Article:
Buckley, AM orcid.org/0000-0002-2790-0717, Moura, IB and Wilcox, MH (2022) The potential of microbiome replacement therapies for Clostridium difficile infection. Current Opinion in Gastroenterology, 38 (1). pp. 1-6. ISSN 0267-1379

https://doi.org/10.1097/MOG.0000000000000800

Current Opinion in Gastroenterology

The potential of microbiome replacement therapies for C. difficile infection

--Manuscript Draft--

Manuscript Number:	
Full Title:	The potential of microbiome replacement therapies for C. difficile infection
Article Type:	Review Article
Corresponding Author:	Anthony Buckley Leeds, UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Anthony Buckley
First Author Secondary Information:	
Order of Authors:	Anthony Buckley
	I.B. Moura
	M.H. Wilcox
Order of Authors Secondary Information:	

The potential of microbiome replacement therapies for *C. difficile* infection

A.M. Buckley[1,2], I.B. Moura[1] and M.H. Wilcox[1,3]*

[1]Healthcare-Associated Infections Group, Leeds Institute of Medical Research, Faculty of Medicine and Health, University of Leeds, Leeds LS1 9JT U.K.

[2]Microbiome and Nutritional Science Group, School of Food Science, Faculty of Food Science and Nutrition, University of Leeds, Leeds LS1 9JT U.K.

[3]Microbiology, Leeds Teaching Hospital NHS Trust, Old Medical School, Leeds General Infirmary, Leeds LS1 3EX U.K.

*Corresponding author: Prof Mark Wilcox, Healthcare-Associated Infections group, Old Medical School, Leeds General Infirmary, Leeds LS1 3EX U.K. Email: mark.wilcox@nhs.net, Tel: +44 113 392 6125

Key words

C. difficile, microbiome therapies, bile acids, nutritional competition

Abstract

Purpose of review

There is a paradox when treating *C. difficile* infection (CDI); treatment antibiotics reduce *C. difficile* colonisation but cause further microbiota disruption and can lead to recurrent disease. The success of faecal microbiota transplants (FMT) in treating CDI has become a new research area in microbiome restorative therapies, but are they a viable long-term treatment option?

Recent findings

C. difficile displays metabolic flexibility to use different nutritional sources during CDI. Using microbiome therapies for the efficient restoration of bile homeostasis and to reduce the bioavailability of preferential nutrients will target the germination ability of *C. difficile* spores and the growth rate of vegetative cells. Several biotechnology companies have developed microbiome therapeutics for treating CDI, which are undergoing clinical trials.

Summary

There is confidence in using restorative microbiome therapies for treating CDI after the demonstrated efficacy of FMT, where several biotechnology companies are aiming to supply what would be a 'first in class' treatment option. Efficient removal of *C. difficile* from the different intestinal biogeographies should be considered in future microbiome therapies. With the gut microbiota implicated in different diseases, more work is needed to assess the long-term consequences of microbiome therapies.

Key points

- Treatment antibiotics are associated with an unacceptably high rate of infection recurrence.
- Faecal microbiota transplantation has been used successfully to treat recurrent CDI but carries risks of transplanting opportunistic pathogens and potential unknown long-term consequences.
- Rational inclusion of microbe candidates against all steps in the disease process are needed to maximise therapeutic efficacy.
- Several biotechnology companies have developed biotherapies for treating recurrent CDI, some of these show promising results from Phase III clinical trials.

Introduction

The intestinal microbiota provides a range of functions to the human host; aiding food digestion, regulating our immune system, and protection from pathogenic microorganisms – a term called colonisation resistance. Broad-spectrum antibiotics, although essential to treat infections caused by an unknown aetiological agent, target different bacteria and have been shown to dramatically impact in the commensal microbiota. Broad-spectrum antibiotics can: lower the gut microbial diversity(1), impact digestion of foods(2), cause an imbalance in the regulation of our immune system(3), and can increase the carriage of pathogenic microorganisms or those harbouring antimicrobial resistance genes(4,5).

Clostridium difficile is the main cause of antibiotic-associated diarrhoea and causes significant morbidity and mortality, contributing a healthcare cost burden of over €3 Billion in the EU and $4.8 billion in the US(6,7). *C. difficile* infection (CDI) is a toxin-mediated disease that can cause pseudomembranous colitis and toxic megacolon, which can be fatal. Onset of CDI is associated with the consumption of different antibiotic classes, for example 3rd generation cephalosporins (ceftriaxone), fluoroquinolones (ciprofloxacin), and clindamycin(8). Current treatment options include metronidazole and vancomycin, but these are associated with high rates of recurrent infections (25%); however, the probability of developing a second and third recurrent episode escalates subsequently(9). Multiple antibiotic treatments have a detrimental impact on microbial diversity, impairing the colonisation resistance ability of the microbiota. Replenishing this ecological niche, thereby reinstating colonisation resistance, is the basis for microbiome replacement therapies.

Faecal microbiota transplantation; successes and pitfalls

Faecal microbiota transplantation (FMT) involves the transplantation of faeces from a suitable donor to a recipient; it was first used in 1953 in the treatment of pseudomembranous colitis(10). For the last 40 years, FMT treatment has been clinically used to treat recurrent CDI cases, helping to restore microbiota diversity and composition within a recipient. However, determining the efficacy of FMT treatment, especially during early clinical trials, is difficult due to the number of variables. For example, donor screening methodologies, using different routes of FMT administration, number of FMT doses given, prior antibiotic treatments administered, and variations in timing between FMT doses, all can affect the reported efficacies(11). However, a weighted systematic review by Tariq *et al*.(12) reported that the clinical cure rate after a single oral FMT dose was 76.1% (66.4%-85.7%), and multiple doses of FMT can increase cure rate. A randomised controlled clinical trial determined the efficacy of vancomycin treatment with/without FMT for the treatment of recurrent CDI; a resolution rate of 81% was reported in the FMT group whilst those receiving vancomycin only had a resolution rate of 31%(13). This shows that clinical cure towards recurrent CDI can be achieved through microbiome therapies where antibiotics alone cannot.

The use of an untargeted microbiome therapy, such as FMT, can have downstream safety implications for the recipient. Improper screening can lead to the transfer of pathogens or undesirable genetic elements, such as antimicrobial resistance determinants or the colon cancer linked PKS toxin. In 2019, two FMT recipients for recurrent CDI treatment subsequently developed severe illness due to the transplantation of multidrug *E. coli* due to improper screening of donor faeces; this was fatal in one recipient(14). This led to a safety alert from the Food and Drug Administration (FDA) regarding the use of FMT. In 2020, six patients that received FMT therapy also contracted enteropathogenic *E. coli* and Shiga

toxin-producing *E. coli* (STEC) from the donors' stools; two patients died from diarrhoea associated with STEC infections. This led to an urgent recall of the identified donor faecal samples and an FDA safety alert. A standardised protocol for enhanced screening of donors is essential for the effective treatment, and safety and wellbeing of CDI patients.

Studies into the long-term efficacy of FMT highlighted that, although efficacious in clinical cure, long-term relapse following FMT treatment occurs in 8-18% of recipients(15,16). CDI relapse in these cases was highly associated with post-FMT antibiotic consumption; however, this included antibiotics that are not associated with induction of CDI, such as penicillin(16). Furthermore, transplantation of an unknown mix of microbes could have unintended consequences for the recipient. For example, there is a link between the gut microbiota and obesity and comorbidities(reviewed in(17)), and microbiota transplantation to cure recurrent CDI could lead to the recipient suffering from weight gain.

General considerations for microbiome therapies for recurrent CDI

The functional role of the intestinal microbiota is so essential that many researchers believe the microbiota should be described as an organ of the body. Understanding its roles will pave the way for the rational design of safe microbiome-based therapeutics. This is not an exhaustive list but meant to highlight the considerations needed.

Changing the nutritional landscape of the gut. The gut microbiota is a source of vitamin production and essential for the breakdown of ingested foods, providing our body with essential nutrients. Changes in the gut microbiota can result in different metabolic diseases, such as obesity. Microbial fermentation of dietary fibres produces short chained fatty acids

(SCFA; acetate, propionate, and butyrate), a primary energy source for colonocytes, which induce apoptosis of colon cancer cells, and help epithelial cells maintain the hypoxia gut environment(18). However, not all microbial metabolites are beneficial to the host. Microbial conversion of phosphatidylcholine to trimethylamine is positively associated with major cardiovascular events(19). This highlights the diet-microbiota-host complexity and further research is needed to establish safe microbiome-based therapies.

Impact on immune system homeostasis. The maturity of the immune system co-evolves with the gut microbiota, which enables the body to respond against infection whilst maintaining tolerance to the normal microbiota. Intestinal macrophages do not produce pro-inflammatory cytokines in response to microbial stimuli binding to Toll-like receptors(20). Some *Clostridium* species, particularly those belonging to clusters IV and XIVa, can promote regulatory T cell accumulation in the colon, to help maintain immune homeostasis(21). Perturbations in the microbiota can have consequences for autoimmune diseases, such as Inflammatory Bowel Disease and Type I diabetes.

Scientific basis for microbial therapies targeting CDI

Preventing recurrent CDI is likely to involve interventions at multiple steps during the disease process that microbiome-based therapies need to consider. Of note, microbiome therapies may need to work in conjunction with antibiotics, as the low microbial diversity caused by antibiotic therapy may increase the engraftment of the transplanting microbiome, enhancing protection.

***C. difficile* spore germination.** Germination of *C. difficile* spores is the first step during CDI. Some amino acids and primary bile acids, but not secondary bile acids, are potent

germinating signals. The conversion of human intestinal 1° to 2° bile acids is performed by a range of indigenous microbial species, and their absence is key for the commencement of CDI(22). This bile acid conversion is catalysed by bile salt hydrolases and the 7α-dehydroxylation (7αDH) enzyme; 7αDH enzymes are only found in a few known *Clostridium* and *Eubacterium* species. Antibiotic consumption can deplete those microbes responsible for bile acid conversion, leading to an accumulation of 1° bile acids and spore germination. Supplementation with *Clostridium scindens* restored bile acid homeostasis, reducing the pool of 1° bile acids and preventing *C. difficile* spore germination in mice(23). However, mucosal biofilms are known to harbour vegetative *C. difficile* cells, which play a role in recurrent CDI(24); thus, identifying microbes that can inhibit *C. difficile* growth will be beneficial. As sessile *C. difficile* cells are a potential reservoir for recurrent infections, therapies able to displace *C. difficile* from the sessile communities would be highly desirable.

Competition for preferred growth nutrients. The expansion of *C. difficile* vegetative cells is fuelled through the fermentation of amino acids, initially proline and glycine(25,26). As this resource becomes depleted, *C. difficile* undergoes metabolic reprogramming to utilise other amino acids and carbon sources funnelled into the central carbon pathway(27). Providing microbes that can compete for this amino acid reservoir, such as *Clostridium Bifermentans*, has been shown to prevent a lethal infection(28). Other nutritional sources utilised by *C. difficile* include, sorbitol(29), haeme(30), ethanolamine(31), and indole(32); thus, microbes competing for these nutritional sources will limit or prevent expansion of *C. difficile* vegetative cells. Interestingly, host mucin can be metabolised by members of the sessile population, such as *Bacteroides thetaiotaomicron*, releasing monosaccharides, such as sialic acid(33), N-acetylglucosamine(34), and succinate(35), which, in the absence of other scavenging microbes, are utilised by *C. difficile* to drive expansion. Metabolism of a wide

range of nutrients for energy highlights the metabolic flexibility employed by *C. difficile* during outgrowth.

Reduce toxin potency. Strains of *C. difficile* can produce up to three toxins, where it is recognised that TcdA and TcdB are responsible for majority of clinical symptoms; thus, inclusion of antagonistic microbes against the effects of these toxins is another consideration. A secreted serine protease produced by *Saccharomyces boulardii*, a non-pathogenic yeast, can cause proteolytic digestion of TcdA and TcdB(36). Some *Bifidobacterium* and *Lactobacillus* species, such as *L. delbrueckii* and *L. kefr* can antagonise the cytotoxic effect of these toxins(37,38). Similarly, Valdes-Varela *et al*.(39) noted that strains of *Bifidobacterium longum* and *B. breve* depleted toxins from *C. difficile* culture supernatants, and this effect is potentially mediated through unknown secreted factors from *Bifidobacterium* strains(24,40). A defined microbial consortium, MET-1, reduced gut inflammation by reducing the amount of TcdA without affecting *C. difficile* viability(41).

Rational targeting of each step in the disease process will enhance microbiome-based therapies for treatment of CDI. However, further understanding of microbiota-*C. difficile* interactions at the different intestinal biogeographies may help rationalise their inclusion/exclusion in microbiome-based therapies.

Microbiome therapies contributing towards successful resolution of CDI

The high efficacy of FMT treatment has been well established, but can a similar efficacy be achievable using a more defined or targeted mix of microbes? Recovered microbial strains from FMT donors have been combined as a therapy for *in vivo* treatment of recurrent CDI. A

simple mixture of six phylogenetically diverse bacteria were selected as a bacteriotherapy based on abundance; these were able to displace *C. difficile* in a recurrent mouse model to resolve disease(42). Similarly, inclusion of 33 bacterial isolates from a stool sample were developed as a 'proof-of-concept' microbiome therapy that successfully treated two patients whom had previously failed multiple rounds of antibiotic chemotherapy; this microbial mixture successfully resolved recurrent CDI in both patients(43). These studies show that simple microbiota cohorts can be effective in preventing recurrent infections.

Screening faecal communities for their ability to exclude *C. difficile* colonisation has been used to design microbiome therapies. Several panels of simplified faecal communities were assessed *in vitro* to reduce *C. difficile* levels and, after another round of simplification of those anti-*C. difficile* communities, communities were assessed *in vivo* to reduce *C. difficile* disease(44). Community designations FS2B and FS2C both protected mice from *C. difficile* disease; FS2C communities caused a reduction in *C. difficile* levels. These communities were associated with enhanced recovery of the indigenous microbiota.

Another rationale for inclusion of microbial species into putative microbiome therapies is using metagenomic analysis of cultured faecal isolates to provide functional predictions to antagonise *C. difficile in vitro*. Using this approach, Ghimire *et al*.(45) found 66 species that inhibited *C. difficile* growth when co-cultured; most inhibiting species were found to be acetate or butyrate producers. Testing 256 combinations of these species showed the importance of microbial composition and abundance in inhibiting *C. difficile*; with a composition of 12 bacterial species effectively inhibited *C. difficile in vitro* growth.

The design of some microbiome therapies has focused on a particular nutrient resource, such as mucin. Highly glycosylated mucins are the dominant structural component of

mucus, and a vital nutrient source for gut microbes. Exploiting this niche enhances *C. difficile* disease progression; mucin monosaccharides act as a chemoattractant for *C. difficile* to colonise the mucus layer(46). A five-member consortium (BacMix) was selected based on capacity to metabolise mucin-derived monosaccharides, consisting of *Akkermansia muciniphila, Ruthenbacterium lactatiformans, Alistipes timonensis, Muribaculum intestinale,* and *Bacteroides* sp.(34). Although members of the BacMix consortium colonised mice, this resulted in a modest decrease in *C. difficile* colonisation, suggesting that either *C. difficile* can compete for this nutritional niche or that *C. difficile* is able to adapt to different nutritional sources.

The inclusion of microbes into microbiome therapies has been based on mathematical modelling to replicate the ecological principals of FMT treatment. Here, Xiao *et al*.(47) used the classical generalised Lotka-Volterra model to simulate the FMT process with input data from preclinical mouse data and a clinical trial of FMT treatment. They proposed a cocktail of 14 species, including two direct inhibitors of *C. difficile* (*C. scindens* and *Roseburia hominis*), to reduce *C. difficile* colonisation.

The pursuit of microbiome-based therapies for CDI: overview of biotechnology companies

With 258,000 and 172,000 CDI cases in the US and Europe, respectively, novel treatments for CDI are urgently needed; a mission being undertaken by several biotechnology companies. Microbiome therapies for the treatment of CDI can help prevent further patient morbidity and the need for additional antibiotic treatment; an overuse of antibiotics leading to the development of resistance. However, microbiome therapies are not without pitfalls.

As a ‘first in class’ medical product, any microbiome therapy would need to be well tolerated, undergo rigorous safety testing, and show superiority over standard treatment options. Nevertheless, several biotechnology companies have made great progress in this area with several products in the clinical testing pipeline (**Table 1**).

Seres Therapeutics Inc. has developed a microbiome therapeutic for the prevention of recurrent CDI based on sporulating bacteria from the Firmicutes phylum taxa, SER-109. A Phase I clinical trial indicated that SER-109 had a favourable safety profile and successfully prevented recurrent CDI with a clinical resolution of 97% in 30 participants(48). Engraftment of spores was associated with the restoration of bile acid homeostasis, increasing the concentration of 2° bile acids(49). Despite a setback during Phase II clinical trial, SER-109 has registered positive topline results in a Phase III clinical trial. However, as a stool preparation, the composition and abundance of spores within SER-109 can vary; thus, Seres have developed a defined spore microbiome therapy, SER-265, which is currently in Phase I clinical trials.

RBX2660 (Rebiotix Inc./Ferring Pharmaceuticals) is a suspension of healthy donor microbiota for the resolution of recurrent CDI. Unlike SER-109, RBX2660 is composed of different bacterial phyla and is delivered by an enema rather than an oral route(50). In a Phase III clinical trial, RBX2660 successfully met primary endpoint, demonstrating superior efficacy vs placebo (70.4% vs 58.1%) at eight weeks post treatment(51). As a further development of RBX2660, RBX7455 is a standardised, lyophilised, orally administered live biotherapeutic that displayed good human tolerance and efficacy at preventing recurrent CDI(52).

CP101 (Finch Therapeutics) is a lyophilised, orally administered biotherapeutic constructed from healthy donor faeces. CP101 met primary endpoint in a Phase II clinical trial (PRISM3), with 74.5% achieving sustained clinical cure 8 weeks after administration.

VE303 (Vedanta Biosciences) is an orally administered rationally-designed bacterial commensal therapy, comprised of 8 distinct species belonging to Clostridium clusters IV, XIVa, and XVII. Administration of VE303 restored microbiome composition after antibiotic-induced dysbiosis and was well tolerated in Phase I trials; Phase II trials are underway.

Conclusions

The intestinal microbiome acts as a gateway for preventing recurrent CDI; but continual antibiotic treatments limit microbial recovery allowing *C. difficile* expansion. There is clear rationale for the use of microbiome therapies in the treatment of CDI; however, striking the right blend and balance of microbes is key for success. Rationale inclusion of microbes based on inhibition at all the steps in the infection process is likely to enhance efficacy. There are several promising microbiome candidates lead by biotechnology companies undergoing clinical trials, and early results indicate superiority over vancomycin treatment alone. There are still questions over the long-term effect of microbiome therapies on the human host, as manipulating the microbiome can have grave consequences for the host.

Acknowledgments

None

Conflicts of interest

AMB has received research funding from Seres Therapeutics Inc.. IBM has received funding to attend conferences from Techlab Ltd. MHW has received honoraria for consultancy work, financial support to attend meetings and research funding from Astellas, AstraZeneca, Abbott, Actelion, Alere, AstraZeneca, Bayer, bioMérieux, Cerexa, Cubist, Da Volterra, Durata, Merck, Nabriva Therapeutics plc, Pfizer, Qiagen, Roche, Seres Therapeutics Inc., Synthetic Biologics, Summit and The Medicines Company.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

▪ of special interest

▪▪ of outstanding interest

1. Zimmermann P, Curtis N. The effect of antibiotics on the composition of the intestinal microbiota - a systematic review. J Infect. 2019 Dec 1;79(6):471–89.

2. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. BMJ. 2018 Jun 13;361:k2179.

3. Wu H-J, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes. 2012 Jan 1;3(1):4–14.

4. Rooney CM, Sheppard AE, Clark E, Davies K, Hubbard ATM, Sebra R, et al. Dissemination of multiple carbapenem resistance genes in an in vitro gut model simulating the human colon. J Antimicrob Chemother. 2019 Jul 1;74(7):1876–83.

5. Buckley AM, Moura IB, Altringham J, Ewin D, Clark E, Bentley K, et al. The use of first-generation cephalosporin antibiotics, cefalexin and cefradine, is not associated with induction of simulated Clostridioides difficile infection. J Antimicrob Chemother. 2021 Sep;

6. Aguado JM, Anttila VJ, Galperine T, Goldenberg SD, Gwynn S, Jenkins D, et al. Highlighting clinical needs in Clostridium difficile infection: The views of European healthcare professionals at the front line. J Hosp Infect. 2015;90(2):117–25.

7. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, et al. Burden of *Clostridium difficile* Infection in the United States. N Engl J Med. 2015;372(9):825–34.

8. Slimings C, Riley T V. Antibiotics and hospital-acquired Clostridium difficile infection: update of systematic review and meta-analysis. J Antimicrob Chemother. 2014 Apr;69(4):881–91.

9. Sheitoyan-Pesant C, Abou Chakra CN, Pépin J, Marcil-Héguy A, Nault V, Valiquette L. Clinical and Healthcare Burden of Multiple Recurrences of Clostridium difficile Infection. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2016 Mar;62(5):574–80.

10. EISEMAN B, SILEN W, BASCOM GS, KAUVAR AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery. 1958 Nov;44(5):854–9.

11. Wilcox MH, McGovern BH, Hecht GA. The Efficacy and Safety of Fecal Microbiota Transplant for Recurrent Clostridium difficile Infection: Current Understanding and Gap Analysis. Open forum Infect Dis. 2020 May;7(5):ofaa114.

*This article provides a framework for future clinical trials to robustly determine microbiome therapy efficacy and safety.

12. Tariq R, Pardi DS, Bartlett MG, Khanna S. Low Cure Rates in Controlled Trials of Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection: A Systematic Review and Meta-analysis. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2019 Apr;68(8):1351–8.

13. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile. N Engl J Med. 2013 Jan 16;368(5):407–15.

14. DeFilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, et al. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant. N Engl J Med. 2019 Oct 30;381(21):2043–50.

*This article first showed that improper screening of faecal donor providers for FMT therapy can have grave consequences.

15. Mamo Y, Woodworth MH, Wang T, Dhere T, Kraft CS. Durability and Long-term Clinical Outcomes of Fecal Microbiota Transplant Treatment in Patients With Recurrent Clostridium difficile Infection. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2018 May;66(11):1705–11.

16. Allegretti JR, Kao D, Phelps E, Roach B, Smith J, Ganapini VC, et al. Risk of Clostridium difficile Infection with Systemic Antimicrobial Therapy Following Successful Fecal Microbiota Transplant: Should We Recommend Anti-Clostridium difficile Antibiotic Prophylaxis? Dig Dis Sci. 2019;64(6):1668–71.

17. Tseng C-H, Wu C-Y. The gut microbiome in obesity. J Formos Med Assoc. 2019;118:S3–9.

18. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, et al. Microbiota-Generated Metabolites Promote Metabolic Benefits via Gut-Brain Neural Circuits. Cell. 2014 Jan 16;156(1):84–96.

19. Tang WHW, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk. N Engl J Med. 2013 Apr 24;368(17):1575–84.

20. Smythies LE, Shen R, Bimczok D, Novak L, Clements RH, Eckhoff DE, et al.

Inflammation anergy in human intestinal macrophages is due to Smad-induced IkappaBalpha expression and NF-kappaB inactivation. J Biol Chem. 2010 Jun;285(25):19593–604.

21. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011 Jan;331(6015):337–41.

22. Jukes CA, Ijaz UZ, Buckley A, Spencer J, Irvine J, Candlish D, et al. Bile salt metabolism is not the only factor contributing to Clostridioides (Clostridium) difficile disease severity in the murine model of disease. Gut Microbes. 2020 May;11(3):481–96.

23. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, et al. Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. Nature. 2014;517(7533):205–8.

24. Normington C, Moura IB, Bryant J, Ewin D, Clark E, Kettle M, et al. Biofilms harbour Clostridioides difficile, serving as a reservoir for recurrent infection. npj Biofilms Microbiomes. 2021;7(1):16.

*This article demonstrates that biofilms harbouring *C. difficile* play a role in recurretn disease.

25. Janoir C, Denève C, Bouttier S, Barbut F, Hoys S, Caleechum L, et al. Adaptive strategies and pathogenesis of clostridium difficile from In vivo transcriptomics. Infect Immun. 2013;81(10):3757–69.

26. Lopez CA, McNeely TP, Nurmakova K, Beavers WN, Skaar EP. Clostridioides difficile proline fermentation in response to commensal clostridia. Anaerobe.

2020;63:102210.

27. Hofmann JD, Otto A, Berges M, Biedendieck R, Michel A, Becher D, et al. Metabolic Reprogramming of *Clostridioides difficile* During the Stationary Phase With the Induction of Toxin Production. Front Microbiol. 2018;9:1970.

28. Girinathan B, Dibenedetto N, Worley J, Peltier J, Lavin R, Delaney D, et al. The mechanisms of *in vivo* commensal control of *Clostridioides difficile* virulence. BioRxiv. 2020;

29. Pruss KM, Sonnenburg JL. C. difficile exploits a host metabolite produced during toxin-mediated disease. Nature. 2021;593(7858):261–5.

30. Knippel RJ, Wexler AG, Miller JM, Beavers WN, Weiss A, de Crécy-Lagard V, et al. Clostridioides difficile Senses and Hijacks Host Heme for Incorporation into an Oxidative Stress Defense System. Cell Host Microbe. 2020 Sep 9;28(3):411-421.e6.

31. Nawrocki KL, Wetzel D, Jones JB, Woods EC, McBride SM. Ethanolamine is a valuable nutrient source that impacts Clostridium difficile pathogenesis. Environ Microbiol. 2018 Apr 1;20(4):1419–35.

32. Charles D, Kimberly P-P, Dayna B, L. DH, J. TP. Clostridium difficile Modulates the Gut Microbiota by Inducing the Production of Indole, an Interkingdom Signaling and Antimicrobial Molecule. mSystems. 2021 Oct 4;4(2):e00346-18.

33. Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature. 2013;502(7469):96–9.

34. Pereira FC, Wasmund K, Cobankovic I, Jehmlich N, Herbold CW, Lee KS, et al. Rational

design of a microbial consortium of mucosal sugar utilizers reduces Clostridiodes difficile colonization. Nat Commun. 2020;11(1):5104.

35. Ferreyra JA, Wu KJ, Hryckowian AJ, Bouley DM, Weimer BC, Sonnenburg JL. Gut microbiota-produced succinate promotes *C. difficile* infection after antibiotic treatment or motility disturbance. Cell Host Microbe. 2014;16(6):770–7.

36. Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. Saccharomyces boulardii protease inhibits the effects of Clostridium difficile toxins A and B in human colonic mucosa. Infect Immun. 1999 Jan;67(1):302–7.

37. Banerjee P, Merkel GJ, Bhunia AK. Lactobacillus delbrueckii ssp. bulgaricus B-30892 can inhibit cytotoxic effects and adhesion of pathogenic Clostridium difficile to Caco-2 cells. Gut Pathog. 2009;1(1):8.

38. Carasi P, Trejo FM, Pérez PF, De Antoni GL, Serradell M de los A. Surface proteins from Lactobacillus kefir antagonize in vitro cytotoxic effect of Clostridium difficile toxins. Anaerobe. 2012;18(1):135–42.

39. Valdés-Varela L, Hernández-Barranco AM, Ruas-Madiedo P, Gueimonde M. Effect of Bifidobacterium upon Clostridium difficile Growth and Toxicity When Co-cultured in Different Prebiotic Substrates . Vol. 7, Frontiers in Microbiology . 2016. p. 738.

40. Wei Y, Yang F, Wu Q, Gao J, Liu W, Liu C, et al. Protective Effects of Bifidobacterial Strains Against Toxigenic Clostridium difficile. Vol. 9, Frontiers in Microbiology . 2018. p. 888.

41. Martz SL, Guzman-Rodriguez M, He S-M, Noordhof C, Hurlbut DJ, Gloor GB, et al. A human gut ecosystem protects against C. difficile disease by targeting TcdA. J

Gastroenterol. 2017 Apr;52(4):452–65.

42. Lawley TD, Clare S, Walker AW, Stares MD, Connor TR, Raisen C, et al. Targeted Restoration of the Intestinal Microbiota with a Simple, Defined Bacteriotherapy Resolves Relapsing *Clostridium difficile* Disease in Mice. PLoS Pathog. 2012;8(10).

43. Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, et al. Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: 'RePOOPulating' the gut. Microbiome. 2013;1:1–12.

44. M. AJ, C. PE, James C, I. LA, A. BR, B. YV. Identification of Simplified Microbial Communities That Inhibit Clostridioides difficile Infection through Dilution/Extinction. mSphere. 2021 Oct 4;5(4):e00387-20.

**This article demonstartes that simple faecal communities are capable of restricting *C. difficile* growth and disease *in vivo*

45. Sudeep G, Chayan R, Supapit W, Linto A, Abhijit M, Chan KM, et al. Identification of Clostridioides difficile-Inhibiting Gut Commensals Using Culturomics, Phenotyping, and Combinatorial Community Assembly. mSystems. 2021 Oct 4;5(1):e00620-19.

**This article characterises those indigenious microbiota capable of inhibiting *C. difficile in vitro*

46. Engevik MA, Engevik AC, Engevik KA, Auchtung JM, Chang-Graham AL, Ruan W, et al. Mucin-Degrading Microbes Release Monosaccharides That Chemoattract Clostridioides difficile and Facilitate Colonization of the Human Intestinal Mucus Layer. ACS Infect Dis. 2021 May 14;7(5):1126–42.

47. Xiao Y, Angulo MT, Lao S, Weiss ST, Liu Y-Y. An ecological framework to understand

the efficacy of fecal microbiota transplantation. Nat Commun. 2020;11(1):3329.

48. Khanna S, Pardi DS, Kelly CR, Kraft CS, Dhere T, Henn MR, et al. A Novel Microbiome Therapeutic Increases Gut Microbial Diversity and Prevents Recurrent *Clostridium difficile* Infection. J Infect Dis. 2016;214(2):173–81.

49. McGovern BH, Ford CB, Henn MR, Pardi DS, Khanna S, Hohmann EL, 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 an Off Publ Infect Dis Soc Am. 2021 Jun;72(12):2132–40.

**This article showcases the first successful Phase III clinical trial with a designed biotherapeutic.

50. Dubberke ER, Lee CH, Orenstein R, Khanna S, Hecht G, Gerding DN. Results From a Randomized, Placebo-Controlled Clinical Trial of a RBX2660-A Microbiota-Based Drug for the Prevention of Recurrent Clostridium difficile Infection. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2018 Sep;67(8):1198–204.

*This article demonstrates the clinicl application of a taxonomically diverse biotherapeutic to prevent recurrent CDI disease

51. Ferring and Rebiotix Present Landmark Phase 3 Data Demonstrating Superior Efficacy of Investigational RBX2660 Versus Placebo to Reduce Recurrence of C. difficile Infection [Internet]. 2021. Available from: https://www.ferring.com/ferring-and-rebiotix-present-landmark-phase-3-data-demonstrating-superior-efficacy-of-investigational-rbx2660-versus-placebo-to-reduce-recurrence-of-c-difficile-infection/

52. Khanna S, Pardi DS, Jones C, Shannon WD, Gonzalez C, Blount K. RBX7455, a Room

1 Temperature-Stable, Orally-Administered Investigational Live Biotherapeutic, is Safe,
2 Effective, and Shifts Patients' Microbiomes in a Phase 1 Study for Recurrent
3 Clostridioides difficile Infections. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2020
4 Sep;

5

Table 1. Selected biotechnology companies with microbiome base therapies targeting CDI

Company	Product	Status
Seres Therapeutics	SER-109	Phase III
Rebiotix	RBX2660	Phase III
Vedanta Biosciences	VE303	Phase II
Finch Therapeutics	CP101	Phase II
NuBiyota	MET-2	Phase I
Rebiotix	RBX7455	Phase I
Seres Therapeutics	SER-262	Phase I

1