Clinical Utility and Alterations in Bacterial Flora in Fecal Microbiome Transplantation

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Abstract

Dysbiotic states of gut ecology can be altered directly by transplantation of fecal preparation from healthy donors to patients with therapeutic intent. This paper assesses this fecal microbiota transplantation (FMT) in two respects: (1) the bacterial shifts in patient microbiomes with FMT and (2) clinical outcomes and variables of FMT. The PubMed database was searched using the MeSH terms "Feces/microbiology," "Microbiota," and "Transplantation." Thirteen papers found examined clinical outcomes and variables of FMT, and eight assessed metagenomic data and bacterial composition in the peri-FMT period. FMT was reported to have high cure rates in *Clostridium difficile* infection (CDI), generally increased levels of members of the phyla *Bacteriodetes* and *Firmicutes*, and decreased levels of members of *Proteobacteria*. Therapeutic FMT alters bacterial composition from a dysbiotic state pre-FMT to a healthy commensal state post-FMT in CDI patients, but further studies are necessary to understand its role in the treatment of other diseases.

Keywords: Clostridium infections; Microbiology; Feces; Fecal Microbiota Transplantation;, Gastrointestinal tract; Humans; Drug effects [Subheading]; Microbiota; Gastrointestinal Microbiome (Source: MeSH, NLM).

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Introduction

Fecal microbiota transplantation (FMT) is an increasingly common therapeutic procedure in which fecal material is taken from a healthy donor and transferred to a recipient with the intention of beneficially altering the composition of bacteria in the recipient's gut. While interest in FMT has risen because of its simplicity and efficacy, the emergence of modern molecular techniques has improved and reshaped the understanding of FMT. Specifically, the advent of 16S bacterial specific ribosomal RNA sequencing, driven by the Human Microbiome Project, has allowed for the elucidation of the composition of intestinal bacteria and their dynamics, particularly in relation to disease.[1]

The understanding of the role of intestinal bacteria has shifted in the last two decades from passive by-standers to commensal maintainers of homeostasis and health. Numerous studies have demonstrated associations between intestinal microbiome composition and diseases, both intestinal and extra-intestinal. Among these are bacterial colonic overgrowth, metabolic derangements, auto-immune and inflammatory disorders, psychiatric and neurological disorders, and cardiovascular disorders.[1]

However, despite the establishment of intestinal dysbioses in such a broad spectrum of disease, of these conditions, only a handful have been treated with FMT and assessed systematically in patients. Notably, patient data have been published related to recurrent *Clostridium difficile* colitis, metabolic syndrome, ulcerative colitis, Crohn's disease, chronic fatigue syndrome, thrombocytopenic purpura, Parkinson's disease, and multiple sclerosis.[2-9] The size and scope of the studies and reports involved are limited, however, and of the disorders listed, *Clostridium difficile* infection (CDI) has had the most clinical data establishing its credibility as a standard therapy.

As the number of clinical trials and patient data continue to grow, the compositional landscape of the flora involved in the normal gut and its pathological states have become more refined. Many of the more recent studies involving FMT have characterized rapid, dynamic shifts in bacterial composition charted through time. The increasing prevalence of FMT has allowed other variables to be more thoroughly examined as well; FMT in immunocompromised patients and methods of FMT have both been assessed. This paper will examine both the metagenomic data gathered from published articles assessing therapeutic FMT and the clinical efficacy of FMT in the currently documented literature. Ultimately, the particular bacterial strains associated with a 'healthy' microbiome and a pathological, dysbiotic microbiome will be compared with regards to FMT for various disease states through time and between donor and recipient. The outcomes of therapeutic FMT will also be discussed, and documented variables in the literature will be assessed for a pattern of clinical success.

Restated, this paper seeks to answer the simple questions: "How does FMT affect gut bacteria? How well does it work? What makes it work better?" These questions are fundamental in establishing the basis of FMT therapy as a viable procedure and will be addressed systemically by reviewing the current relevant literature. While this analysis of the literature gives insight into the current evidence and understanding of FMT, it is worth mentioning that our understanding of the mechanisms and the complexities of the microbe-gut interaction remains relatively nascent.

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Search Strategy and Selection Criteria

Goals of search

The goal of the search strategy used was to identify two types of articles in the PubMed database: those with published clinical data regarding the efficacy of FMT therapeutically and those with metagenomic data demonstrating shifts in bacterial composition associated with the use of FMT. Since the aim of this paper was to answer questions regarding variables about the FMT procedure, care was taken to filter for publications specifically assessing FMT rather than profiling microbiota associations alone.

Search terms

The emerging nature of therapeutic FMT warranted additional search methods to procure all relevant literature. Because "FMT" (or "Fecal Microbiota Transplantation") is not a unique MeSH term, other MeSH terms were initially used to maximize inclusion of desired articles and minimize inclusion of unrelated articles. The MeSH terms used were: "Feces/microbiology," "Microbiota," and "Transplantation;" these terms identified 4 desired articles when filtered for clinical trials only searching the entire PubMed database.[10-13]

Selection and analysis

After identification of these major articles, additional articles were selected by two means. The first method of finding additional articles was by reviewing the citations in each paper. Papers that met the inclusion criteria of being an FMT-related clinical trial published from 2010 to present were included. The second method used the "Cited by" option in the PubMed database to identify papers that cited each of the papers included, and the inclusion criteria were again applied.

Two exceptions to these criteria were made. Because of their size and scope, two systematic reviews of FMT were included. The first was published in November 2011 by Gough, et al., and included 27 case series and reports of outcomes of FMT in CDI.[14] The second was published in August 2014 by Colman, et al., and included 18 case series and reports of FMT in IBD.[5]

Each paper included was analyzed for the data provided that addressed the original research goals. Two evidence tables were compiled from this; one detailing the clinical aspects of FMT and the other detailing the microbiome compositional analysis in the peri-FMT period. When the original papers did not provide the level of detail required to properly compare data, their supplementary material was assessed for the relevant information and added to the tables. Supplementary data was re-analyzed to formulate similar data presentation from papers.

Results

Ultimately, 4 papers were returned with the initial MeSH term search, and 17 papers were included through indirect citation, with a total of 21 papers assessed. Of these, 19 met the inclusion criterion. Thirteen papers were related to the clinical efficacy of FMT, and 8 papers were related to the effects of FMT on the composition of patients' microbiomes. The types of papers assessed are categorized in ***Table 1***.

Clinical aspects of fecal microbiota transplantation

Transplantation of fecal material from donor to recipient has been clinically reported in a variety of capacities. The exact methods of FMT, sample selection, patient selection, and efficacy are all variables of the procedure that have been assessed. Recent papers have examined these variables of FMT for the treatment of CDI as well as inflammatory bowel disease (IBD).

Fecal microbiota transplantation in recurrent Clostridium difficile infection

Cure rates using fecal transplantation for recurrent *Clostridium difficile* infection

Until recently, FMT was reported in the literature only through case series as successful in the treatment of CDI. The first randomized control trial testing was published in January 2013, and it validated what had been established through interventional observation: FMT is as effective, if not more effective, for treating CDI as traditional antibiotic therapy. The study (n=43) by van Nood, et al., demonstrated that FMT was able to cure CDI with an overall rate of 94%, while conventional vancomycin therapy and vancomycin therapy with bowel lavage fell short, with cure rates of 31% and 23%, respectively.[15]

Virtually every case series published prior to this trial regarding CDI and FMT has reported cure rates in a similar range. A case series charting the long term outcomes of FMT in CDI patients had an overall cure rate of 98% (n=77), and another case series examining the outcomes of FMT in immunocompromised patients had an overall cure rate of 89%.[13,16] Other studies examining procedural variables of FMT, such as a combined small and large intestinal transplantation (n=27), and a frozen pill-based delivery of FMT (n=20) had success rates of 100% and 90%, respectively.[10,17] Prior to these studies, Gough, et al., performed a systematic review of all case series and reports pooled data into a meta-analysis of reported FMTs, finding that 92% of patients indexed were successfully treated with FMT for CDI with one or multiple transplantations.[13]

Protocol of transplantation for recurrent *Clostridium difficile* infection

The concept of FMT involves delivery of donor bacteria to a recipient. The protocol of this delivery has not been standardized,

Table 1. Article types and numbers.

Level	Description
Level 0	Preclinical studies– including experimental studies and animal models
Level 1	Randomized controlled trials
Level 2	Non-randomized controlled trial – a prospective (pre-planned) study with a predetermined eligibility criteria and outcome measures
Level 3	Observational studies with controls- includes retrospective, case-control studies, and cohort studies
Level 4	Observational studies without controls – includes cohort studies without controls, case series without controls, case studies without controls
Level 5	Systematic reviews and meta-analyses

and techniques vary widely. While data have been published vetting the efficacy of fecal transplantation via nasoduodenal tube, combined colonoscopy, encapsulated oral formulation, and enema for the treatment of CDI, thus far only one randomized control trial has been conducted to evaluate the optimal route of fecal administration.[10,15,17,18] In this study (n=20) by Youngster, et al., outcomes of FMT performed by delivery via nasogastric (NG) tube and colonoscopy were compared in an open-label, randomized trial.[18] Both methods had high cure rates of 80% and 100%, respectively, with the major limitation being the sample size. The same research group further established efficacy of an oral, encapsulated FMT, with a cure rate of 90% in a similarly small sample size of 20 patients.[10]

Other parameters of the FMT protocol have not been as thoroughly examined, but have been mentioned by clinical investigators as potentially having an effect on clinical outcomes.[14] Sample preparation of donor feces generally involves dilution and filtration; homogenization in a blender system was not reported to alter microbiome composition of the sample.[17] Critically, the optimal volumes and concentrations of FMT have yet to be established, as a failure of colonization with low amounts could possibly lead to a failure of FMT therapy.

Candidates for therapeutic fecal transplantation for recurrent *Clostridium difficile* infection

Fecal microbiota transplantation has been shown to be largely effective in the treatment of recurrent *C. difficile* infection. Largely, adult populations were treated, with the mean age of these patients in ***Table 2*** being 55.9 (SD=18.4). More females (n=351) than males (n=242) were treated for CDI with FMT in the papers included. However, there were no studies that established any meaningful variable to successful FMT therapy for CDI. Variables such as age, sex, previous recurrence of CDI, antibiotic regimen, and proton pump inhibitor therapy were examined in most papers. One study found some association between patient-reported health scores and FMT outcomes.[10]

Russell, et al., reported a case series of pediatric patients (n=10) who underwent FMT for the treatment of CDI.[19] Of the 7 patients with uncomplicated CDI, a cure rate of 100% was reported. In 3 patients, CDI was compounded on by IBD in which 2 of these patients were cured, but the third was refractory to treatment. Overall, the authors noted that while FMT aided in the resolution for CDI, the underlying IBD was unaffected.

Another critical variable assessed by one study (n=80) was the immune status of patients undergoing FMT.[16] Since the fundamental nature of fecal transplantation requires the introduction of foreign organisms into a host, the theoretical complication of infection is possible. It is speculated that this risk could be higher in patients with compromised immune status. Kelly, et al., examined outcomes in a population of patients that included patients with solid organ transplants on immunosuppressive therapy, patients with HIV/AIDS, patients on systemic immunosuppressants for inflammatory conditions such as IBD and rheumatoid arthritis, patients with chronic, end-stage diseases such as cirrhosis, ESRD, COPD, and cancers, many of whom were receiving anti-neoplastic therapy. FMT in these patients had an overall cure rate of 89% for CDI. The other major outcome measured to assess the safety of the procedure in this population was a number of serious adverse events, including unplanned hospitalizations, deaths, life-threatening experiences, and other important medical events. Fifteen percent of the study participants experienced a serious adverse event, and two patients died. However, the authors stressed the critical nature of these patients and that adverse events that occurred in patients receiving FMT were not related to the procedure. Due to the similar cure rate to previous studies and the nature of complications observed in patients, the authors concluded that immunocompromised patients were at no risk of complication and that FMT was safe in such a population.[16]

Fecal microbiota transplantation in inflammatory bowel disease

IBD is a term used to describe two inflammatory conditions of the gastrointestinal system, ulcerative colitis (UC) and Crohn's disease. While fairly high cure rates of FMT therapy have been reported for CDI, results of FMT in IBD have been mixed. Clinical data supporting the use of FMT in IBD is limited. To date, two published randomized trials have examined FMT in IBD.[20,21] One systematic review pooling data from 18 studies was published in August 2014, which included the data from the mentioned randomized control trial.[5]

Outcomes of fecal transplantation in patients with inflammatory bowel disease

Colman, et al., pooled data from 18 case series and reports to systematically review outcomes of IBD patients receiving FMT.[5] Included in this assessment of the literature are three studies in ***Table 1***, one randomized control trial that is still underway with preliminary results published and two case series examining pediatric populations, and two studies from ***Table 3***.[4,20,22-24] The systematic review included data published up until May 2014, pooling data from 119 patients suffering from IBD. Statistical analysis performed on the data found that 22% of Crohn's patients and 60.5% of UC patients achieved clinical remission. Overall, 45% of patients with IBD in the 18 included studies of the review achieved remission.

The first randomized control trial (n=70) evaluating the outcomes of FMT against a placebo in patients with UC were conducted by Moayyedi, et al., in 2015. Rates of remission and changes in the severity of UC were assessed. The protocol involved weekly infusion of fecal material from healthy donors, and the outcomes were in line with what has thus far been reported in the literature regarding remission achieved by FMT in IBD. FMT did not have any advantage over water enema in severity reduction, but had some success in disease remission. Twenty four percent of patients who had received weekly fecal transplantations for 6 weeks had clinically achieved remission at week 7, whereas only 5% of patients who had received a water enema achieved remission.[20]

The second randomized control trial (n=50) by Rossen, et al., published in July 2015 had similar findings and parameters to the aforementioned UC randomized control trial. The rates of remission and clinical response, defined through improvement in the Simple Clinical Colitis Index (SCCAI) were addressed. The protocol involved two infusions of either autologous or donor fecal preparation, with the second infusion given three weeks after the first. Follow-up at 6 and 12 weeks after FMT revealed

Table 2. Clinical evaluation of fecal microbiota transplantation.

Paper	Date, Design, Level, (n), First author	Inclusion criteria	Objective	Measures	Findings
Systematic Review of Intestinal Microbiota Transplantation (Fecal Bacteriotherapy) for Recurrent *Clostridium difficile* Infection.[14]	November 2011. Systematic literature review. Level 5 n = 317 Gough, Ethan	Publications documenting infusion of stool from a healthy donor into an unhealthy human subject for CDI. No duplicate cohorts included. Mean age: 53 F: 193, M: 124	Perform a full systematic review of all available FMT procedures for CDI (27 case series and reports), which, at the time of publication, was not before assessed.	• *Failure*: continued occurrence or recurrence of disease after transplantation. • *Cessation*: complete cessation of symptoms or diagnostic verification of no disease. • *Relapse*: resolution with return of symptoms. • Deaths, due to illness unrelated illness as reported by authors. • FMT procedure parameters.	92% of patients treated had resolution; 89% with single treatment and 5% with treatment after failure or relapse. 11% had a relapse event. Better outcomes observed with: • Transplant from a related donor. • Rectal catheter route. • Antibiotics and lavage before FM. o This also had a high relapse rate. • Larger (>500 mL) FMT used for transplantation.
Long-Term Follow-Up of Colonoscopic Fecal Microbiota Transplant for Recurrent *Clostridium difficile* Infection.[16]	July 2012. Retrospective case series. Level 2 n = 77 Brandt, Lawrence J	Recurrent *C. difficile* colitis refractory to medical therapy, FMT therapy. Mean age: 65 F: 56, M: 21	Determine long-term cure rates of FMT in patients with *C. difficile* infection; mean of follow up was 17 months. Assess antibiotic use post-FMT.	• *Primary cure*: resolution of diarrhea within 90 days. • *Secondary cure*: resolution with additional antibiotics with or without repeat FMT. • Patient-reported diarrhea, abdominal pain, fatigue, weight change.	Primary cure in 70 of 77 patients (91%), secondary cure in 76 of 77 patients (98%). Patients reported improvement and resolution in all survey parameters. Two patients reported improvement in other conditions and 4 patients reported development of new diseases. 30 of 77 patients required antibiotic use unrelated to CDI after FMT; of these 8 had a recurrence of CDI.
Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile.[15]	January 2013. Randomized control trial, open-label (NTR1177). Level = 2 n = 43, 2 lost van Nood, Els	Patients with CDI defined as at least one relapse while using antibiotic therapy and positive toxin assay. Mean age: 69.6 F: 18, M: 24	Compare three protocols for treatment of recurrent CDI: nasoduodenal FMT, antibiotic therapy, and antibiotic therapy with bowel lavage. Assess diversity of microbiome.	• *Cure*: resolution of diarrhea without relapse within 10 weeks of FMT. • Simpson reciprocal index of diversity.	• 13/16 (81%) of patients in the FMT group were cured with one FMT; 2/3 (66%) patients requiring more than one FMT were cured after relapse. o Overall cure rate for FMT was 15/16 (94%). • 4/13 (31%) of patients in vancomycin antibiotic therapy group were cured. • 3/13 (23%) of patients were cured in the vancomycin-lavage arm. • Microbiome diversity was low pre-FMT and resembled donor's high diversity after FMT.
Safety, Tolerability, and Clinical Response After Fecal Transplantation in Children and Young Adults With Ulcerative Colitis.[22]	June 2013. Case series. Level 2 n = 10 Kunde, Sachin	Patients with ulcerative colitis (UC), with an UC index between 15 and 65; this excluded cases of fulminant colitis and *C. difficile* colitis. Mean age: 15.2 F: 4, M: 6	Evaluate safety and tolerability of FMT (with no bowel preparation) in a pediatric population. Assess effect of FMT on clinical severity of UC.	• Adverse events. • Serious adverse events. • Tolerance, defined as ability to retain enema. • Clinical response in terms of UC index activity - questionnaire.	Adverse events reported included abdominal pain, bloating, diarrhea, blood in stool, fatigue, and fever. • No serious adverse events were noted. • One patient had intolerance (and was excluded from further therapy). 78% of patients showed clinical response in 1 week. 66% maintained this improvement at 1 month. 33% of patients had clinical remission after a week. Overall, median UC index activity decrease was statistically significant (p=0.03), and the overall level of improvement did not correlate with the initial level of UC index activity.
Fecal Transplant for Recurrent Clostridium difficile Infection in Children with and without Inflammatory Bowel Disease.[19]	May 2014. Case series. Level 2 n = 10 Russell, George	Pediatric patients with recurrent C. difficile colitis; 3/10 patients had IBD. Mean age: 7.8 F: 4, M: 6	Assess the efficacy of FMT in a pediatric population with IBD.	• Relapse rate • Adverse events • Time to relapse • Follow-up	Patients without IBD (7/7) had 100% resolution. 2/3 (67%) of patients with IBD had resolution of *C.difficile* colitis, though underlying IBD activity was not affected. No serious adverse events were noted, though: • Short term, self-resolving complications included abdominal pain, bloating, cramping, and diarrhea. • One patient experienced mucoid stools for 2 weeks after FMT.
Fecal Microbiota Transplant for Relapsing *Clostridium difficile* Infection using a Frozen Inoculum from Unrelated Donors: a Randomized, Open-label, Controlled Pilot Study.[18]	June 2014. Randomized control trial. Level 1 n = 20 Youngster, Ilan	Patients with CDI with at least 3 relapses after antibiotic treatment, Mean age: 54 F: 11, M: 9	Compare the clinical efficacy of FMT delivered by nasogastric (NG) tube and colonoscopy.	• *Cure*: cessation of diarrhea for at least 8 weeks. • Relapse rate. • Adverse events. • Self-reported health questionnaire. • Shannon diversity index.	In the colonoscopy group, 6/10 (60%) of patients were cured with one FMT. • Overall cure rate for colonoscopy: 100%. In the NG tube group, 8/10 (80%) were cured with one FMT. • Overall cure rate for NG tube: 80%. Of the remaining 6 patients requiring a second FMT, one refused further FMT and 5 elected to have a second FMT via NG tube; of these, 4 were cured. Health score was reported higher in colonoscopy patients. Diversity was unaffected by route of FMT.
Fecal Microbiota Transplant for Treatment of *Clostridium difficile* Infection in Immuno-compromised Patients.[13]	July 2014. Retrospective case series. Level 2 n = 80 Kelly, Colleen R	Compromised immune status. • IBD with immuno-suppressant therapy. • Solid organ transplant recipients. • Severe or end stage chronic conditions. o HIV/AIDS. o Cancer. o Others. Mean Age: 53 F: 38, M: 42	Assess the long term cure rates and adverse events of FMT in immuno-compromised patients with C. difficile infection; mean follow up was 11 months. Parameters within 12 weeks.	• Mortality. • Hospitalizations. • Adverse events (AEs): any untoward medical occurrence, seemingly causal or not. • Serious adverse events (SAEs): death, life-threatening experience, unplanned hospitalization, or important medical event. • CDI recurrence and cure rates.	Resolution in 62 of 80 patients (78%) with single FMT; 8 of 12 remaining patients cured with second FMT; overall cure rate of 89%. SAEs seen in 12 patients in 12 weeks. • Two deaths. o Due to pneumonia after resolution of diarrhea. o Due to aspiration 1 day after FMT. • 10 hospitalizations. o One due to abdominal pain after FMT (directly related). o Four with IBD flares after FMT. o Other events deemed unrelated. ▪ Cerebrovascular event. ▪ Influenza, no progression. ▪ Catheter infection. ▪ Hip fracture. ▪ Pancytopenia, fever, encephalopathy. Authors conclude no increased risk of FMT in this patient population.

Table 2 (continue). Clinical evaluation of fecal microbiota transplantation.

Paper	Date, Design, Level, (n), First author	Inclusion criteria	Objective	Measures	Findings
Fecal Microbiota Transplantation as Therapy for Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis.[5]	August 2014. Systematic review. Level 5 n = 119 Colman, Ruben J	Publications assessing FMT in the treatment and management of IBD. Age range: 7-64	Perform a full systematic review of all available FMT procedures for IBD (18 case series and reports included).	• Clinical remission: defined by a Mayo score › 2. • UC outcomes. • Crohn's outcomes.	54/119 (45%) of demonstrated remission after FMT. • 22% of UC patients had remission. • 60.5% of Crohn's patients had remission.
Efficacy of Combined Jejunal and Colonic Fecal Microbiota Transplantation for Recurrent *Clostridium difficile* Infection.[17]	September 2014. Prospective case series. Level 2 n = 27 Dutta, Sudhit	Patients with three recurrences of C. difficile colitis treated with FMT therapy. Mean age: 64.5 F: 22, M: 5	Measure clinical outcomes of FMT with a protocol involving simultaneous delivery of transplant to both colon and jejunum.	• *C. difficile* toxin. • Symptomatic relief. • Time to resolution. • Body mass index. • Taxonomic composition of FMT. • Effect of homogenization of FMT sample.	Clinical efficacy • 27 of 27 patients had resolution of colitis and negative toxin 1 to 3 months after FMT. • 88.2% had resolution of abdominal pain. • 100% had resolution of bloating. • 1-3 days were reported for time to resolution. • Homogenization had no significant effect on the composition of microbiota (‹0.3% change in composition). • Dual delivery of FMT to jejunum and colon was performed with 100% clinical success. Metagenomic analysis • Phylum: Firmicutes. o Lachnospiraceae: increase. • Phylum: Proteobacteria. o Enterobacteriales: decrease.
Oral, Capsulized, Frozen Fecal Microbiota Transplantation for Relapsing *Clostridium difficile* Infection.[10]	November 2014. Prospective case series. Level 2 n = 20 Youngster, Ilan	Patients with at least 3 recurrences of CDI. Median age: 64.5 F: 9, M: 11	Assess the clinical safety of oral, encapsulated FMT in CDI and determine its efficacy, particularly as an alternative to fresh stool preparations.	• Resolution of diarrhea without relapse. • FMT-related adverse events. • Daily bowel movements. • Self-reported general health survey score and GI survey score.	Overall, in the 8 week study period, 18/20 patients achieved a resolution (90%) of their CDI-related diarrhea • 14/20 patients had resolution with administration of FMT on first administration. • 6/20 patients were given a second FMT after recurrence of CDI after 7 days; of these 5/6 had resolution of CDI in the 8-week study with one of the 5 have a relapse. • 1/20 patient had unresolved CDI after 8 weeks. Secondary outcomes • Bowel movements decreased from 5/day to 1/day. • General self-health score improved from 5 to 8. • GI self-health score improved from 4.5 to 8. • No variables were associated with FMT outcomes other than the pre-FMT general self-health score. • Age, sex, previous recurrence number, antibiotic regimen, and gastric acid suppression therapy were not associated with outcomes.
Fecal Microbial Transplant via Nasogastric Tube for Active Pediatric Ulcerative Colitis.[23]	January 2015. Case series. Level 2 n = 4 Suskind, David	Male pediatric patients with active UC without *C.difficile* infection. Patients were medicated for UC. Mean age: 14.5	Assess the role of FMT via nasogastric (NG) tube in UC management.	• UC index activity score at follow up at 2, 6, and 12 weeks post-FMT. • Adverse events. • Serum CRP and stool calprotectin.	2/4 (50%) of patients developed C. difficile diarrhea despite being tested prior to FMT and despite donor screening for evidence of *C. difficile*; treatment successfully cured these patients. No change in UC index was found in any patient 2 weeks after follow up; no change in calprotectin or CRP seen. Authors state no clinical benefit of NG tube.
Findings From a Randomized Controlled Trial of Fecal Transplantation for Patients With Ulcerative Colitis.[21]	July 2015. Randomized control trial. Level 1 n = 50 Rossen, Noortje	Patients with mild to moderate ulcerative colitis - Simple Clinical Colitis Index (SCCAI) ≥ 4, ≤ 11 - on maintenance therapy.	Compare outcomes of FMT in UC patients with either autologous or donor feces with follow-up at 6 and 12 weeks.	• Clinical remission of UC: SCCAI ≤2 • Clinical response defined as change in SCCAI ≥1.5. • Adverse events.	Primary outcome • Clinical remission in 7/23 (30.4%) patients who underwent healthy donor FMT vs 8 of 25 (32%) patients who underwent autologous FMT. Secondary outcomes • Clinical response in 11 of 23 (47.8%) patients with donor FMT vs 13 of 25 (52%) patients with autologous FMT. • Adverse events non-significant with spontaneous resolution within 2 days of FMT.
Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial.[20]	July 2015. Randomized control trial. Level 1 n = 63 Moayyedi, Paul	Patients with active ulcerative colitis (Mayo score › 4) without CDI.	Compare outcomes of FMT given weekly for 6 weeks in UC patients with water-enema placebo given weekly for 6 weeks.	• Remission of UC: Mayo score ‹ 2. • Change in Mayo score.	9/27 (33%) patients in FMT enema group achieved remission. Remission rates of UC did not differ between placebo group and FMT group.

Table 3. Bacterial shifts in intestinal microbiome composition.

Paper	Date, Design, Level, (n), First author	Inclusion criteria	Objective	Measures	Findings
Transfer of Intestinal Microbiota From Lean Donors Increases Insulin Sensitivity in Individuals With Metabolic Syndrome.[3]	October 2012 Randomized control trial Level 1 n = 18, each group with 9 Vrieze, Anne	Male Caucasians with BMI > 30 and fasting plasma glucose > 5.6 Mean age: 47	Assess effect of lean donor FMT in obese patient with metabolic parameters after 6 weeks. Determine alterations of intestinal microbiome composition with allogeneic and autologous FMT. Quantify diversity of microbiomes in microbiome samples	• Insulin sensitivity via hyperinsulinemic-euglycemic clamp • Intestinal microbiome composition as determined by sequencing and analysis • Fecal short chain fatty acids • Simpson reciprocal index of diversity	Obese subjects had increased insulin sensitivity and bacterial diversity after FMT, especially those related to butyrate production; total amount of bacteria was not significantly different • 16 different groups of bacteria were increased with allogeneic FMT compared to before FMT o Phylum: *Firmicutes. Roseburia intestinalis; Ruminococcus bromii, R. callidus, R. lactaris*, ▪ R. gnavus; Sporobacter termitidis; Eubacterium siraeum; Anaerotruncus colihominis; Clostridium nexile*, C. ramosum, C. symbosium, C. sphenoides*; Aneurinibacillus; Coprobacillus catenaformis*; Dorea formicigenerans*.* o Phylum: *Proteobacteria. Oxalobacter formigenes** • Of these 16, six (*) groups were at significantly different levels between the two FMT groups. Diversity was increased post-FMT in patient samples; this was found to be associated with improved insulin sensitivity.
High-Throughput DNA Sequence Analysis Reveals Stable Engraftment of Gut Microbiota Following Transplantation of Previously Frozen Fecal Bacteria.[26]	March 2013 Prospective case series Level: 2 n = 3 Hamilton, Matthew J	Fecal samples of CDI patients treated with frozen FMT before and after transplantation	Observe changes in microbiome composition of CDI patients before and after FMT. Compare donor composition to FMT recipient composition. Assess temporal changes in microbiome composition up to 4 months.	• Genetic sequencing of DNA from fecal samples • Operational taxonomic units (OTUs) derived from sample sequences • Shannon diversity index	OTUs from the three patients were low pre-FMT with an average of 296 and high post-FMT with an average of 948 for all three patients. Donor samples were characterized by higher levels of *Bacteroidetes* and *Firmicutes* and lower levels of *Proteobacteria, Actinobacteria* and *Verrucomicrobia* • Phylum: *Bacteroidetes* o *Bacteroidaceae; Bacteroides; Porphyromonadaceae; Parabacteroides; Rikenellaceae; Alistipes* • Phylum: *Firmicutes* o *Lachnospiraceae; Ruminococcaceae; Erysipelotrichaceae; Unclassified Clostridiales* Pre-FMT samples were characterized by higher levels of *Proteobacteria* and *Firmicutes*, with considerable variation among the three patients; notably, many of the bacteria were classified into: • Phylum: *Proteobacteria* o *Enterobacteriaceae. Klebsiella; Salmonella; Escherichia/Shigella; Kyuvera; Parasutterella* • Phylum: *Firmicutes* o *Lactobacillaceae. Lactobacillus; Veillonellaceae. Veillonella; Enterococcus; Erysipelotrichaceae* Post-FMT samples were characterized by increases in levels of Bacteriodetes and Firmicutes, with profiles similar to donor samples. Post-FMT composition remained stable in patients 1 and 2 over the course of observation and resembled donor and initial profiles. Post-FMT composition in patient 3 was dynamic; 20 days post-FMT the composition resembled donor profiles but at subsequent time points the composition of Proteobacteria (especially genus: Escherichia/Shigella) shifted: • Day 28: 10%, day 36: 50%, day 68: 18%, day 90: 28% This was attributed to development and treatment with antibiotics of urinary tract infection in patient 3; post-FMT composition was not regained within the observation period.
Microbiota Dynamics in Patients Treated with Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection.[2]	November 2013 Prospective case series Level 2 n = 14 Song, Yang	Patients with three recurrences of C. difficile colitis treated with FMT therapy. Donors were chosen by patients. Note: one treated patient relapsed and successfully re-treated with FMT. Mean age: 62.7 F: 12, M: 2	Infer changes in the microbiome composition before and after FMT therapy at several discrete time points	• Genetic sequencing of fecal samples from both FMT recipient and donor • Samples analyzed for operational taxonomic units (OTUs); each OTU is the equivalent to one taxonomic species o 14 donor samples o 8 donor samples after FMT o 11 patient samples pre-FMT o 17 samples from 8 of the treated patients post-FMT • Relative abundance of specific taxa of bacterial species using Metastats • Diversity as inferred by Shannon index of diversity	1321 OTUs identified; 65% of these were unique to both post-FMT and donor groups; 35% were not identified in the pre-FMT samples. OTUs increased significantly after FMT procedure as compared to before procedure; equated to an increase in microbial diversity (Shannon index of diversity and Wilcoxon rank sum, p<0.01). No difference in diversity was noted between donors and post-FMT groups. • In the patient with relapse, the measured diversity was low relative to healthy donor samples; upon re-treatment, this was corrected. • The composition of the microbiome of the relapse patient was similar to that of other post-FMT patients and donor compositions. • Due to the short nature of the relapse, authors hypothesized that the existence or lack of existence of specific microbiome colonizers reflective of long term CDI and short CDI. Bacteria from 3 taxonomic orders, all from two total phyla, altered in relative abundance; post-FMT samples were near but not exactly matching donor samples • Phylum: *Firmicutes* o *Clostridiales. Lachnospiraceae; Peptostreptococcaceae; Ruminococcaceae* o *Lactobacillales. Enterococcaceae; Streptococcaceae* • Phylum: *Proteobacteria* o *Enterobacteriales. Klebsiella* The variation in these was as follows after FMT: • *Lactobacillales: significant decrease. Clostridiales: significant increase. Enterobacteriales: decrease* Streptococcus members were found in higher amounts in the post-FMT group than the donor group, implicating a role in the increased susceptibility of post-FMT patients to recurrence of C. difficile colitis; not statistically significant. Patients' intestinal profiles were analyzed temporally; variation of profiles was not found as analyzed by two different statistical methods to 20 weeks. The diversity of the post-FMT group was stable and comparable up to a year in patients; authors described the changes over time as shifting in the direction of an already established profile and increasing in concentration of existing bacteria rather than shifting to alternate profiles as measured by mean UniFrac phylogenetic values.
Species and Genus Level Resolution Analysis of Gut Microbiota in Clostridium Difficile Patients Following Fecal Microbiota Transplantation.[25]	April 2014 Case series Level: 2 n = 3 Shankar, Vijay	Patients with recurrent CDI with failure of treatment with antibiotics.	Assess changes of microbiome composition in CDI patients before and after FMT, and compared to donors through time.	• Genetic sequencing and microarray analysis of fecal samples from recipient pre- and post-FMT and donor, confirmed by fluorescence in situ hybridization (FISH). • Stability through time assessed by Spearman correlation. • Changes in diversity of microbiome assessed by Shannon index.	Pre-FMT samples had low levels of diversity, whereas donor and post FMT samples had higher levels of diversity as assessed by the Shannon diversity index; after day 3, post-FMT profiles converged to a composition to donor. Temporally, the microbiome composition of patients remained stable with a Spearman correlation of 0.87 over 4 months of sampling; no major shifts in composition occurred. Specific constituents were identified as abundant in samples; these were divided into groups with the phyla and genera listed below. • Donor samples, group 1: an average of the abundance in all these genera was <1% in pre-FMT samples and higher in donor and post-FMT samples at 3% o *Bacteriodetes. Bacteriodes* o *Firmicutes. Holdemania; Coprococcus; Faecalibacterium; Subdoigranulum; Roseburia; Blautia; Papillibacter.* o Other: *Akkermansia* • Abundant in all pre-FMT patient samples but not in donor samples, group 2: an average of the abundance in all these genera was 6-12% in pre-FMT samples and lower in donor and post-FMT samples at 0% o *Firmicutes. Veillonella; Lactobacillus* o *Proteobacteria. Escherichia/Shigella; Raoultella; Enterobacter* • Abundant in some pre-FMT patient samples, group 3: in the indicated patients below, elevated levels of these genera were 4-30%, and all were less than 3% in post-FMT and donor samples o *Firmicutes. Zymophilus* (2 + 3); *Streptococcus* (1 + 2); *Enterococcus* (3) o *Proteobacteria. Klebsiella* (2 + 3); *Haemophilus* (1) • Inconsistently abundant in samples, group 4: *Bifidobacterium* levels were elevated in samples both pre-FMT (patient 1, 18%) and donor (patient 3, 13%), inconsistently, while levels of Lactococcus varied, but not as dramatically elevated in donor samples (patient 1 and 3, 2%) and pre-FMT (3%) o *Actinobacteria. Bifidobacterium. Firmcutes. Lactococcus* On a species level, the trends followed the genera; specific species that increased after FMT: • *Bacteriodes fragilis, B. ovatus, B. uniformis; Faecalibacterium prausnitzii; Clostridium bartlettii; Dorea longicatena; Holdemania filiformis; Roseburia intestinalis; Ruminococcus obeum* Species that were detected in high quantities in pre-FMT samples: • *Bifidobacterium adolescentis; Escherichia coli; Klebsiella pneumonia; Bifidobacterium adolescentis; Enterococcus faecium; Lactobacillus salivarius*

Table 3 (continue). Bacterial shifts in intestinal microbiome composition.

Paper	Date, Design, Level, (n), First author	Inclusion criteria	Objective	Measures	Findings
Recovery of the Gut Microbiome following Fecal Microbiota Transplantation.[11]	June 2014 Prospective case series Level: 2 n = 14 Seekatz, Anna M	Patients with C.difficile infection with two prior infections of C. difficile colitis and failed antibiotic therapy. Mean age: 57.4 F: 11, M: 3	Infer changes in the microbiome composition before and after FMT therapy with regards to CDI.	• Genetic sequencing of fecal samples from both FMT recipient and donor. • Samples analyzed for operational taxonomic units (OTUs); each OTU is the equivalent to one taxonomic species o 14 samples pre-FMT o 14 donor samples o 16 post-FMT samples • Relative abundance of specific taxa of bacterial species using Metastats. • Diversity as inferred by Shannon index of diversity.	1796 OTUs identified; these represented 164 genera – post-FMT and donor composition was more similar than as compared to pre-FMT levels as calculated by a similarity index. The bacteria that had the most significant compositional changes were: • Phylum: *Firmicutes. Ruminococcaceae: Faecalibacterium, Subdoligranulum, Flavonifactor; Lachnospiracea: Blautia, Lachnospiracea, Anerostipes, Clostridium XIVb, Roseburia, Dorea, Coprococcus, Clostridium XIVa; Acidaminococcaceae: Acidaminococcus; Clostridiaceae: Clostridium sensu stricto; Streptococcaceae: Streptococcus; Lactobacillaceae: Lactobacillus; Peptostreptococcaceae: Clostridium XI; Enterococcaceae: Enterococcus; Veillonellaceae: Dialister; Erysipelotrichaceae: Erysipelotrichaceae, Clostridium XVIII.* • Phylum: *Bacteriodetes. Bacteriodaceae: Bacteriodes; Rikenellaceae: Alistipes; Porphyromonadaceae: Parabacteroides, Barnesiella; Prevotellaceae: Prevotella.* • Phylum: *Proteobacteria. Enterobacteriaceae: Cronobacter; Sutterellaceae: Parasutterella; Pasteurellaceae.* Pre-FMT composition heavily favored Proteobacteria species • An average of 48.2% of OTUs – Cronobacter and another member (unclassified) of Enterobacteriaceae composed to bulk of OTUs pre-FMT • Post-FMT, these levels dropped to 0.12% of the composition of samples Post-FMT and donor samples had elevated levels of OTUs from *Firmicutes* and *Bacteroidetes* • *Firmicutes* o Major components: *Blautia, Lachnospiraceae*, an unclassified member, and *Faecalibacterium*; OTU composition: Donor: 65.4%, post-FMT: 51.6% • *Bacteroidetes* o OTU composition: Donor: 32.8%, post-FMT: 32.2%; Major components: *Bacteroides* and *Alistipes* Post-FMT, diversity was found to increase in samples from CDI patients but not to the level found in donor samples; possible partial recovery diversity post-FMT
Alteration of Intestinal Dysbiosis by Fecal Microbiota Transplantation Does not Induce Remission in Patients with Chronic Active Ulcerative Colitis.[4]	September 2013 Prospective case series Level: 2 n = 6 Kump, Patrizia	Patients with chronic, active UC refractory to treatment; patients were on regimens of 5-ASA and steroids. Mean age: 36.2 F: 3, M: 3	Assess the potential for FMT in the patient population of chronic, active UC. Note: no pre-FMT antibiotic was used.	• UC score judged with Mayo score • Fecal calprotectin levels and serum CRP • Genetic analysis of FMT samples • Patterns of response to FMT indicated by microbiome composition via UniFrac distance to assess similarity • Diversity as inferred by Shannon index of diversity	No patient achieved remission in the follow-up interval of 90 days; at 1 year, 50% had remission. 2 weeks post-FMT, all patients reported a decrease in stool frequency; 4/6 patients by day 30 increased. No change in fecal calprotectin or serum CRP FMT had a temporary increase in diversity, peaking at day 7 and declining thereafter. 4 patterns of response were observed: 1. Microbiome converged to donor (3/6 patients) 2. Microbiome converged to donor then reversed 3. Microbiome changed unrelated to donor/self 4. Microbiome resembled donor before FMT and changes were minor; the authors noted that the 4th pattern of response was noteworthy; this patient had the best response to FMT. Compositional analysis of microbiome profiles showed a decreasing abundance of *Proteobacteria* from day 0 to day 90 and an increasing colonization by *Bacteroides* and *Firmicutes*. 12 bacterial species of 64 identified represented 85% of OTUs in all samples; 3 of these bacterial families had statistically significant changes post-FMT in stool samples and 5 in mucosal samples– changes are given baseline to day 7 to day 30 to day 90 assessed via Metastas Stool samples • Phylum: *Bacteriodetes* o *Bacteroidaceae*: increase from 0.9% to 12.2% to 13.5% to 23.0% to 19.6% • Phylum: *Firmicutes* o *Lactobacillales*: decrease from 5.7% to 0.1% (day 7) to 0.1%. *Enterococcaceae* • Phylum: *Proteobacteria* o *Enterobacteriales. Enterobacteriaceae*: decrease from 25.8% to 3.3% (day 30) to 0.1% Mucosal samples • Phylum: *Firmicutes* o *Lactobacillales*: decrease from 6.5% to 0.2% (day 7) to 0.1%. *Enterococcaceae* o *Bacillales*: increase from 0% to 1.2%. *Turicibacteraceae* • Phylum: *Bacteriodetes* o *Bacteriodales. Bacteroidaceae*: increase from 1.5% to 16.0% to 11.9% to 18.2% to 15.0% o *Clostridiales* family XIII: increase from 0% to 1.0% • Phylum: *Proteobacteria* o *Enterobacteriaceae*: decrease from 20.7% to 0.8% (day 30) to 0.1%
Temporal Bacterial Community Dynamics Vary Among Ulcerative Colitis Patients After Fecal Microbiota Transplantation.[24]	September 2013 Prospective case series Level = 2 n = 5 Angelberger, Sieglinde	Patients with chronic UC Mean age: 34.3 F: 2, M: 3	Analyze changes in microbiome composition over time in UC patients after FMT. Relate clinical correlation of symptoms to microbiome.	• Remission • Mayo endoscopic score • Genetic analysis of microbiome composition using OTUs o Bacteria were sampled at times points at follow up to track changes in composition • Similarity as determined by UniFrac distance and Pearson correlation • QIIME software parameters for diversity o Alpha and beta diversity	No patients achieved remission in a follow up of 12 weeks; one patient had an improvement in the Mayo score and two experienced worsening. A transient shift to donor microbiota after FMT was detected, but the longevity of this shift was highly variable among patients; the single patient that responded to FMT had persistent shift 12 weeks to follow up. • Pre-FMT samples had low levels of phylum diversity o Elevated levels of: *Enterobacteriaceae, Enterococccaceae* o Depressed levels of: *Lachnospiraceae, Ruminococcaceae, Bacteroidaceae* • Post-FMT samples of UC patients were taken at several time points and varied significantly from patient to patient o Samples from patients 1, 3, and 5 shifted to donor profile ▪ Samples from patient 3, the lone responder, had a similar compositional profile to donor 12 weeks post-FMT; OTUs related four species were found to stabilize at different time points throughout the observation in this patient • Clostridium spiroforme • Roseburia faecis • Bacteroides ovatus • Faecalibacterium prausnitzii. Samples from patients 1 and 5 had marked dissimilarity to donor after 2-4 weeks o Samples from patient 4 did not indicate alteration in microbiome to donor profile; data on patient 2 was unavailable The dynamics of the microbiome composition were shifting, and certain groups of bacteria peaked at distinct times, suggesting colonization of the gut to be a slow, iterative process.
Reset of a Critically Disturbed Microbial Ecosystem: Faecal Transplant in Recurrent *Clostridium Difficile* Infection.[12]	February 2014 Retrospective case series (using data from NTR1177) Level: 4 n = 9 Fuentes, Susana	Patients with CDI defined as at least one relapse while using antibiotic therapy and positive toxin assay. Mean age: 69.6 F: 18, M: 24	Analyze the microbiome composition of patients with recurrent CDI and the changes therein before and after FMT. Assess temporal variation post-FMT in microbiome composition. Determine a preferential donor composition for FMT by comparison of FMT from the same donor to a different donor. Determine composition of microbiome specifically protective against CDI	• Genetic sequencing via microarray of DNA isolated from fecal samples • Shannon index of diversity • Similarity index • Networks of occurrence of as determined by Spearman correlation • Pearson correlation • Microbiome composition as compared to healthy, vancomycin-treated volunteers	FMT increased levels of diversity in CDI patients; this was maintained throughout the observation period of 70 days and increased to levels comparable to donors Shifts in composition occurred immediately after FMT, and resembled donor composition. • Pre-FMT samples had large levels of Proteobacteria species, as well as 3 to 50 fold levels of bacteria related to Bacilli members, Lactobacillus plantarum, and Streptococcus intermedius; these levels dropped off after FMT • Post-FMT, Bacteroidetes and butyrate-producing bacteria from Clostridium clusters IV and XIVa were increased • 41% of the genera were significantly different between donor samples and pre-FMT samples • This value dropped to 13% post-FMT at day 70 • Composition profiles remained stable after day 14 Samples from donor 4 (D4) were used in FMT of 4 patients; these patients had similarity indices higher to each other than to patients that received FMT from other donors • FMT recipients from D4 also had higher similarity at day 70 compared to their donor than FMT recipients from other donors, but this was not significant statistically • Authors hypothesize some microbiome members may have properties that allow for such a shift; D4 samples expressed higher levels of Bacteroides *o B. intestinalis* *o B. plebeius* *o B. uniformis* Samples of CDI patients on vancomycin assessed against healthy volunteers on vancomycin showed majorly decreased levels of Bacteroidetes and Firmicutes; levels of butyrate-producer Megasphaera elsdenii was also less in CDI patient samples

results congruent with data regarding FMT in IBD thus far. Seven of 23 (30.4%) patients who underwent healthy donor FMT had clinical remission of UC, as compared to 8 of 25 (32%) controls who underwent autologous FMT. One secondary outcome examined clinical response defined by change in the SCCAI, finding 11 of 23 (47.8%) patients with donor FMT had some measurable response whereas 13 of 25 (52%) patients of autologous transplantation also had some response. The adverse events noted in this randomized control trial were not significant and resolved spontaneously within 2 days.[21]

Two published case series assessed FMT for the treatment of IBD in pediatric populations, with a mean age of 15.0.[22,23] A total of 14 patients treated between the two case series. Another case series examined outcomes of FMT for CDI in 3 patients with UC, noting remission of CDI but no effect of FMT on UC clinically.[19] A pooled analysis on both FMT case series by Colman, et al., indicated a remission rate of 64.1% in these pediatric populations overall.[5]

Compositional analysis of the intestinal microbiome in therapeutic fecal transplantation

Many studies have linked compositional dysbioses of intestinal bacteria to a variety of disease states.[1] While the evidence of FMT efficacy in the resolution of CDI and other conditions is becoming more validated through clinical intervention and observation, the exact therapeutic mechanisms of the procedure are not fully elucidated. With the overarching goal of FMT being alteration of these pathological states to a point to symbiotic homeostasis, understanding the changes and dynamics of bacterial flora after FMT has begun to shed light on the effects of the procedure.

The large driving force in clarifying the compositional changes of the human microbiome has been the advancement of analytical technology. At the turn of the century, the Human Microbiome Project was created to begin a categorization of the various niches of bacteria commensal to humans.[1] Out of this came the development of 16S ribosomal sequencing, a method that sequences the hypervariable portion of the 16S portion of the 30S prokaryotic ribosomal RNA subunit. The high-throughput ability of the sequencing method created allowed for the development of catalogues of bacterial signatures across a number of body sites. Data from these catalogues has been used to generate operational taxonomic units (OTUs), small phylogenetic units clustered by sequence similarity. OTU clusters generated from samples have been equated to species, and OTU analysis has allowed for characterization of taxonomic composition of samples.[17]

This compositional analysis has been used to characterize the intestinal microbiome and reveal on a more detailed level the bacterial alterations of FMT.

Compositional changes associated with fecal transplantation

Compositional analyses of data from the peri-FMT period have been performed on samples from patients with CDI, IBD, and metabolic syndrome in 8 studies.[3,4,11,12,17,24-26] Findings are broadly categorized by proportional changes in three phyla: *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*. Members of these phyla on the family, genus, and species level as documented in the literature are included in ***Table 3***. Post-FMT, every study documented increases in members of *Bacteriodetes* and *Firmicutes* in CDI samples. Members of *Proteobacteria* post-FMT in CDI patient samples were decreased in all studies. Findings in patients with UC were not generalizable, but were found to be less consistent than those of CDI.

Temporal changes in composition after fecal transplantation

Samples taken at various time points have allowed for the observation of the dynamics in the peri-FMT period. Microbiome profiles before and after FMT have characterized compositional changes in the phyla listed above. In addition to describing these changes, six studies sequenced and analyzed multiple samples a year past the FMT procedure.[2,4,12,24-26]

Dynamics of the microbiome after fecal transplantation in Clostridium difficile infection

Four studies in ***Table 3*** examined alterations of bacterial flora in patients with CDI.[2,12,25,26] Song, et al., did not find any significant alterations in proportions of compositional flora after 20 weeks.[2] Shankar, et al., found stability in patient microbiomes after 4 months, with an increasing similarity of post-FMT samples to donor samples with time.[25] Comparable results were demonstrated by Fuentes, et al., in patients after 70 days, with samples post-FMT converging to donor profiles; particular donor profiles were also found to impart greater similarity post-FMT with time.[12]

Hamilton, et al, observed stability in two of three patients treated with FMT over 90 days.[26] In their third patient, significant alterations were seen over the course of the study. These alterations were found to coincide with patient dosing of antibiotics for treatment of a urinary tract infection. In this patient, a composition similar to the FMT donor was not regained in the observation period.

Dynamics of the microbiome after fecal transplantation in inflammatory bowel disease

Two studies characterized dynamics of bacterial flora in patients with IBD, specifically with UC.[4,24] Kump, et al., described four patterns of changes in six patients with UC treated with FMT.[4] In three of the treated patients, the intestinal microbiome converged to donor composition. Of the other treated patients, one patient had a microbiome composition convergence to donor composition, but subsequent reversal. In another patient, compositional changes in the microbiome appeared to be unrelated to the pre-FMT composition or to the donor composition. In the last patient, pre-FMT bacterial composition and donor composition were similar, and changes post-FMT were minor. Compositional analysis of microbiome profiles showed a decreasing abundance of *Proteobacteria* from day 0 to day 90 and an increasing colonization by *Bacteroides* and *Firmicutes*. At 90 days, none of the UC patients had remission of their disease, but at follow up one year post-FMT, three of the six treated patients had remission.[4]

Angelberger, et al., treated five patients suffering from UC with FMT and observed changes in the bacterial flora over the course of 12 weeks.[24] A transient shift to donor microbiota after FMT was detected in patients, but the longevity of this shift was highly variable. Samples from three patients shifted to a composition resembling donor bacterial composition. Of these three patients, one patient had a similar compositional profile

to the donor 12 weeks post-FMT and experienced clinical benefit of the FMT therapy. Samples from the other patients with donor shifts had marked dissimilarity to the donor after 2-4 weeks. Other patients in the study did not have a compositional shift in their microbiome after FMT that resembled donor composition.[24]

Compositional diversity of microbiomes

Diversity of the bacterial communities found in samples from the peri-FMT period and from donors was measured via three primary modalities in studies assessed: the Shannon index of diversity, the Simpson index of diversity, and alpha and beta diversities.

Studies documenting shifts in diversity in CDI patients consistently found increases in diversity after FMT therapy.[2,11,12,15,25,26] Additionally, diversity was found to increase to levels approaching donor diversity over time. Vrieze et al., 2012 assessed FMT in patients with metabolic syndrome and also found diversity to be increased post-FMT in patient samples.[3] This increase was found to be associated with improved insulin sensitivity. Diversity in microbiomes of patients with UC who underwent FMT were found to be varied. Kump et al., 2013 indicated an initial increase in diversity immediately after FMT, but declining levels thereafter throughout the duration of the observation period.[4] Angelberger et al., did not have clear metrics of diversity.[24]

Discussion

Fecal microbiota transplant as an effective therapy

Published clinical data assessing the clinical efficacy of FMT as a cure for CDI is well established in the reviewed studies. Cure rates ranged from 89-100%, and FMT was shown to be superior to antibiotics in a controlled trial in CDI patients. Additionally, no paper attributed any direct adverse outcomes of FMT. This was especially highlighted in patients with compromised immune status, a population more at risk with such a procedure.

However, FMT was not as consistently successful in the treatment of IBD, with much lower cure rates. The pathogenesis and disease characteristics of IBD, both Crohn's and UC, differ significantly from CDI. Specifically, both IBDs are chronic conditions characterized by high levels of inflammation with multifactorial and incompletely understood etiology, whereas CDI is intrinsically caused by an acute disruption of the microbiome, often precipitated by antibiotic use. Because FMT is associated with an acute restoration of a healthy microbiome composition, it may directly treat CDI. Alternatively, the variable response to FMT seen in patients with IBD indicates the acute therapeutic modality of FMT may not suit the chronicity of the disease.

To highlight this point, some remission of UC was documented over placebo in a trial that treated patients with several FMTs over weeks. Both 2015 randomized control trials of FMT in UC had modest cure rates and similar outcomes, despite different frequencies of serial FMTs, with one being once every three weeks for a total of two infusions and the other being weekly for six weeks.[20,21] The multiple rounds of microbiome restoration may have had some effect on the chronic inflammation of IBD. However, other trials without multiple rounds of FMT for the treatment of IBD did not have lesser cure rates than the randomized trial, casting doubt on this interpretation.

Associations of fecal transplantation with clinical outcomes

The clinical variables by the papers reviewed examined age, sex, immune status, antibiotic regimen, CDI recurrence, gastric acid suppression therapy, and patient perception of treatment.[3,10,13-16,18,24] Surprisingly, no correlation of FMT outcomes and variables were established other than with patient perception of health that was self-reported and is likely due to three factors. The first is the limited sample size of each study. Due to the emerging nature of therapeutic FMT, there are not a substantial number of FMT trials conducted to warrant establishment of patterns of clinical success. The second relates to study design. Currently, clinical researchers seek to establish the credibility of FMT as a viable and easy therapy, with more concern with the overall clinical outcomes and the microbiome shifts associated with FMT. Practically, after more research and trials on FMT have been conducted, objectives will shift to other clinical variables. The third reason there has been no concrete correlation of certain variables with outcomes is the high success rate of FMT in CDI. The vast majority of FMT trials have been conducted in patients with CDI, and, of these, few failures of resolution have been reported. These low failure rates coupled with the already small sample size in the studies reviewed leave little room for variables to be firmly tied to clinical success and failure.

Safety of fecal transplantation

The transplantation of fecal material from donor to recipient is, by definition, introduction of foreign biological material into a host. The transfer of bacteria raises the possibility of infection, particularly if the recipient is already in a diseased state. FMT in patients with systemic immune suppression, such as HIV/AIDS, or patients on immunosuppressive drugs with solid organ allografts or inflammatory conditions such as rheumatoid arthritis, could theoretically result in infection through the donor material. Kelly et al., 2014 concluded after a multicenter, retrospective case series of 80 patients that there was no increased risk of FMT in the immunocompromised after assessing adverse events, the nature of the events, and overall critical nature and complexity of said patients.[13]

Safety of the FMT procedure is partially warranted by rigorous donor screening, and while each center performing FMT has a slightly different protocol, communicable diseases are generally paneled and screened. These include cytomegalovirus, hepatitis B and C, HIV, and syphilis serologically and *C. difficile, Salmonella, Shigella, Yersinia, E. coli*, and *Campylobacter*. Stools are also tested for helminths and parasites.[3,4,22] Much like any other transplantation protocol, standardization of FMT would allow for a more rigorous approach to its assessment and evaluation. As its prevalence increases, this becomes more of a necessity for the safety of patients undergoing FMT.

Alterations of bacterial flora in dysbiotic microbiomes

While a variety of diseases have been associated with microbiome dysbiosis, only a handful of these have been treated by FMT in a clinical trial, reviewed here. The alterations in the composition of microbiomes for metabolic syndrome, IBD, and, most thoroughly, CDI, have been documented in ***Table 3***.

In lean donors, researchers observed an appreciable increase in insulin sensitivity in patients with metabolic syndrome in a randomized control trial.[3] Interestingly, other metabolic para-

meters measured were not altered, such as lipid profiles and body composition. In patients suffering from IBD treated with FMT, metrics to assess outcomes of FMT were mainly the Mayo score of severity for UC, patient-reported health scores, and endoscopy.[4,5,16,18-20,22,24] FMT was not consistently associated with remission. In CDI, FMT has much evidence for its efficacy in the resolution of diarrhea.

The three phyla reported to have significant changes in these patient groups were *Firmicutes*, *Bacteriodetes*, and *Proteobacteria*. In all three FMT-treated disease states, members within these phyla shifted in similar directions.[2-4,11,12,24-26]

Firmicutes members *Lachnospiraceae*, *Ruminococceae, Erysipelotrichaceae, Peptostreptococcaceae, Veillonellaceae, Acidaminococcaceae, Streptococcaceae, Enterococcaceae, Lactobacillaceae*, and *Clostridiaceae* were found to be increased in more than one study.[11,25,26] *Bacteriodetes* members *Bacteriodaceae, Porphyromonadaceae*, and *Rikenellaceae* were found to be increased after FMT in more than one study.[11,26] *Proteobacteria* members of *Enterobacteriaceae* were found to be decreased after FMT.[2,4,11,24-26]

These shifts were stable in successful therapeutic FMT, and declined in the majority of UC cases of IBD analyzed by studies. Particularly, the fact that stability of the typical post-FMT shifts persisted in the lone UC patient in the study by Angelberger et al., in 2013 supports this notion.[24]

Shortcomings of microbiome analysis in fecal transplantation

The major limitation of the compositional analyses was their inter-relatability. While having similar findings, each study had different metrics of data presentation. In particular, reporting by studies on specific species and its shifts were limited by the resolution of the analytic method used. Some studies were able to report genus- and family-level resolution, whereas others were able to report species-level resolution.[3,4,11,12,17,24-26] As the technology of microbiome analysis advances, sequencing and analysis will become more standardized and distinct, allowing for more readily comparable data sets.

Another shortcoming of studies reviewed for microbiome analysis was the small patient populations in each study. Increasing interest in FMT, as well as advances in technology will allow for larger populations of patients to undergo sequencing and compositional analysis, increasing the power of the findings of these studies.

Future perspectives

While the efficacy of FMT in CDI is fairly high, several studies remarked on the fact that no clinically proven optimal protocol exists. Variables of the procedure have been assessed briefly with no clinical impact, and further randomized control trials with larger populations are needed to properly assess the impact of protocol variability on clinical outcomes.

Ample microbiome studies have provided evidence for links between dysbiotic gut bacteria and disease. As research in this field progresses, clinical trials of therapeutic FMT for diseases other than IBD, CDI, and metabolic syndrome can evaluate the true potential of microbiome compositional alteration in clinical medicine.

Conclusion

FMT is slowly gaining traction as a treatment option for disease. Evidence backing its therapeutic value exists for CDI, but further elucidation of its effects in IBD and other diseases is required.

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