Clostridioides difficile Infection: Landscape and Microbiome Therapeutics

Brent J. Gawey, MD, and Sahil Khanna, MBBS

Department of Medicine, Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota

Corresponding author:
Dr Sahil Khanna
Mayo Clinic
200 First Street SW
Rochester, MN 55905
Tel: (507) 266-4347
Fax: (507) 293-3917
E-mail: Khanna.sahil@mayo.edu

Abstract: *Clostridioides difficile* infection (CDI) is the leading cause of hospital-acquired diarrhea and is common in the community. Both younger individuals who may be healthy otherwise and older individuals with comorbid conditions are at risk for developing CDI, with the predominant risk factor being antibiotic use. Unlike other gastrointestinal infections, CDI is not self-limited, requires antimicrobial therapy, and tends to recur at high rates even without additional risk factor exposure. The goals of CDI management include controlling active symptoms and using a recurrence prevention strategy such as a narrow-spectrum antibiotic, tapered and pulsed regimens, antibody-based therapies (directed against toxin B), or microbiome restoration. In recent years, fecal microbiota transplantation (FMT) has been the most used modality to prevent recurrent CDI with high cure rates. Heterogeneity, lack of scalability, and serious adverse events from FMT have led to development of standardized microbiota restoration therapies (MRTs). The US Food and Drug Administration has approved 2 stool-derived MRTs for prevention of recurrent CDI: fecal microbiota, live-jslm, an enema-based therapy; and fecal microbiota spores, live-brpk, an oral therapy. A phase 3 trial for a synthetic oral MRT is underway. This article outlines the pathophysiology and treatment of CDI, focusing primarily on the gut microbiome and standardized MRTs.

Keywords
Diarrhea, *Clostridioides difficile,* fecal microbiota transplantation, live biotherapeutics, microbiome, microbiota restoration therapy

Clostridioides difficile is a spore-forming, anaerobic gram-positive bacteria that colonizes the human gastrointestinal (GI) tract.[1,2] First discovered in 1935, *C difficile* was initially thought to be a commensal member of the gut microbiota.[3] It was not until the 1970s that its association with clinically significant diarrheal illness was understood.[4] In the following years, *C difficile* infection (CDI) quickly emerged as the most common cause of infectious diarrhea in hospitalized patients.[5] Currently, worldwide, the estimated incidence of CDI is 49.36 per 100,000 population/year with a concerning increase both in hospital and community rates.[6] In the United States, *C difficile* has remained a continued public health concern, labeled an urgent threat by the Centers for Disease Control and Prevention (CDC) in 2019.[7] In 2017, CDI was diagnosed in nearly a half-million Americans, with almost half requiring

hospitalization and over a quarter of those patients dying during their admission.[8] Although the national burden of CDI and associated hospitalizations decreased from 2011 to 2017, community-acquired CDIs, first recurrences, and in-hospital deaths remained at a stable high and did not change significantly.[8] The most recent CDC data show that mortality continues to be an ongoing challenge, as 1 in 11 patients older than 65 years die within 1 month of diagnosis of health care–associated CDI each year.[9]

Previously, CDI was believed to be primarily a hospital-acquired disease; however, recent data suggest that up to 40% to 50% of all CDIs can be community acquired.[2,10] CDIs are occurring in patients without traditional risk factors, such as advanced age and recent antibiotic use, at increased rates.[5,6,11,12] Of major concern in the treatment of CDI is relapse, with approximately 1 in 6 patients experiencing recurrent infection in the 2 to 8 weeks following an initial episode.[9,13] In patients with at least 1 recurrence, the risk of subsequent recurrences increases to 45% to 65%.[14] This often precipitates a cycle involving escalation in antibiotic intensity and frequency, with significant morbidity and mortality.[15]

C difficile has major implications not only clinically but also economically for an already strained US health care system.[16] In 2014, health care costs associated with CDI in US hospitals, long-term care facilities, and long-term acute care hospitals were estimated at $4.7 billion, while the estimated cost of CDI in the community was $725 million, totaling approximately $5.4 billion. Previous studies from 1997 to 2012 estimated attributable costs of $5.4 to $6.3 billion per year in the United States for CDI.[17] In health care facilities, an estimated $3.2 billion is spent on initial CDI and $1.5 billion on recurrent episodes.[18] Other studies have estimated the annual cost associated with recurrent CDI (rCDI) in the United States to be $2.8 billion.[19] Estimates of global economic burden are not clear but likely far exceed the aforementioned costs.

The clinical and economic burden of CDI is of major concern both in the United States and globally. Preventing CDI recurrence has become a key focus in ongoing studies due to the increased mortality rates and costs to health care institutions associated with recurrent infections. This article reviews the pathophysiology and current treatment landscape of CDI and explores an alternative paradigm to the treatment of rCDI, with evaluation of microbiota-based therapeutics, especially the novel standardized microbiota restoration therapies (MRTs).

Pathophysiology

C difficile is a gram-positive anaerobe capable of forming spores resistant to antibiotics, heat, and alcohol-based sanitization. These spores can remain viable for up to 5 months on dry surfaces, and have the capacity to rapidly spread in settings where proper cleaning methods are not used.[20] Infection with *C difficile* follows 3 phases: transmission of spores, disturbance to the gut microbiome, and colonization and proliferation in the GI tract resulting in an immune-mediated response to exotoxin production.

In the environment, *C difficile* exists as a dormant spore, with host transmission following oral ingestion. In the community setting, this is typically via the fecal-oral route, whereas in the hospital setting, it is usually secondary to contact with contaminated surfaces. Normally, *C difficile* is kept in low numbers by an intact gut microbiome through competition for nutrients and attachment sites,[1] as well as production of bacteriocins, which directly inhibit *C difficile* proliferation.[21] Exposures or diseases that decrease the diversity of the gut microbiome are potential risk factors for *C difficile* proliferation and toxin production within the GI tract, leading to CDI.[5,22]

There are numerous risk factors associated with CDI. All are linked to dysbiosis of the gut microbiome, typically characterized by a loss in species diversity. The main risk factor for developing CDI is exposure to antibiotics. The risk varies by antibiotic class; the highest risk is associated with clindamycin, fluoroquinolones, third- or fourth-generation cephalosporins, and carbapenems.[23,24] Antibiotics significantly alter the commensal intestinal flora that normally suppress *C difficile* overgrowth. *C difficile* is resistant to many commonly used antibiotics because of the loss of competitive inhibition and competition for nutrients as well as changes in the microenvironment resulting from collateral antibiotic damage to commensal flora, which has opened a new ecologic niche that allows for *C difficile* proliferation.[25] Other risk factors associated with an altered gut microbiome include advanced age (≥65 years),[26] kidney disease,[27] inflammatory bowel disease,[28] chemotherapy,[29] obesity,[30] and gastric acid suppression through use of proton pump inhibitors[31,32] or gastric bypass surgery.[33]

Once *C difficile* gains a niche for proliferation, it can enter its vegetative form to elicit infection.[34] It is worth noting that *C difficile* exists in toxigenic and nontoxigenic strains, and only toxigenic strains cause CDI.[35] The main virulence factors in toxigenic strains of *C difficile* are endotoxins A and B.[36] Toxin A is an enterotoxin that induces cytoskeleton disruption of enterocytes, leading to apoptosis.[37] Toxin B is a cytotoxin that exerts its apoptotic effect through pore formation in the enterocyte membrane.[38] A third endotoxin, binary toxin,[39] is thought to increase the virulence of toxins A and B. All 3 toxins are proinflammatory and capable of producing a clinical syndrome ranging from mild diarrhea to life-threatening conditions, including pseudomembranous colitis and toxic megacolon.[40]

Table 1. Antibiotic and Antibody Treatment Recommendations for CDI

Classification by severity and recurrence	Antibiotic or antibody therapy	Dosage and duration
Nonfulminant infection[a]		
Initial episode	Vancomycin	125 mg PO 4 times daily for 10-14 days
	Fidaxomicin	200 mg PO twice daily for 10 days
	Bezlotoxumab	Single 10 mg/kg infusion (if ≥1 risk factor for recurrence)[b] in addition to vancomycin or fidaxomicin
First recurrence[c]	Vancomycin	Pulse-tapered PO vancomycin for 6-8 weeks
	Fidaxomicin	200 mg PO twice daily for 10 days
	Bezlotoxumab	Single 10 mg/kg infusion in addition to vancomycin or fidaxomicin
Second or subsequent recurrence[c]	Vancomycin	Pulse-tapered PO vancomycin for 6-8 weeks
	Vancomycin + rifaximin	Vancomycin 125 mg PO 4 times daily for 10 days followed by rifaximin 400 mg 3 times daily for 20 days
	Fidaxomicin	200 mg PO twice daily for 10 days
	Bezlotoxumab	Single 10 mg/kg infusion in addition to vancomycin or fidaxomicin
Fulminant infection[a]		
Initial episode or recurrence	Vancomycin	500 mg PO 4 times daily for 10-14 days
	Metronidazole	500 mg IV 3 times per day for 10-14 days

CDI, *Clostridioides difficile* infection; IV, intravenous; PO, oral.

[a]CDI is considered fulminant if hypotension, shock, paralytic ileus, and/or toxic megacolon is present. CDI in patients without any of these findings is classified as nonfulminant and is treated based on the number of recurrent episodes.
[b]Risk factors for recurrence include age >65 years, CDI in the past 6 months, current systemic antibiotic use, fulminant CDI, and medical comorbidities (eg, inflammatory bowel disease and therapeutic or pathologic immunosuppression).
[c]Antibiotic choice should be based on antibiotics used during prior infection to avoid exposure to the same agent.

Recurrent Infection

Following an initial CDI, patients can experience recurrent disease secondary to relapse from the initial infection or to infection by a new strain of *C difficile*.[41] Although the pathophysiology underlying recurrence is still largely unknown, it is thought that recurrence is secondary to sustained disturbance of the gut microbiota following initial antibiotic therapy, as antibiotics used to treat CDI can lead to further gut microbial dysbiosis.[14] Altered bile acid metabolism,[42] poor antibody response to *C difficile* toxins,[43] and other factors, similar to the factors that increase risk of initial infection such as advanced age (≥65 years) and proton pump inhibitor usage, are also associated with rCDI.[44,45]

Current Treatment Landscape

The existing paradigm in treating CDI is centered on antibiotic therapy. Generally, all patients with symptomatic disease are recommended to receive antibiotics, with type and duration dependent upon severity of CDI and number of recurrent episodes.[46,47] The first step when deciding on treatment of CDI is to determine whether the patient meets the criteria for fulminant infection. CDI is considered fulminant if hypotension, shock, paralytic ileus, and/or toxic megacolon is present. CDI in patients who do not have any of these findings is classified as nonfulminant and treated based on the number of recurrent episodes.

Antibiotic and antibody treatment recommendations for CDI are listed in Table 1. In patients with fulminant disease, high-dose oral vancomycin and intravenous metronidazole are recommended.[46] First episodes of nonfulminant CDI are treated with the oral antibiotics vancomycin or fidaxomicin.[46] Fidaxomicin has similar outcomes in treating initial CDI and is associated with significantly lower risk of recurrent infection when compared with vancomycin.[48,49] Generally, a 10-day course is recommended for nonfulminant cases.

rCDI is defined as a positive stool specimen with reappearance of symptoms (loose or watery stools for 2

days) within 8 weeks of prior resolution.[46] In patients with nonfulminant CDI experiencing their first recurrent episode, pulse-tapered oral vancomycin for 6 to 8 weeks or oral fidaxomicin for 10 days is recommended. In patients with more than 1 recurrence, pulse-tapered oral vancomycin for 6 to 8 weeks, standard-dose oral vancomycin for 10 days followed by rifaximin for 20 days, or fidaxomicin for 10 days unless used for prior episodes is recommended. In these patients with more than 1 recurrence, evaluation for fecal microbiota transplantation (FMT) should be considered.[50] Notably, FMT has been shown to be more effective for the treatment of rCDI than both vancomycin[51] and fidaxomicin.[52] Further, *C difficile* strains with decreased susceptibility to vancomycin have started to emerge.[53]

In patients with rCDI, a one-time infusion of bezlotoxumab, a monoclonal antibody against *C difficile* toxin B, can be given concurrently with antibiotics.[54,55] Additionally, bezlotoxumab can be added to standard antibiotics in patients with CDI and more than 1 risk factor for recurrence, including CDI within the last 6 months, age 65 years or older, immunocompromised status, and severe infection (white blood cell count >15,000 cells/μL or creatinine >1.5 mg/dL).[50]

The Microbiome and *C difficile*

The human GI tract is home to an estimated 100 trillion microorganisms, including bacteria, archaea, fungi, and viruses.[56] The collective gene expression of these microorganisms is termed the microbiome, which contributes 10- to 360-fold more genetic information than the human genome.[57] These microorganisms make up a robust community that plays an important physiologic role in host biology, including nutrient digestion, endocrine signaling, vitamin and hormone synthesis, and bile acid metabolism.[58] As mentioned earlier, the native microbiota inhibit proliferation of foreign strains of bacteria, such as *C difficile,* through competition for nutrients, niche occlusion on gut wall attachment sites, production of antimicrobial metabolites, and alteration of bile acid composition.[57,59]

Although the gut microbiota can be health-promoting in a setting of balanced symbiosis, deleterious effects can occur in the setting of gut microbiota disruption (dysbiosis). Alteration of the composition and function of the microbiome can result from age, diet, acid-suppressing medications, systemic illness, and most notably broad-spectrum antibiotic use.[57,60] Ultimately, exposures or states that alter the underlying gut microbiome can be an attributable risk factor for CDI.

Specific changes in gut microbiota composition that occur in CDI include reduction in Firmicutes and Actinobacteria.[61] These bacteria normally inhibit *C difficile* from adhering to the intestinal epithelium. Additionally, these microbes have direct antimicrobial action on *C difficile* through induction of a proinflammatory response and production of antimicrobial peptides.[62,63] Dysbiosis-induced shifts in microbial metabolism are also not without consequence.[64,65] Loss of diversity leads to decreased bacterial transformation of primary bile acids into secondary bile acids. A high level of secondary bile acids normally inhibits *C difficile* spore germination; however, this shift in composition allows germination and pathogenic proliferation of *C difficile* spores.[42,66] This alteration of bile acid composition is a known risk factor for both initial CDI and rCDI.[67,68] Another concerning shift in microbial metabolism associated with gut dysbiosis is a decrease in microbial-produced short-chain fatty acids (SCFAs). SCFAs have been shown to directly inhibit *C difficile* growth through multiple in vivo and in vitro pathways.[69,70]

Fecal Microbiota Transplantation

In patients being treated with antibiotics for CDI, up to 30% will experience at least 1 episode of rCDI, with risk of recurrence increasing in each subsequent episode.[46,71] Notably, patients with rCDI have reduced species diversity in the gut microbiota, and antibiotic-induced changes can persist over time.[61,72] Despite evidence suggesting that microbiome disruption is a crucial pathophysiologic factor in CDI and rCDI, microbiota restoration is not currently included in the routine treatment of CDI.

The most used method for gut microbiota restoration is FMT, which involves the instillation of donor stool into the GI tract of a recipient. Current guidelines recommend use of FMT in CDI for patients experiencing 3 or more episodes of nonsevere CDI treated with appropriate first-line therapy (vancomycin or fidaxomicin) and 2 or more prior episodes of CDI requiring hospitalization.[46] Additionally, FMT can be considered when patients have severe CDI or fulminant CDI with insufficient clinical improvement after 48 to 72 hours of maximal medical therapy.[46,73]

Success and Pitfalls

Largely, microbiome restoration with FMT has been a success in the treatment of rCDI. In one study of rCDI, success rates of 90% with FMT and 40% with standard antibiotic regimens were seen.[74] In another study, clinical resolution of rCDI was seen in 92% of patients receiving FMT compared with 42% and 19% of patients who received fidaxomicin and vancomycin, respectively.[52] A review of 317 patients showed resolution of rCDI and persistent disease in 92% of cases in patients receiving FMT.[75]

Table 2. Microbiota Restoration Therapies for Prevention of Recurrent *Clostridioides difficile* Infection

Composition and route of administration	Product	Recurrence	Current status
Stool-derived enema	Fecal microbiota, live-jslm; formerly RBX2660	Second/third	Received FDA approval for prevention of recurrence
Stool-derived oral capsule	CP101	Second/third	Clinical development has been discontinued
	MET-2	Second/third	Phase 2 is being planned
	RBX7455	Second/third	Phase 3 is being planned
	Fecal microbiota spores, live-brpk; formerly SER-109	Third	Received FDA approval for prevention of recurrence
Synthetic oral capsule	SER-262	First	No active trials
	VE303	First/second/third	Phase 3 is underway

FDA, US Food and Drug Administration.

A systematic review of 480 patients treated with FMT for rCDI found a cure rate of 85% across all studies.[76]

FMT not only has clinical efficacy in treating rCDI, but also has the ability to restore or increase gut microbiota diversity, restore colonization resistance, and increase secondary bile acids—important pathophysiologic components underlying recurrent infection.[51,77-79] This restoration of protective gut function can help break the cycle of rCDI by reinstituting competitive resistance to *C difficile* spore germination and proliferation. Importantly, these changes are noted to persist for years after treatment.[80,81]

Despite its demonstrated efficacy, there are several important considerations when assessing FMT as a therapeutic modality in the treatment of rCDI. First, FMT has no standardized protocol, and its implementation often varies by institution.[82] Without a standardized methodology, there are variations in donor recruitment, screening, and testing as well as stool handling, processing, and composition, adding heterogeneity to FMT and the treatment of rCDI. Stool contains far more than just microorganisms; bile acids, SCFAs, and proteins also can influence colonization and resistance to *C difficile*. Access to FMT is equivocal across institutions, and establishing and managing a stool bank is logistically difficult. Further, most protocols call for stool to be used within 6 hours following defecation or thawing if frozen.[60] To date, FMT is not approved by the US Food and Drug Administration (FDA) and is used under enforcement discretion.

Beyond quality control issues, there are also safety concerns. Minor side effects primarily affecting the GI tract typically resolve in days to weeks and include abdominal discomfort and altered bowel patterns (diarrhea and constipation).[83] Serious side effects are less common; however, they currently provide the greatest limitation to FMT therapy.[84] Of greatest concern is transmission of infectious agents from donor stool to the recipient. Accordingly, donor stool requires extensive screening for multidrug-resistant organisms and enteric pathogens, although systemic screening for bloodborne infection should also be done. Most recently, this has included exclusion of patients with emerging diseases, such as COVID-19 and monkeypox.[85,86] Additionally, individuals with comorbid diseases linked to microbiome disruption, such as obesity, type 2 diabetes mellitus, cancer, and inflammatory bowel disease, should be excluded.[84]

Despite extensive screening measures, transmission of infectious agents from asymptomatic donors during FMT has been previously reported.[87] The FDA recently reported on 6 patients in whom enteric infections developed following FMT for rCDI.[88] These events were related to improper screening of donor stool.[89] These safety concerns and others have been thoroughly addressed by the FDA.[90,91]

Outside of infection risks, there are procedural risks associated with FMT. Instillation of donor stool is commonly performed via colonoscopy, which introduces the risk of bleeding and perforation. Similar risks occur with endoscopic administration. Patients must be able to safely undergo these procedures.[60]

Standardized Microbiota Restoration Therapies

Despite the therapeutic efficacy of FMT in the treatment of rCDI, many questions remain unanswered, and large-scale trials are needed to address these issues. The heterogeneity of the current practice is a deterrent to scalability

and approval of FMT. Many of the risks associated with FMT are related to the wholesale transfer of stool from donor to recipient. To address these challenges, standardized MRTs have been developed, allowing for scaling of therapy and control over the entirety of the transplanted material. Ideally, these therapeutics will provide improved mechanistic insight, allowing for more targeted, refined therapy in the future.

MRTs can be categorized into 3 groups: stool-derived enema-based therapy, stool-derived oral therapies, and synthetic (nonstool-derived) oral therapies (Table 2). These therapies standardize the aspects of FMT, leading to an elimination of the heterogeneity of donor screening, testing, and stool processing. Many of these therapeutics contain a defined set of bacteria, limiting the broad untargeted restoration approach seen with FMT. Ideally, this will provide novel insight into understanding the mechanisms behind the efficacy of microbiome repletion in rCDI and prevent many of the safety concerns seen in FMT. Further, this standardized approach will allow for scaling of clinical trials to assess the efficacy of microbiome restoration more thoroughly.

Stool-Derived Enema-Based Therapy

The FDA has approved the stool-derived enema-based therapy fecal microbiota, live-jslm (Rebyota, Rebiotix), formerly RBX2660, to treat rCDI. Initially, a phase 2 trial of fecal microbiota, live-jslm assessed participants with 3 or more episodes of CDI but did not reach statistical significance between active product and placebo; there were no differences between 1 and 2 doses.[92] A follow-up phase 3 trial enrolled patients with 2 or more episodes of rCDI treated with standard-of-care antibiotics or patients with 2 or more episodes of severe CDI requiring hospitalization. Upon Bayesian analysis (incorporating data from the phase 2 trial), the phase 3 trial showed statistically significant resolution of CDI at 8 weeks in patients receiving the enema MRT, when compared with placebo (70.6% and 57.5%, 99.1% posterior probability of superiority).[93] In both trials, the enema MRT significantly increased diversity of the fecal microbiome when compared with baseline measurements, with composition reflecting that of fecal microbiota, live-jslm. Data demonstrated that these changes were durable to 24 months after treatment.[88,94] Safety profiles were similar between placebo and the enema MRT. Published data suggest similar efficacy.

Stool-Derived Oral Therapies

CP101 (Finch Therapeutics) is a stool-derived oral capsule made up of a broad consortium of microbiota. A phase 2 trial (PRISM3) enrolled adults who had experienced 3 or more episodes of CDI or those with 2 or more episodes of CDI deemed high risk for recurrence (aged ≥65 years). All patients had previously received standard-of-care antibiotics. At both 8 weeks and 24 weeks posttreatment, CP101 provided a statistically significant improvement in the prevention of rCDI compared with placebo. Patients treated with CP101 also showed significantly increased microbiome diversity when compared with placebo at week 1, which was sustained at 8 weeks. A follow-up phase 2 trial (PRISM-EXT) found that 80.3% of participants who received CP101 had absence of rCDI at 8 weeks and 78.8% of participants had absence of rCDI at 24 weeks. No treatment-related adverse effects were noted.[95] However, clinical development has been discontinued.

MET-2 (NuBiyota) is a stool-derived oral capsule with a consortium of 40 lyophilized commensal bacteria species. In a phase 1 trial, patients with 2 or more episodes of CDI in 12 months following standard oral antimicrobial therapy were given MET-2. At day 40, 79% of patients receiving MET-2 did not have rCDI, which increased to 95% 40 days after receiving a second dose. There were no serious adverse events or deaths seen in this trial. At 130 days, 84% of patients did not have rCDI, suggesting sustained response. Stool analysis showed increased microbiota diversity and increased abundance of MET-2–containing bacteria during final analysis when compared with baseline.[96]

RBX7455 (Rebiotix) is a stool-derived oral capsule created from the enema-based fecal microbiota, live-jslm, in which aliquots of the MRT are freeze dried into oral capsules. A phase 1 dose de-escalation study included patients with 2 or more episodes of CDI. At 8 weeks, rCDI was avoided in 90% of participants in the high-dose group, 80% of participants in the medium-dose group, and 100% of patients in the low-dose group. Microbiome analysis showed that patients demonstrating treatment response had microbiomes more similar to RBX7455. RBX7455 also had the added benefit of home administration.[97] A phase 3 trial is currently being planned.

The FDA approved fecal microbiota spores, live-brpk (Vowst, Seres Therapeutics), formerly SER-109, a stool-derived oral capsule composed of a defined consortium of Firmicutes spores, for rCDI. This is important as stool-derived consortia often do not have defined populations of microbiota, posing future regulatory challenges. Patients with 3 or more CDI episodes within 9 months were included in a phase 1 trial. At 8 weeks, 55.9% of patients receiving the oral MRT had CDI resolution, which was not significantly different from placebo (46.6%; P=.4). However, statistical significance was seen when patients were stratified by age. Assessment of the trial revealed that dosing was likely suboptimal and asymptomatic patients were misdiagnosed with the active infection group.[98] A follow-up phase 3 trial using a higher dose than the phase 2 trials targeted patients with 3 or more episodes of CDI

with the qualifying episode diagnosed with a toxin-based assay within 9 months. At 8 weeks, 88.9% of the participants receiving the oral MRT did not have rCDI when compared with 58.7% in the placebo group (P<.001). Bacterial species in fecal microbiota spores, live-brpk were detected in stool at 1 week, suggesting engraftment. Additionally, analysis of stool from patients treated with the oral MRT revealed increased secondary bile acid concentration, which is known to inhibit *C difficile* spore germination.[99]

Synthetic Oral Therapies

SER-262 (Seres Therapeutics) is a synthetic oral capsule composed of 12 strains of commensal bacteria in spore form. In a phase 1 trial, patients with 1 prior episode of CDI or prior resolution of CDI with antibiotic treatment were included. Although safety of SER-262 was comparable to that of placebo, this trial failed to show any significant difference between treatment groups, and clinical development has been halted.[100]

VE303 (Vedanta Biosciences) is a synthetic oral capsule containing 8 live *Clostridia* species. A phase 2 trial including patients with prior CDI or their first episode of CDI at high risk for recurrence (≥65 years) found significant reduction in rCDI episodes in 86.2% of participants receiving a high dose of VE303 and 54.5% in the placebo group (P=.007).[101] Phase 1a/b data in healthy individuals showed VE303 is safe and well-tolerated at all doses tested. Further, VE303 strains were detected in stool of participants, suggesting engraftment and increased microbiota diversity.[102] Importantly, the synthetic formulation creates opportunities for global level scaling of a standardized therapeutic and eliminates reliance on heterogenous donor stool. Additionally, VE303 is produced as a powdered product from bacterial cell banks that can be easily standardized and scaled. A phase 3 trial is underway.

Future Considerations for Microbiota Restoration Therapies

When assessing MRTs for rCDI, it is important to consider their fit in the current care model for CDI. Currently, only VE303 and SER-262 have been studied for use following 1 episode of CDI. Five MRTs have been studied for use following a second episode of CDI: fecal microbiota, live-jslm; CP101; VE303; MET-2; and RBX7455. All the MRTs (FDA approved and in development), with the exception of SER-262, have been studied for use in patients following a third recurrence. Future trials supporting the clinical effectiveness and cost-effectiveness of these novel therapeutics are warranted to further support the ideologic shift of introducing microbiome restoration earlier into the treatment of CDI.

Considering route of administration, both oral and enema/colonoscopy delivery of MRT have been effective at treating rCDI. Oral administration offers a noninvasive, lower-cost option that can be self-administered at home or in the outpatient setting. Home-based delivery may also mitigate contagious spread during active infection. In comparison, enema/colonoscopy delivery allows for direct intestinal inoculation, limiting precolonic digestive alterations. However, these routes are more invasive, potentially requiring sedation, and are associated with procedural complications such as bowel perforation and bleeding.

Current Recommendations for Live Biotherapeutics

Although indications differ by professional society, in general, FMT is recommended for patients with CDI or rCDI in whom antibiotic therapy had failed. MRTs are only indicated for prevention of rCDI in individuals who have previously completed antibiotic treatment. The newly FDA-approved MRTs are not indicated for treatment of active infection, and both FMT and MRT are not recommended as first-line therapies for initial episodes of CDI. In addition, no prophylactic options exist for primary CDI. Given the promising evidence of MRT in treating rCDI, future studies should explore its potential to treat initial CDI infections and assess its efficacy as a primary prophylactic agent for CDI, particularly in high-risk hospitalized patients, such as those who are receiving high-risk antibiotics, in an immunosuppressed state, or have a history of CDI. Clinical practice could be transformed by identifying hospitalized patients at high risk for CDI and prophylactically restoring gut microbial diversity using MRT before the risk of CDI increases during their hospitalization.

Conclusion

CDI and rCDI remain significant challenges in health care, each significantly contributing to morbidity and mortality. As the pathophysiology of CDI and rCDI becomes better characterized, evolution of treatment paradigms should follow, targeting the underlying gut microbiome disruption that serves as the nidus for infection. The emergence of *C difficile* strains with decreased susceptibility to vancomycin emphasizes the urgency of this paradigm shift in CDI treatment. Although restoration of microbial diversity through FMT has emerged as a promising therapy, it has limitations and notable risks. Emerging MRTs provide more standardized microbial reconstructions and a potentially safer alternative to FMT. Results are encouraging for the use of MRT in

rCDI treatment, with many studies showing significantly reduced rCDI at 8 weeks and restored gut microbial diversity. Further, many of these changes were sustainable over time, and safety profiles of MRT were similar to those of placebo controls. Although current antibiotic therapy is relatively effective in treating active CDI, the current care model does not address the dysbiosis underlying the disease. Use of MRT directly acts on the pathophysiology of CDI and provides a bright future in the therapeutic approach to CDI. Going forward, a balanced approach of antibiotic stewardship, gut microbiome restoration, and proper infection control will be needed to reduce the prevalence of CDI. Through incorporation of microbiome restoration earlier in treatment, transition from a reactive model to a more proactive, integrated model can be leveraged to not only improve patient outcomes, but also restore gut microbial balance, the sequelae of which could be remarkably profound.

Disclosures

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References

1. Burke KE, Lamont JT. *Clostridium difficile* infection: a worldwide disease. *Gut Liver.* 2014;8(1):1-6.
2. Guh AY, Kutty PK. *Clostridioides difficile* infection. *Ann Intern Med.* 2018;169(7):ITC49-ITC64.
3. Gerding DN, Johnson S, Peterson LR, Mulligan ME, Silva J Jr. *Clostridium difficile*-associated diarrhea and colitis. *Infect Control Hosp Epidemiol*. 1995;16(8):459-477.
4. Kelly CP, Pothoulakis C, LaMont JT. *Clostridium difficile* colitis. *N Engl J Med*. 1994;330(4):257-262.
5. Khanna S, Pardi DS. *Clostridium difficile* infection: new insights into management. *Mayo Clin Proc.* 2012;87(11):1106-1117.
6. Balsells E, Shi T, Leese C, et al. Global burden of *Clostridium difficile* infections: a systematic review and meta-analysis. *J Glob Health*. 2019;9(1):010407.
7. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: US Department of Health and Human Services, CDC; 2019.
8. Guh AY, Mu Y, Winston LG, et al; Emerging Infections Program *Clostridioides difficile* Infection Working Group. Trends in U.S. burden of *Clostridioides difficile* infection and outcomes. *N Engl J Med*. 2020;382(14):1320-1330.
9. Centers for Disease Control and Prevention. What is *C. diff*? https://www.cdc.gov/cdiff/what-is.html. Updated September 7, 2022. Accessed April 12, 2023.
10. Chitnis AS, Holzbauer SM, Belflower RM, et al. Epidemiology of community-associated *Clostridium difficile* infection, 2009 through 2011. *JAMA Intern Med.* 2013;173(14):1359-1367.
11. Khanna S, Baddour LM, Huskins WC, et al. The epidemiology of *Clostridium difficile* infection in children: a population-based study. *Clin Infect Dis*. 2013;56(10):1401-1406.
12. Khanna S, Pardi DS, Aronson SL, et al. The epidemiology of community-acquired *Clostridium difficile* infection: a population-based study. *Am J Gastroenterol*. 2012;107(1):89-95.
13. Cornely OA, Miller MA, Louie TJ, Crook DW, Gorbach SL. Treatment of first recurrence of *Clostridium difficile* infection: fidaxomicin versus vancomycin. *Clin Infect Dis.* 2012;55(suppl 2):S154-S161.
14. McFarland LV, Elmer GW, Surawicz CM. Breaking the cycle: treatment strategies for 163 cases of recurrent *Clostridium difficile* disease. *Am J Gastroenterol*. 2002;97(7):1769-1775.
15. Olsen MA, Yan Y, Reske KA, Zilberberg MD, Dubberke ER. Recurrent *Clostridium difficile* infection is associated with increased mortality. *Clin Microbiol Infect.* 2015;21(2):164-170.
16. Gupta A, Ananthakrishnan AN. Economic burden and cost-effectiveness of therapies for *Clostridiodes difficile* infection: a narrative review. *Therap Adv Gastroenterol.* 2021;14:17562848211018654.
17. Zhang S, Palazuelos-Munoz S, Balsells EM, Nair H, Chit A, Kyaw MH. Cost of hospital management of *Clostridium difficile* infection in United States-a meta-analysis and modelling study. *BMC Infect Dis*. 2016;16(1):447.
18. Desai K, Gupta SB, Dubberke ER, Prabhu VS, Browne C, Mast TC. Epidemiological and economic burden of *Clostridium difficile* in the United States: estimates from a modeling approach. *BMC Infect Dis.* 2016;16:303.
19. Rodrigues R, Barber GE, Ananthakrishnan AN. A comprehensive study of costs associated with recurrent *Clostridium difficile* infection. *Infect Control Hosp Epidemiol.* 2017;38(2):196-202.
20. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis.* 2006;6:130.
21. Rea MC, Dobson A, O'Sullivan O, et al. Effect of broad- and narrow-spectrum antimicrobials on *Clostridium difficile* and microbial diversity in a model of the distal colon. *Proc Natl Acad Sci U S A.* 2011;108(suppl 1):4639-4644.
22. Stecher B, Hardt WD. Mechanisms controlling pathogen colonization of the gut. *Curr Opin Microbiol.* 2011;14(1):82-91.
23. Owens RC Jr, Donskey CJ, Gaynes RP, Loo VG, Muto CA. Antimicrobial-associated risk factors for *Clostridium difficile* infection. *Clin Infect Dis.* 2008;46(suppl 1):S19-S31.
24. Slimings C, Riley TV. Antibiotics and hospital-acquired *Clostridium difficile* infection: update of systematic review and meta-analysis. *J Antimicrob Chemother.* 2014;69(4):881-891.
25. Deshpande A, Pasupuleti V, Thota P, et al. Community-associated *Clostridium difficile* infection and antibiotics: a meta-analysis. *J Antimicrob Chemother*. 2013;68(9):1951-1961.
26. Wu YL, Xu J, Rong XY, Wang F, Wang HJ, Zhao C. Gut microbiota alterations and health status in aging adults: from correlation to causation. *Aging Med (Milton).* 2021;4(3):206-213.
27. Liu F, Xu X, Chao L, et al. Alteration of the gut microbiome in chronic kidney disease patients and its association with serum free immunoglobulin light chains. *Front Immunol*. 2021;12:609700.
28. Issa M, Ananthakrishnan AN, Binion DG. *Clostridium difficile* and inflammatory bowel disease. *Inflamm Bowel Dis*. 2008;14(10):1432-1442.
29. Wei L, Wen XS, Xian CJ. Chemotherapy-induced intestinal microbiota dysbiosis impairs mucosal homeostasis by modulating toll-like receptor signaling pathways. *Int J Mol Sci*. 2021;22(17):9474.
30. Bishara J, Farah R, Mograbi J, et al. Obesity as a risk factor for *Clostridium difficile* infection. *Clin Infect Dis*. 2013;57(4):489-493.
31. Bruno G, Zaccari P, Rocco G, et al. Proton pump inhibitors and dysbiosis: current knowledge and aspects to be clarified. *World J Gastroenterol*. 2019;25(22):2706-2719.
32. Cao F, Chen CX, Wang M, et al. Updated meta-analysis of controlled observational studies: proton-pump inhibitors and risk of *Clostridium difficile* infection. *J Hosp Infect.* 2018;98(1):4-13.
33. Li JV, Ashrafian H, Sarafian M, et al. Roux-en-Y gastric bypass-induced bacterial perturbation contributes to altered host-bacterial co-metabolic phenotype. *Microbiome.* 2021;9(1):139.
34. Fettucciari K, Marconi P, Marchegiani A, Fruganti A, Spaterna A, Bassotti G. Invisible steps for a global endemy: molecular strategies adopted by *Clostridioides difficile. Therap Adv Gastroenterol*. 2021;14:17562848211032797.
35. Schäffler H, Breitrück A. *Clostridium difficile* — from colonization to infection. *Front Microbiol*. 2018;9:646.
36. Lyras D, O'Connor JR, Howarth PM, et al. Toxin B is essential for virulence of *Clostridium difficile. Nature.* 2009;458(7242):1176-1179.
37. Di Bella S, Ascenzi P, Siarakas S, Petrosillo N, di Masi A. *Clostridium difficile* toxins A and B: insights into pathogenic properties and extraintestinal effects. *Toxins (Basel).* 2016;8(5):134.
38. Abt MC, McKenney PT, Pamer EG. *Clostridium difficile* colitis: pathogenesis and host defence. *Nat Rev Microbiol.* 2016;14(10):609-620.
39. Cowardin CA, Buonomo EL, Saleh MM, et al. The binary toxin CDT enhances *Clostridium difficile* virulence by suppressing protective colonic eosinophilia. *Nat Microbiol.* 2016;1(8):16108.
40. Leffler DA, Lamont JT. *Clostridium difficile* infection. *N Engl J Med.* 2015;372(16):1539-1548.

41. Figueroa I, Johnson S, Sambol SP, Goldstein EJ, Citron DM, Gerding DN. Relapse versus reinfection: recurrent *Clostridium difficile* infection following treatment with fidaxomicin or vancomycin. *Clin Infect Dis.* 2012;55(suppl 2):S104-S109.
42. Allegretti JR, Kearney S, Li N, et al. Recurrent *Clostridium difficile* infection associates with distinct bile acid and microbiome profiles. *Aliment Pharmacol Ther*. 2016;43(11):1142-1153.
43. Solomon K, Martin AJ, O'Donoghue C, et al. Mortality in patients with *Clostridium difficile* infection correlates with host pro-inflammatory and humoral immune responses. *J Med Microbiol.* 2013;62(Pt 9):1453-1460.
44. Deshpande A, Pasupuleti V, Thota P, et al. Risk factors for recurrent *Clostridium difficile* infection: a systematic review and meta-analysis. *Infect Control Hosp Epidemiol*. 2015;36(4):452-460.
45. Garey KW, Sethi S, Yadav Y, DuPont HL. Meta-analysis to assess risk factors for recurrent *Clostridium difficile* infection. *J Hosp Infect.* 2008;70(4):298-304.
46. McDonald LC, Gerding DN, Johnson S, et al. Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis*. 2018;66(7):987-994.
47. Kelly CR, Fischer M, Allegretti JR, et al. Correction to: ACG Clinical Guidelines: Prevention, Diagnosis, and Treatment of *Clostridioides difficile* Infections. *Am J Gastroenterol*. 2022;117(2):358.
48. Louie TJ, Miller MA, Mullane KM, et al; OPT-80-003 Clinical Study Group. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *N Engl J Med*. 2011;364(5):422-431.
49. Okumura H, Fukushima A, Taieb V, Shoji S, English M. Fidaxomicin compared with vancomycin and metronidazole for the treatment of *Clostridioides (Clostridium) difficile* infection: a network meta-analysis. *J Infect Chemother.* 2020;26(1):43-50.
50. Johnson S, Lavergne V, Skinner AM, et al. Clinical Practice Guideline by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA): 2021 Focused Update Guidelines on Management of *Clostridioides difficile* Infection in Adults. *Clin Infect Dis.* 2021;73(5):e1029-e1044.
51. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile. N Engl J Med*. 2013;368(5):407-415.
52. Hvas CL, Dahl Jørgensen SM, Jørgensen SP, et al. Fecal microbiota transplantation is superior to fidaxomicin for treatment of recurrent *Clostridium difficile* infection. *Gastroenterology*. 2019;156(5):1324-1332.e3.
53. Darkoh C, Keita K, Odo C, et al. Emergence of clinical *Clostridioides difficile* isolates with decreased susceptibility to vancomycin. *Clin Infect Dis*. 2022;74(1):120-126.
54. Carlson TJ, Gonzales-Luna AJ, Garey KW. Fulminant *Clostridioides difficile* infection: a review of treatment options for a life-threatening infection. *Semin Respir Crit Care Med*. 2022;43(1):28-38.
55. Wilcox MH, Gerding DN, Poxton IR, et al; MODIFY I and MODIFY II Investigators. Bezlotoxumab for prevention of recurrent *Clostridium difficile* infection. *N Engl J Med*. 2017;376(4):305-317.
56. Qin J, Li R, Raes J, et al; MetaHIT Consortium. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* 2010;464(7285):59-65.
57. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. 2014;157(1):121-141.
58. Robinson CJ, Bohannan BJ, Young VB. From structure to function: the ecology of host-associated microbial communities. *Microbiol Mol Biol Rev.* 2010;74(3):453-476.
59. Nord CE, Kager L, Heimdahl A. Impact of antimicrobial agents on the gastrointestinal microflora and the risk of infections. *Am J Med*. 1984;76(5A):99-106.
60. Khanna S. Microbiota replacement therapies: innovation in gastrointestinal care. *Clin Pharmacol Ther.* 2018;103(1):102-111.
61. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile*-associated diarrhea. *J Infect Dis*. 2008;197(3):435-438.
62. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat Rev Immunol.* 2013;13(11):790-801.
63. Britton RA, Young VB. Role of the intestinal microbiota in resistance to colonization by *Clostridium difficile. Gastroenterology.* 2014;146(6):1547-1553.
64. Franzosa EA, Hsu T, Sirota-Madi A, et al. Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. *Nat Rev Microbiol*. 2015;13(6):360-372.
65. Smith AB, Jenior ML, Keenan O, et al. Enterococci enhance *Clostridioides difficile* pathogenesis. *Nature.* 2022;611(7937):780-786.
66. Mullish BH, Allegretti JR. The contribution of bile acid metabolism to the pathogenesis of *Clostridioides difficile* infection. *Therap Adv Gastroenterol.* 2021;14:17562848211017725.
67. Reed AD, Theriot CM. Contribution of inhibitory metabolites and competition for nutrients to colonization resistance against *Clostridioides difficile* by commensal Clostridium. *Microorganisms.* 2021;9(2):371.
68. Sun R, Xu C, Feng B, Gao X, Liu Z. Critical roles of bile acids in regulating intestinal mucosal immune responses. *Therap Adv Gastroenterol*. 2021;14:17562848211018098.
69. Ouyang ZR, Niu XR, Wang WG, Zhao JH. The role of short-chain fatty acids in *Clostridioides difficile* infection: a review. *Anaerobe*. 2022;75:102585.
70. Gregory AL, Pensinger DA, Hryckowian AJ. A short chain fatty acid-centric view of *Clostridioides difficile* pathogenesis. *PLoS Pathog*. 2021;17(10):e1009959.
71. Kelly CP. Can we identify patients at high risk of recurrent *Clostridium difficile* infection? *Clin Microbiol Infect.* 2012;18(suppl 6):21-27.
72. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol*. 2008;6(11):e280.
73. Kelly CR, Yen EF, Grinspan AM, et al. Fecal microbiota transplantation is highly effective in real-world practice: initial results from the FMT National Registry. *Gastroenterology*. 2021;160(1):183-192.e3.
74. Gupta A, Cifu AS, Khanna S. Diagnosis and treatment of *Clostridium difficile* infection. *JAMA.* 2018;320(10):1031-1032.
75. Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent *Clostridium difficile* infection. *Clin Infect Dis.* 2011;53(10):994-1002.
76. Kelly CR, Khoruts A, Staley C, et al. Effect of fecal microbiota transplantation on recurrence in multiply recurrent *Clostridium difficile* infection: a randomized trial. *Ann Intern Med*. 2016;165(9):609-616.
77. Grehan MJ, Borody TJ, Leis SM, Campbell J, Mitchell H, Wettstein A. Durable alteration of the colonic microbiota by the administration of donor fecal flora. *J Clin Gastroenterol*. 2010;44(8):551-561.
78. Sandhu A, Chopra T. Fecal microbiota transplantation for recurrent *Clostridioides difficile,* safety, and pitfalls. *Therap Adv Gastroenterol*. 2021;14:17562848211053105.
79. Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME J*. 2007;1(1):56-66.
80. Saha S, Mara K, Pardi DS, Khanna S. Durability of response to fecal microbiota transplantation after exposure to risk factors for recurrence in patients with *Clostridioides difficile* infection. *Clin Infect Dis.* 2021;73(7):e1706-e1712.
81. Broecker F, Kube M, Klumpp J, et al. Analysis of the intestinal microbiome of a recovered *Clostridium difficile* patient after fecal transplantation. *Digestion.* 2013;88(4):243-251.
82. Bafeta A, Yavchitz A, Riveros C, Batista R, Ravaud P. Methods and reporting studies assessing fecal microbiota transplantation: a systematic review. *Ann Intern Med.* 2017;167(1):34-39.
83. Saha S, Mara K, Pardi DS, Khanna S. Long-term safety of fecal microbiota transplantation for recurrent *Clostridioides difficile* infection. *Gastroenterology.* 2021;160(6):1961-1969.e3.
84. Dailey FE, Turse EP, Daglilar E, Tahan V. The dirty aspects of fecal microbiota transplantation: a review of its adverse effects and complications. *Curr Opin Pharmacol*. 2019;49:29-33.
85. Khanna S, Pardi D. Fecal microbiota transplantation for recurrent *Clostridioides difficile* infection: the COVID-19 era. *Am J Gastroenterol*. 2020;115(7):971-974.
86. Owais R, Iqbal M. Monkeypox and fecal microbiota for transplantation (FMT): an unprecedented risk? *Ann Med Surg (Lond)*. 2022;82:104779.
87. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N Engl J Med*. 2019;381(21):2043-2050.
88. Ferring and Rebiotix present landmark phase 3 data demonstrating superior efficacy of investigational RBX2660 versus placebo to reduce recurrence of *C. difficile* infection [press release]. Saint-Prex, Switzerland and Roseville, MN: Ferring Pharmaceuticals; May 21, 2021.
89. US Food and Drug Administration. Important safety alert regarding use of fecal microbiota for transplantation and risk of serious adverse reactions due to transmission of multi-drug resistant organisms. Silver Spring, MD: US Food and Drug Administration; 2019.
90. US Food and Drug Administration. Information pertaining to additional safety protections regarding use of fecal microbiota for transplantation—screening and testing of stool donors for multi-drug resistant organisms. Silver Spring, MD:

US Food and Drug Administration; 2019.
91. US Food and Drug Administration. Safety alert regarding use of fecal microbiota for transplantation and risk of serious adverse events likely due to transmission of pathogenic organisms. Silver Spring, MD: US Food and Drug Administration; 2020.
92. Dubberke ER, Orenstein R, Khanna S, Guthmueller B, Lee C. Final results from a phase 2b randomized, placebo-controlled clinical trial of RBX2660: a microbiota-based drug for the prevention of recurrent *Clostridioides difficile* infection. *Infect Dis Ther.* 2023;12(2):703-709.
93. Khanna S, Assi M, Lee C, et al. Efficacy and safety of RBX2660 in PUNCH CD3, a phase III, randomized, double-blind, placebo-controlled trial with a Bayesian primary analysis for the prevention of recurrent *Clostridioides difficile* infection. *Drugs.* 2022;82(15):1527-1538.
94. Orenstein R, Dubberke ER, Khanna S, et al. Durable reduction of *Clostridioides difficile* infection recurrence and microbiome restoration after treatment with RBX2660: results from an open-label phase 2 clinical trial. *BMC Infect Dis*. 2022;22(1):245.
95. Khanna S, Kelly CR, Louie T, et al. CP101, an investigational orally administered microbiome therapeutic, increases intestinal microbiome diversity and prevents recurrent *C. difficile* infection: results from a randomized, placebo-controlled trial. *Am J Gastroenterol.* 2021;116:S57. Abstract S131.
96. Kao D, Wong K, Franz R, et al. The effect of a microbial ecosystem therapeutic (MET-2) on recurrent *Clostridioides difficile* infection: a phase 1, open-label, single-group trial. *Lancet Gastroenterol Hepatol.* 2021;6(4):282-291.
97. Khanna S, Pardi DS, Jones C, Shannon WD, Gonzalez C, Blount K. RBX7455, a non-frozen, orally administered investigational live biotherapeutic, is safe, effective, and shifts patients' microbiomes in a phase 1 study for recurrent *Clostridioides difficile* infections. *Clin Infect Dis*. 2021;73(7):e1613-e1620.
98. McGovern BH, Ford CB, Henn MR, et al. SER-109, an investigational microbiome drug to reduce recurrence after *Clostridioides difficile* infection: lessons learned from a phase 2 trial. *Clin Infect Dis*. 2021;72(12):2132-2140.
99. Feuerstadt P, Louie TJ, Lashner B, et al. SER-109, an oral microbiome therapy for recurrent *Clostridioides difficile* infection. *N Engl J Med.* 2022;386(3):220-229.
100. Ford C, Litcofsky K, McGovern B, et al. 1503. Engraftment of investigational microbiome drug, SER-262, in subjects receiving vancomycin is associated with reduced rates of recurrence after primary *Clostridium difficile* infection (CDI). *Open Forum Infect Dis*. 2019;6(suppl 2):S547-S548.
101. Vedanta announces positive topline phase 2 data for VE303 in high-risk *C. difficile* infection and exercise of $23.8 million option by BARDA [press release]. Cambridge, MA: Vedanta Biosciences; October 5, 2021.
102. Dsouza M, Menon R, Crossette E, et al. Colonization of the live biotherapeutic product VE303 and modulation of the microbiota and metabolites in healthy volunteers. *Cell Host Microbe*. 2022;30(4):583-598.e8.