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Fecal Microbial Therapy – Promises and Pitfalls

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Abstract

A rapidly-expanding range of diverse human diseases are now associated with perturbations to the gastrointestinal microbiome. Fecal microbial transfer (FMT) has been used with high rates of efficacy to treat gastrointestinal microbiome perturbation associated with recurrent *Clostridium difficile* infection, and is now being considered for other indications. Here we discuss the gut microbiome, review published and on-going studies using FMT as a treatment modality for human disease, consider the regulatory aspects of FMT and outline some factors that should be considered in cases where this therapeutic strategy is being contemplated.

Keywords

Gastrointestinal microbiome; fecal microbiota transfer; clinical trials; clinical efficacy

Introduction

The rapidly developing field of microbiome research, i.e. studies of the diverse microbial communities, their genomes and interactions within and on the human host, has increased our appreciation for the impact of microbial community composition and function on a variety of human diseases ranging from metabolic [1] to neurological [2] and respiratory [3-5] disorders. Diseases as diverse as asthma and autism have links to perturbed gastrointestinal microbiota composition [2, 6], implicating the gut microbiome as a major mediator of host health status. These diseases are typically characterized by loss of microbial diversity coupled with species overgrowth and as a result, depletion of critical microbial functions necessary for maintaining host health. A classic example of gastrointestinal microbiome perturbation and species outgrowth is evident in *Clostridium difficile* overgrowth, a common occurrence in hospitalized patients treated with antimicrobials. Reduced microbiome diversity as a consequence of antimicrobial administration permits overgrowth of opportunistic *C. difficile*, which, with increasing frequency, does not respond to subsequent antimicrobial therapy targeted to this species e.g. vancomycin. Because of the growing number of recalcitrant *C. difficile* infection (CDI) cases and the diminishing impact of antimicrobial therapy, alternative therapeutic strategies

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have been examined for treatment of such patients. Recently fecal microbial transplant (FMT), i.e. transfer of stool (containing both microbes and the bioactive molecules they produce) from a healthy donor to a CDI patient has been employed to treat patients with high rates of efficacy [7]. The success of this approach has increased interest in expanding the therapy to other diseases in which a gut microbiome dysbiosis is known or suspected to play a role in disease development. Here we discuss the current state of science behind FMT, including background on the gut microbiome, approaches to transfer fecal material as a therapeutic modality and a review of the studies performed to date as well as those currently in the active stages of patient enrollment.

The Gastrointestinal microbiome

The recent expansion of the field of human microbiome research has largely been fuelled by technological advances to profile the diversity of organisms present (biomarker gene e.g 16S rRNA-based microbiota profiling), their collection of genomes (metagenomics), transcriptional activity (metatranscriptomics) as well as the dominant products biosynthesized (metaproteomics, metabolomics) by these microbial communities. These studies, though still largely at the descriptive stage, have demonstrated the staggering diversity of organisms that inhabit humans, with the greatest burden housed in the lower gastrointestinal tract. This large “microbial organ” houses an approximate one trillion bacterial cells and estimates place the number of species present anywhere from 800 to 40,0000 species of microbes [8]. Fungi, Archaea, Protozoa, Bacteriophage and viral species are also detected in this niche, though the majority of studies to date have focussed on the bacterial fraction of these communities. These organisms represent a thriving microbial bioreactor, producing essential metabolites for the host such as vitamin K and hormones, as well as degradative enzymes capable of digesting a range of otherwise indigestible dietary fibers. Developmental studies in mice have demonstrated a role for the microbiome in appropriate mucosal and immunological development; germ-free animals develop physiologically aberrant gastrointestinal-associated lymphoid tissue (GALT; [9]). More recent murine studies have shown that the composition of the gut microbiome is distinct in a murine model of autism-spectrum disorder that, compared to control animals, exhibits distinct behavioral patterns, implicating bioactive molecules produced by the microbial community in neurological function [2]. The emerging implication is that this co-evolved microbial bioreactor may play a significantly larger role in defining a wide variety of human physiological attributes than previously appreciated. By extension, novel therapeutic strategies to manipulate these communities towards a more beneficial composition and function, may prove highly efficacious in treatment of a broad spectrum of human diseases.

Given the key role the microbiome plays in the human host, it is predictable that several studies have described microbiome disturbances associated with a variety of chronic inflammatory diseases. Characteristically, diseases such as Inflammatory Bowel Disease (IBD; [10]), Type II diabetes [11] and even Chronic Sinusitis (CRS; [12]) have demonstrable collapse of the normal microbial community structure, depletion of species with the capacity to produce anti-inflammatory molecules such as short chain fatty acids, and enrichment of pathogenic species. Several studies, particularly those in the gastrointestinal tract have clearly demonstrated the capacity of discrete species within the

microbiome to induce distinct host immune responses. For example, *Clostridia* species belonging to Clades IV or XIV induce anti-inflammatory T-regulatory cells (T-regs; [13]), while segmented filamentous bacteria (SFB), a colonizer of the murine ileum, induces proliferation of Th17 cells in the terminal ileum lamina propria [14]. The latter species was identified using high-resolution comparative microbiome profiling, which identified SFB as one of the most highly enriched species in the ileum of mice with a preponderance of Th17 cells [14], indicating the utility of such approaches to move beyond description of the community towards identification of key species that influence particular host phenotypes. Beyond their role in influencing immune responses, studies of the sinus mucosa have revealed that species such as *Corynebacterium tuberculostearicum*, which had never previously been considered to be pathogenic (because of its prevalence as a commensal skin inhabitant), can, in the context of a disturbed and species-depleted microbiome, behave in a pathogenic manner [12]. This suggests that the pattern of microbial co-colonization, defines the behavior of bacterial species in a given niche and that competition in more diverse communities may serve to prevent species outgrowth and inflammatory or infectious disease development.

Fecal Microbial Transplant (FMT)

The concept that microbial community composition influences the abundance and behavior of its component members, is strongly supported by reports of the efficacy of fecal microbial transplant or FMT, as a viable therapeutic option for patients with recalcitrant *C. difficile* infection (CDI). Provision of a diversity of mircoorganisms and their products associated with a healthy gut microbiome to a patient with CDI, leads, with high frequency, to infection remission. This suggests that either the microbes, their products, or a combination of these factors sufficiently reduce the numbers and activity of the pathogenic species and modulate host immune responses in a manner that affords clinical efficacy. FMT has enjoyed a recent revival, largely due to our increasing understanding of the critical role played by the gut microbiome in providing crucial functions that influence host immune activation status. However, clinical medicine's first published report of the potential therapeutic benefits of stool transplant appears in the literature in 1958, when Eiseman and colleagues described the use of an adjunctive, enema-delivered stool as treatment for pseudomembranous colitis. [15] The use of fecal material for treatment of gastrointestinal disorders has been recorded historically. Accounts widely reported by German Soldiers in the 1940's, during their African campaign, describe the native Arabian population ingesting fresh camel stool as an effective means of preventing dysentery, a practice that had been passed down through the generations [16].

Though it enjoyed popularity amongst the medical profession in the 40's and 50's, the advent of an ever-expanding repertoire of broad-spectrum antimicrobials in the 50's and 60's superseded the use of FMT. However, as the rate of antibiotic-resistant infection has significantly increased over the past several decades, alternative approaches to treating resistant infections are now paramount. More recently, FMT has resurfaced as a highly efficacious therapeutic option for treatment of CDI refractory to traditional antimicrobial therapy. Approximately 20% of patients treated for primary CDI, develop recurrent antimicrobial resistant CDI [17-20] and are at significantly higher risk for developing

additional infections. The mortality rate associated with CDI is high and represents a substantial health care burden. One Canadian study examining appropriate approaches to accurately quantify CDI-attributable deaths in adults found, using death within 30 days of infection as a marker for CDI-attributable death, that 80% of deaths in their cohort of CDI patients were directly or strongly attributable to CDI [21]. This percentage increased to 86% if clinical recurrences were considered [21]. Largely due to the limited options to treat CDI and the high degree of efficacy observed upon FMT treatment, coupled with a greater public awareness of the role the gut microbiome plays in promoting host health, FMT has been recently widely promoted in both the scientific and lay press, and is rapidly being adopted as a therapeutic option for CDI and potentially for other diseases and disorders in which disturbances in the gut microbiome are described.

FMT Procedure

The procedure for FMT varies across practitioners and no single standardized protocol has been widely adopted. Donor fecal material has been used with success from both recipient selected donors (typically family members) as well as universal (non-familial) donors. Fresh and frozen specimens have been used for FMT procedures; both exhibit similar efficacy [22]. At a minimum, selection of donors entails screening stool for pathogens with tests for toxigenic *C. difficile*, ova and parasites as well as bacterial culture and antibiotic sensitivity. In research settings, more in depth testing of donors is often pursued including serologic studies for hepatitis A, B and C, HIV types 1 and 2 and syphilis. Additional stool testing extends to assessments for *Giardia*, *Cryptosporoidum* as well as *Isospora* parasites and gastrointestinal viral pathogens such as rotavirus. *Helicobacter pylori* screening of donors is also recommended. Donors are typically excluded if they have been treated with antibiotics within 3 months of FMT. Because of the potential serious implications of altering the intestinal microbiome, some researchers recommend even more rigorous criteria and exclude donors with chronic medical conditions including atopy, chronic fatigue, obesity, inflammatory bowel disease, irritable bowel syndrome and other conditions [23].

The process of FMT requires a healthy donor to provide feces to the recipient patient. Processing donor stool for FMT requires that the material be liquefied by resuspension in any of a variety of solutions. Water, milk or most commonly non-bacteriostatic saline have been used in published reports. Resuspension approaches range from simply mixing the constituents in a beaker to homogenizing stool and fluid in a sterile bench-top blender to create the slurry. The slurry is then typically filtered to remove larger particulate matter and facilitate delivery. At least 50g of stool is recommended, and is typically resuspended in 250-300 ml fluid [24]. In preparation for FMT, recipients are often advised to discontinue antibiotics 1-3 days prior to the procedure. Regardless of route of administration, most recipients are given large bowel lavage with four liters of polyethylene glycol to decrease microbial contents of the large intestine prior to FMT. On the day of the procedure, it is common practice for patients to be administered loperamide, a piperidine derivative opioid, which acts on μ-opioid receptors to increase residence time of the transferred fecal material in the gut [25].

Several modalities have been used in the delivery of FMT, including nasoduodenal infusion, retention enemas and administration through a colonoscope. All three techniques have been shown to be effective in the treatment of recurrent CDI [25]. In delivery of FMT by colonoscope, the entire colonic mucosa can be visualized allowing for the identification of potential comorbid conditions. Additionally, biopsies can be obtained for histologic evaluation at the time of the procedure. Proximal colonic instillation through the biopsy channel of the colonoscope may be advantageous since the entire length of colonic mucosa is exposed to and repopulated with donated microbes. Risks associated with colonoscopy are minimal but the cost of this procedure exceeds that of retention enema and nasoduodenal administration. At this time, no clear consensus exists as to which is the optimal mode for delivery of FMT [26].

Regulatory Aspects of FMT

On a daily basis physicians use many drugs and surgical procedures that have not been approved for the purpose for which they are administered. However, the Food and Drug Administration (FDA) has been particularly rigorous in regulating the use of live microbes as a treatment modality, primarily because of concerns associated with administration of live microorganisms. As a result, the agency has required a much higher approval level for products such as probiotics and FMT. In an effort to enforce regulations, the FDA initially imposed a mandatory shutdown of all FMT and associated research that did not have a full Investigational Drug (IND) approval in place. For those interested in obtaining such an approval, the following link is provided which outlines steps to ensure compliance with FDA regulations http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM153222.pdf. The IND approval process typically takes 12-18 months to obtain, with defined period of response set out from the FDA in which they must review the application and make recommendations. For most FDA-approved drugs an IND waiver is granted if the drug is to be studied for a different indication, but this did not apply, at least at the outset to microbial-based therapeutics, including FMT. Following the publication of a high-impact manuscript demonstrating high rates of efficacy of FMT for CDI [7], in July 2013, the FDA revised their requirements for FMT stating, “We, FDA, are informing members of the medical and scientific community, and other interested persons that we intend to exercise enforcement discretion regarding the investigational new drug (IND) requirements for the use of fecal microbiota for transplantation (FMT) to treat *Clostridium difficile* (*C. difficile*) infection not responding to standard therapies”. FDA intends to exercise this discretion provided that the treating physician obtains adequate informed consent from the patient or his or her legally authorized representative for the use of FMT products. Informed consent should include, at a minimum, a statement that the use of FMT products to treat *C. difficile* is investigational and a discussion of its potential risks. FDA intends to exercise this discretion on an interim basis while the agency develops appropriate policies for the study and use of FMT products under IND.” (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm361379.htm) Currently, this FDA ruling only extends to CDI; full IND applications are necessary for all

other applications of FMT or probiotics, which has resulted in the majority of on-going probiotic and FMT research being conducted outside the U.S.

FMT studies and trials

In an effort to assess the current literature in this field, a review was conducted in September 2013, via Pubmed, using the search terms “fecal OR stool OR feces OR microbiota AND transplant*”, resulting in recovery of 1,530 citations. These were then filtered to only include clinical trials, resulting in a total of 86 publications. The large majority of reports on FMT examined the role for FMT in treating CDI but a more recent trend in the published literature indicated growing interest in other opportunities for application of this treatment for diseases such as inflammatory bowel disease or obesity. [25, 27-29]

In 2009, Bakken published a review examining FMT for Recurrent CDI infection and identified 13 published case reports encompassing a total of 100 patients [16]. Approximately 25% of patients received FMT via upper GI tract delivery methods, such as nasogastric tube, while the remainder received bacteriotherapy via a variety of lower tract delivery methods, such as colonoscopy. The review found an approximately 90% reported cure rate of CDI via FMT. A subsequent review by Gough *et al.* included all but one of the articles in the Bakken review but also included 15 additional studies that meet their inclusion criteria, resulting in a total sample size of 317 patients [24]. Although the majority of reports were journal articles, they also included letters (15%), abstracts (12%), and unpublished data (3%). Both reviews were comprised entirely of case series or case reports. Even with the additional 217 patients, Gough *et al*. reported a nearly identical rate of resolution of CDI at 92%. The most recent review by Kassam *et al*., published in 2013 included 11 studies [30]. This was a methodologically robust review that only included full peer reviewed studies and did not limit by language. As expected, at the time of the review they identified no randomized controlled trials (RCTs) and their total population was 273 patients. Although only two of the studies were the same as those identified by Bakken, they also reported a 90% cure rate across this metadata set. Hence, based on various reviews of the literature using variable numbers of patients across different study, the preponderance of evidence indicates that FMT is at least, 90% efficacious in curing recurrent CDI, though the caveat that none of these studies were randomized controlled trials but instead care series or reports, should be noted.

At the time of writing this article, there is only one published randomized controlled trial (RCT) that examined the role of FMT in recurrent CDI [7]. In an eloquent design that included two control arms and a third group receiving FMT, active treatment included a vancomycin regimen (500 mg orally four times per day for 4 days), followed by bowel lavage and subsequent infusion of a solution of donor feces through a nasoduodenal tube. The primary outcome was resolution of diarrhea from CDI without relapse in 10 weeks. Control arms received either the standard vancomycin regimen (500 mg orally four times per day for 14 days) or the standard vancomycin regimen with bowel lavage. The data safety monitoring board requested that the study be halted prematurely after 13 of 16 patients in the FMT group had resolution of CDI, following a single FMT treatment. The three remaining patients in this group received a second infusion, following which, two of the three

responded with full resolution of their CDI. In contrast, resolution of CDI occurred in only 4 of 13 patients receiving vancomycin alone and 3 of 13 patients receiving vancomycin with bowel lavage, indicating a significant increase in efficacy in the FMT-treated group ($P < 0.001$; [7]). Similarly to the case report reviews, the patients studied in this RCT had many comorbid conditions (mean age of FMT group = 73) and all but 8 of the 43 enrolled in the trial had CDI relapses more than once prior to enrollment.

Although, only one RCT of FMT has been published, the recent interest in bacteriotherapy is reflected in the increasing number of on-going trials. As of October 2013, clinicaltrials.gov lists 46 trials using the terms “fecal” and “transplant”, though only 19 are relevant to the topic of this article. Nine of those studies are being conducted in the United States. One U.S. study anticipates enrolling 100 participants, while a separate trial aims to include 53 subjects, the remaining seven studies are considerably smaller and plan to enroll a total of 107 participants. One Canadian trial (NCT01372943) plans to transfer synthetic stool, a recently published mix of thirty-three culturable bacterial species from healthy stool, including members of the Acidaminococcus, Bacteroides, Bifidobacterium, Clostridia, Eubacterium. As expected, all of the other studies are using a variety of donors and methods of delivering the stool to the recipients. The studies are examining the role of FMT for a variety of conditions. One study is examining the efficacy of FMT for treatment of diabetes, nine for CDI and the remainder for inflammatory bowel disease. Only three studies in the U.S are examining the FMT for treatment of inflammatory bowel disease, with a cumulative total of 40 participants across all three studies. A review of the NIH database appears that there is only of FMT study currently funded by the NIH, through their R21 mechanism i.e. pilot, exploratory research. This study represents a Phase I safety study, examining at the role of FMT to treat recurrent CDI.

FMT for non-CDI indications – Concerns and Considerations

Though it has primarily been used with great efficacy for treatment of recalcitrant CDI, the growing number of diseases characterized by gut microbiome dysbiosis has led to rising enthusiasm regarding the application of FMT to treatment of a variety of diseases beyond CDI. Though again this is a nascent field, a limited number of studies with small cohort sizes have examined the application of FMT as a therapeutic modality for other gastrointestinal disorders that are characterized by a dysbiotic microbiome, such as inflammatory bowel disease. Of the published studies to date, efficacy has been variable. In one recent small study of five IBD patients, FMT resulted in fever and a temporary increase of C-reactive protein. While the numerically dominant bacteria in the donor feces were deemed to have established in the recipients, the effectiveness of therapy and the stability of resulting community that developed in the patients varied greatly across participants. Only one patient exhibited a positive clinical response 12 weeks post-FMT and this patient exhibited successful colonization by donor-derived *Faecalibacterium prausnitzii*, *Rosebura faecis*, and *Bacteroides ovatus* [31]. Similar results were noted in a study of pediatric and young adult IBD patients who underwent FMT [32]. Again, in this study, establishment of the donor microbiome in the recipient patient was variable, as was longevity of the resulting microbiome and reported efficacy. Moreover, these patients also recorded mild to moderate, though self-limiting adverse events at the time of treatment [32].

Part of the issue of FMT for currently non-indicated ailments is the overwhelming lack of understanding of human gastrointestinal microbiome function in the context of host genetics and environmental exposures (particularly diet). These factors exert significant impact on the composition and by extension, function of the gut microbiome, though they are not readily considered in FMT studies. For example, risk genes associated with inflammatory bowel disease such as Nod2 and ATGL13, are associated with the presence of particular bacterial species within the fecal microbiome [33]. However, these risk alleles, while frequently associated with the disease in European populations, do not recapitulate in Asian IBD patient cohorts, suggesting that ethnically distinct populations may possess discrete microbial assemblages that require distinct community rehabilitation strategies to afford optimal efficacy. Moreover, diet, because it represents the largest carbon and nitrogen source for the microbial communities resident in the gastrointestinal tract, is one of the most influential factors on community composition and function [34]. A large existing body of work has identified a variety of non-digestible food ingredients, known as prebiotics, that promote the growth of beneficial species. However to date no studies have considered either post-FMT dietary restrictions, or supplementation with specific prebiotics as a means to further enhance the establishment and longevity of the donor community in the recipient patient.

To further complicate microbiome rehabilitation efforts, the composition of the microbial community is distinct and specific to particular regions of the gastrointestinal tract in healthy individuals. This has implications for diseases that manifest at particular sites in the GI tract. For example, though it can manifest without ileal involvement, Crohn's disease often presents in the ileum, which houses a compositionally and functionally distinct microbiome to that of the colon. Therefore, supplementation with fecal material, which largely represents microbial species adapted to life and function in the distal colon, may not provide the appropriate species to competitively colonize the ileum. Clearly, clinicians need to determine where a patient's disease predominates as those with primarily distal colonic disease and would presumably benefit most from FMT. Other important considerations include the age and gender of the donor and recipient. Gut microbiome composition is dynamic and changes with age [35, 36], and has been shown in separate studies to be related to the degree of immune activation in individuals with underlying disease [37]. Given our lack of knowledge regarding the long-term implications of FMT, it is incumbent upon those in the field to consider age- and gender-matching donors with recipients, particularly when the recipients are pediatric patients who are still in the developmental stage of life.

Many questions remain unanswered about FMT, including appropriate testing of donor material, appropriate FMT delivery and when it is best indicated. Due to the lack of RCTs and the inherent microbial variability of donated fecal material, it is difficult to properly assess acute adverse events. Most of the case reports do not address even acute adverse events. When adverse events are reported they are generally reported as un-attributable to FMT. Amongst the adverse effects reported, included a flare of ulcerative colitis that had been inactive for twenty years [27], and bacteremia in a patient with Crohn's disease and CDI [38]. However, one of the most important issues is the long-term implication of this therapeutic strategy. In general studies are not designed to address long-term safety and can only address immediate adverse events. As we learn more about the microbiome, this may

provide us with specific biomarkers, either host or microbial-derived, that predict long-term outcomes. One multi-institutional study did try to examine long term complications by following up patients (n= 77) at least 3 months (mean=17 months) after their FMT for CDI [39]. Similar to other studies, this cohort was sickly, with patients reporting a mean of 11 months duration of experiencing symptoms prior to FMT, and, on average five conventional antimicrobial regimens. The average cure rate of initial FMT treatment was 91% - almost identical to that reported in other studies. The survey found that 97% of patients reported they would repeat FMT for CDI, two patients associated FMT with an improvement in allergic sinusitis and arthritis, while four patients reported a new medical condition, peripheral neuropathy, Sjogrens, idiopathic thrombocytopenic purpura, and rheumatoid arthritis. Seven patients were deceased by the follow-up survey but family members participated in the survey, and death was not believed attributable to FMT [39].

Clearly manipulation of the gastrointestinal microbiome has profound implications for both gastrointestinal diseases as well as those that manifest at sites remote from the gastrointestinal tract. Approaches such as FMT that rehabilitate perturbed microbial ecosystems within the human host have obvious promise and open up a host of new possibilities for treatment of a range of recalcitrant diseases. However, for the reasons outlined in this article, great caution must be urged in considering FMT for indications other than CDI. Moreover, use of this therapeutic modality, should be considered a first step towards development of rationally designed next-generation probiotics. Ultimately, development of therapeutic microbial communities based on a solid understanding of the mechanistic basis of how these organisms afford protection in a given anatomical niche and the long-term implications of such supplementation interventions represents the most practical approach to microbiome manipulation as a viable therapy for human disease.

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References

1. Karlsson F, et al. Assessing the human gut microbiota in metabolic diseases. Diabetes. 2013; 62(10): 3341–9. [PubMed: 24065795]
2. Hsiao EY, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell. 2013; 155(7):1451–63. [PubMed: 24315484]
3. Huang YJ, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. J Allergy Clin Immunol. 2011; 127(2):372–381. e1–3. [PubMed: 21194740]
4. Zemanick ET, et al. Inflammation and airway microbiota during cystic fibrosis pulmonary exacerbations. PLoS One. 2013; 8(4):e62917. [PubMed: 23646159]
5. Han MK, et al. Significance of the microbiome in obstructive lung disease. Thorax. 2012; 67(5): 456–63. [PubMed: 22318161]
6. Fujimura KE, et al. House dust exposure mediates gut microbiome Lactobacillus enrichment and airway immune defense against allergens and virus infection. Proc Natl Acad Sci U S A. 2014; 111(2):805–10. [PubMed: 24344318]

7. van Nood E, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med. 2013; 368(5):407–15. [PubMed: 23323867]
8. Frank DN, Pace NR. Gastrointestinal microbiology enters the metagenomics era. Curr Opin Gastroenterol. 2008; 24(1):4–10. [PubMed: 18043225]
9. Rhee KJ, et al. Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. J Immunol. 2004; 172(2):1118–24. [PubMed: 14707086]
10. Walker AW, et al. High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. BMC Microbiol. 2011; 11:7. [PubMed: 21219646]
11. Vaarala O. Human intestinal microbiota and type 1 diabetes. Curr Diab Rep. 2013; 13(5):601–7. [PubMed: 23934614]
12. Abreu NA, et al. Sinus microbiome diversity depletion and Corynebacterium tuberculostearicum enrichment mediates rhinosinusitis. Sci Transl Med. 2012; 4(151):151ra124.
13. Nagano Y, Itoh K, Honda K. The induction of Treg cells by gut-indigenous Clostridium. Curr Opin Immunol. 2012; 24(4):392–7. [PubMed: 22673877]
14. Ivanov II, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009; 139(3):485–98. [PubMed: 19836068]
15. Eiseman B, et al. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery. 1958; 44(5):854–9. [PubMed: 13592638]
16. Bakken JS. Fecal bacteriotherapy for recurrent Clostridium difficile infection. Anaerobe. 2009; 15(6):285–9. [PubMed: 19778623]
17. Fekety R, et al. Recurrent Clostridium difficile diarrhea: characteristics of and risk factors for patients enrolled in a prospective, randomized, double-blinded trial. Clin Infect Dis. 1997; 24(3): 324–33. [PubMed: 9114180]
18. McFarland LV, et al. Recurrent Clostridium difficile disease: epidemiology and clinical characteristics. Infect Control Hosp Epidemiol. 1999; 20(1):43–50. [PubMed: 9927265]
19. McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. Dig Dis. 1998; 16(5):292–307. [PubMed: 9892789]
20. McFarland LV, Elmer GW, Surawicz CM. Breaking the cycle: treatment strategies for 163 cases of recurrent Clostridium difficile disease. Am J Gastroenterol. 2002; 97(7):1769–75. [PubMed: 12135033]
21. Hota SS, et al. Determining mortality rates attributable to Clostridium difficile infection. Emerg Infect Dis. 2012; 18(2):305–7. [PubMed: 22305427]
22. Hamilton MJ, et al. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. Am J Gastroenterol. 2012; 107(5):761–7. [PubMed: 22290405]
23. Brandt LJ, Reddy SS. Fecal microbiota transplantation for recurrent clostridium difficile infection. J Clin Gastroenterol. 2011; 45(Suppl):S159–67. [PubMed: 21992957]
24. Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. Clin Infect Dis. 2011; 53(10):994–1002. [PubMed: 22002980]
25. Bakken JS, et al. Treating Clostridium difficile infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol. 2011; 9(12):1044–9. [PubMed: 21871249]
26. Borody TJ, Brandt LJ, Paramsothy S. Therapeutic faecal microbiota transplantation: current status and future developments. Curr Opin Gastroenterol. 2014; 30(1):97–105. [PubMed: 24257037]
27. De Leon LM, Watson JB, Kelly CR. Transient flare of ulcerative colitis after fecal microbiota transplantation for recurrent Clostridium difficile infection. Clin Gastroenterol Hepatol. 2013; 11(8):1036–8. [PubMed: 23669309]
28. Borody TJ, et al. Treatment of ulcerative colitis using fecal bacteriotherapy. J Clin Gastroenterol. 2003; 37(1):42–7. [PubMed: 12811208]
29. Borody TJ, et al. Bacteriotherapy using fecal flora: toying with human motions. J Clin Gastroenterol. 2004; 38(6):475–83. [PubMed: 15220681]

30. Kassam Z, et al. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. Am J Gastroenterol. 2013; 108(4):500–8. [PubMed: 23511459]
31. Angelberger S, et al. Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal microbiota transplantation. Am J Gastroenterol. 2013; 108(10):1620–30. [PubMed: 24060759]
32. Kunde S, et al. Safety, tolerability, and clinical response after fecal transplantation in children and young adults with ulcerative colitis. J Pediatr Gastroenterol Nutr. 2013; 56(6):597–601. [PubMed: 23542823]
33. Frank DN, et al. Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. Inflamm Bowel Dis. 2011; 17(1):179–84. [PubMed: 20839241]
34. Wu GD, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011; 334(6052):105–8. [PubMed: 21885731]
35. Palmer C, et al. Development of the human infant intestinal microbiota. PLoS Biol. 2007; 5(7):e177. [PubMed: 17594176]
36. Costello EK, et al. Bacterial community variation in human body habitats across space and time. Science. 2009; 326(5960):1694–7. [PubMed: 19892944]
37. Vujkovic-Cvijin I, et al. Dysbiosis of the gut microbiota is associated with hiv disease progression and tryptophan catabolism. Sci Transl Med. 2013; 5(193):193ra91.
38. Quera R, et al. Bacteremia as an adverse event of fecal microbiota transplantation in a patient with Crohn's disease and recurrent Clostridium difficile infection. J Crohns Colitis. 2013
39. Brandt LJ, et al. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection. Am J Gastroenterol. 2012; 107(7):1079–87. [PubMed: 22450732]