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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.

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- 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 *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, *Clostridioides difficile* infection, dysbiosis, fecal microbiota transplantation, microbiota, microbiota-based therapy

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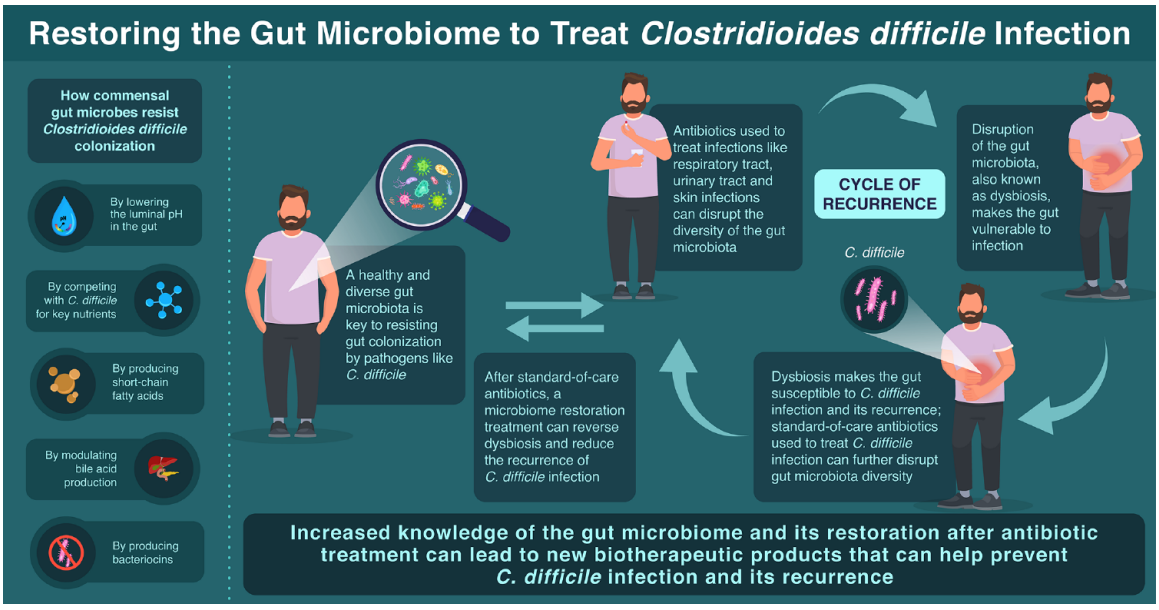

Infographic: Restoring the Gut Microbiome to Treat *Clostridioides difficile* Infection.

Introduction

The human gut microbiota comprises a diverse group of microorganisms that inhabit the gastrointestinal tract and includes bacteria, viruses, and fungi.[1] Collectively with the gastrointestinal environment that it inhabits, the gut microbiome[1,2] is critical to maintaining host health, including immune system regulation,[3,4] epithelial barrier support,[5,6] and metabolic regulation such as energy acquisition.[7] Disruption of the healthy composition of microbiota results in dysbiosis and has been associated with a range of gastrointestinal and non-gastrointestinal diseases.[8–14] Restoring the gut microbiota to a more diverse, healthier, and balanced composition, the condition known as eubiosis,[13,15] 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).[16] 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.[16,17] 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.[16,18] 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.[16,19] Because interindividual variation in the types of genera and species in the human gut occurs,[19–21] 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.[22–24] The composition of the adult gut microbiome is influenced by many factors.[25–35] 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

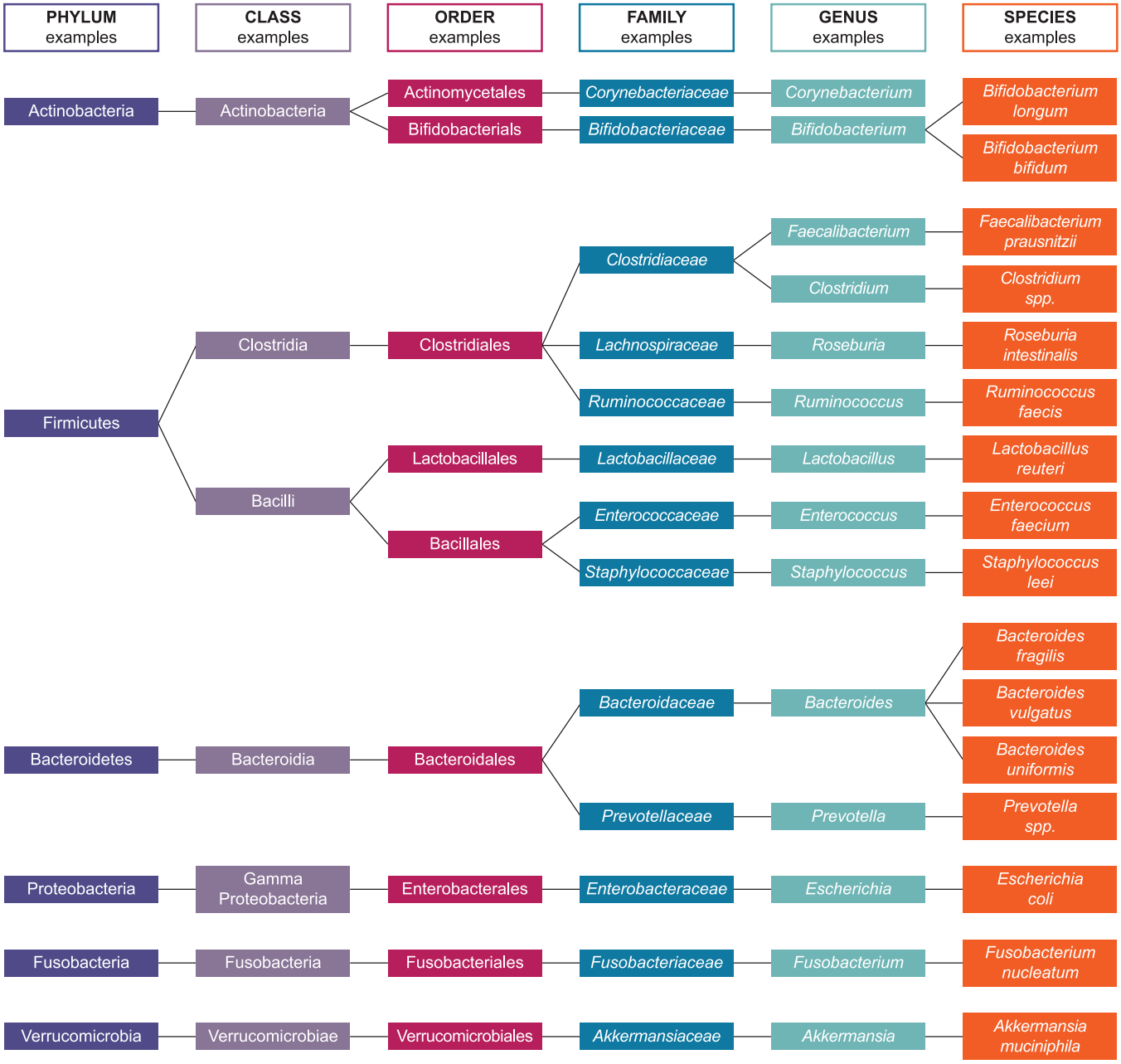

Figure 1. Composition of common gut microbiota. Taxonomically, bacteria are classified into phyla, classes, orders, families, genera, and species. The Firmicutes and Bacteroidetes phyla are the two most common bacterial phyla in the gut.[16]

the composition of the gut microbiome, thus influencing its functionality.[30,31] 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.[22]

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).[8–15,36,37] Perhaps most relevant to decreased colonization resistance are medications, particularly antibiotics,

Table 1. Common terminology defined.

Term	Definition
Microbiota[1]	The diverse group of microorganisms, including bacteria, archaea, viruses, and fungi, found in and on multicellular organisms
Microbiome[1,2]	The collective community of microorganisms and their activity within their environment
Eubiosis[12,13]	A healthy, balanced state of the microbiome
Colonization resistance[8,9]	Gut microbiota provide resistance to colonization of the gut by exogenous organisms
Dysbiosis[10–12]	Disruption of the healthy composition, abundance, diversity, and functionality of the microbiome

which are known to drastically change the microbiota, resulting in disruption of major potentially beneficial bacteria.[35,38,39] 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 *C. difficile*.[32,38] 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.[32,38] 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.[17,40,41] 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).[36,37,42] 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[36,43,44] or attachment sites on the gut epithelium,[11,17,45,46] to produce antibacterial substances[36,47,48] and to modulate the host immune response[3,4] such as inducing immunoglobulin A secretion, which inhibits colonization of the gut by potential pathogens.[49,50] This review will focus on mechanisms by which the gut microbiome restoration could influence colonization resistance against the pathogen *C. difficile*.

Clostridioides difficile infection

C. difficile is an anaerobic, Gram-positive bacterium that exists in both vegetative and spore forms.[14,51] Spores are ubiquitous in the environment and are the infectious form of *C. difficile*.[14,51,52] Spores are highly resistant to some antibiotics and environmental factors including oxygen, disinfectants (including ethanol-based hand sanitizers), high temperature, and ultraviolet light.[14,52] They can contaminate the environment around patients and can persist for years.[53] Once ingested by the host,[14] under the right conditions, normally dormant spores can germinate in the gut into replicating, metabolically active vegetative cells.[54–58] If the strain of *C. difficile* contains genes for toxin production (i.e. is toxigenic), vegetative cells will produce toxins in the colon that ultimately lead to disease,[14,52,53,59] defined by symptoms ranging from diarrhea and gastrointestinal distress, to more severe forms including toxic megacolon, pseudomembranous colitis, and even death.[60,61] The toxins responsible for disease include Toxin A (TcdA) and Toxin B (TcdB), with some strains producing a binary toxin (or *C. difficile* transferase) which enhances its virulence

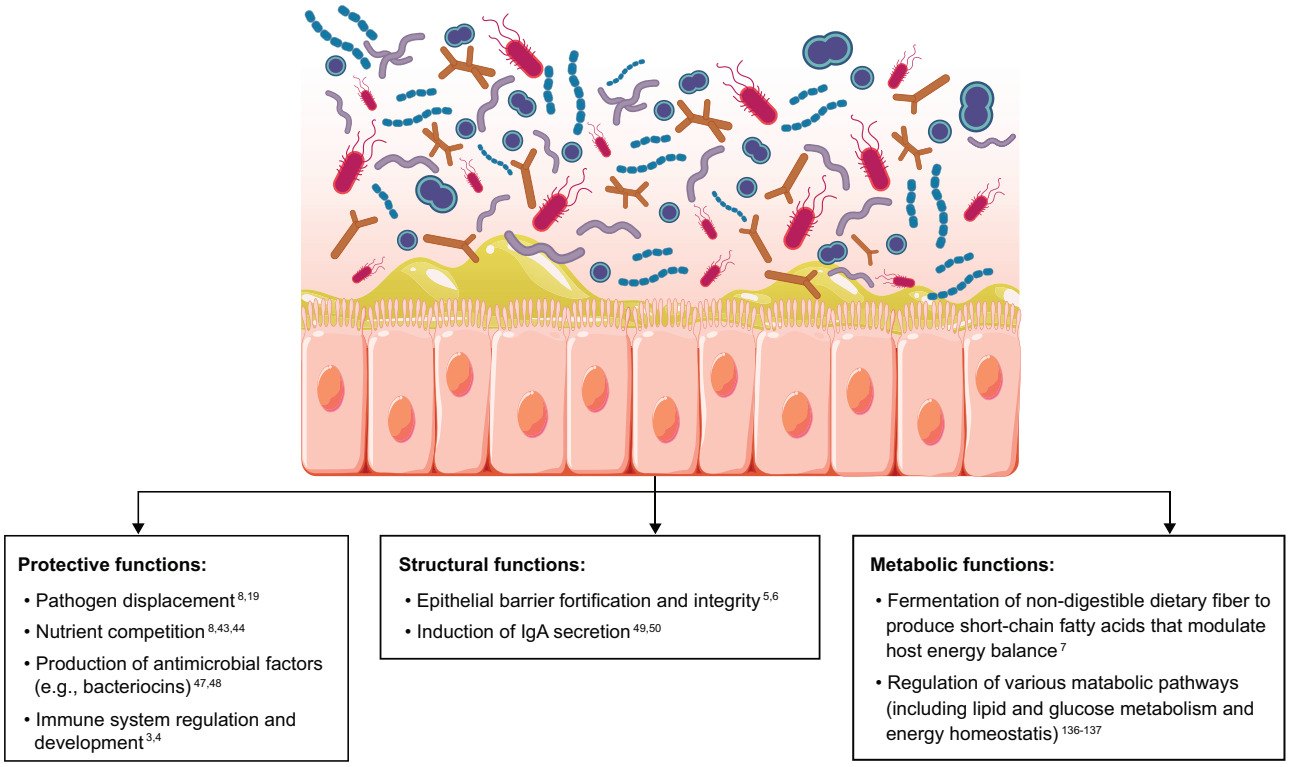

Figure 2. Protective, structural, and metabolic functions of the gut microbiota to promote overall health. A healthy microbiota consists of a broad consortium of organisms that promote overall health through protective, structural, and metabolic functions. IgA, immunoglobulin A.

(Figure 3).[60,62,63] 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.[53,60]

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.[64] Even as healthcare-associated infections have decreased slightly in the last 10 years, some reports suggest an overall increase in community-related diagnoses.[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.[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.[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.[14,65,68,69] These recurrences can have a profound impact on the lives of patients and be

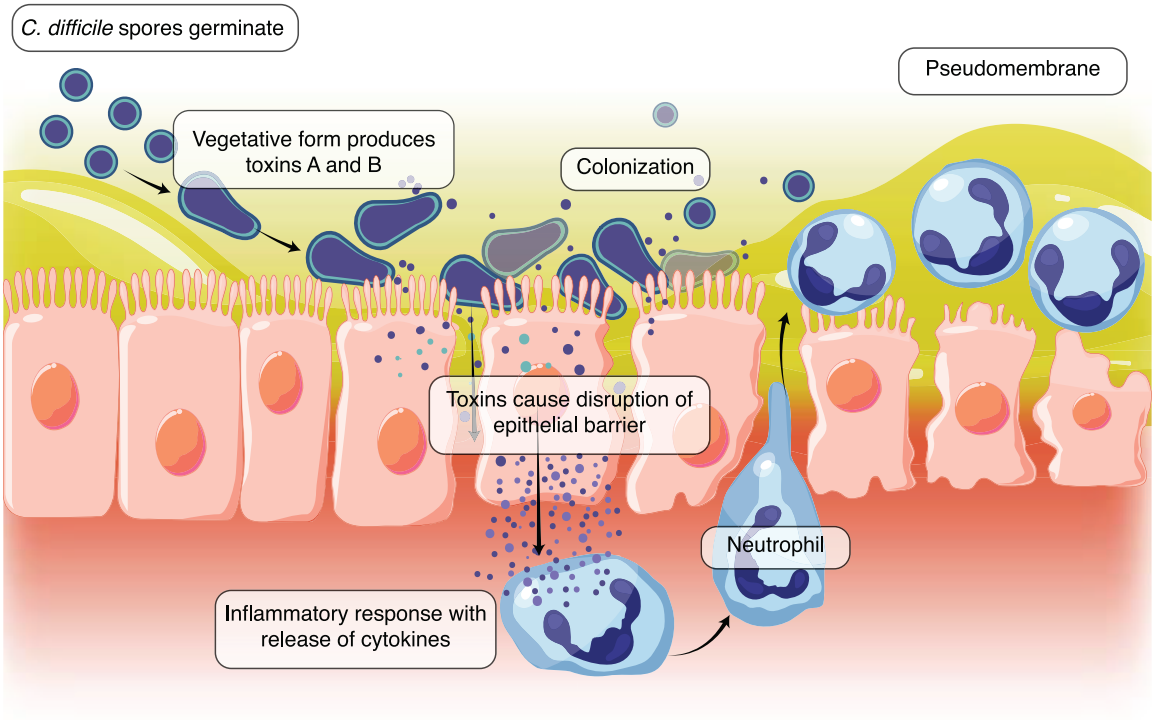

Figure 3. The pathogenesis of *Clostridioides difficile*. *C. difficile* spores germinate into toxin-producing vegetative cells, disrupting the intestinal epithelial barrier and resulting in a host inflammatory response.[14,53,60] Escalation of the inflammatory response with neutrophil influx results in the formation of a pseudomembrane.[53,60]

severely disabling.[70] 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.[71] 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.[71] 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.[72]

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.[73] 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.[74] 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).[75,76] In particular, Firmicutes members such as *Lachnospiraceaea* and *Ruminococcaceae* families are decreased, whereas *Enterococcus* genera increased.[76] Decreased gut microbiota diversity and resilience is associated with the severity of CDI and is a risk factor for recurrent CDI.[77] Furthermore, the composition of the gut microbiome prior to CDI may also predict the response to treatment and subsequent recurrence risk.[78]

Antibiotic therapy disrupts a eubiotic gut microbiome, reducing microbiota diversity and

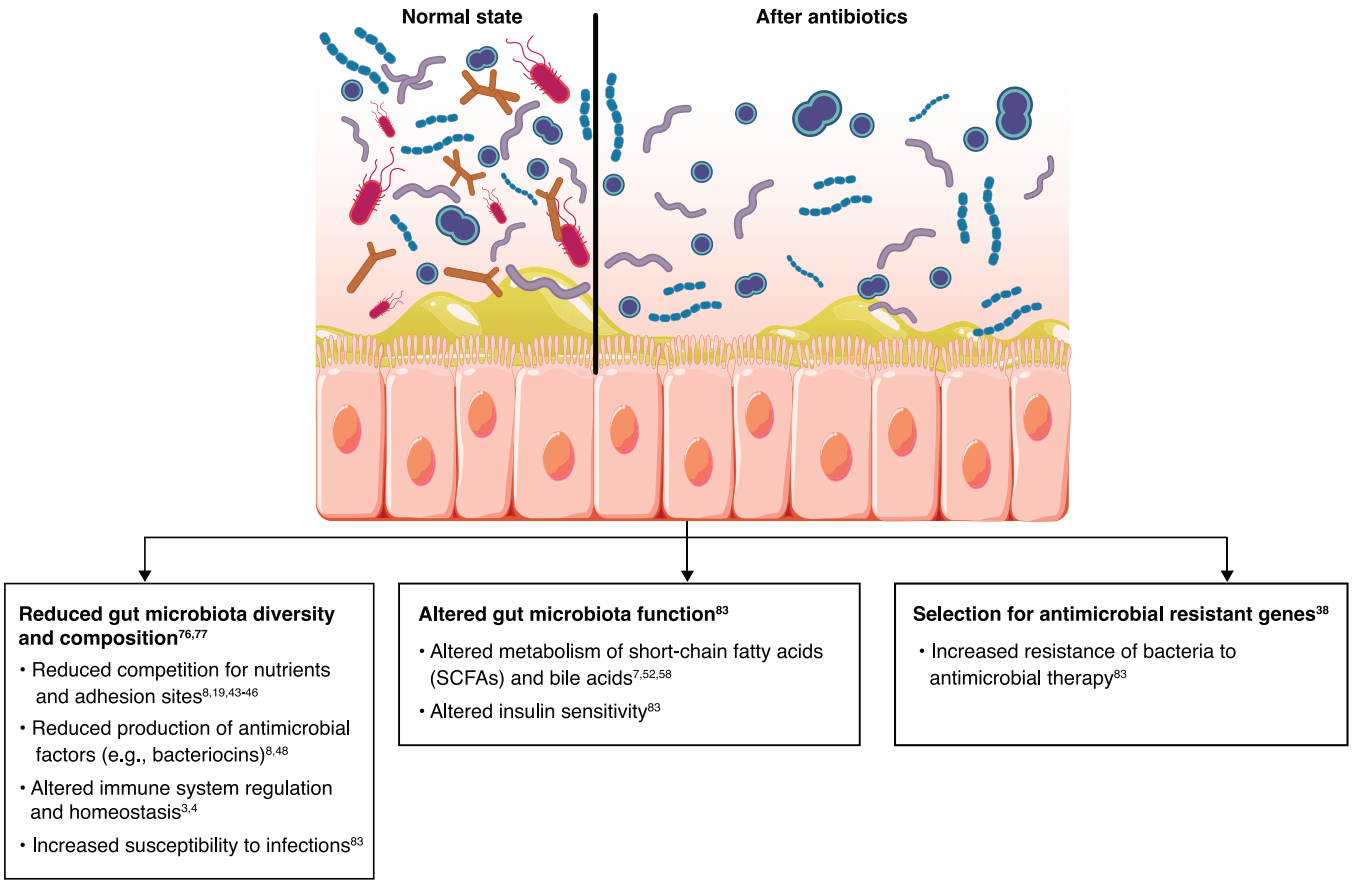

Figure 4. Effect of antibiotics on gut microbiota. Antibiotics disrupt the gut microbiota; reduce diversity, composition, and function; reduce colonization resistance against potential pathogens such as *Clostridioides difficile*; and select for antimicrobial resistant organisms and genes.

colonization resistance, which, in turn, may lead to AAD, with or without CDI.[10,38,79] Not all antibiotics have the same effect on the gut microbiota, as they have different modes of action.[76] Broad-spectrum antibiotics do not discriminate between pathogens and commensal gut bacteria[80,81] and this may affect 30% of the gut bacteria, contributing to loss of microbiota diversity.[82,83] 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,[10,81] with positive links to conditions such as obesity, asthma, allergies, inflammatory bowel disease, and adverse effects on cognition, behavior, and emotional outcomes.[10,81] 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.[10,79] 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.[80,82]

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*[38] and multidrug-resistant *Klebsiella pneumoniae*.[84] Fidaxomicin is a narrow-spectrum antibiotic recommended as a first-line treatment option for an initial and recurrent CDI.[85] Unlike vancomycin, fidaxomicin may have a more limited effect on commensal bacteria in the gut. Fewer recurrences occur with oral fidaxomicin than with vancomycin.[14,85] The lower rate of recurrent CDI with fidaxomicin may be because fidaxomicin effectively inhibits *C. difficile* toxin production, inhibits spore production, and

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.[51] Recurrent CDI may also be caused by a new infection with a different *C. difficile* strain.[86] *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 infection[56] 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 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.[134,135] Successful restoration of the gut microbiome may also decrease the abundance of antibiotic-resistant organisms and antibiotic resistance genes.[38,136] 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.[8,9] 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.[17,36,43–45] 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*.[36,45,81,137] 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.[38] Restoration of a healthy microbiome is critical to break the vicious cycle of recurrent CDI.[107] Although effective as a treatment option to prevent recurrent CDI, current FMT processes for donor recruitment, stool selection, and processing are not standardized.[117,119] Newer biotherapeutic formulations currently in development have more standardized manufacturing processes[107,133] and have shown promising results in phase III clinical studies in preventing *C. difficile* recurrence and restoring the gut microbiome,[134,135] 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.

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