics in children for the prevention of dental caries, although fewer studies support their use in periodontits, gingivitis, halitosis or oral candidiasis [118–135]. Similarly, multiple studies find benefit of intravaginal probiotics for the management of bacterial vaginosis, but the evidence is less strong for vulvovaginal candidiasis [136–141]. Although few in number, several publications comment on the potential benefit of probiotics for the prophylaxis or treatment of infectious, rheumatologic and psychiatric diseases, all with limited success with the possible exception of upper respiratory tract infections, which has some promising findings [142–155]. The wide breadth of probiotics research is presented in Box 1.

Conclusions

This review describes two potential interventions – FMT and probiotics – aimed at restoring the normal gut flora. As the intestinal microbiome and its alteration is intricately linked with both gastrointestinal and nongastrointestinal disease processes, characterizing this association is critical. In many ways, FMT is the 'ultimate probiotic,' although as described above is significantly more invasive than oral intake of a daily probiotics capsule. For these reasons, directly comparing these two interventions is challenging. Although the aim of FMT and probiotics is similar, FMT has demonstrated significantly more success in the management of recurrent CDI and possibly IBD. The data for probiotics are less strong, likely due to a lower viable bacterial dose reaching the intestine in contrast to a direct deposit of multistrain stool to an FMT recipient. As research progresses and gut flora is further characterized in patients, a long-term goal would be to develop a true probiotic that matches the bacterial strains seen in FMT at the appropriate dose and reaching the targeted destination. Ideally, this noninvasive, more publically acceptable, effective treatment could then be applied to other gastrointestinal and nongastrointestinal diseases and evaluated with randomized controlled trials.

There are multiple areas of potential research to further elaborate on the relationship between microbiome alteration and disease. In the setting of CDI, there is information lacking on the significance of gender, age, ethnicity and geographic location. For example, it is unknown if there are significant differences in the microbiome of an elderly, African-American woman from the USA compared with a middle-aged, Caucasian man in Europe compared with a young Chinese woman living in Asia. Furthermore, with increasing prevalence of community-acquired CDI, the microbiome of affected individuals living in the community is likely different from hospital-acquired cases. Specific to FMT, there is a need for additional randomized controlled trials, including as first-line therapy for CDI, as management for other gastrointestinal diseases, and as adjuvant therapy to other currently accepted treatments. Additionally, there are ongoing discussions about the potential benefit of frozen stool, synthetic stool and the utility of stool banks to ease the identification of stool donors. As we learn more about the relationship between gut flora and the immune system, it would also be beneficial to better understand immunoglobulin levels and other serum biomarkers pre- and post-FMT. As safety is paramount, there is a need for studies to examine the long-term risks and benefits of FMT and probiotics, including in immunocompromised hosts. On a broader, public health level, there is an opportunity to also discuss the impact on health policy (e.g., who should pay for donor screening costs) and regulatory impacts (e.g., what is the role of the Food and Drug Administration) on the interventions described above (Box 2).

Expert commentary

Recurrent CDI is an excellent example of alteration of the microbiome, which leads to disease. The use of probiotics has had limited success in treatment of this illness. By contrast, FMT has been shown to be very effective and confirmed in one randomized controlled trial. The use of FMT requires regulation, and the FDA has recently announced that any practitioner must submit an Investigational New Drug application to perform it. FMT, which involves putting filtrate from a whole stool specimen from a healthy

Box 2. Future research questions.

***Clostridium difficile* infection**
- Analysis of gender differences in CDI and recurrent CDI
- Evaluation of ethnic differences and geographic populations (USA versus Europe versus Asia)
- Analysis of aging effects on the microbiome
- Surveillance for increasing community-acquired strains of *C. difficile*

Fecal microbiota transplant
- Donor stool acquisition and preparation
  - Clinical efficacy of fresh versus frozen stool
  - Development of synthetic stool
  - Responsibility of identifying a donor: transplant recipient versus healthcare provider (i.e., establishing stool banks)
- Effectiveness
  - Additional randomized controlled trials assessing colonoscopic FMT
  - Clinical trials examining benefit of adjuvant therapies in combination with FMT (e.g., probiotics, monoclonal antibodies, antibiotics and vaccination)
  - Clinical trials of FMT as first-line therapy for severe CDI cases
  - Clinical trials evaluating FMT benefits in other diseases, including IBD and constipation-predominant IBS
  - Analysis of host immune responses, including after treatment with FMT (i.e., alterations and sustainability of serum immunoglobulin levels)
- Health policy
  - Responsibility for donor screening costs – donor vesus recipient
- Safety
  - Long-term follow-up of patients post-FMT for monitoring of outcomes and safety
  - Characterization of FMT risks in immunocompromised recipients

Probiotics
- Additional analysis of most effective strains and adequate doses for the management of various diseases
- Characterization of the risks in immunocompromised hosts
- Consideration of FDA regulation given potential risks and benefits

individual into the patient, is an effective but relatively crude way to treat this infection. Ultimately, we believe that researchers will identify the key important bacteria necessary to restore colonization resistance and they will become available for therapy. The role of the microbiome in the pathophysiology of and treatment of other diseases requires much further research and well-designed controlled trials before any manipulation of the fecal microbiome can be considered in these diseases.

Five-year view

The underlying hypothesis that the microbiome impacts host health is important, and emerging data suggest that alteration either through FMT or probiotics can alter clinical disease. However, the treatment is fundamentally primitive in that it simply re-populates bacterial species. It does not alter the recipient interaction (e.g., inflammatory or regulatory immune responses) with the bacterial populations. The evolution of this field should optimize the mode and manner of delivery; and in addition, involve a further understanding of host response, including identifying patients who are at particularly increased risk for CDI or developing intestinal inflammation. Included in that analysis is examining differences in gender responses, ethnic and geographic populations, older individuals and immunocompromised patients. Pre-existing serum IgG responses to toxin A predicted response to immunization against *C. difficile*. Does FMT alter recipient serum IgG (or IgE or IgA) and does that effect persist and protect against recurrent disease? Do lower levels of serum IgG predict recurrent CDI? Is there a threshold above which IgG is protective? Immune responses to influenza vaccination decrease with age – are there similar decreases in mucosal responses to pathogens such as *C. difficile*? Would that result clinically in less or more severe colitis? In 5 years, we expect that the combination of standardizing optimal delivery and a deeper understanding of host immune responses will significantly move this field forward.

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Key issues

- Fecal microbiota transplant (administered via nasogastric tube, enema, colonoscopy) appears safe with the appropriate donor screening, with clinical efficacy approaching 95% for recurrent *Clostridium difficile* infection.
- Limited data suggest possible efficacy of fecal microbiota transplant for treatment of inflammatory bowel disease and constipation-predominant irritable bowel syndrome.
- For prevention of *Clostridium difficile* infection, some multistrain probiotics have demonstrated efficacy.
- Patients with mild-to-moderate ulcerative colitis and pouchitis demonstrated decreased symptoms when treated with probiotics (VSL#3) alone and in combination with anti-inflammatory or immune-modulatory medications
- Treatment with probiotics has resulted in decreased global irritable bowel syndrome symptoms, reduced surrogate markers of inflammation, improved cholesterol levels and variable clinical effects on allergic diseases.
- Given the infectious risks, probiotics should be used cautiously, if at all, in immunosuppressed individuals

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