REVIEW

Microbiota-Based Live Biotherapeutic Products for *Clostridioides Difficile* Infection- The Devil is in the Details

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Abstract: *Clostridioides difficile* infection (CDI) remains a significant contributor to healthcare costs and morbidity due to high rates of recurrence. Currently, available antibiotic treatment strategies further disrupt the fecal microbiome and do not address the alterations in commensal flora (dysbiosis) that set the stage for CDI. Advances in microbiome-based research have resulted in the development of new agents, classified as live biotherapeutic products (LBPs), for preventing recurrent CDI (rCDI) by restoring eubiosis. Prior to the LBPs, fecal microbiota transplantation (FMT) was available for this purpose; however, lack of large-scale availability and safety concerns have remained barriers to its widespread use. The LBPs are an exciting development, but questions remain. Some are derived directly from human stool while other developmental products contain a defined microbial consortium manufactured ex vivo, and they may be composed of either living bacteria or their spores, making it difficult to compare members of this heterogenous drug class to one another. None have been studied head-to head or against FMT in preventing rCDI. As a class, they have considerable variability in their biologic composition, biopharmaceutic science, route of administration, stages of development, and clinical trial data. This review will start by explaining the role of dysbiosis in CDI, then give the details of the biopharmaceutical components for the LBPs which are approved or in development including how they differ from FMT and from one another. We then discuss the clinical trials of the LBPs currently approved for rCDI and end with the future clinical directions of LBPs beyond *C. difficile*.

Keywords: *Clostridioides difficile* infection, microbiome, microbiome therapeutic, indirect treatment comparison

Introduction

The study of bacteria and their role in disease has been queried by humans for centuries. In the last two decades, advances in genomic sequencing technology have accelerated understanding of the gut microecosystem and led to a surge in microbiome-based research.[1–3] The quest to harness the power of the human microbiome into a commercially available product has resulted in a proverbial gold rush in drug development. Biotechnology startups have worked to discover novel microbiome-based drug candidates, and those with exceptional promise have been acquired by large pharmaceutical companies with the capital to bring them to commercial development.[4,5] In 2022 and 2023, the Food and Drug Administration (FDA) issued the first approvals of such agents for the prevention of recurrent *Clostridioides difficile* infection (rCDI) in the United States (US).[6] These drugs, derived from the human gut microbiome, have been designated a new class of medication called live biotherapeutic products (LBPs) or microbiome-based therapeutics (MBTs). Prior to these drugs, the only option for rCDI was fecal microbiota transplant (FMT). The FDA defines an LBP as a biological product that: 1) contains live organisms, such as bacteria; 2) is applicable to the prevention, treatment, or cure of a disease or condition of human beings; 3) is not a vaccine; and 4) as a general matter, is not administered by injection.[7] FDA approval of two LBPs represents a major milestone in the path from bench to bedside for drug development; however, significant questions remain about this drug class. The LBPs currently approved or under investigation are either derived directly from human stool or contain a defined microbial component isolated from

human stool during development and then processed for manufacture ex vivo.[8–16] None of the LBPs have been studied either head-to head or against FMT in preventing rCDI. There is variability among the LBPs in their composition, biopharmaceutical science, dosage form design, and stages of development.[3,17] Lastly, the human clinical trials for these agents in preventing rCDI had heterogeneity in study design, patient population, and clinical endpoints which make their comparison difficult. In this review, we aim to clear up some of this confusion by discussing the LBPs and explaining how they differ not only from conventional FMT but from one another. First, we will explain the role of the gut microbiome in *Clostridioides difficile* infection (CDI) and the morbidity of its recurrence. Next, we provide a history of stool-based therapies and explain the differences between LBPs and FMT. Last, we discuss the biopharmaceutics and clinical trials of the LBPs currently on the market and being investigated for approval, as well as their future directions.

Methodology

The literature search for this review was completed on July 1st, 2023, utilizing the keyword, "difficile" in the title/abstract section of PubMed. To capture important future drugs for CDI in early clinical development, the clinical trial database was also queried utilizing, "difficile" in the "condition of disease" term search on August 1st, 2023. Detailed information about the National Clinical Trial (NCT) numbers cited in this manuscript can be searched on the clinicaltrials.gov database.[18] Therapies falling under the broad category of LBT or MBT were included as defined by the FDA definition.[7] Drugs and investigational agents for CDI or rCDI falling out of this category were excluded including the following: antibiotic containing therapies, novel small molecules under investigation as antibiotics for CDI, immunoglobulin-based therapies (monoclonal or polyclonal antibodies) including those aimed at neutralizing *C. difficile* toxins, inhibiting growth, or preventing germination to the adult vegetative form, charcoal-based colon-specific antibiotic inactivators, and therapeutic beta-lactamase inhibitors.

Role of the Gut Microbiome in Colonization Resistance and *Clostridioides Difficile* Infection

The microbiome of human mucous membranes constitutes a micro-ecosystem containing trillions of microorganisms including bacteria, fungi, viruses, and bacteriophages.[19,20] Over 1000 known species of bacteria reside in the human intestine, but upwards of 90% are from two main phyla; the Firmicutes (which include the *Clostridioides, Lactobacillus, Bacillus)*, and the Bacteroidetes which include *Bacteroides* species.[19,20] Disruption in these bacterial communities (dysbiosis) has been correlated to a broad range of negative health effects including gastrointestinal conditions like rCDI and inflammatory bowel disease (IBD), as well as extraintestinal conditions such as obesity and depression.[8,19–21] When healthy and balanced, this population of microorganisms provides resistance to colonization of the gut from exogenous pathogens through a variety of mechanisms known as colonization resistance. These microbiota compete for key nutrients, produce inhibitory bile acids and short-chain fatty acids, lower the enteric luminal pH, and produce bacteriocins.[20,22] This colonization resistance is crucial to understanding the pathophysiology of CDI and rCDI.[8,19,22,23]

The mechanism by which dysbiosis causes a loss of colonization resistance and predisposes to initial CDI and rCDI is increasingly being understood. Antibiotic use leads directly to dysbiosis by reducing the microbial diversity, or relative abundance of this ecosystem and is the most strongly associated risk factor for precipitating an initial episode of CDI.[24–27] Changes in sleep, diet, lifestyle, and immune senescence that occur during aging also modify the microbiome.[28] Gastric acid suppressant medications change the gut microecosystem pH and cause an imbalance in bacterial species resulting in increased proportions of *Enterococcus, Streptococcus*, and *Staphylococcus* species in older adults.[29] Bile acid metabolism is also crucial in the human gut because alterations in the concentrations of primary and secondary bile acids as a result of dysbiosis are directly related to the risk of developing CDI after ingestion of *C. difficile* spores.[30–35] Primary bile acids are produced by hepatocytes and converted into secondary bile acids by commensal anaerobic bacteria in the human intestine.[32–34] The concentrations of these bile acids substantially determine whether an ingested *C. difficile* spore will remain dormant or will germinate into its metabolically active vegetative form as a gram-positive anaerobic rod.[22,32–35] Hosts with higher levels of microbiome-derived secondary bile acids are more resistant to developing CDI after spore ingestion in both animal and human studies, whereas those with higher concentrations of primary bile acids are more

susceptible to CDI.[31–34] If the *C. difficile* spore germinates under the influence of primary bile acids, and the strain expresses genes for exotoxin production (Toxin A, Toxin B, or binary toxin), then active CDI occurs with symptoms ranging from mild diarrhea to toxic megacolon and death.[30] Thus, alterations in the commensal gut microbiome and its metabolites set the stage for pathogens like *C. difficile* to cause disease as well as recur.

Burden and Morbidity of Recurrent *Clostridioides Difficile* Infection

Standard treatment of CDI includes administering antibiotics to target *C. difficile* while supporting the patient with resuscitative measures and surgical assessment as needed.[36] Bezlotoxumab (a monoclonal antibody directed against *C. difficile* exotoxin B) offers protection against future recurrence by providing passive temporary immunity to toxin.[36,37] Unfortunately, recurrence is common because none of the standard treatment strategies correct the underlying pathophysiology of dysbiosis and loss of colonization resistance that led to CDI. Around 35% of patients who experience an initial CDI will go on to have rCDI, and approximately 65% with a first recurrence will experience additional recurrences.[38–40] Morbidity, severity of illness, and complications such as sepsis and the need for surgical intervention increase with each recurrent episode.[41] A 2021 analysis of claims data found that in patients with three or more recurrences of CDI, rates of subtotal colectomy or diverting loop ileostomy within a year were 10%.[41] These cases require considerable expenditure from patients and the health system with prolonged lengths of stay (median 33 days), yet have high rates of in-hospital mortality of 36–80%[42] Aside from the burden of critical illness and medical complications, the toll of ongoing diarrhea experienced by rCDI patients can be substantial. Ongoing diarrhea prevents patients from performing their usual activities and engaging in social events which leads to emotional distress and reduced quality of life.[43,44] A recent systematic review and cost synthesis analysis estimated the per-patient per-year rCDI attributable cost is $67,837 to $82,268.[45] Ultimately, CDI recurrence is common and increasingly morbid with each episode in a self-perpetuating cycle. The lack of a standardized easily obtained therapy aimed at breaking this cycle of recurrence by restoring eubiosis to the gut microbiome has long been a missing piece of CDI management.

Historical Context of Fecal Microbiota Transplantation and Live Biotherapeutic Products

In the 4th century AD, Chinese alchemist Ge Hong described a treatment for food poisoning made by mixing herbs with the feces of a healthy person who consumed a diet of grain and fruit.[46] Hong's description of this, "Yellow Soup", in his ancient text, "Baopuzi" (抱朴子), may have been the first recorded use of FMT.[46–48] In 1958, Eismann et al published the first scientific manuscript describing FMT in a case series of 4 patients with pseudomembranous enterocolitis cured using fecal enemas.[49] It would not be until the late 1970s that *C. difficile* was recognized as the pathogen responsible for antibiotic-associated diarrhea and pseudomembranous colitis.[50,51] Incidence rates and severity of CDI increased throughout the US in the 1990s and early 2000s when the hypervirulent NAP1 strain of *C. difficile* emerged as a major public health issue.[38,52] By 2013, CDI was deemed an urgent threat by the Centers for Disease Control and Prevention.[53] During this time, FMT re-emerged as a non-conventional solution for CDI and rCDI.

FMT is a heterogenous process that involves harvesting stool from a healthy donor and transplanting it into the gastrointestinal tract of a recipient patient. FMT can be given by rectal enema, nasogastric tube, esophagogastroduodenoscopy (EGD), colonoscopy, or filled capsules.[54–58] Medical centers began establishing FMT programs to meet patient demand; however, no standardized donor stool procurement process initially existed. Patients were asked to identify their own donors (often a healthy partner, friend, or family member).[59,60] As use of FMT increased, the FDA took note of the lack of regulation and safety concerns associated with this practice and determined that FMT met the legal definition of a "drug" (since it was being used to prevent, treat, or cure a human disease or condition).[61] In addition, since FMT had not been FDA approved for any clinical indication, it constituted an investigational drug that required providers to hold an investigational new drug (IND) permit.[61] This led to significant administration burdens, not only for physicians treating rCDI with FMT, but also for clinical pharmacists tasked with creating policies to store or procure FMT.[61] As a result, a joint society recommendation was released in 2013 from the Infectious Diseases Society of America (IDSA) and the American College of Gastroenterology (ACG) to guide the screening of donors and petition the FDA to relax its

enforcement of IND applications and their burden on physicians treating acutely ill patients.[62] Later that year, the IND application requirement was relaxed, and an IND was encouraged but not required for use of FMT to treat rCDI.[63] Even with the relaxation of absolute requirements for an IDN, this process remained heterogenous. First, donors and their stool are tested for a variety of infectious diseases and the donated stool is quarantined, then certain screening tests are repeated prior to administration to the recipient. The logistics of this process have proven to be difficult, costly, and time consuming; resulting in donors not being identified or stool being discarded after donation.[60,61,64] Furthermore, there was much heterogeneity in the manufacturing process for donated stool itself. FMT programs varied in where the donated samples were blended for administration (in a laboratory verses a clinical space), the time from donation to administration, the diluent choice (normal saline versus water), stool mass and volume administered, and infection control procedures.[64] As a result of these difficulties, stool banks (such as OpenBiome) emerged as a means to address the limitations of procuring stool with a heterogenous patient-selected donor model. OpenBiome offered a centralized facility to screen donors, process stool, and store FMT preparations for use by clinicians and researchers.[65,66] In order to meet FDA requirements, OpenBiome distributes investigational FMT preparations manufactured by the University of Minnesota under an IND application to physicians who are registered as, "Clinical Partners."[67] As part of the application, OpenBiome provides assurance of appropriate product storage and shipment, and clinicians must agree to assume the potential risk of any infectious agents not detected by the screening assays employed by OpenBiome and to notify the company within 24 hours of any adverse events.[67] Despite having this stool bank to obtain product, most academic physicians do not have the regulatory experience to partake in this process, and FMT has remained a second-line treatment for rCDI.[63] In addition, ongoing safety concerns of donor stool and a paucity of reliable access out of academic centers have further hampered FMT use.[68,69] The clinical space, oversight, and resources to run an FMT practice have limited it to large academic centers with research experience.[64,68] FMT has not traditionally been available for primary care doctors or sub-specialists caring for patients in rural areas without access to a larger medical center.

The clinical efficacy of FMT reported in the medical literature varies greatly. Over the preceding decades, a multiple of observational studies, systematic reviews, and clinical trials have been published giving FMT through various administration forms, to different patient populations, and for different indications (rCDI versus fulminant CDI). In terms of efficacy for rCDI, a meta-analysis of observational studies found efficacy rates of around 85%.[70] The efficacy rates of FMT in clinical trials have shown more variation. For example, FMT efficacy rates for rCDI were much higher than placebo in a 2016 randomized clinical trial (90% vs 62.5%, respectively).[71] Conversely, a clinical trial the following year showed efficacy rates of FMT given via enema for rCDI to be 44% versus 54% in a vancomycin taper group.[72] A subsequent systematic review and meta-analysis of FMT found overall lower cure rates of around 67% in randomized trials (95% CI, 54.2%–81.3%, p < 0.001).[73] Cure rates were even lower in a subgroup analysis of patients who received FMT via enema (66%) compared to colonoscopy (87%).[73] The evidence for treating fulminant CDI with FMT is sparse and limited to mostly case-reports or small case-series. In a 2021 systematic review and meta-analysis of FMT for fulminant CDI, only one of the ten included studies was a randomized trial.[74]

As mentioned previously, widespread use of FMT has also been limited by safety concerns. Safety events including gram-negative bacteremia and aspiration of feculent material have been reported.[75–78] In 2019, the FDA issued a safety alert after two immunocompromised patients developed invasive disease with extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* linked to FMT.[79,80] FMT centers began screening for ESBL organisms in subsequent donations as a result of this tragedy. A reactive strategy of responding by adding additional testing for infectious pathogens as they arise is less ideal than a proactive strategy; however, it is impossible to anticipate most emerging infections. SARS-CoV-2 and mpox (formerly monkey pox) have continued to disrupt FMT programs and limit the availability of FMT to the masses.[81] The logistic difficulties limiting the availability of FMT have led some patients to pursue dangerous do-it-yourself stool preparations with no physician oversight.[82]

Research and development of commercially available LBPs have emerged as a way to overcome these limitations by expanding access for patients outside of academic centers. It is exciting to imagine a future where a patient might simply undergo a single procedure such as a rectal administration or have specific bowel preparation before swallowing pills on an empty stomach and restore eubiosis; however, the biopharmaceutics needed to deliver these organisms outside of FMT remains quite complex. Most of the beneficial microbiota are obligate anaerobes or have various degrees of aerotolerance

which make engineering their survival outside of the stool or laboratory complicated.[83] Lyophilization (also known as freeze-drying) is used in biotechnology to preserve microorganisms and offered one possible solution for prolonging shelf stability of bacteria. Unfortunately, the membrane integrity and survival after lyophilization is suboptimal for many bacteria particularly for some gram negatives.[84] As a result, scientists have had to develop new technology or other solutions (such as using spores) to overcome these limitations and bring new products to market.

LBPs and FMT: Similarities, Differences, and the Intricacies of Biopharmaceutical Drug Development

There are two commercially available off-The-shelf FMT stool bank products available in the US; MTP-101LR (for rCDI) and MTP-101LF (for fulminant CDI) from Open Biome.[67] Both are delivered as a cryobag of 35 mL FMT for delivery via colonoscopy, sigmoidoscopy, enema, nasogastric tube or EGD, and neither are FDA approved.[67] The MTP-101LF suspension contains $\geq 5\times10^{11}$ bacteria while the MTP-101LF suspension for fulminant CDI contains $\geq 2.5\times10^{12}$ bacteria (5-fold higher number of bacteria).[85] Both contain human fecal matter sourced from qualified healthy traceable donors after screening for a panel of 29 transmissible pathogens including bacteria, viruses, and protozoa.[85] Some FMT centers in the US have used freezing techniques to lyophilize FMT for administration in capsules; however, none are commercially available drug products.[86–89]

Commercially available LBPs have the potential to increase patient access to therapies capable of restoring the microbiome, but there are still some open questions about this drug class. The goal of an LBP is to inoculate the recipient's intestinal microflora and restore eubiosis. Two broad differences to consider when comparing the LBPs to traditional FMT are the procurement process and the concept of selective manufacturing. Current LBPs are donor-derived (meaning they rely on thoroughly screening donor stool as the original source for their microbiologic components), while other developmental products are designed consortia (meaning they are cultivated in a laboratory).[15,90–92] It should be noted that this distinction can be somewhat blurred and difficult to discern due to the proprietary nature of manufacturing. For example, some products described as designed consortiums were originally derived from the stool of a healthy donor but then subsequent manufacturing is done through in vitro proliferation of purified intestinal bacterial cultures.[92,93] The donor-derived LBPs are similar to FMT in that they too require rigorous testing of donor stool to minimize the risk of transmitted pathogens. There are currently two donor-derived LBPs approved for rCDI; REBYOTA RBL™ (formerly RBX2660), VOWST, VOS ™(formerly SER-109), and two others which progressed into clinical trials but have since been halted from further development (RBX7455, and CP-101).[11–15,94] The biologic components of these agents, which vary greatly when compared to FMT and to each other, will now be discussed.

In November 2022, the first FDA approved LBP was REBYOTA (fecal microbiota, live – jslm), formerly RBX2660 from Rebiotix, a Ferring pharmaceuticals company. REBYOTA is manufactured from human fecal matter sourced from qualified healthy traceable donors after screening for a panel of 29 transmissible pathogens including bacteria, viruses, and protozoa.[95] The fecal microbiota suspension is filtered and processed in a pre-defined ratio with a solution of polyethylene glycol (PEG) 3350 and saline into a 150 mL enema. Each enema is verified to contain between 1×10^8 and 5×10^{10} colony forming units (CFU) per mL of fecal microbes including >1×10^5 CFU/mL of *Bacteroides*.[95] The product requires storage at −80°C and is then thawed and then administered rectally via retention enema. No bowel preparation before the enema is required and it is administered as a one-time dose by any HCP. The second FDA approved LBP is VOWST (fecal microbiota spores, live-brpk) formerly SER-109. In 2021, Seres and Nestlé Health Science entered into an agreement to commercialize VOWST in the US and Canada, with FDA approval following in April 2023.[96] VOWST is sourced from qualified donors after screening for pathogens; however, it is composed of spores rather than an isolated consortium of specific bacteria. The donated stool suspension is treated with ethanol to kill living vegetative organisms and the slurry is filtered to remove solids and isolate spores from the Firmicutes phyla such as *Bacilli* and *Clostridia*.[13,97] The purified spores are resistant to gastric acid and are formulated into capsules each containing between 1×10^6 and 3×10^7 Firmicutes spore CFU in glycerol and saline.[97] VOWST requires an initial dose of 10 ounces of magnesium citrate as a bowel washout for the previously consumed antibiotics, which is then followed by three consecutive days of four capsules orally once daily on an empty stomach.[97]

A third LBP which progressed to clinical trials, RBX7455, is from the same manufacturer of REBYOTA.[12] RBX7455 is derived from the approved RBX2660 suspension and treated with a proprietary formulation of lyoprotectant and

cryoprotectant excipients to stabilize the lyophilized bacteria at room temperature and against gastric acid into capsule form.[12] Phase 1 trials of RBX7455 (NCT02981316) were published in 2021 with plans for further study; however, subsequent trials have not occurred.[12] A fourth donor-derived LBP, CP101, was developed by Finch Therapeutics.[94] Finch had partnered with Takeda Pharmaceutical Company in 2019 to develop several microbiome-based products including products they patented as Full-Spectrum Microbiota ® and Rationally Selected Microbiota®.[98] CP101 was developed as a lyophilized capsule of, "full-spectrum microbiota containing diverse microorganisms", and received FDA designations as both a breakthrough therapy and fast track status.[94,98,99] The exact bacterial components and CFU per dose contained in CP101 were never specified; however, Finch does hold patents for *C. difficile* treatment containing a mixture of Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes.[100] CP101 was studied for rCDI and completed its Phase 2 trial (PRISM3) in 2021.[99] In January 2023, Finch announced the decision to discontinue the Phase 3 trial of CP101 and halt all further development.[101]

All of the donor-derived LBPs may potentially contain donor-derived food allergens and carry a risk of transmitting known infectious agents, though this risk is considerably mitigated and no cases of food allergen events have been published to date.[8,11,12,14,95,97] Similar to FMT, the donated stool used in LBPs is susceptible to emerging pathogens that may initially go undetected due to incorrect assay selection or a delay in recognition and development of a screening test.[102] The manufacturing process for LBPs requires additional procedures beyond deep-freezing such as lyophilization or inactivation with ethanol. Whether the risk of infectious transmission of an emerging pathogen will be lower or mitigated by these additional manufacturing steps is unknown. The package inserts of both REBYOTA and VOWST state that the exact mechanisms of action have not been established, though both are approved for use in rCDI based on safety and efficacy data which will be discussed.[95,97]

In contrast to both donor-derived LBPs, designed LBPs have standardized compositions processed by batch culture for individual strains of microbes that are rationally defined and combined in a specific formulation.[90,92] The term "rationally" designed or defined is often used by the companies to self-describe these agents based on the inclusion of only certain bacterial species selected for manufacture in a laboratory environment based on their proposed biologic functions. As of late 2023, all the designed LBPs (VE303, NTCD-M3, ADS024, MET-2, and SER-262) remain under clinical investigation. The first of these, VE303, was developed by Vedanta Biosciences based on the ability of commensal *Clostridium* species to increase secondary bile acids and short chain fatty acids associated with colonization resistance against *C. difficile*.[103,104] VE303 is a defined consortium of eight commensal strains of clonally derived and distinct *Clostridium* species (5 strains from *Clostridia* cluster XIVa, 2 from cluster IV, and 1 from cluster XVII) manufactured into an enteric capsule. Each capsule contains 1×10^8 CFU of lyophilized bacteria from each species for a total 8×10^8 CFU of bacteria in 400mg combined with sucrose, histidine, yeast extract, cysteine, and other excipients.[103,104] Clinical Phase 2 trials of VE303 are complete (NCT03788483). A phase 3 trial of VE303 was planned to initiate in 2023 but is yet to be listed on clinicaltrials.gov.

Another designed LBP under investigation is non-toxigenic *C. difficile* strain M3 (NTCD-M3), formerly known as VP20621. It was previously observed that strains of *C. difficile* lacking the genes for toxin production could prevent CDI upon exposure to a toxigenic strain in animal models and healthy human volunteers.[105,106] Takeda (Shire) pharmaceuticals completed Phase 2 trials in 2015 on an oral capsule formulation of NTCD-M3 (NCT01259726).[106] Initially, there was a concern about utilizing non-toxigenic strains such as NTCD-M3 due to in vitro data demonstrating that a toxigenic *C. difficile* strain was able to pass its toxin genes to a nontoxic strain CD37 via horizontal gene transfer.[107] In 2022, the passive gene transfer experiment was replicated on NTCD-M3 using the same toxin donor strain and no toxin gene transfer occurred.[108] Additionally, this phenomenon has not been documented in humans and was not reported in the phase 2 trials of NTCD-M3. A phase 1 colonization study using NTCD-M3 in healthy Dutch volunteers was planned to commence in 2023; however, no other trials of NTCD are currently registered (NCT05693077).

A third designed LBT, called MET-2, was developed by Microbial Ecosystem Therapeutics (a merger between Takeda Pharmaceuticals and NuBiyota LLC).[109,110] Prior to MET-2, MET-1 (formerly known as RePOOPulate) was developed as a stool substitute designed to be an alternative to FMT.[111] MET-1 contained a defined microbial consortium of 33 bacterial strains in 100 mL (3.5 x 10^9 CFU/mL) made from purified intestinal bacterial cultures originally derived from the stool of a single healthy human donor.[111] MET-2 contains a proprietary consortium of 40 species from strains which are then purified and combined as a lyophilized product into capsules for oral delivery.[93] Phase 1 trials of MET-2

for treating CDI were completed and published in 2021 (NCT02865616).[93] This oral lyophilized product falls somewhere between a donor-derived LBP and designed LBP in that the bacterial species were initially isolated from the stool of a healthy screened donor, but subsequently manufactured independently of the donor thus eliminating potential risks introduced by changes in donor health.[93,110] Phase 2 trials of MET-2 are planned.

A fourth designed LBP under active study is ADS024 (formerly ART24). ADS024 is a strain of *Bacillus velezensis* owned by Artugen Therapeutics which merged with Bacainn Therapeutics in 2022 to form Adiso Therapeutics.[112] Adiso classifies ADS024 as a single strain LPB, or SS-LBP, since it is composed of a single strain of *B. velezensis*.[113] *B. velezensis* has been shown to combat *C. difficile* through two main mechanisms; by directly killing it via inhibition of translation and membrane permeabilization and by reducing toxicity through proteases capable of degrading *C. difficile* toxin.[9] Phase 1 trials administering capsules containing lyophilized ADS024 were completed in October 2022 (NCT04891965). A February 2023 review of emerging CDI therapies notes that Artugen currently holds two patents on ADS024, one listing a composition of lyophilized ART24 spores and another composed of spores or a vegetative form of the bacteria along with edible legumes.[114–116] In addition, clinicaltrials.gov lists a study for *B. velezensis* designated DSM3384 (DifProtecTM) sponsored by Novozymes A/S, a subsidiary of the Novo Nordisk Foundation (NCT05606159).[117] DifProtec TM is patented as a probiotic capsule rather than an LBP, but analogous to ADS024 it contains *Bacillus velezensis*. A table summarizing the LBPs approved or in clinical trials including their formulation and administration parameters is summarized in Table 1.

Table 1 Live Biotherapeutic Products Approved or in Clinical Trials for Preventing Recurrent *Clostridioides Difficile* Infection

Product Type	Product name; *Company*	Composition And Storage Administration Parameters	Current Status
Donor-derived enema	**REBYOTA (RBX2660)**[11] *Rebiotix, Ferring Pharmaceuticals*	• Fecal microbiota, live-jslm • Rectal suspension containing between 1×10^8 and 5×10^{10} CFU per mL of fecal microbes including $>1\times10^5$ CFU/mL of *Bacteroides* • Requires storage at −80°C and is then thawed and administered via retention enema Dose: 150 mL rectal suspension one time	FDA Approved
Donor-derived oral capsule	**VOWST (SER-109)**;[15,91,118] *Seres Therapeutics*	• Fecal microbiota spores, live-brpk • Narrow consortium or Firmicutes spores derived from donated stool suspension which is treated with ethanol to kill living organisms and the slurry is then filtered to remove solids and isolate spores from the Firmicutes phyla • Formulated into capsules each containing 1×10^6 - 3×10^7 Firmicutes spore CFU • Requires 10 ounces of magnesium citrate as a bowel washout preparation • Dose: four capsules orally once daily on an empty stomach for three days	FDA Approved
	RBX7455[12] *Rebiotix, Ferring Pharmaceuticals*	• Fecal microbiota, live-jslm • Derived from RBX2660 suspension containing $\geq10^7$ live bacteria / mL and treated with a proprietary formulation of lyoprotectant and cryoprotectant excipients to stabilize the lyophilized bacteria at room temperature and against gastric acid in capsule form. • Delivered as doubly encapsulated V-caps® stored at 2 to 8°C until dispensing.	Phase 2 trial complete, halted further development
	CP-101[94,119] *Finch Therapeutics*	• Oral Full-Spectrum MicrobiotaTM (broad consortium) • Lyophilized capsule of, full-spectrum microbiota containing diverse microorganisms	Further development halted

(Continued)

Table 1 (Continued).

Product Type	Product name; *Company*	Composition And Storage Administration Parameters	Current Status
Designed oral capsules	**VE-303;**[103,104] *Vendanta Biosciences*	● A defined consortium of 8 commensal strains of clonally derived and distinct *Clostridium* species (5 strains from *Clostridia* cluster XIVa, 2 from cluster IV, and 1 from cluster XVII) ● Enteric capsules each containing 1×10^8 CFU of lyophilized bacteria from each species for a total 8×10^8 CFU of bacteria in 400mg combined with sucrose, histidine, yeast extract, cysteine, and other excipients	Phase 2 trial complete
	NTCD-M3 (VP20621);[105,120] *Shire pharmaceuticals*	● Enteric capsules containing a non-toxigenic *C. difficile* strain M3	Phase 2 trial complete
	MET-2;[93] *Microbial Ecosystem Therapeutics*	● A proprietary consortium of 40 commensal bacterial species from strains which are then purified and combined as a lyophilized product into capsules for oral delivery	Phase 1 trial complete
	ART24 (ADS024)[9] *Adiso Therapeutics*	● A single-strain of *Bacillus velezensis* lyophilized and administered via capsule	Phase 1
	DSM33864 DifProtec(TM)[117] *Novozymes A/S*	● A single strain probiotic dietary supplement capsule containing *Bacillus velezensis*	Phase = N/A
	SER-262[121] *Seres Therapeutics*	● Cultivated Eubacterial Spore Suspension, Encapsulated Ecobiotic® ● A rationally designed, multi-strain microbiome therapeutic produced synthetically by in vitro anaerobic fermentation to produce commensal bacteria in spore form	Phase 1 complete

Abbreviation: CFU, colony-forming units.

The definition of an LBP may seem straight forward, but the distinctions between the LBP products can be confusing for providers unfamiliar with this drug class. Some LBPs are composed of broad microbiota consortia (either spores or bacteria), while others contain a single phylum or even a single species.[12,93,94,99,110,122] Some LBPs are delivered via enema, while others are administered as oral capsules after lyophilization which may or may not require a bowel preparation before-hand. It makes logical sense to consider a product with a set number of bacterial species distinct from one with hundreds or thousands, however, there is no consensus on the where this distinction lies and LBPs currently exist on a spectrum. In a scientific sense, some LBPs may not be drastically different from an FMT, but in a practical sense these products are quite different. Lyophilization of an FMT for oral administration required patients to take large numbers of capsules (average 27–40) in one dose whereas the oral LBPs require far fewer capsules.[14,86] Furthermore, the FDA approved LBPs can be ordered like any other medication and produced in facilities capable of mass production using good manufacturing practice standards. They not only expand availability for patients but also transfer the oversight and liability in procuring a microbiome product from the physician to the drug manufacturer and FDA.[8,17] In a similar vein, the distinction between a narrow spectrum LBT verses a probiotic can also seem arbitrary. Single strain commensal bacterial such as *Lactobacillus* species have long been used by patients with gastrointestinal issues, yet these probiotic products have always been classified as dietary supplements rather than drugs since they have not undergone the FDA approval regulatory process. Similarly, DifProtec (which contains *B. velezensis*) is listed in the clinical trials website as a probiotic dietary supplement (NCT05606159). In contrast, ADS024 is classified as a single strain LBP, yet it contains only *B. velezensis* (NCT04891965). As the biopharmaceutics and clinical use of this heterogenous drug class expands, our definitions and categories of LBPs may continue to be refined over time.

Clinical Trials of the FDA Approved LBPs (REBYOTA and VOWST)

REBYOTA and VOWST were both recently FDA approved for prevention of rCDI based on clinical trial data demonstrating safety and efficacy.[11,14] Neither agent has been compared head to head or to bezlotoxumab or FMT in their ability to prevent rCDI. There was considerable heterogeneity between the trials leading to the FDA approval of these agents which must be discussed. REBYOTA (formerly RBX 2660) was studied in the PUNCH CD trials. This started with a phase 1 open-label, noncomparative study in 2016 showing that it was safe and effective in preventing rCDI in patients who had had two or more recurrences (at least 3 life time episodes), or two episodes requiring hospitalization.[123] After two enemas of REBYOTA, 87% (27/31) had no further recurrences at 8 weeks.[123] This study was followed by a multi-center open-label phase 2 (PUNCH CD2) trial including a similar patient population (≥2 previous rCDI episodes or ≥2 severe CDI requiring hospitalization).[124] One cohort of patients were given up to 2 doses of REBYOTA (143 participants received 2 doses and 6 participants received one dose) and compared to a cohort of historical matched controls. REBYOTA patients had 79% treatment success compared to 31% success in the historic group at 8 weeks (P<0.0001).[124] Durability of this protective response at 6, 12, and 2 years was demonstrated in a subsequent publication.[125] A phase 2B, placebo-controlled, dose-ranging study followed, showing favorable recurrence rates after a single dose of REBYOTA; in the per-protocol population, 19% (3/24) of participants had recurrence after one dose of drug and one dose of placebo, compared to 52% (13/31) of those who received two doses placebo (p = 0.017).[126] These results culminated in PUNCH CD3, the randomized, double-blind phase 3 trial comparing REBYOTA to placebo that gained FDA approval.[11] Inclusion criteria were less strict than the prior PUNCH CD trials in that patients had ≥1 previous recurrences (at least 2 lifetime episodes), or to recruitment challenges, the FDA proposed with an analysis of the primary endpoint (absence of CDI diarrhea at 8 weeks) using a Bayesian hierarchical model that borrowed information from the phase IIb trial, PUNCH CD2. Within 30 days of enrollment, participants had to have tested positive for CDI by polymerase chain reaction (PCR), enzyme immunoassay (EIA), or another assay. Notably, 73% of total patients had positive PCR testing at inclusion, and only 17% had positive glutamate dehydrogenase (GDH) (20% in the REBYOTA arm and 11.5% in the placebo arm). Statistical modeling showed that 71% of participants treated with REBYOTA and 58% of participants treated with placebo remained free of CDI recurrence through 8 weeks, meeting the threshold for superiority to placebo determined by Bayesian analysis.[11] This 13.1 percentage difference in blinded treatment success corresponds to a 99.1% posterior probability that REBYOTA is superior to Placebo (95% Credible Interval: 2.3, 24.0).[11] In the per protocol analysis, 72% (120/167) of REBYOTA patients had treatment success compared to 62% (48/78) of placebo patients.[11] The observed treatment difference at 8 weeks was maintained at 6 months across all analysis populations (ITT and per protocol). After confirmation of treatment failure, 65 participants (41 who had received REBYOTA and 24 who had received placebo) were subsequently given REBYOTA in an open-label treatment arm. Treatment success at 8 weeks occurred in 54% (22/41) in the group that had already received blinded REBYOTA and 63% (15/24) who had received blinded placebo.[11] Most adverse effects were mild to moderate gastrointestinal side effects such as abdominal pain, diarrhea, or bloating and were similar between drug and placebo. No new or unexpected events were reported and no pathogen transfer from donor to recipient, product-related significant adverse events, or procedure-related events occurred.[11] In a subsequent safety analysis from five prospective clinical trials (PUNCH CD, PUNCH CD2, PUNCH Open-Label) and two Phase III trials (PUNCH CD3, PUNCH CD3-OLS) including 978 patients, no cases of bacteremia, fungemia, or treatment-related infections occurred.[127] In a secondary analysis of PUNCH CD3 patients comparing quality of life scores at weeks 1, 4, and 8, REBYOTA treated patients showed significantly greater improvements in mental health domains than those receiving placebo.[128]

Notable limitations of the PUNCH CD3 trial include the high success rate in the placebo arm and the diagnostic inclusion criteria. One hypothesis for the high rates of placebo response is the influence of positive PCR as the most common diagnostic modality for inclusion. The PCR assay is for detecting toxin genes. This method can be overly sensitive since it cannot determine whether the genes are being transcribed and cannot

distinguish between living and dead organism containing the gene; none-The-less, patients presenting with diarrhea may have PCR testing as part of their diagnosis.[129] Seventy-three percent of participants included in PUNCH CD3 had positive PCR as their CDI confirmation method.[11] While this approach is less rigorous to avoid false-positive results, it is practical for real-world practice. About one-third of patients enrolled in PUNCH CD3 had had only one rCDI occurrence at inclusion and thus may have been at lower baseline risk for recurrence compared to cohorts with two or more recurrences prior to inclusion.[11] If included patients did not truly have rCDI due to the diagnostic testing, then this could perhaps explain the high rates of clinical success in the placebo group. Another limitation is the paucity of patients who were treated with fidaxomicin, which is known to have lower rates of recurrence compared to vancomycin and metronidazole.[36] The study randomization was stratified by antibiotics used for the qualifying CDI event (vancomycin alone, vancomycin in combination with another antibiotic, fidaxomicin alone, or other); however, the trial was conducted prior to more recently updated IDSA guidelines preferentially recommending fidaxomicin as first-line treatment over oral vancomycin both for the first episode of CDI and for rCDI. About 6% (17/267) of participants received fidaxomicin for their qualifying rCDI event.[11] Whether the clinical success of REBYOTA would be as robust in a cohort treated with fidaxomicin is unknown. Lastly, some patients who experience rCDI such as those with immunocompromise or IBD were excluded from participating; however, subsequent data has shown promising results for such patients.[130] Outcomes on 94 patients who were excluded from PUNCH CD3 due to immunocompromise or bowel disease conditions were later given RBX2660 under FDA discretion. Results presented at the ACG 2021 National Meeting showed efficacy of 83% and no significant safety events.[130]

VOWST (formerly SER-109) was studied in the ECOSPOR trials starting with ECOSPOR I, a phase 1 trial conducted at four US medical centers.[131] Participants in ECOSPOR I had ≥3 laboratory-confirmed CDI episodes in the previous 12 months. SER-109 manufactured for the trial was taken from seven adult donors after screening and processing which included a deep freeze to −80°C followed by homogenization, filtration, ethanol washing and several steps of centrifugation to isolate Firmicutes spores.[131] Twenty-six of 30 (87%) participants met the primary efficacy end point of no recurrence at 8 weeks. Three patients with self-limiting diarrhea and positive CDI testing had clinical resolution without antibiotics bringing overall clinical success to 97% (29/30 participants). The gut microbiota of participants were also studied to verify whether the Firmicutes spores had engrafted and confirmed a diversified microbiome and no outgrowth of non–spore-forming bacteria after VOWST treatment.[131] A significant setback occurred during ECOSPOR II, the subsequent phase 2 double-blind trial comparing VOWST to placebo in patients with 3 or more CDI episodes within 9 months. Rates of rCDI at 8 weeks were lower in the VOWST arm versus placebo (44% vs 53%), however, this did not meet statistical significance (NCT02437487).[13] This was attributed to the use of PCR testing for diagnosis in most participants, as well as engraftment kinetics which suggested that VOWST was suboptimally dosed (1×10^8 spores).[13] In the following phase 2b/3 trial (ECOSPOR III), the trial design and dose of VOWST were adjusted based on these previously observed issues. The daily dose of spores contained in the four VOWST capsules was increased three-fold (3×10^7 spores contained in a total of four capsules), and the doses were given for three consecutive days instead of once.[14,16] VOWST capsules were taken on an empty stomach after a bowel preparation of 10 ounces of magnesium citrate was administered the night before treatment initiation to limit inactivation of the spores. Participants had three or more episodes of CDI within 12 months, and the inclusive qualifying acute episode had to be diagnosed with a positive *C. difficile* toxin test by EIA or cell cytotoxicity neutralization assay.[14] CDI recurrence was significantly lower with VOWST compared to placebo at 8 weeks (12% versus 40%, respectively; $P<0.001$).[14] Results were consistent in analyses with stratification according to age and antibiotic received (vancomycin versus fidaxomicin). Vancomycin was given prior to VOWST in 133/182 (73%) patients, and fidaxomicin was given to 49/182 (27%). Risk of recurrence was reduced in the VOWST cohort regardless of previous antibiotic regimen (relative risk, 0.41 [95% CI, 0.22 to 0.79] with vancomycin and 0.09 [95% CI, 0.01 to 0.63] with fidaxomicin).[14] Durability and safety at 24 weeks were subsequently shown in an open-label single-arm trial. (ECOSPOR IV)[132,133] The most common significant adverse events were mild to moderate gastrointestinal complaints and were observed at rates similar to that of placebo.[14,132,133]

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A notable strength of ECOSPOR III was the strict inclusion criteria for toxin testing which ensured appropriate candidate selection and accuracy of the definition of recurrence; however, real-world diagnosis of rCDI is seldom this stringent. While encouraging, it is unknown if these robust results would be demonstrated in routine clinical practice. ECOSPOR IV attempted to address this by separating patients into two cohorts: one including ECOSPOR III rollover patients and another less strict cohort of de novo patients with at least 1 CDI recurrence diagnosed by any detection method.[132] This included 69/263 (26%) patients who had their rCDI episode diagnosed using PCR alone.[132] Rates of rCDI at 8 weeks were low, even in patients with a first recurrence (6.5%) or enrollment based on positive PCR results (4.3%).[132] Rates of fidaxomicin use were higher in ECOSPOR III than PUNCH CD3 (30% versus 6%, respectively), but neither trial was powered to detect superiority in only those patients.[11,14] Similar to the REYBOTA trials, patients who were immunocompromised or had IBD were excluded from the VOWST trials. Investigational use in an expanded access program is ongoing (NCT02437500).[134]

Given the heterogeneity in data used to approve these agents based on their performance against placebo, it is impossible to compare their clinical efficacy directly. Both appear to have only mild to moderate gastrointestinal side effects and no major safety events, though post-marketing surveillance is ongoing.[127,132] Some patients may be interested in an oral regimen that can be taken discreetly at their home, while others may prefer a one-time treatment in a clinical setting. A recent survey of LBP-naïve subjects with prior CDI showed that 87% were likely to consider a rectally administered treatment and that patients who had received a rectal LBP found it easy, quick, and appealing due to lack of bowel preparation.[135] Future financial analysis studies may show the high cost of these products ($17,500 USD for VOWST and $9100 USD for REBYOTA) is worth avoiding a high expenditure hospitalization for rCDI where the direct attributable medical cost can range from $67,837 to $82,268.[45,136] Budget impact analysis of potential cost savings for new LBPs is already being published.[137] Similar analyses advocating for bezlotoxumab have been performed and yet pharmacy budgets have not been increased to account for inpatient use and payors continue to impede prescribing it without prior authorization.[138,139] Whether an increased number of therapeutic options for rCDI will influence the costs of existing agents on the market remains to be seen. Ultimately, the choice of LBP selected for rCDI prevention will depend on patient preference, prescriber opinion, and cost. A summary of REBYOTA and VOWST clinical trial parameters is shown in Table 2.

Table 2 Clinical Trial Data for REBYOTA and VOWST

Trial Name (Drug)	Number of Prior CDI Episodes	Prior CDI Treatment	Diagnostic Test Used to Identify CDI	Antibiotic Use Through The Follow-Up Period (8 weeks)	Surgical and ICU Exclusions	Immunologic and Medical Exclusions
PUNCH CD3 (REBYOTA)	● Required ≥ 1 recurrence after a primary episode or ≥ 2 severe episodes within the last 1 year	● Standard-of-care antibiotic therapy (defined as 10 to 21 days of treatment with vancomycin [125 mg QID] and/or fidaxomicin [200 mg BID])	● Any positive stool test for the presence of toxigenic *C. difficile* within 30 days prior to enrollment	● Did not allow oral vancomycin, metronidazole, fidaxomicin, rifaximin, nitazoxanide or IVIG through the 8-week follow-up unless prescribed by a treating investigator during the study as a result of recurrent CDI diagnosis	● Excluded intra-abdominal surgery within the prior 60 days ● Did not exclude patients in the ICU	● Excluded patients with high medical risks and clinically significant co-morbid disease per the opinion of the investigator ● Excluded patients with ANC<1000 cells/ml^3

(*Continued*)

Table 2 (Continued).

Trial Name (Drug)	Number of Prior CDI Episodes	Prior CDI Treatment	Diagnostic Test Used to Identify CDI	Antibiotic Use Through The Follow-Up Period (8 weeks)	Surgical and ICU Exclusions	Immunologic and Medical Exclusions
ECOSPOR III (VOWST)	● Required ≥ 3 episodes of CDI within the previous 9 months, inclusive of the current episode	● Standard-of-care antibiotic therapy (defined as 10 to 21 days of treatment with vancomycin [125 mg QID] and/or fidaxomicin [200 mg BID])	● Positive C. difficile stool sample tested by a toxin assay preferably performed by the central laboratory	● Allowed antibiotic treatment. Patients were excluded at study start if projected to receive antibiotics during the 8-week period post-randomization	● Excluded major gastrointestinal surgery (eg Bowel resection / diversion) within 3 months prior to enrollment ● Excluded prior total colectomy or bariatric surgery (Prior appendectomy or cholecystectomy allowed) ● Excluded patients admitted or expected to be admitted to ICU	● Excluded patients with high medical risks and clinically significant co-morbid disease per the opinion of the investigator ● Excluded patients with ANC<500 cells/ml^3

Note: [a] This table only includes key inclusion/exclusion criteria that were determined to be different between the two trials. For full inclusion/exclusion criteria, please refer to the respective protocols of the trials.
Abbreviations: CDI, *Clostridioides difficile* Infection; ICU, Intensive Care Unit.

Future Directions and Conclusion

The LBPs are important new tools in our armamentarium against CDI, but some questions remain. FMT has traditionally only been prescribed by subspecialists in gastroenterology and infectious diseases, but LBPs can be prescribed by any provider. The impact of practitioners with little experience using microbiome-based treatments prescribing these drugs is unknown.[17] As we approach four years from the start of the SARS-CoV2 pandemic, we are left to wonder if an unknown pathogen may be encountered in the future which would require additional screening in the LBP manufacturing process. Bioscience companies and pharmaceutical companies have merged to gain the capital needed to bring LBPs to the market, but the exact components are sometimes proprietary emerging biotechnology. It is difficult to provide full information to patients for informed consent when some of these products are ambiguous about the exact species contained therein.

The LBPs continue to push the boundaries of their potential by branching into conditions beyond CDI. As of late 2023, there are clinical trials sponsored by several companies with a foothold in the LBP market. Ferring pharmaceuticals is studying RBX7455 before surgery in operable breast cancer (NCT04139993), Vedanta Biosciences is investigating VE303 for hepatic encephalopathy (NCT04899115), and VE 202 for ulcerative colitis (NCT05370885), and Seres therapeutics is studying SER-155 for preventing graft-versus host disease in hematopoietic stem cell transplantation (NCT04995653) and antimicrobial-resistant bacterial infections.[140–143] Early pre-trial exploration of the bidirectional relationship between the gut microbiome and other conditions including depression, obesity, and other malignancies are also already underway.[21,144]

LBPs such as REBYOTA & VOWST, appear poised to strike the perfect balance between traditional healing and emerging science; restoring eubiosis as nature intended while simultaneously giving patients the guarantee of safety that comes inherently with an FDA-approved medication. These products are an exciting addition to our limited options for rCDI. Whether their widespread availability will result in unfettered use is unknown. Post-marketing surveillance will be crucial for monitoring their appropriate use and detecting rare safety events or latent events not seen in clinical trials. Even the greatest breakthroughs in medicine must be tempered with cautious optimism, healthy skepticism, and ongoing data collection. As the idiom goes, *the devil is in the details*.

Disclosure

Dr Glenn Tillotson is a consultant for Ferring Pharmaceuticals, Spero Therapeutics, and Taro Pharmaceuticals, outside the submitted work. The author reports no other conflicts of interest in this work.

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