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SUPPLEMENT ARTICLE

Fecal Microbiota Transplantation and Microbial Therapeutics for the Treatment of *Clostridioides difficile* Infection in Pediatric Patients

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Clostridioides difficile infection (CDI) is the most common cause of antibiotic-associated diarrhea and has high rates of recurrent disease. As a disease associated with intestinal dysbiosis, gastrointestinal microbiome manipulation and fecal microbiota transplantation (FMT) have evolved as effective, although relatively unregulated therapeutics and not without safety concerns. FMT for the treatment of CDI has been well studied in adults with increasing data reported in children. In this review, we discuss the current body of literature on the use of FMT in children including effectiveness, safety, risk factors for a failed FMT, and the role of FMT in children with comorbidities. We also review emerging microbial therapeutics for the treatment of rCDI.

Key words. fecal microbiota transplantation; fecal transplantation; pediatric *Clostridioides difficile*.

Clostridioides difficile (*C. difficile*) is an anaerobic, spore-forming, Gram-positive bacillus, and the number one cause of antibiotic-associated and nosocomial diarrhea [1]. Antibiotic use is the primary risk factor for *C. difficile* infection (CDI) related to its impact on the intestinal microbiome, which includes communities of commensal and symbiotic microorganisms in the host intestine. Dysbiosis, the development of an imbalance or disturbance of microorganisms often following antibiotic therapy, creates an environment that is hospitable for *C. difficile* colonization, germination, and toxin production [2]. While antibiotics are often a successful first-line treatment, *C. difficile* has a strong predilection to recur. Recurrence rates are 20%-30% in children and adults after a single episode of CDI and up to 60% in those with one or more recurrences [3]. Risk factors such as malignancy, recent surgery, antibiotic exposure, inflammatory bowel disease (IBD), and the presence of a tracheostomy tube are all associated with increased risk of recurrent *Clostridioides difficile* infection (rCDI) in children, presumably related to their contributions to persistent intestinal dysbiosis [4–6]. Patients with intestinal microbiota compositional changes, such as low bacterial diversity, have an increased susceptibility to rCDI [7]. The reasons for this are likely multifactorial, including the "colonization resistance" that is provided by a healthy intestinal microbiome through direct microbial competition, as well as the contribution of the intestinal microbiome on bile acid metabolism, which impact *C. difficile* spore germination and vegetative cell growth in the gut [8].

Fecal microbiota transplantation (FMT) is the transfer of stool from a healthy donor to a symptomatic patient. FMT was used to successfully treat pseudomembranous colitis as early as the 1950s, two decades prior to the discovery of *C. difficile* as the causative agent [9]. The primary understanding of the therapeutic mechanism of FMT is that it improves intestinal dysbiosis by transferring a diverse healthy microbiome from donor to recipient. Bacteriophages, metabolites, repair of the mucosal immune system, and short-chain fatty acids may also play a key role in FMT success [10], although the exact mechanism of treatment remains poorly understood.

In 2013, the first randomized controlled trial (RCT) demonstrated FMT was significantly more effective than vancomycin for rCDI [11]. A recent 2020 meta-analysis of 45 studies demonstrated that 84% of patients show clinical improvement following a single FMT and 91% if FMT is repeated [12]. Of note, although many societies have recommended specific screening and treatment protocols, the preparation and use of FMT remains poorly standardized and warrants attention [13, 14]. FMT remains an investigational therapeutic and can only be performed under "therapeutic discretion" for the treatment of CDI or through an investigational new drug (IND) application based on Food and Drug Administration (FDA) recommendations.

Only recently, the body of literature supporting FMT in the treatment of pediatric CDI has expanded. Here we review the

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current literature investigating the role of FMT for rCDI in pediatric patients and discuss emerging microbial therapeutics for the treatment of rCDI.

USE OF FMT IN CHILDREN WITH RCDI

There are important differences between adult and pediatric CDI. Pediatric patients have distinct risk factors, onset of symptoms, duration of disease, and CDI-related complications [15]. Asymptomatic colonization with *C. difficile* occurs more commonly among young children, and severe disease in children is markedly less frequent than in adults [16]. The pediatric intestinal microbiome, especially in younger children, differs from that in adults. Bacterial diversity increases with age and interpersonal variation is significantly greater among children than among adults [17]. There is rising interest in identifying the differences in the disease processes and treatment responses in adult and pediatric populations. Much of the published literature regarding FMT for the treatment of CDI in children stems from case reports (excluded from this review) and case series that have called attention to important questions for the pediatric population including effectiveness, safety, and the role of FMT in children with comorbidities. Recently, larger multicenter studies have started to address these initial questions and improve the understanding of the efficacy and safety of FMT in children (Table 1).

In children, the cure rates of FMT for rCDI are 80%-90%, similar to what has been previously identified in adults [18, 19]. In 2015, Hourigan et al published the first pediatric single-center study with a cure rate of 100% following initial FMT in 8 children [20]. Brumbaugh et al reported FMT cure rate by underlying disease status in 42 children at a single center; 94% of the healthy children, 75% of medically complex children, and 54% of children with IBD had a successful initial FMT ($P = .04$) [21]. Recently, a large retrospective multicenter pediatric study by Nicholson et al investigated cure rates and outcomes of FMT in children with rCDI [22]. Of the 335 children who underwent FMT, 271 (81%) had a successful first FMT which improved to 87% if FMT was repeated.

A 2021 meta-analysis in adults identified significant predictors of FMT failure including (i) the use of non-CDI antibiotics pre-FMT, (ii) severe CDI, (iii) the presence of IBD, (iv) poor quality of colonoscopy preparation (poor visibility not allowing full volume of the fecal infusion or visualization of the bowel mucosa), and (v) inpatient location at the time of FMT [23]. Female sex, previous hospitalization, and surgery before FMT have also been recognized as risk factors for a failed FMT in prior adult studies [24]. In contrast, the lack of a feeding tube, fewer episodes of CDI prior to FMT, and FMT performed via colonoscopy have been demonstrated as predictors of adult FMT success [25]. In children, Nicholson et al similarly identified the lack of a feeding tube (odds ratio [OR]: 2.08, 95% confidence interval [CI]: 1.05-4.11), delivery via colonoscopy (OR: 2.41, 95% CI: 1.26-4.61), and a lower number of CDI episodes prior to undergoing FMT (OR: 1.20, 95% CI: 1.04-1.39) as predictors of a successful pediatric FMT based on stepwise logistic regression [22]. Notably, the use of fresh vs thawed, previously frozen, stool (OR: 2.66, 95% CI: 1.39-5.08) was also identified as an independent predictor of FMT success. Prior adult RCTs have not demonstrated a statistically significant difference between the use of fresh vs frozen stool samples for FMT [18, 26]. Nicholson et al theorized that potential shifts in the microbiome or metabolome that occur during the freeze-thaw cycle may make frozen donor stool less appropriate for pediatric patients due to their unique intestinal microbiome. Although 16S rRNA sequencing has demonstrated the stability of FMT products when stored at −80°C for 6 months, this does not confirm the viability of organisms [27]. Alternatively, it is possible that there was a closer age-match between donor and recipient when FMT was performed with fresh stool (family members and sibling donors) vs from a stool bank (adult only donors) and this may have influenced the results. A recent study demonstrated improved effectiveness of FMT for the treatment of IBD when there was a smaller age difference between donor and recipient age (0- to 10-year difference vs ≥11-year difference, $P = .003$) [28]. Future prospective FMT studies are needed to further identify best practices for FMT in children with CDI.

SAFETY OF FMT IN CHILDREN

Although generally described as a well-tolerated procedure, the practice of FMT remains poorly standardized and long-term outcomes remain unknown. Until recently, most serious adverse events (SAEs) related to FMT in adults included outcomes primarily related to the procedure itself such as intestinal perforation related to colonoscopy and aspiration pneumonia in the setting of upper FMT delivery [29, 30]. The most common non-severe adverse events (AEs) from FMT identified in a meta-analysis of 18 studies included short-lived bloating/flatulence, abdominal pain/cramping, and diarrhea [31]. More recently, additional attention to the safety of FMT occurred after a 2019 FDA safety alert detailing the acquisition of extended-spectrum beta-lactamases (ESBL) producing *Escherichia coli* in 2 immunocompromised (IC) patients, 1 of whom died. Although neither patient received FMT for rCDI, the FMT donor was found to be positive for ESBL *E. coli* with clonality confirmed by whole-exome sequencing of the ESBL isolates from the donor and recipients [32]. Additional safety concerns have developed in the setting of the COVID-19 pandemic, with the identification of SARS-CoV-2 in feces [33]. Concerns over the potential transmission of virulent pathogens through the use of FMT have called attention to the importance of donor screening and surveillance.

Table 1. Summary of Pediatric Studies for Fecal Microbiota Transplantation for Recurrent *Clostridioides difficile* Infection

Author, Year of Publication, Location	Study Type	Age	Sample Size	Route of Delivery	Donor Stool Selection	Efficacy Rate of Single FMT	Adverse Events
Aldrich et al, 2020, USA	Retrospective, single center	1-21 years	10	Colonoscopy (59%), NJ tube (25%), both EGD and colonoscopy (8%), gastrostomy tube (8%)	Patient selected (100%)	80%	Unknown
Cho et al, 2020, USA	Retrospective, single center	9-18 years	8 with IBD	Colonoscopy (100%)	Patient selected (25%) or commercial bank (75%)	75%	One patient was hospitalized post-FMT; had influenza unrelated to FMT
Nicholson et al, 2020, USA	Retrospective, multicenter	11 months-23 years	335 (120 with IBD)	Colonoscopy (76.6%), NG/gastrostomy (9.1%), ND/NJ/J-tube (8.9%), capsule (3.8%), enema (1.1%), sigmoidoscopy (0.5%)	Patient selected (43.3%), commercial stool bank (29.6%), local stool bank (26.6%)	80.9%	5.7% reported diarrhea, pain, and/or bloating; 4.7% had serious adverse events (2 hospitalizations related to FMT and 5 possibly related)
Zeky et al, 2020, USA	Retrospective, single center	1-18 years	34	Gastrostomy tube/NG tube (82.3%), enema (2.9%), endoscopy (14.7%)	Patient selected (100%)	73.5%	21% of patients from the hospital setting and 27% of outpatients reported non-severe adverse events including abdominal pain, bloating, diarrhea not related to CDI, and emesis during FMT delivery
Hourigan et al, 2019, USA	Prospective, multicenter	2-20 years	9	Colonoscopy (100%)	Patient selected (33.3%) or commercial bank (66.6%)	100%	One patient had prolonged noninfectious diarrhea that resolved
Brumbaugh et al, 2018, USA	Retrospective, single center	1-18 years	42 (13 had IBD)	NG tube, gastrostomy tube, oral capsules	Commercial bank (100%)	71%	13% with self-limited vomiting
Fareed et al, 2018, USA	Prospective, multicenter	21 months-18 years	15; 12 with follow-up	Colonoscopy (93%), NJ tube (7%)	Commercial bank (100%)	100%	33% had abdominal pain at the 3-month follow-up visit
Li et al, 2018, China	Prospective, single center	6 months-12 years	11	NI tube (81.2%), rectal tube (18.8%)	Patient selected (100%)	63.6%	Non-severe adverse events were reported in 36.4% of children including transient diarrhea, mild abdominal pain, transient fever, and vomiting
Zhang et al, 2018, China	Retrospective, single center	4 months-16 years	49	NJ tube, nasogastric tube, and enema	Patient selected (46.7%) and commercial (53.3%)	Unknown	The most common non-severe adverse events were abdominal pain, diarrhea, fever, and vomiting; 4% had hematochezia and hematemesis post-FMT
Hourigan et al, 2015, USA	Prospective, single center	6-17 years	8 (5 with IBD)	Colonoscopy (100%)	Patient selected (100%)	100%	Transient mild abdominal pain occurred in 2 patients; 1 patient without IBD had prolonged diarrhea, fecal urgency and intermittent fecal incontinence after FMT
Kronman et al, 2015, USA	Retrospective, single center	1-13 years	10	NG tube (70%), NJ tube (20%), ND tube (10%)	Patient selected (100%)	90%	50% with vomiting immediately following the procedure
Kelly et al, 2014, USA	Retrospective, multicenter	6-16 years	5 IC children (out of 80 IC patients)	Colonoscopy (75%)	Unknown	78% in adults and children	Not specified in children. Death occurred in 2 patients within 12 weeks of FMT, one of which was the result of aspiration during sedation for FMT administered via colonoscopy; the other was unrelated to FMT

Abbreviations: CDI, *Clostridioides difficile* infection; EGD, esophagogastroduodenoscopy; FMT, fecal microbiota transplantation; IBD, inflammatory bowel disease; IC, immunocompromised; J-tube, jejunostomy; ND, nasoduodenal; NG, nasogastric; NI, nasointestinal; NJ, nasojejunal.

In the pediatric literature, the most common AEs described include mild and transient vomiting related to the procedure, abdominal pain or cramps, bloating, and flatulence (Table 1). In a large pediatric study, Nicholson et al reported that 17/335 (5.1%) of children had SAEs following FMT. Only 2 events, hospitalizations for aspiration pneumonia and vomiting/dehydration, respectively, were thought to be FMT-related [22]. There were no deaths in the study. Notably, no acquisition of multidrug-resistant organisms such as ESBL *E. coli* attributable to FMT has been reported in the pediatric literature to date.

Long-term safety outcomes remain unknown at this time and may be particularly relevant in our pediatric patients. Presumably, microbiomes that are healthy in the donor could be deleterious in the recipient, which provides a challenge for screening donors. As FMT alters the intestinal microbiome and metabolome, which also influences immune function and hormonal development, the future risk of chronic conditions warrants attention. Notably, Hourigan et al found that FMT decreased the prevalence of antimicrobial resistance (AMR) genes and regenerated a healthy microbial milieu post-FMT; however, there was also acquisition of tetracycline AMR genes in recipients with FMT [34]. In addition, both clearance and transmission of potential procarcinogenic bacteria with FMT from adult donors to pediatric recipients have been described [35]. Further research on the long-term repercussions of these changes is needed and additional donor screening may be warranted.

FMT IN SPECIAL POPULATIONS

Inflammatory Bowel Disease

In adult patients with IBD, a recent meta-analysis with 9 cohort studies demonstrated an 81% initial cure rate of CDI with FMT and no difference in success rates amongst those with or without IBD [36]. However, concerns over the safety of FMT in patients with IBD are relevant, with post-FMT IBD flares reported in up to 22.7% of adult patients with IBD [37]. Children with IBD have increased CDI incidence, disproportionately higher rates of initial antibiotic treatment failure, and greater rates of recurrence [6, 38]. In addition, those with CDI are more likely to have a severe course of their underlying IBD, higher rates of colectomy, and in-hospital mortality [39]. A likely mechanism for both the increased incidence of CDI and rates of rCDI is the underlying dysbiosis associated with IBD [40].

In 2019, Cho et al evaluated 8 pediatric patients with IBD and found FMT to be an effective treatment option for rCDI with a cure rate of 75% [41]. One patient had an SAE with vomiting and fever 2-hour post-FMT requiring hospitalization. However, this was ultimately attributed to influenza and felt to be non-FMT-related. Furthermore, although FMT gives sustained *C. difficile* eradication in children with and without IBD, FMT-restored microbiome diversity is only maintained in children without IBD but returns to pre-FMT baseline by 6 months in those with IBD [20]. In a recent large retrospective study (Nicholson et al, abstract only [42]), children with IBD were no less likely to have a successful first FMT than those without IBD (77% vs 83%, P = .22). Additionally, successful FMT did not differ in children with Crohn disease vs ulcerative colitis (78% vs 75%, P = .87). Children with IBD were as likely to have an SAE with FMT vs children without IBD (3.6% vs 0.09%, P = .09). Admission for an IBD flare post-FMT accounted for all of the SAEs in the children with IBD (4/112, 3.6%). The authors noted that despite good effectiveness in children with IBD, a careful discussion of risk vs benefit was warranted.

Immunocompromised Patients Without IBD

IC pediatric patients are also at higher risk for primary and rCDI [43]. Hospitalized children with solid organ transplant (SOT) and CDI have increased rates of additional infections such as cytomegalovirus and graft-vs-host disease compared to hospitalized children with SOT without CDI [44]. Among pediatric patients with cancer, CDI has been associated with an increased risk of death from all causes [45]. Many factors contribute to the risk of CDI in patients with cancer or SOT including increased length of hospital stay and prolonged use of antibiotics [46].

In 2014, Kelly et al evaluated FMT for rCDI in 80 IC patients, including 5 pediatric patients. Results demonstrated an overall cure rate of 89% with no infectious complications [47]. Non-severe AEs occurred in 12 (15%) patients and included self-limiting diarrhea, abdominal pain, and bloating. Twelve (15%) patients had SAEs which included (i) 2 deaths (1 FMT-related) and (ii) 10 hospitalizations (5 FMT-related) during the 12-week follow-up period. In 2020, Conover et al reported experience in 24 IC children who underwent FMT for the treatment of rCDI (abstract only [48]). Children were considered as IC if they had a primary immunodeficiency or were taking immunosuppressive medications during the 3 months prior to FMT. Patients with IBD were excluded. In this study, FMT was curative in 20 (83%) of pediatric IC patients, while 4 (17%) had an episode of rCDI in the 3-month follow-up period. Interestingly, 11 (46%) patients required a total of 13 hospitalizations during the 12-week follow-up period of which 4 (31%) were likely FMT-related. While there were no deaths or infectious complications related to FMT, this study illuminates the possibility of severe complications related to FMT in this high-risk population.

Emerging Data on Biotherapeutics

The current use of FMT is fraught with technical challenges including poorly standardized applications and procedures. Biotherapeutics, loosely defined as drug therapy products where the active substance is extracted from a biological, are exciting alternatives to FMT with the potential for improved standardization, practicality, and safety (Table 2). Multiple products are currently being evaluated for their potential safety and efficacy in the treatment of CDI, although notably, none are currently being trialed in children or adolescents. ECOSPOR III is a phase III trial

Table 2. Investigational Biotherapeutic Options for Recurrent *C. difficile* Infection

Name	Study Sponsor	Administration	Product Description	Trial Phase in Adults
SER-109	Seres Therapeutics	Oral capsule	Purified Firmicute spores from healthy donors	3
RBX2660	Rebiotix	Enema	Broad consortium of standardized intestinal microbes	3
VE303	Vedanta Biosciences	Oral capsule	Lyophilized product of 8 clonal human commensal bacterial strains	2
CP101	Finch Therapeutics	Oral capsule	Lyophilized intact microbiome community from healthy human donors	2-extension

that has been completed for SER-109, an investigational oral therapeutic consisting of a consortium of bacterial spores (purified Firmicute bacteria) from healthy donors (NCT03183128). After partitioning the targeted bacteria from the stool of healthy human donors, the SER-109 manufacturing performs ethanol treatment which inactivates vegetative bacteria (eg, Listeria, Salmonella, Staphylococcus, or Enterococcus). This step serves to reduce the risk of pathogen transmission not detected during screening, but could also have implications for reducing other vegetative pathogens and impacting efficacy. In the ECOSPOR III trial, patients were randomized 1:1 to receive either SER-109 or placebo, after standard-of-care antibiotic treatment for rCDI. Unpublished results in 182 patients showed 11.1% of patients administered SER-109 experienced a CDI recurrence, vs 41.3% of placebo patients. Seres Therapeutics, the study sponsor, is continuing to gather data to support the safety of SER-109 in an ongoing open-label study (NCT03183141) and is in discussion with the FDA regarding regulatory approval and Biological License Application (BLA) submission. Additionally, RBX2660 is a regulated suspension of standardized intestinal microbes administered as an enema that is currently in phase III clinical trials (NCT03244644). Phase II study results found that 2 RBX2660 doses spaced 1 week apart were not superior to placebo, but a single dose of RBX2660 was significantly better than placebo (NCT02299570) [49]. Two trials, PUNCH CD3 and PUNCH CD3-OLS, are evaluating the safety and efficacy of the enema in patients who have had at least 1 recurrence of CDI after a primary episode and have completed at least 1 round of standard-of-care oral antibiotic therapy or have had at least 2 episodes of severe CDI resulting in hospitalization (NCT03244644, NCT03931941). The estimated open-label study completion date is the summer of 2022.

VE303 is an orally administered live biotherapeutic product consisting of 8 types of clonal human commensal bacteria strains selected for their ability to provide colonization resistance to *C. difficile*. Phase 1a/1b clinical trial results in healthy volunteers demonstrated a favorable safety profile and accelerated gut microbiota restoration when used following vancomycin administration [50]. Phase II trials (CONSORTIUM) will compare high and low doses of VE303 to inactive placebo (NCT03788434). Unlike VE303, CP101 is an orally administered freeze-dried stool donation-based capsule that encompasses the complete intestinal microbiome community. In a phase II study (PRISM3), the drug prevented rCDI in 76/102 (74.5%) of patients, compared with 59/96 (61.5%) taking placebo (Allegretti et al, abstract only [51]). Phase II and III study results for VE303 and CP101 are expected in 2021 and 2022, respectively. Microbiota-based biotherapeutics that expand on the FMT mechanism are encouraging. However, completion of the clinical trials, particularly those involving children, and further clinical development is warranted to determine the optimal dosing strategy and long-term safety profile.

CONCLUSION

Although severe CDI in children is rare, rCDI is a common pediatric condition associated with significant morbidity. Modulation of the intestinal microbiome, through FMT or alternative biotherapeutics, has emerged as an important therapeutic strategy for CDI. There is a growing body of literature evaluating FMT as a therapeutic modality in children, demonstrating good effectiveness and an overall favorable safety profile. Moving forward, FMT standardization and refinement, clinical trials enrolling pediatric patients, and additional long-term safety data will be critically important to improve the care of children with CDI.

Notes

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