The role of the gut microbiome in colonization resistance and recurrent Clostridioides difficile infection

Anna Maria Seekatz, Nasia Safdar and Sahil Khanna

Abstract
The species composition of the human gut microbiota is related to overall health, and a healthy gut microbiome is crucial in maintaining colonization resistance against pathogens. Disruption of gut microbiome composition and functionality reduces colonization resistance and has been associated with several gastrointestinal and non-gastrointestinal diseases. One prime example is Clostridioides difficile infection (CDI) and subsequent recurrent infections that occur after the development of systemic antibiotic-related dysbiosis. Standard-of-care antibiotics used for both acute and recurrent infections do not address dysbiosis and often worsen the condition. Moreover, monoclonal antibodies, recommended in conjunction with standard-of-care antibiotics for the prevention of recurrent CDI in patients at high risk of recurrence, reduce recurrences but do not address the underlying dysbiosis. Fecal microbiota transplantation (FMT) is an evolving therapeutic strategy in which microbes are harvested from healthy donor stool and transplanted into the gut of a recipient to restore the gut microbiome. Although effective in the prevention of recurrent CDI, some existing challenges include screening and the standardization of stool acquisition and processing. Recent safety alerts by the US Food and Drug Administration raised concern about the possibility of transmission of multidrug-resistant organisms or severe acute respiratory syndrome coronavirus 2 via FMT. Increased knowledge that microbes are beneficial in restoring the gut microbiome has led to the clinical development of several newer biotherapeutic formulations that are more regulated than FMT, which may allow for improved restoration of the gut microbiome and prevention of CDI recurrence. This review focuses on mechanisms by which gut microbiome restoration could influence colonization resistance against the pathogen C. difficile.

Plain language summary
The Role of the Gut Microbiome in Clostridioides difficile Infection

Introduction:
- A rich and diverse gut microbiome is key to immune system regulation and colonization resistance against pathogens.
- A disruption in the gut microbiome composition can make the gut more vulnerable to diseases such as Clostridioides difficile infection (CDI), caused by the bacterium C. difficile.
- CDI management presents a therapeutic dilemma, as it is usually treated with antibiotics that can treat the infection but also can damage the microbiome.
• Treatment of CDI using antibiotics can further reduce microbial diversity and deplete beneficial bacteria from the gut leading to a condition called dysbiosis.
• Antibiotic treatment can be followed by therapies that restore the gut microbiota, boost colonization resistance, and prevent the development of antimicrobial resistance.
• It is important to evaluate treatment options to determine their safety and effectiveness.

Methods:
• The researchers provided an overview of the mechanisms that the gut microbiome uses to prevent colonization of the gut by pathogens.
• They subsequently reviewed the efficacy and shortcomings of the following treatments for CDI:
  - Antibiotics
  - Monoclonal antibodies
  - Fecal microbiota transplantation (FMT)

Results:
• Commensal intestinal bacteria prevent colonization of the gut by pathogens using mechanisms such as:
  - Competition for key nutrients
  - Production of inhibitory bile acids
  - Short-chain fatty acid production
  - Lowering the luminal pH
  - Production of bacteriocins
• Antibiotic therapy is recommended as a standard treatment for CDI. However, patients are vulnerable to recurrent CDI after discontinuation of the therapy.
• Monoclonal antibodies that inactivate \textit{C. difficile} toxins may be recommended along with antibiotics to prevent recurrent CDI. However, this approach does not restore the microbiome.
• FMT is one method of microbial restoration, where stool is harvested from a healthy donor and transplanted into a patient’s colon.
• Although FMT has shown some efficacy in the treatment of recurrent CDI, the procedure is not standardized.
• Safety concerns have been raised about the possibility of transmission of multidrug-resistant pathogens via FMT.

Conclusion:
• Treatment methods that can efficiently restore the diversity of the gut microbiome are crucial in preventing recurrence of CDI.

Keywords: bacteria, \textit{Clostridioides difficile} infection, dysbiosis, fecal microbiota transplantation, microbiota, microbiota-based therapy

Received: 17 March 2022; revised manuscript accepted: 4 October 2022.
Introduction

The human gut microbiota comprises a diverse group of microorganisms that inhabit the gastrointestinal tract and includes bacteria, viruses, and fungi. Collectively with the gastrointestinal environment that it inhabits, the gut microbiome is critical to maintaining host health, including immune system regulation, epithelial barrier support, and metabolic regulation such as energy acquisition. Disruption of the healthy composition of microbiota results in dysbiosis and has been associated with a range of gastrointestinal and non-gastrointestinal diseases. Restoring the gut microbiota to a more diverse, healthier, and balanced composition, the condition known as eubiosis, represents a novel therapeutic target to combat many conditions known to be influenced by the microbiome, such as infections caused by the healthcare-associated pathogen, *Clostridioides difficile*.11

Bacteria in the gut are taxonomically classified into phyla, classes, orders, families, genera, and species (Figure 1). More than 2000 microbial species have been identified in the human gut, classified into 12 different phyla, of which more than 90% belong to the Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria phyla. Broadly, studies demonstrate that a healthy gut microbiota is dominated by a diversity of members from the Bacteroidetes and Firmicutes phyla, with a lower abundance of Proteobacteria and Actinobacteria. Genera such as *Lactobacillus*, *Bacillus*, *Clostridium*, and *Ruminococcus* within the Firmicutes phylum and *Bacteroides* and *Prevotella* within the Bacteroidetes phylum are frequently associated with good health. Because interindividual variation in the types of genera and species in the human gut occurs, providing a specific definition of what constitutes a healthy microbiota is complicated.

The composition of the gut microbiome is dynamic, characterized by rapid changes in the first 3 years of life, followed by a period of relative stability, and then a gradual shift again from mid-to-late adulthood. The composition of the adult gut microbiome is influenced by many factors. Diet in particular has been demonstrated to influence microbiota composition and, thus, its role in disease development. For example, the typical Western-type animal-based diet that is high in fat and low in plant-based fiber has been shown to decrease microbial diversity in the gut and change

Infographic: Restoring the Gut Microbiome to Treat *Clostridioides difficile* Infection.
the composition of the gut microbiome, thus influencing its functionality. In addition to environmental factors, aging has been shown to impact gut microbiome composition. A gradual shift in microbiome composition and species diversity has been observed even in healthy elderly individuals, including a decline in core taxa within health-associated Bacteroidetes phyla.

A healthy, balanced gut microbiome provides resistance to colonization of the gut by exogenous organisms and prevents expansion of potential pathogenic organisms within the gut through a variety of mechanisms, a property known as colonization resistance (Table 1). Perhaps most relevant to decreased colonization resistance are medications, particularly antibiotics,
which are known to drastically change the microbiota, resulting in disruption of major potentially beneficial bacteria. Dysbiosis due to antibiotic use commonly results in a shift in dominant phyla accompanied by an increase in Proteobacteria, frequently associated with loss of colonization resistance to pathogens including \textit{C. difficile}. Although the compositional and functional dynamics between different intestinal bacterial phyla may predispose an individual to possible opportunistic infections and diseases, understanding the specific mechanisms and interactions within the gut microbiome that influence colonization resistance can aid in the development of innovative microbiota-derived therapeutics to prevent and treat infections and diseases associated with dysbiosis.

The abundance and diversity of ‘healthy’ commensal microbes within the human body is one metric used to define a healthy microbiome and has been demonstrated to be impacted by antibiotic use. In the gut, high microbial diversity is commonly linked to overall health and wellness, whereas low microbial diversity has been associated with development of diseases such as obesity and inflammatory bowel disease. Maintaining high microbial diversity has been demonstrated to provide colonization resistance against many external pathogens and is thought to provide resilience via multiple mechanisms (Figure 2). However, underlying the simple definition of microbial diversity or composition is a broad consortium of microorganisms that compete against each other and potential pathogens for nutrients or attachment sites on the gut epithelium to produce antibacterial substances and to modulate the host immune response such as inducing immunoglobulin A secretion, which inhibits colonization of the gut by potential pathogens. This review will focus on mechanisms by which the gut microbiome restoration could influence colonization resistance against the pathogen \textit{C. difficile}.

\textbf{Clostridioides difficile infection} \textit{C. difficile} is an anaerobic, Gram-positive bacterium that exists in both vegetative and spore forms. Spores are ubiquitous in the environment and are the infectious form of \textit{C. difficile}. Spores are highly resistant to some antibiotics and environmental factors including oxygen, disinfectants (including ethanol-based hand sanitizers), high temperature, and ultraviolet light. They can contaminate the environment around patients and can persist for years. Once ingested by the host, under the right conditions, normally dormant spores can germinate in the gut into replicating, metabolically active vegetative cells. If the strain of \textit{C. difficile} contains genes for toxin production (i.e. is toxigenic), vegetative cells will produce toxins in the colon that ultimately lead to disease defined by symptoms ranging from diarrhea and gastrointestinal distress, to more severe forms including toxic megacolon, pseudomembranous colitis, and even death. The toxins responsible for disease include Toxin A (TcdA) and Toxin B (TcdB), with some strains producing a binary toxin (or \textit{C. difficile} transferase) which enhances its virulence.

| Term | Definition |
|------|------------|
| Microbiota\textsuperscript{1} | The diverse group of microorganisms, including bacteria, archaea, viruses, and fungi, found in and on multicellular organisms |
| Microbiome\textsuperscript{1,2} | The collective community of microorganisms and their activity within their environment |
| Eubiosis\textsuperscript{12,13} | A healthy, balanced state of the microbiome |
| Colonization resistance\textsuperscript{8,9} | Gut microbiota provide resistance to colonization of the gut by exogenous organisms |
| Dysbiosis\textsuperscript{10–12} | Disruption of the healthy composition, abundance, diversity, and functionality of the microbiome |
The internalization of TcdA by intestinal epithelial cells triggers cytoskeletal changes that lead to the disruption of tight junctions and loosening of the epithelial barrier, cell death, and/or the production of inflammatory factors that attract neutrophils. The disruption of the tight junctions allows for the translocation of TcdA and TcdB across the epithelium, where they can further induce inflammatory cytokine production in phagocytes and mast cells. This leads to escalation of the inflammatory response due to neutrophil and lymphocyte influx, which results in further damage to the intestinal lining and the potential formation of a pseudomembrane.

Figure 2. Protective, structural, and metabolic functions of the gut microbiota to promote overall health. A healthy microbiota consists of a broad consortium of organisms that promote overall health through protective, structural, and metabolic functions. IgA, immunoglobulin A.

The estimated burden of primary Clostridioides difficile infection (CDI) cases annually in the United States exceeds 450,000, with most infections classified as healthcare associated.\textsuperscript{64} Even as healthcare-associated infections have decreased slightly in the last 10 years, some reports suggest an overall increase in community-related diagnoses.\textsuperscript{64,65} A CDI case was classified as community associated if the C. difficile-positive stool specimen was collected on an outpatient basis or within 3 days after hospital admission in a person with no documented overnight stay in a healthcare facility in the preceding 12 weeks. All other CDI cases not meeting these criteria were classified as healthcare associated.\textsuperscript{65,66} The economic burden associated with CDI is substantial, with the total estimated annual cost of all CDI cases in the United States amounting to $5.4 billion dollars.\textsuperscript{67} In addition to primary infection, it is estimated that 20–30% of patients experience disease recurrence after a first infection (defined as occurring 8 weeks or less after the previous episode), with the risk of recurrence increasing after every episode. The risk of recurrence after two infections is 40–50%, and greater than 60% after three infections.\textsuperscript{14,69,68,69} These recurrences can have a profound impact on the lives of patients and be
severely disabling. A 2017 survey of patients experiencing recurrent CDI revealed symptoms of severe diarrhea and severe exhaustion in 58.5% and 30.7% of respondents, respectively. Patients with severe diarrhea were three times more likely to have days of inactivity compared to patients with low or moderate diarrhea severity. More than half of responders reported that they were most concerned about getting sick again, and between 22% and 32% of responders changed their behavior, avoiding public places and eating out less. A US population study analyzing the impact of active and previous CDI on the daily lives of patients showed that the physical, psychological, social, and financial impact could be devastating, even after the acute infection has passed.

The role of antibiotics in CDI

*C. difficile* is responsible for most of the severe cases of antibiotic-associated diarrhea (AAD) and the development of colitis. Although prospective studies in patients prior to CDI are limited by sample availability, studies in animal models demonstrate profound effects on the microbiota by antibiotics that induce susceptibility to CDI. Patients who contract CDI or suffer from other cases of AAD have demonstrated decreased overall diversity and altered gut microbiome composition, with decreases in the normally abundant Bacteroidetes and Firmicutes phyla that are distinct from patients who do not contract CDI (Figure 4). In particular, Firmicutes members such as *Lachnospiraceae* and *Ruminococcaceae* families are decreased, whereas *Enterococcus* genera increased. Decreased gut microbiota diversity and resilience is associated with the severity of CDI and is a risk factor for recurrent CDI. Furthermore, the composition of the gut microbiome prior to CDI may also predict the response to treatment and subsequent recurrence risk.
colonization resistance, which, in turn, may lead to AAD, with or without CDI. Not all antibiotics have the same effect on the gut microbiota, as they have different modes of action. Broad-spectrum antibiotics do not discriminate between pathogens and commensal gut bacteria and this may affect 30% of the gut bacteria, contributing to loss of microbiota diversity. Broad-spectrum antibiotics and macrolides have also been shown to change the gut microbiota composition in children and neonates and early use of antibiotics has been associated with detrimental effects on health, with positive links to conditions such as obesity, asthma, allergies, inflammatory bowel disease, and adverse effects on cognition, behavior, and emotional outcomes. Amoxicillin–clavulanate and cefixime are associated with up to 25% and 20% of AAD cases, respectively, followed by other cephalosporins, fluoroquinolones, clindamycin, azithromycin, clarithromycin, erythromycin, and tetracycline.

Gut microbiome recovery after antibiotic-induced dysbiosis may show some resilience to recover to its original state but the recovery is often incomplete and may take months and years in some cases. Antibiotics used to treat CDI (e.g. vancomycin and metronidazole) disrupt the gut microbiome further and can select for antibiotic-resistant organisms such as vancomycin-resistant Enterococci and multidrug-resistant Klebsiella pneumoniae. Fidaxomicin is a narrow-spectrum antibiotic recommended as a first-line treatment option for an initial and recurrent CDI. Unlike vancomycin, fidaxomicin may have a more limited effect on commensal bacteria in the gut. Fewer recurrences occur with oral fidaxomicin than with vancomycin. The lower rate of recurrent CDI with fidaxomicin may be because fidaxomicin effectively inhibits C. difficile toxin production, inhibits spore production, and

Figure 4. Effect of antibiotics on gut microbiota. Antibiotics disrupt the gut microbiota; reduce diversity, composition, and function; reduce colonization resistance against potential pathogens such as Clostridioides difficile; and select for antimicrobial resistant organisms and genes.
improves preservation of the gut microbiome compared to vancomycin after treatment for a primary CDI.70

Since antibiotic treatment for CDI (particularly vancomycin) disrupts the gut microbiome, it is logical that the recommended standard-of-care antibiotic treatment for CDI does not correct dysbiosis, and it is a prominent risk factor for recurrent infections.38 Changes in the gut microbiome due to antibiotics or other causes ultimately impact functions necessary to maintain eubiosis in the host. For colonization resistance against C. difficile in particular, functional changes in the gut can impact the trajectory of CDI at multiple points of C. difficile pathogenesis, such as spore germination, vegetative outgrowth, or toxin production, subsequently influencing disease development, severity, and recurrence. For instance, antibiotic-induced disruption of the gut microbiome creates an environment where spores can overgrow and cause C. difficile colonization and infection, including recurrent CDI.14,38 The resistance of the C. difficile spore form to some antibiotics enables it to persist in the gut after treatment of CDI, which can result in recurrent CDI.54 Recurrent CDI may also be caused by a new infection with a different C. difficile strain.80 C. difficile recurrence is also likely in patients who have multiple C. difficile strains at primary infection.87,88

Mechanisms of colonization resistance

Colonization resistance against potential pathogens via the gut microbiome is maintained through several mechanisms.36,45,52 Potentially pathogenic bacteria compete with commensal intestinal bacteria for available nutrients. Therefore, utilization of key nutrients by the resident bacteria in the gut prevents colonization by pathogens.36,43,44 For spore-forming pathogens like C. difficile, multiple colonization resistance mechanisms may be necessary to prevent both spore germination and outgrowth of vegetative cells.

Alterations of the gut microbiome by antibiotics in particular induce a loss in microbiota diversity,38 which ultimately alters gut microbiota metabolism, especially with regard to the production of bile acid and nutrients.52,58 A healthy gut microbiome, particularly Clostridium species belonging to the Firmicutes phyla, has an important role to play in bile acid metabolism. Primary bile acids produced in the liver are deconjugated and transformed by certain species in the gut microbiome.16,36 Clostridium species in the gut are responsible for the production of two main secondary bile acids, deoxycholic acid and lithocholic acid.36 These secondary bile acids inhibit the growth of several pathogenic bacteria, including C. difficile.36,54 Furthermore, conversion to secondary bile acids depletes the pool of primary bile acids, which are known to induce spore germination of C. difficile.36,54 Bile acid conversion has been demonstrated to be important in CDI, particularly in the development of primary infection56 and in human patients, recovery from CDI is correlated with recovery of secondary bile acids.57

In addition to depleting bile acid converters, antibiotic-induced loss of diversity alters the nutrient landscape of the gut. Depletion of commensal bacteria by antibiotic treatment results in an excess of these nutrients, which can then be used by C. difficile to grow.52 For instance, sialidase-producing commensal bacteria in the gut cleave sugar from glycosylated protein to produce free sialic acid.52,89 Primary fermenters also break down complex carbohydrates and fiber into organic acids such as succinate.90 Both these metabolites are used as energy sources by commensal bacteria.52,90 Similarly, C. difficile is capable of metabolizing amino acids in the gut via a process known as Stickland fermentation.91 An excess of amino acids, which is normally metabolized by commensal bacteria, has been correlated with susceptibility to both primary and recurrent infection.92 C. difficile metabolism is also intricately connected to toxin production, which can influence disease severity and sustain colonization. In mice, C. difficile has been demonstrated to leverage toxin-mediated damage for its own nutritional advantage, providing new sources of nutrients for its own survival in the gut.93

Alterations to gut resources can also be directly influenced by diet. Most evidence for the role of diet in CDI have been conducted in mice, although some dietary correlations to CDI susceptibility have been demonstrated in humans.94,95 Diets high in fat and/or protein have been demonstrated to exacerbate CDI in mouse models of disease, potentially by influencing the available nutrient pool for resident microbes.95-97 In contrast, diets high in carbohydrates, specifically fiber, may alleviate disease or even directly influence C. difficile via the production of butyrate (summarized below).96,98 Recently, a very
low-calorie diet was also observed to influence susceptibility to CDI.99 *C. difficile* requires proline for growth, and a gut environment low in proline has been demonstrated to decrease CDI severity.100 Conversely, a diet high in zinc has been demonstrated to lower the threshold of susceptibility in mice.96 Mice fed diets with higher fat and/or sugar content incur increased susceptibility to CDI.97 Collectively, these studies highlight a potential, albeit complex, role for dietary manipulation of CDI status. Future therapeutic strategies targeting the microbiome for treatment of CDI may benefit from including a dietary perspective.

One particular group of gut metabolites, modified by both gut microbes and diet, that has been associated with decreased susceptibility to CDI are short-chain fatty acids. These metabolites are produced through bacterial fermentation of indigestible carbohydrates or dietary fiber and have an important role to play in maintaining colonization resistance.36 In particular, the short-chain fatty acid butyrate is a main energy source for colonic epithelium cells,36,101 is known to improve fatty acid butyrate is a main energy source for intestinal epithelial barrier function,17 and can modify the host immune response and provide anti-inflammatory effects.17,41,102 A Western-type diet low in dietary fiber and rich in animal fat and sugar could therefore potentially result in a decrease in short-chain fatty acid production and the benefits thereof.17 Short-chain fatty acids have also been demonstrated to prevent the growth or virulence of pathogenic organisms.101,103 As observed with secondary bile acids, recovery from CDI has been correlated with recovery of short-chain fatty acid production.104 Mice fed a fiber-rich diet demonstrate higher colonization resistance against *C. difficile*.98 Although the exact mechanism against *C. difficile* directly remains unknown, butyrate has been demonstrated to combat inflammation from *C. difficile* toxin, attenuating disease severity, in mice.104

Metabolic activity of gut microbiota promotes a largely anaerobic environment, which suppresses pathogen virulence.101,105 Specific gut microbiota species are also able to produce metabolites called bacteriocins, that have bactericidal activity against potential pathogens.36,48 Other mechanisms of colonization resistance against exogenous microorganisms include the protective role of mucus layers of the gut, and the potential role of bacteriophages that target only specific bacterial strains, thereby minimizing the impact on commensal microbiota. Further studies on the therapeutic use of bacteriophages will increase our understanding of their contribution to colonization resistance in humans.36,46

**Restoration of the gut microbiome**

Management of CDI presents a clinical dilemma, creating a need for antibiotic-sparing treatments that restore the intestinal microbiota, enhance colonization resistance, and do not select for development of antimicrobial resistance.38 Antibiotic therapy (fidaxomicin and vancomycin) is recommended as standard-of-care by current 2021 IDSA/SHEA guidelines and 2021 ACG guidelines as treatment for primary CDI, as well as recurrent CDI.85,106 Yet patients who have received antibiotics remain vulnerable to the development of recurrent CDI for at least 3 months after discontinuation of the antibiotic therapy.107 Dysbiosis associated with antibiotic use may persist for 1–2 years.81,107 Therefore, restoration or preservation of a healthy microbiome is critical to break the vicious cycle of recurrent CDI.107

One option for CDI treatment includes targeting toxin with non-antibiotic approaches to reduce damage to potentially beneficial microbes. Monoclonal antibodies that target and inactivate *C. difficile* toxins, such as bezlotoxumab, are recommended in conjunction with standard-of-care antibiotics to prevent recurrent CDI in patients at high risk of recurrence. However, bezlotoxumab has no direct effect on *C. difficile* and does not restore the microbiome.85,108,109

Direct restoration of beneficial microbes and their metabolites is an optimal treatment for prevention of recurrence. Different probiotics have been proposed to be used in conjunction with antibiotics to aid gut microbiota restoration, including *Saccharomyces boulardii*, *Lactobacillus*, or *Bifidobacterium* species.110,111 However, results on efficacy of probiotic use demonstrate moderate or inconclusive benefit.112,113 Given the role of diet in modulating the gut microbiome, diet has also been proposed as a potential approach to reduce recurrent disease. Diets high in fiber, for instance, have been shown to increase diversity and benefit symptoms caused by other gastrointestinal conditions such as irritable bowel syndrome.114 Despite the above discussed animal studies investigating the influence of diet on CDI, studies investigating...
diet modulation in human patients have not been conducted. Dietary changes to restore the gut microbiome thus remain to be investigated.

An example of a highly successful method of microbiome restoration to combat recurrent or refractive CDI is fecal microbiota transplantation (FMT).115,116 FMT is an evolving therapeutic strategy whereby stool is harvested from a healthy donor and transplanted into a patient’s colon to restore the gut microbiome to a healthier state.117 It is a complex intervention that involves multiple components, including donor selection and screening of stool, choice of method of transplantation, and use of stool banks.117 Stool preparations can be fresh or freeze–thawed, and methods of transplantation include colonoscopy, an oral capsule, nasogastric delivery, or an enema.118,119

FMT is recommended as a treatment option after a second or further recurrence of CDI by current 2021 IDSA/SHEA guidelines and 2021 ACG guidelines, to prevent further recurrence of CDI.85,106 It is highly effective in the treatment of recurrent CDI, with reported efficacy between 60% and 90% after a single treatment.119 Although FMT is a robust treatment option for recurrent CDI, its value in treating primary CDI remains to be determined. Antibiotics are standardly employed to treat patients with primary CDI;85 however, the established link between increased antibiotic exposure and increased likelihood of CDI recurrence,120 as well as the limited efficacy of standard-of-care antibiotics,121 necessitates improved therapeutic strategies. A recent small-scale clinical trial evaluated the use of FMT as a treatment for primary CDI and found that FMT may be an alternative to antibiotic therapy.122 In addition, moderate quality evidence from randomized controlled trials indicated that FMT is more effective in patients with C. difficile-associated diarrhea than vancomycin or placebo.123 Further research is underway to determine the efficacy of newer antibiotics for primary CDI and prevention of future recurrences, as well as vaccines and antibiotic-sparing therapies for CDI management.124

There are some challenges with regard to screening and standardization of methods used to harvest stool and processing of FMT.117 Current FMT processes for donor recruitment, stool selection, and processing are not standardized.117 A 2017 systematic review to examine the methods and reporting of studies evaluating FMT identified 85 eligible studies for assessment.117 Of these studies, 89% did not describe the eligibility criteria of donors or characteristics of donors,117 98% did not describe the methods used to collect donor stool,117 and 80% did not describe the type of stool used for infusion (whether it was fresh or frozen) or the volume infused.117 Furthermore, recent safety alerts issued by the Food and Drug Administration concerning the possible transmission of multidrug-resistant organisms or severe acute respiratory syndrome coronavirus 2 via FMT highlight the need for standardized screening and processing methods.125–131

It may soon be possible to measure the successful restoration of the microbiome and predict treatment response. Possible biological markers for dysbiosis and successful gut microbiome restoration have been investigated in clinical trials. The Microbiome Health Index is an investigational tool that captures changes in the relative abundance of taxonomic classes known to be involved in microbiome health and colonization resistance (Bacteroidia and Clostridia), and those associated with antibiotic-induced dysbiosis (Gammaproteobacteria and Bacilli).18 Khanna et al.78 prospectively examined pre-treatment stool samples from individuals with their first CDI episode and concluded that the gut microbiome signature may predict treatment response and recurrence risk, potentially aiding in identification of individuals who may benefit from earlier alternative treatment. A prospective longitudinal study conducted by Lee et al.132 among patients with ulcerative colitis found definitive differences in the microbiota community structure and characteristics between recurrent CDI and non-recurrent CDI patients, which may be useful to predict risk of recurrent CDI.

Increased knowledge about the most beneficial composition of bacteria to administer to restore the gut microbiota led to the clinical development of several newer live biotherapeutic formulations.107 These investigational formulations have more standardized production methodologies.107,133 The exact underlying mechanisms through which live biotherapeutic products replace the microbiota to restore the microbiome are currently unknown, although many of the colonization resistance mechanisms discussed...
previously represent potential microbial targets. Preliminary results from a phase III, randomized, placebo-controlled clinical trial involving a liquid preparation containing a broad consortium of live microbiota (delivered via enema), as well as phase III trial results involving an oral microbiome therapeutic capsule containing purified spores, show promising results in reducing *C. difficile* recurrence and restoring the gut microbiome. Successful restoration of the gut microbiome may also decrease the abundance of antibiotic-resistant organisms and antibiotic resistance genes. Further research within other disease areas may determine whether gut microbiome restoration is also beneficial in the management of gastrointestinal diseases associated with dysbiosis such as inflammatory bowel disease, irritable bowel syndrome, and colon cancer. As our understanding of this rapidly evolving disease area grows, live microbiota replacement therapies may become ever more targeted to support colonization resistance against potential pathogenic organisms such as *C. difficile*, to reduce recurrent disease, and to promote overall health.

**Conclusion**

A healthy gut microbiome consists of a broad consortium of microorganisms that compete against potential pathogens and each other for resources such as nutrients and adhesion receptors on gut epithelium, and are able to produce antibacterial substances. Disruption of the diversity and abundance of the gut microbiota lead to dysbiosis and a lack of colonization resistance, that make the gut more susceptible to colonization by pathogenic organisms such as *C. difficile*. Treatment of acute and recurrent CDI often presents a clinical dilemma, as standard-of-care antibiotics do not restore the intestinal microbiota or colonization resistance and may select for development of antimicrobial resistance. Restoration of a healthy microbiome is critical to break the vicious cycle of recurrent CDI. Although effective as a treatment option to prevent recurrent CDI, current FMT processes for donor recruitment, stool selection, and processing are not standardized. Newer biotherapeutic formulations currently in development have more standardized manufacturing processes and have shown promising results in phase III clinical studies in preventing *C. difficile* recurrence and restoring the gut microbiome, paving the way forward for the reduction of recurrent CDI.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Author contribution(s)**

Anna Maria Seekatz: Conceptualization; Writing – original draft; Writing – review & editing.

Nasia Safdar: Conceptualization; Writing – original draft; Writing – review & editing.

Sahil Khanna: Conceptualization; Writing – original draft; Writing – review & editing.

**Acknowledgements**

Medical writing and editorial assistance were provided by Shandré Pieterse, MD, at ApotheCom (Yardley, PA, USA) and was funded by Ferring Pharmaceuticals Inc.

**Funding**

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Medical writing and editorial assistance were funded by Ferring Pharmaceuticals Inc.

**Competing interests**

Dr. Khanna declared research grants from Rebiotix, Inc (a Ferring Company), Seres, Vedanta, and Finch and consulting fees from Shire Plc, Immuron, Jetson, and Niche.

Dr. Seekatz declared consulting fees from Finch.

Dr. Safdar declared funding from the National Institutes of Health (NIH), Veterans Affairs (VA), and Agency for Healthcare Research and Quality (AHRQ).

**Availability of data and materials**

Not applicable.
References

1. Berg G, Rybakova D, Fischer D, et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome 2020; 8: 103.

2. Whipp J M, Lewis K and Cooke R. Mycoparasitism and plant disease control. In: Beemster ABR, Bollen GJ, Gerlagh M, et al. (eds) Fungi in biological control systems. Manchester: Manchester University Press, 1988, pp.161–187.

3. Mazmanian SK, Liu CH, Tzianabos AO, et al. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005; 122: 107–118.

4. Ivanov II, Frutos Rde L, Manel N, et al. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe 2008; 4: 337–349.

5. Cario E, Gerken G and Podolsky DK. Toll-like receptor 2 controls mucosal inflammation by regulating epithelial barrier function. Gastroenterology 2007; 132: 1359–1374.

6. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 2004; 118: 229–241.

7. Macfarlane S and Macfarlane GT. Regulation of short-chain fatty acid production. Proc Nutr Soc 2003; 62: 67–72.

8. Carding S, Verbeke K, Vipond DT, et al. Dysbiosis of the gut microbiota in disease. Micro Ecol Health Dis 2015; 26: 26191.

9. Buford TW. (Dis)Trust your gut: the gut microbiome in age-related inflammation, health, and disease. Microbiome 2017; 5: 80.

10. Ramirez J, Guarner F, Bustos Fernandez L, et al. Antibiotics as major disruptors of gut microbiota. Front Cell Infect Microbiol 2020; 10: 572912.

11. Goldberg E, Amir I, Zafra M, et al. The correlation between clostridium-difficile infection and human gut concentrations of bacteroidetes phyllum and clostridial species. Eur J Clin Microbiol Infect Dis 2014; 33: 377–383.

12. Bien J, Palagani V and Bozko P. The intestinal microbiota dysbiosis and clostridium difficile infection: is there a relationship with inflammatory bowel disease? Therap Adv Gastroenterol 2013; 6: 53–68.

13. Bajinka O, Tan Y, Abdelhalim KA, et al. Extrinsic factors influencing gut microbes, the immediate consequences and restoring eubiosis. AMB Express 2020; 10: 130.

14. Smits WK, Lyras D, Lacy DB, et al. Clostridium difficile infection. Nat Rev Dis Primers 2016; 2: 16020.

15. Iebba V, Totino V, Gagliardi A, et al. Eubiosis and dysbiosis: the two sides of the microbiota. New Microbiol 2016; 39: 1–12.

16. Rinninella E, Raoul P, Cintoni M, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms 2019; 7: 14.

17. Thursby E and Juge N. Introduction to the human gut microbiota. Biochem J 2017; 474: 1823–1836.

18. Blount K, Jones C, Walsh DM, et al. Development and validation of a novel microbiome-based biomarker of post-antibiotic dysbiosis and subsequent restoration. Front Microbiol 2021; 12: 781275.

19. Sorbara MT, Littmann ER, Fontana E, et al. Functional and genomic variation between human-derived isolates of lachnospiraceae reveals inter-and intra-species diversity. Cell Host Microbe 2020; 28: 134.e4–146.e4.

20. Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. Nature 2011; 473: 174–180.

21. Zhu A, Sunagawa S, Mende DR, et al. Inter-individual differences in the gene content of human gut bacterial species. Genome Biol 2015; 16: 82.

22. Wilmanski T, Diener C, Rappaport N, et al. Gut microbiome pattern reflects healthy ageing and predicts survival in humans. Nature Metab 2021; 3: 274–286.

23. Roswall J, Olsson LM, Kovatcheva-Datchary P, et al. Developmental trajectory of the healthy human gut microbiota during the first 5 years of life. Cell Host Microbe 2021; 29: 765.e3–776.e3.

24. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 2015; 17: 690–703.

25. Huurre A, Kalliomäki M, Rautava S, et al. Mode of delivery-effects on gut microbiota and humoral immunity. Neonatology 2008; 93: 236–240.
26. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA 2010; 107: 11971–11975.

27. Pannaraj PS, Li F, Cerini C, et al. Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. JAMA Pediatr 2017; 171: 647–654.

28. O’Sullivan A, Farver M and Smilowitz JT. The influence of early infant-feeding practices on the intestinal microbiome and body composition in infants. Nutr Metab Insights 2015; 8: 1–9.

29. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011; 334: 105–108.

30. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014; 505: 559–563.

31. Lee M and Chang EB. Inflammatory bowel diseases (IBD) and the microbiome-searching the crime scene for clues. Gastroenterology 2021; 160: 524–537.

32. Theriot CM, Bowman AA and Young VB. Antibiotic-induced alterations of the gut microbiota alter secondary bile acid production and allow for Clostridium difficile spore germination and outgrowth in the large intestine. mSphere 2016; 1: e00045–e00115.

33. Beamish LA, Osornio-Vargas AR and Wine E. Air pollution: an environmental factor contributing to intestinal disease. J Crohns Colitis 2011; 5: 279–286.

34. Biedermann L, Zeitz J, Mwinyi J, et al. Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans. PLoS One 2013; 8: e59260.

35. Zhermakova A, Kurilshikov A, Bonder MJ, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science 2016; 352: 565–569.

36. Ducarmon QR, Zwittink RD, Hornung BVH, et al. Gut microbiota and colonization resistance against bacterial enteric infection. Microbiol Mol Biol Rev 2019; 83: e00007-e00019.

37. McBurney MJ, Davis G, Fraser CM, et al. Establishing what constitutes a healthy human gut microbiome: state of the science, regulatory considerations, and future directions. J Nutr 2019; 149: 1882–1895.

38. Langdon A, Schwartz DJ, Bulow C, et al. Microbiota restoration reduces antibiotic-resistant bacteria gut colonization in patients with recurrent clostridioides difficile infection from the open-label PUNCH CD study. Genome Med 2021; 13: 28.

39. Falony G, Joossens M, Vieira-Silva S, et al. Population-level analysis of gut microbiome variation. Science 2016; 352: 560–564.

40. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012; 486: 207–214.

41. Monda V, Villano I, Messina A, et al. Exercise modifies the gut microbiota with positive health effects. Oxid Med Cell Longev 2017; 2017: 3831972.

42. Lozupone CA, Stombaugh JI, Gordon JI, et al. Diversity, stability and resilience of the human gut microbiota. Nature 2012; 489: 220–230.

43. Degnan PH, Barry NA, Mok KC, et al. Human gut microbes use multiple transporters to distinguish vitamin B12 analogs and compete in the gut. Cell Host Microbe 2014; 15: 47–57.

44. Oliveira RA, Ng KM, Correia MB, et al. Klebsiella michiganensis transmission enhances resistance to enterobacteriaceae gut invasion by nutrition competition. Nature Microbiol 2020; 5: 630–641.

45. Lawley TD and Walker AW. Intestinal colonization resistance. Immunology 2013; 138: 1–11.

46. Sicard JJ, Le Bihan G, Vogeleer P, et al. Interactions of intestinal bacteria with components of the intestinal mucus. Front Cell Infect Microbiol 2017; 7: 387.

47. Vaishnava S, Behrendt CL, Ismail AS, et al. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. Proc Natl Acad Sci USA 2008; 105: 20858–20863.

48. Rea MC, Sit CS, Clayton E, et al. Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against Clostridium difficile. Proc Natl Acad Sci USA 2010; 107: 9352–9357.

49. Macpherson AJ and Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 2004; 303: 1662–1665.

50. Massacand JC, Kaiser P, Ernst B, et al. Intestinal bacteria condition dendritic cells to promote IgA production. PLoS One 2008; 3: e2588.
51. Dowle C. Faecal microbiota transplantation: a review of FMT as an alternative treatment for Clostridium difficile infection. *Bio Horizons* 2016; 9: 1–14.

52. Abt MC, McKenney PT and Pamer EG. Clostridium difficile colitis: pathogenesis and host defence. *Nat Rev Microbiol* 2016; 14: 609–620.

53. Rupnik M, Wilcox MH and Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol* 2009; 7: 526–536.

54. Giel JL, Sorg JA, Sonenshein AL, et al. Metabolism of bile salts in mice influences spore germination in Clostridium difficile. *PLoS One* 2010; 5: e8740.

55. Sorg JA and Sonenshein AL. Bile salts and glycine as cogerminants for *Clostridium difficile* spores. *J Bacteriol* 2008; 190: 2505–2512.

56. Theriot CM, Koenigschnetl MJ, Carlson PE, Jr., et al. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to *clostridium difficile* infection. *Nat Commun* 2014; 5: 3114.

57. Buffie CG, Bucci V, Stein RR, et al. Precision microbiome reconstitution restores bile acid mediated resistance to *clostridium difficile*. *Science* 2015; 351: 205–208.

58. Mullish BH and Allegretti JR. The contribution of bile acid metabolism to the pathogenesis of *clostridioides difficile* infection. *Therap Adv Gastroenterol* 2021; 14: 1756284211017725.

59. Fordtran JS. Colitis due to *clostridium difficile* toxins: underdiagnosed, highly virulent, and nosocomial. *Proc (Bayl Univ Med Cent)* 2006; 19: 3–12.

60. Shen A. *Clostridium difficile* toxins: mediators of inflammation. *J Innate Immun* 2012; 4: 149–158.

61. Bartlett JG and Gerding DN. Clinical recognition and diagnosis of *clostridium difficile* infection. *Clin Infect Dis* 2008; 46: S12–S18.

62. Gerding DN, Johnson S, Rupnik M, et al. *Clostridium difficile* binary toxin CDT: mechanism, epidemiology, and potential clinical importance. *Gut Microbes* 2014; 5: 15–27.

63. Pruitt RN, Chambers MG, Ng KK, et al. Structural organization of the functional domains of *clostridium difficile* toxins A and B. *Proc Natl Acad Sci USA* 2010; 107: 13467–13472.

64. Guh AY, Mu Y, Winston LG, et al. Trends in U.S. burden of *clostridioides difficile* infection and outcomes. *N Engl J Med* 2020; 382: 1320–1330.

65. Lessa FC, Mu Y, Bamberg WM, et al. Burden of *clostridioides difficile* infection in the United States. *N Engl J Med* 2015; 372: 825–834.

66. Centers for Disease Control and Prevention. *CDC 2018 Annual report for the emerging infections program for clostridioides difficile infection*. Retrieved February 17, 2022, from https://www.cdc.gov/hai/eip/Annual-CDI-Report-2018.html.

67. Desai K, Gupta SB, Dubberke ER, et al. Epidemiological and economic burden of *clostridium difficile* in the United States: estimates from a modeling approach. *BMC Infect Dis* 2016; 16: 303.

68. Kelly CP. Can we identify patients at high risk of recurrent *clostridioides difficile* infection? *Clin Microbiol Infect* 2012; 18: 21–27.

69. Cornely OA, Miller MA, Louie TJ, et al. Treatment of first recurrence of *clostridium difficile* infection: fidaxomicin versus vancomycin. *Clin Infect Dis* 2012; 55: S154–S161.

70. Al-Jashaami LS and DuPont HL. Management of *clostridium difficile* infection. *Gastroenterol Hepatol* 2016; 12: 609–616.

71. Weaver FM, Trick WE, Evans CT, et al. The impact of recurrent *clostridium difficile* infection on patients’ prevention behaviors. *Infect Control Hosp Epidemiol* 2017; 38: 1351–1357.

72. Lurienne L, Bandinelli PA, Galvain T, et al. Perception of quality of life in people experiencing or having experienced a *clostridioides difficile* infection: a US population survey. *J Patient Rep Outcomes* 2020; 4: 14.

73. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal microbiome in recurrent *clostridium difficile* infection. *J Infect Dis* 2008; 197: 435–438.

74. Schubert AM, Sinani H and Schloss PD. Antibiotic-induced alterations of the murine gut microbiota and subsequent effects on colonization resistance against *clostridium difficile*. *mBio* 2015; 6: e00974.

75. Schubert AM, Rogers MA, Ring C, et al. Microbiome data distinguish patients with *clostridium difficile* infection and non--*C. difficile*-associated diarrhea from healthy controls. *mBio* 2014; 5: e01021–e01014.

76. Berkell M, Mysara M, Xavier BB, et al. Microbiota-based markers predictive of *clostridium difficile* infection. *Nat Commun* 2021; 12: 2241.

77. Seekatz AM, Rao K, Santhosh K, et al. Dynamics of the fecal microbiome in patients with recurrent
and nonrecurrent clostridium difficile infection. *Genome Med* 2016; 8: 47.

78. Khanna S, Montassier E, Schmidt B, et al. Gut microbiome predictors of treatment response and recurrence in primary clostridium difficile infection. *Aliment Pharmacol Ther* 2016; 44: 715–727.

79. Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. *N Engl J Med* 2002; 346: 334–339.

80. Suez J, Zmora N, Zilberman-Schapira G, et al. Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous PMT. *Cell* 2018; 174: 1406.e16–1423.e16.

81. Neuman H, Forsythe P, Uzan A, et al. Antibiotics in early life: dysbiosis and the damage done. *FEMS Microbiol Rev* 2018; 42: 489–499.

82. Francino MP. Antibiotics and the human gut microbiome: dysbioses and accumulation of resistances. *Front Microbiol* 2015; 6: 1543.

83. Dethlefsen L, Huse S, Sogin ML, et al. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 2008; 6: e280.

84. Caballero S, Carter R, Ke X, et al. Distinct but spatially overlapping intestinal niches for vancomycin-resistant enterococcus faecium and carbapenem-resistant klebsiella pneumoniae. *PLoS Pathog* 2015; 11: e1005132.

85. Johnson S, Lavergne V, Skinner AM, et al. Antibiotic expansion of enteric pathogens. *Nature* 2013; 502: 96–99.

86. Johnson S, Adelmann A, Clabots CR, et al. Recurrences of clostridium difficile diarrhea not caused by the original infecting organism. *J Infect Dis* 1989; 159: 340–343.

87. Seekatz AM, Wolfram E, DeWald CM, et al. Presence of multiple clostridium difficile strains at primary infection is associated with development of recurrent disease. *Anaerobe* 2018; 53: 74–81.

88. Johnson S, Adelmann A, Clabots CR, et al. Microbiota-accessible carbohydrates suppress clostridium difficile infection in a murine model. *Nat Microbiol* 2018; 3: 662–669.

89. Ng KM, Ferreyra JA, Higginbottom SK, et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. *Nature* 2013; 502: 96–99.

90. Ferreyra JA, Wu KJ, Hryckowian AJ, et al. Gut microbiota-produced succinate promotes C. difficile infection after antibiotic treatment or motility disturbance. *Cell Host Microbe* 2014; 16: 770–777.

91. Jenior ML, Leslie JL, Young VB, et al. Clostridium difficile colonizes alternative nutrient niches during infection across distinct murine gut microbiomes. *mSystems* 2017; 2: e00063–e00017.

92. Aguirre AM, Yalcinkaya N, Wu Q, et al. Bile acid-independent protection against clostridiodes difficile infection. *PLoS Pathog* 2021; 17: e1010015.

93. Fletcher JR, Pike CM, Parsons RJ, et al. Clostridiodes difficile exploits toxin-mediated inflammation to alter the host nutritional landscape and exclude competitors from the gut microbiota. *Nat Commun* 2021; 12: 462.

94. Lewis S, Burmeister S and Brazier J. Effect of the prebiotic oligofructose on relapse of clostridium difficile-associated diarrhea: a randomized, controlled study. *Clin Gastroenterol Hepatol* 2005; 3: 442–448.

95. Mefferd CC, Bhute SS, Phan JR, et al. A high-fat/high-protein, Atkins-type diet exacerbates clostridiodes (clostridium) difficile infection in mice, whereas a high-carbohydrate diet protects. *mSystems* 2020; 5: e00765–19.

96. Hazleton KZ, Martin CG, Orlicky DJ, et al. Dietary fat promotes antibiotic-induced clostridiodes difficile mortality in mice. *bioRxiv* 2022; 8: 15.

97. Jose S, Mukherjee A, Horrigan O, et al. Bile acid-independent protection against clostridiodes difficile infection in high fat diet-induced obese mice. *Mucosal Immunol* 2021; 14: 500–510.

98. Hryckowian AJ, Van Treuren W, Smits SA, et al. Microbiota-accessible carbohydrates suppress clostridium difficile infection in a murine model. *Nat Microbiol* 2018; 3: 662–669.

99. von Schwartzenberg RJ, Bisanz JE, Lyalina S, et al. Caloric restriction disrupts the microbiota and colonization resistance. *Nature* 2021; 595: 272–277.

100. Battaglioni EJ, Hale VL, Chen J, et al. Clostridioides difficile uses amino acids associated with gut microbial dysbiosis in a subset of patients with diarrhea. *Sci Transl Med* 2018; 10: eaam7019.

101. Rivera-Chávez F, Zhang LF, Faber F, et al. Depletion of butyrate-producing clostridia from
the gut microbiota drives an aerobic luminal expansion of salmonella. Cell Host Microbe 2016; 19: 443–454.

102. Mathewson ND, Jenq R, Mathew AV, et al. Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. Nat Immunol 2016; 17: 505–513.

103. Pinhal S, Ropers D, Geiselmann J, et al. Acetate metabolism and the inhibition of bacterial growth by acetate. J Bacteriol 2019; 201: e00147–19.

104. Fachi JL, Felipe JS, Pral LP, et al. Butyrate protects mice from clostridium difficile-induced colitis through an HIF-1-dependent mechanism. Cell Rep 2019; 27: 750.e7–761.e7.

105. Litvak Y, Mon KKZ, Nguyen H, et al. Commensal enterobacteriaceae protect against salmonella colonization through oxygen competition. Cell Host Microbe 2019; 25: 128. e5–139.e5.

106. Kelly CR, Fischer M, Allegretti JR, et al. ACG clinical guidelines: prevention, diagnosis, and treatment of clostridioides difficile infections. Am J Gastroenterol 2021; 116: 1124–1147.

107. Gonzales-Luna AJ and Carlson TJ. Follow your gut: microbiome-based approaches in the developmental pipeline for the prevention and adjunctive treatment of clostridioides difficile infection (CDI). Curr Infect Dis Rep 2020; 22: 22.

108. Wilcox MH, Gerdning DN, Poxtton IR, et al. Bezlotoxumab for prevention of recurrent clostridium difficile infection. N Engl J Med 2017; 376: 305–317.

109. Merck Sharp and Dohme Corp. Prescribing information: ZINPLAVA (bezlotoxumab) injection. Whitehouse Station, NJ: Merck Sharp & Dohme Corp, 2016.

110. Sinclair A, Xie X, Saab L, et al. Lactobacillus probiotics in the prevention of diarrrhea associated with clostridium difficile: a systematic review and bayesian hierarchical meta-analysis. CMAJ Open 2016; 4: e706–e718.

111. Mills JP, Rao K and Young VB. Probiotics for prevention of clostridium difficile infection. Curr Opin Gastroenterol 2018; 34: 3–10.

112. Goldenberg JZ, Yap C, Lytvyn L, et al. Probiotics for the prevention of clostridium difficile-associated diarrhea in adults and children. Cochrane Database Syst Rev 2017; 12: Cd006095.

113. Allen SJ, Wareham K, Wang D, et al. A high-dose preparation of lactobacilli and bifidobacteria in the prevention of antibiotic-associated and clostridium difficile diarrhoea in older people admitted to hospital: a multicentre, randomised, double-blind, placebo-controlled, parallel arm trial (PLACIDE). Health Technol Assess 2013; 17: 1–140.

114. Yang J, Wang HP, Zhou L, et al. Effect of dietary fiber on constipation: a meta analysis. World J Gastroenterol 2012; 18: 7378–7383.

115. Tariq R, Pardis DS, Bartlett MG, et al. Low cure rates in controlled trials of fecal microbiota transplantation for recurrent clostridium difficile infection: a systematic review and meta-analysis. Clin Infect Dis 2019; 68: 1351–1358.

116. Quraishi MN, Widlak M, Bhala N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory clostridium difficile infection. Aliment Pharmacol Ther 2017; 46: 479–493.

117. Bafeta A, Yavchitz A, Riveros C, et al. Methods and reporting studies assessing fecal microbiota transplantation: a systematic review. Ann Intern Med 2017; 167: 34–39.

118. Ramai D, Zakhia K, Ofoosu A, et al. Fecal microbiota transplantation: donor relation, fresh or frozen, delivery methods, cost-effectiveness. Ann Gastroenterol 2019; 32: 30–38.

119. Kao D, Roach B, Silva M, et al. Effect of oral capsule-vs colonoscopy-delivered fecal microbiota transplantation on recurrent clostridium difficile infection: a randomized clinical trial. JAMA 2017; 318: 1985–1993.

120. Davies K, Lawrence J, Berry C, et al. Risk factors for primary clostridium difficile infection; results from the observational study of risk factors for clostridium difficile infection in hospitalized patients with infective diarrhea (ORCHID). Front Public Health 2020; 8: 293.

121. Peng Z, Jin D, Kim HB, et al. Update on antimicrobial resistance in clostridium difficile: resistance mechanisms and antimicrobial susceptibility testing. J Clin Microbiol 2017; 55: 1998–2008.

122. Juul FE, Garborg K, Brethvauer M, et al. Fecal microbiota transplantation for primary clostridium difficile infection. N Engl J Med 2018; 378: 2535–2536.

123. Moayyedi P, Yuan Y, Baharith H, et al. Faecal microbiota transplantation for clostridium difficile-associated diarrhoea: a systematic
124. Chai J and Lee CH. Management of primary and recurrent clostridium difficile infection: an update. *Antibiotics (Basel)* 2018; 7: 54.

125. US Food and Drug Administration. Safety alert regarding use of fecal microbiota for transplantation and additional safety protections pertaining to SARS-CoV-2 and COVID-19. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/safety-alert-regarding-use-fecal-microbiota-transplantation-and-additional-safety-protections (2020, accessed 29 November 2021).

126. US Food and Drug Administration. Information pertaining to additional safety protections regarding use of fecal microbiota for transplantation—screening donors for COVID-19 and testing for SARS-CoV-2. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/information-pertaining-additional-safety-protections-regarding-use-fecal-microbiota-transplantation-1 (2020, accessed 29 November 2021).

127. US Food and Drug Administration. Information pertaining to additional safety protections regarding use of fecal microbiota for transplantation—screening and testing of stool donors for multi-drug resistant organisms. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/information-pertaining-additional-safety-protections-regarding-use-fecal-microbiota-transplantation (2019, accessed 29 November 2021).

128. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N Engl J Med* 2019; 381: 2043–2050.

129. Zellner C, Sater MRA, Huntley MH, et al. Shiga toxin-producing escherichia coli transmission via fecal microbiota transplant. *Clin Infect Dis* 2021; 72: e876–e880.

130. Khanna S and Kraft CS. Fecal microbiota transplantation: tales of caution. *Clin Infect Dis* 2020; 72: e881–e882.

131. Khanna S and Pardi D. Fecal microbiota transplantation for recurrent clostridioides difficile infection: the COVID-19 era. *Am J Gastroenterol* 2020; 115: 971–974.

132. Lee AA, Rao K, Limsrivilai J, et al. Temporal gut microbial changes predict recurrent clostridioides difficile infection in patients with and without ulcerative colitis. *Inflamm Bowel Dis* 2020; 26: 1748–1758.

133. Rebiotix Inc. Rebiotix announces first patient enrolled in phase 3 clinical trial of RBX2660 for the prevention of recurrent clostridium difficile infection. Roseville, MN: Rebiotix Inc, 2017.

134. Blount K, Walsh DM, Gonzalez C, et al. Treatment success in reducing recurrent clostridioides difficile infection with investigational live biotherapeutic RBX2660 was associated with microbiota restoration: consistent evidence from a phase 3 clinical trial. *Open Forum Infect Dis* 2021; 8: S624–S625.

135. Feuerstadt P, Louie TJ, Lashner B, et al. SER-109, an oral microbiome therapy for recurrent clostridioides difficile infection. *N Engl J Med* 2022; 386: 220–229.

136. Hau H. Antimicrobial resistance genes are reduced following administration of investigational microbiota based therapeutic RBX2660 to individuals with recurrent *Clostridioides difficile* infection.

137. Round JL and Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009; 9: 313–323.