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Guardians of the Gut: Development and Use of Live Biotherapeutic Products for Preventing Recurrent Clostridioides Difficile Infection

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ABSTRACT

Clostridioides difficile infection (CDI) poses significant healthcare costs and morbidity due to its high recurrence rates. Reductions in gastrointestinal microbiome species heterogeneity from antibiotics or other causes result in a loss of colonization resistance that sets the stage for CDI. Antibiotics including vancomycin and fidaxomicin are the standard of care for primary and recurrent non-fulminant CDI (rCDI). Regardless of antibiotic choice, the likelihood of rCDI increases after each episode, and novel treatments are needed to prevent further recurrences. Microbiome research has led to the development of live biotherapeutic products (LBPs) to restore GI colonization resistance and break the cycle of rCDI. While derived from human stool, LBPs differ from fecal microbiota transplant (FMT) and one another. These agents vary in bacterial composition, manufacturing methods, administration routes, and other factors. The Food and Drug Administration has approved two LBPs to prevent rCDI and other LBPs remain in various stages of clinical development. These agents are an exciting addition to the options for preventing rCDI; however, questions remain. This review aims to summarize the development of LBPs and explain how they differ from FMT, explore their clinical utility for preventing rCDI, and address future perspectives and unanswered questions.

Keywords: Clostridioides difficile infection; Microbiome; Live biotherapeutic products

INTRODUCTION

Clostridioides Difficile Infection and the Burden of Recurrence

Clostridioides difficile, an anaerobic gram-positive spore-forming bacterium, accounts for 15%-20% of all cases of antibiotic-associated diarrhea [1]. With just over 220,000 cases annually resulting in approximately 13,000 deaths and accounting for $ 1 billion USD of healthcare costs, Clostridioides difficile infection (CDI) is a major public health concern [2]. C. difficile inhabits the gastrointestinal tracts of people of all age groups, including healthy individuals and those with chronic illnesses. Historically, CDI was associated with hospitalization or residence in a healthcare facility. Recent studies, however, have shown that 41% of CDIs are now acquired by patients in the community without recent hospitalization [3]. These patients may be younger and may or may not have been exposed to antibiotics but have often had other forms of health care exposure, such as visits to a dentist or urgent care center [3]. Prompt diagnosis and initiation of treatment are needed to prevent the development of manifestations such as pseudomembranous colitis, toxic megacolon, ileus, and septic shock [4]. Healthcare providers in the community are essential for diagnosing CDI in this diverse population beyond the hospital setting. Recurrent CDI (rCDI) is common, challenging, and burdensome. About 20%-35% of patients will experience a recurrent infection following an initial episode of CDI, and 40%-65% of patients with a recurrence will go on to have two or more rCDI episodes [5-12]. In addition, the incidence of rCDI has risen disproportionately to the incidence of CDI. Between 2001 and 2012, CDI increased by 46%, while rCDI rose by 189% [13]. The burden of this cycle is financially and clinically staggering.

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Not only do recurrences lead to increased healthcare costs for retesting, readmission, and retreatment, but they also lead to increasingly severe clinical outcomes. In a retrospective analysis of commercial claims data, Feuerstadt and colleagues found that 4.6% of CDI patients undergo colectomy within a year of index CDI, and this increases to 7.3% after the first rCDI and over 10% once a patient has three or more recurrences [14].

Dysbiosis and the Cycle of Recurrent Clostridioides Difficile

The diverse microbiome in the colon consists of approximately 2000 species amounting to trillions of bacteria, fungi, andviruses and 200,000 bacteriophages, which exist in a homeostatic condition known as eubiosis [15]. The gut microbiome provides resistance to colonization of the gut from exogenous pathogens through mechanisms known as colonization resistance. The composition and function of gut microbiota are influenced by age, genetics, environment, chronic disease, medication use, and diet [16]. Disruptions in this complex population of microorganisms (termed dysbiosis) can result in various imbalances in the human gut. In CDI, dysbiosis is characterized by the depletion of beneficial bacterial phyla (Firmicutes, Bacteroidetes, Actinobacteria) as well as an increase in Proteobacteria and alterations in butyrogenic and lactic acid-producing bacterial species [17]. This imbalance in species diversity and chemical microenvironment sets the stage for C. difficile spore germination, outgrowth, and toxin production [18,19]. C. difficile produces toxin A and toxin B, which cause disruption of the enteric cytoskeletal wall and the tight junction between colonic cells, leading to decreased transepithelial resistance and bacterial translocation into the lamina propria and systemic circulation [20]. In response to the C. difficile toxins, intestinal epithelial cells and innate immune cells in the lamina propria release proinflammatory cytokines (IL-12, IL-6, IL-23, IFNγ) and chemokines, which recruit neutrophils to the site of infection [21-24]. This immune response leads to CDI ranging from mild diarrhoea to toxic megacolon [25].

The antibiotics used to treat C. difficile (vancomycin, fidaxomicin, and metronidazole) can kill the bacteria due to their activity against gram-positive rods; however, they do not remove this underlying maladaptive process that sets the stage for infection. Furthermore, metronidazole and vancomycin lower indigenous flora and promote dysbiosis [26,27]. Fidaxomicin causes less dysbiosis due to its narrower antimicrobial profile; However, there has been a much-needed shift to explore alternative treatments that aim to prevent rCDI by restoring gut eubiosis [28]. This has led to a groundswell of microbiome-based research for rCDI, first with fecal microbiota transplantation (FMT) and now with the development of new agents classified as live biotherapeutic products (LBPs).

MICROBIOME-BASED TREATMENTS FOR CDI AND rCDI

History and Clinical Use of Fecal Microbiota Transplantation

FMT involves transferring processed stool from a healthy donor to the gastrointestinal tract of a diseased recipient. It aims to restore gut dysbiosis in CDI by introducing a diverse and healthy microbial community, including live microbes, bioactive compounds, dietary components, phages, and metabolites [29-31]. This symbiotic community helps colonize the intestine, enhancing bacterial diversity and reintroducing missing strains to control C. difficile overgrowth and achieve colonization resistance. FMT has ancient roots far before germ theory was known, dating back to the 4th century when Chinese alchemist Ge Hong was the first to describe a fecal slurry called “yellow soup” for the treatment of dysentery [32]. Isolated case reports followed in more modern times, starting with Eismann and colleagues successfully treating four patients with severe pseudomembranous colitis using FMT in 1958 [33]. The fluoroquinolone-resistant North American pulsed-field gel electrophoresis type 1 (NAP1) strain of Clostridioides difficile (also known as ribotype 027) emerged as the most prevalent strain causing CDI in the 1990s and 2000s, prompting researchers to re-explore FMT as an alternative treatment for CDI [17,34,35]. News outlets and internet media highlighted FMT success stories, further sparking public interest and clinical research [35].

The FDA was initially cautious about FMT, as no standardized donor stool procurement process or rules about screening for the transmission of infectious pathogens existed. FMT had not been FDA approved for any indication but met the legal definition of a “drug” since it was being used to treat or cure a human disease [36]. In 2013, the FDA determined that FMT would be regulated as an Investigational New Drug (IND) [37]. Thisdecision was met with concernfrom patients and physicians that the logistic and bureaucratic hurdles of IND paperwork would be a barrier to its use. A joint society recommendation was released 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 [38]. The FDA later amended this 2013 statement to exercise enforcement discretion, meaning physicians could provide FMT to patients giving informed consent with an IND application [37].

The clinical efficacy of FMT varies depending on the published source. Many observational studies, systematic reviews, and clinical trials have been published, giving FMT in different administration forms and for different indications (rCDI versus fulminant CDI). A 2013 systematic review and meta-analysis of 273 CDI patients in 11 studies treated with FMT found unweighted pooled resolution rates of 89.7% [39]. A later 2017 meta-analysis by Quraishi and colleagues analyzing FMT efficacy specifically in rCDI found 37 studies and a clinical resolution rate of 92%; however, there was variability in recipient preparation, volume administered, and significant differences were seen in the success rates between lower GI and upper GI delivery of FMT [40]. Additionally, cure rates for rCDI using a single dose of FMT appear to vary considerably depending on the trial types analyzed; a 2019 meta-analysis found cure rates of 83% in pooled open-label studies but only 68% in randomized trials [41]. Overall, treatment success with FMT appears to be influenced by transplantation frequency (number of FMT doses and volume) and method; endoscopy

yields the highest clinical effect rate (96.4%), while enema is the least effective (50.2%) [42]. Adverse effects after FMT most often include mild gastrointestinal side effects such as cramping, bloating, or flatulence; however, more severe side effects have been reported [43]. Serious adverse events are rare (<1%), but infection transmission risk exists, especially for immunocompromised recipients. Bacteremia and serious infections with multidrug-resistant gram-negative organisms have been reported [44-48]. Donor and sample screening are crucial to minimize this risk, despite which, unidentified microorganisms may still pose a threat.

Procuring donor stool for transplanting into a recipient is a complex and heterogeneous process. First, the donor is screened for general health and excluded if they have certain conditions or take certain medications [49,50]. If deemed healthy, the donor and stool are tested for various infectious pathogens and the stool is quarantined, then screening is repeated before administration to the recipient. This process is logistically complex, expensive, and may result in the donated stool having to be discarded [49]. Stool biobanks such as OpenBiome have emerged to help clinicians overcome these difficulties by offering a standardized FMT product that is pre-screened and ready for clinical use [51]. OpenBiome maintains an IND application under the oversight of the FDA and manufactures the FMT at the University of Minnesota's Microbiota Therapeutics Program per current Good Manufacturing Practice (cGMP) regulations for use by physicians registered as clinical partners [51]. The stool biobank assures product storage and shipping. Clinicians administering the FMT agree to assume the potential risk of any infectious agents not detected by the standard screening assays and to notify OpenBiome within a day if any adverse events occur [52]. The COVID-19 pandemic in 2020 and Mpox outbreaks in 2022 have required additional screening recommendations [53]. This reactive strategy of adding additional stool screening for infectious pathogens as they arise is less ideal than a proactive strategy; however, it is impossible to anticipate most emerging infections.

Development of Live Biotherapeutic Products (LBPs)

The development of LBPs has been driven by the desire to offer a commercially available microbiome therapy rather than an 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 [54]. Advances in genomic sequencing have led to a better understanding of the diverse microbial communities and interactions in our human microbiome; however, culturing these complex organisms is challenging for many reasons. Most of the bacterial phyla shown to benefit gut eubyosis (Firmicutes, Bacteroidetes, Actinobacteria) are obligate anaerobes that operate within a complex ecosystem where the interdependence of species and lack of aerotolerance make them difficult to culture and survive outside of the gut [54,55]. Freeze-drying, called lyophilization, can offer a solution to preserve microorganisms or their spores and allow for human administration; however, not all bacteria can survive this process [56]. To overcome these and other manufacturing difficulties, collaborative mergers have occurred between small microbiome laboratories and larger pharmaceutical companies to bring such products to the market. The FDA has approved two LBPs, RBX2660 (Rebyota™) and SER-109 (VOWST™), for the treatment of rCDI [57].

Rebyota

In November 2022, the FDA approved FMBL-jslm (Rebyota™), the first LBP for rCDI. It comprises a rectal suspension derived from healthy human stool samples, containing a diverse mixture of trillions of live microbes, particularly targeting the gut microbiome, including Bacteroides strains [58]. Before suspension, the stool undergoes thorough screening for transmissible pathogens and is then combined with a polyethylene glycol (PEG) 3350 solution. Unlike conventional FMT, this therapeutic offers a standardized approach to restoring microbiome diversity, with consistent potency and a uniform route of administration, validated through long-term placebo-controlled trials [59]. Administered as a single 150 mL dose, Rebyota™ contains a minimum of ≥ 1 × 10^5 colony-forming units (CFU)/mL of Bacteroides, with PEG content not exceeding 5.97 g in saline. Healthcare professionals administer the refrigerated preparation rectally to patients in a supine position following bowel cleansing, with the procedure typically taking about 45 minutes, scheduled 24 hours-72 hours after the last CDI antibiotic dose [58].

Approval of FMBL-jslm (Rebyota™) relied on data from the PUNCH CD trials. These trials encompassed a randomized, double-blind, placebo-controlled, multicenter phase 3 study, incorporating findings from the preceding phase 2 study. The data interpretation involved adults experiencing diarrhea with a positive C. difficile stool test and at least two prior CDI episodes within a year. Initially randomized into placebo, single-dose, and double-dose groups, the single-dose group's failure to meet non-inferiority criteria led to Bayesian analysis extrapolation. The analysis revealed a higher recurrence-free rate at 8 weeks (70.6% vs 57.5% for placebo), with over 90% of successful cases remaining recurrence-free after 6 months. Adverse events were monitored for up to 24 months post-treatment across five preliminary clinical studies. Rebyota™ is well-tolerated, with mild gastrointestinal side effects being the most reported. Prominent adverse reactions include abdominal pain (8.9%), diarrhea (7.2%), abdominal distention (3.9%), and flatulence (3.3%) [59,60]. While most events occurred within 2 weeks post-treatment, further understanding of potential transmissible pathogens and food-allergen reactions is needed. As with other approved live biotherapeutic products, caution is warranted in their widespread use due to uncertainties in extrapolating available data to a broader patient population [61].

Vowst

FMSL-brpk (VOWST™) is a novel orally administered bacterial spore suspension recently approved by the FDA. This capsulated

live biotherapeutic product is made by purifying human fecal matter sourced from meticulously screened donors. The fecal material undergoes an ethanol-based treatment to eliminate non-sporulated organisms, followed by filtration to remove residuals and isolate Firmicutes spores. While this process effectively eradicates potential pathogens, non-spore-forming organisms, and residual solids, the risk of infectious commensal fecal flora remains. The resulting product is a standardized consortium of Firmicutes spores engineered to withstand gastrointestinal acids for oral administration. Presented in universal capsules, each package contains 12 capsules, with a recommended daily dose of 4 capsules taken orally on an empty stomach before the first meal [62] Treatment duration spans 3 days, starting 2 days-4 days post-completion of CDI antibiotic therapy. Before treatment initiation, magnesium citrate is administered to purge residual antibiotics from the gastrointestinal system, mitigating unintentional harm to the administered live bio-therapeutic product.

The FDA approval of FMSL-brpk (VOWST™) was grounded on findings from the ECOSPOR trials, which comprised a randomized, double-blind, placebo-controlled trial and a randomized, open-label trial. Eligible adults met criteria for recurrent Clostridioides difficile infection (rCDI), defined as three or more episodes within 12 months. The blinded phase three trial evaluated three daily doses of VOWST™ against placebo in patients with rCDI, establishing a primary efficacy endpoint of no rCDI episodes in the 8 weeks post-treatment. Firmicutes spores engrafted as early as one week post-administration, persisting for up to 8 weeks, alongside elevated concentrations of secondary bile acids observed in the active treatment group. At 8 weeks post-treatment, CDI recurrence rate was 12% in the active group compared to 40% in the placebo group [63]. Subsequent studies affirmed sustained reductions in recurrence risk compared to placebo, with follow-up extending to 24 weeks post-treatment. Notable safety data predominantly encompassed gastrointestinal adverse effects, including abdominal distention (31%), constipation (14.4%), and diarrhea (10.0%) [64,65]. Similar to other live biotherapeutic products and fecal microbiota transplant procedures, VOWST™ carries a risk of transmissible infectious agents, warranting rigorous screening protocols.

CONCLUSION AND FUTURE DIRECTIONS

Rebyota™ and VOWST™ are a welcome new option for breaking the cycle of dysbiosis in rCDI. These drugs represent a significant breakthrough in biopharmaceutics as the first FDA-approved microbiome-based therapies brought to market. Neither of these products are FMTs, but both are derived from donors and may potentially contain donor-derived food allergens. They carry a theoretical risk of transmitting infectious agents that are not screened for. This risk is mitigated substantially by extensive donor screening, and no cases of food allergen events have been published to date [60,63,65,66]. Research is already underway to study LBPs for their potential in other areas, such as inflammatory bowel disease, metabolic disorders, hepatic encephalopathy, and graft versus host disease after stem cell transplant [67-70]. While these drugs are exciting new tools, unanswered questions remain. Specialists in gastroenterology and infectious diseases have traditionally offered FMT, while LBPs can be ordered by any clinician able to prescribe medications. It is unknown what, if any, impact this would have on patients suffering from rCDI, and it is unclear if increased use for off-label indications outside of rCDI prevention will occur. Neither of these drugs is for treatment for active CDI, and they currently have no role in treating patients hospitalized with fulminant CDI. Post-marketing surveillance and registries to follow the use of these agents will be critical for understanding the long-term safety, efficacy, and use of these new agents.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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