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Interactions Between the Gastrointestinal Microbiome and *Clostridium difficile*

Casey M. Theriot[1] and **Vincent B. Young**[2,3]

Vincent B. Young: youngvi@umich.edu

[1]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27607

[2]Department of Internal Medicine, Division of Infectious Disease, University of Michigan, Ann Arbor, Michigan 48109

[3]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan 48109

Abstract

Antibiotics have significant and long-lasting effects on the intestinal microbiota and consequently reduce colonization resistance against pathogens, including *Clostridium difficile.* By altering the community structure of the gut microbiome, antibiotics alter the intestinal metabolome, which includes both host- and microbe-derived metabolites. The mechanisms by which antibiotics reduce colonization resistance against *C. difficile* are unknown yet important for development of preventative and therapeutic approaches against this pathogen. This review focuses on how antibiotics alter the structure of the gut microbiota and how this alters microbial metabolism in the intestine. Interactions between gut microbial products and *C. difficile* spore germination, growth, and toxin production are discussed. New bacterial therapies to restore changes in bacteria-driven intestinal metabolism following antibiotics will have important applications for treatment and prevention of *C. difficile* infection.

Keywords

gut microbiota; *Clostridium difficile*; antibiotics; colonization resistance; bacterial metabolism; bile acids

INTRODUCTION

Study of the microbiome—the vast and complex community of microbes that live in association with a host—is growing exponentially. Spurred on by the activities of the Human Microbiome Project and the European MetaHIT program ([52], [93]), a large number of investigators have studied associations between the status of the microbiome and human health and disease. We are now at a critical juncture in studying the role of the microbiome as related to host health. Whereas early studies focused on finding associations between a particular community structure or global description (e.g., diversity) and a particular health status, causality and mechanism are now key. Attention is broadening to include not just the structure of host-associated microbial communities but also their functions.

In the transition to examining the function of a particular microbiome structure, investigators have turned to a number of strategies ([13]). In particular, much attention has been paid to determining the metabolic activity of a given community. Metagenomic sequencing, cataloging the sum of protein-coding genes in a specific microbial community, can provide insight into the metabolic potential of that community. Metatranscriptome analysis gives an indication of what portion of that metabolic potential is being expressed under a given condition. Proteomics will demonstrate the actual production of proteins, whereas metabolomic analysis can provide direct assessment of the metabolic effects of bacterial and host activities.

In terms of the structure and function of the intestinal microbiome, one area of research interest is to assess the effects of antibiotics on the metabolic activity of this community. The gastrointestinal microbiome has profound effects on host health, and we, along with others, have been examining this community in the setting of antibiotic-associated diarrhea and colitis, in particular colitis due to infection with the bacterial pathogen *Clostridium difficile*. In this review we discuss the relationship between *C. difficile* infection (CDI) and the intestinal microbiome. In particular, we focus on studies that have examined the metabolic activities of the gut microbiome that are responsible for establishing and maintaining colonization resistance against *C. difficile*, and how alteration of the community by antibiotics leads to CDI susceptibility.

C. DIFFICILE AND ANTIBIOTIC-ASSOCIATED DIARRHEA

C. difficile is an anaerobic, gram-positive, spore-forming, toxin-producing bacillus first isolated by Hall & O'Toole ([46]) from the gut of a healthy newborn. Greater interest in this organism developed when it was demonstrated to be the causative agent of most cases of infectious postantibiotic colitis. CDI results in a range of clinical disease; more severe cases lead to pseudomembranous colitis (PMC), toxic megacolon, and/or death ([6, 78, 115]). In the United States there were approximately 500,000 cases of CDI, resulting in 29,000 deaths, in 2011 alone ([71]). Annual health care costs due to this nosocomial infection have reached $4.8 billion in US acute-care facilities ([33]). The main risk factor for CDI is antibiotic usage ([81, 91]).

CDI represents a subset of the gastrointestinal disease that can occur in the setting of antibiotic administration. Antibiotic-associated diarrhea (AAD) is a commonly reported side effect of administration of antibacterial medications. The incidence of AAD is unknown, but it is estimated that between 5% and 35% of patients taking antibiotics will develop diarrhea ([8, 49, 80]). Antibiotics associated with a higher rate of diarrhea generally have a broader spectrum of antimicrobial activity and include clindamycin, cephalosporins, and ampicillin/amoxicillin ([8]). Previous studies have implicated a number of pathogens associated with AAD cases, including *Clostridium perfringens, Staphylococcus aureus, Klebsiella oxytoca, Candida* spp., and *Salmonella* spp. ([49, 80]). However, the most common pathogen associated with infectious AAD is *C. difficile*, which is responsible for an estimated 10–20% of all AAD cases ([7, 8, 60]).

In 1974, Tedesco et al. ([118]) conducted a large, prospective study of clindamycin-associated colitis, which had become endemic in many hospitals. They noted that administration of clindamycin in 200 consecutive patients resulted in diarrhea in 21% and that the incidence of endoscopy-diagnosed pseudomembranous colitis was 10%. This study led to a search for an infectious cause of this colitis, which culminated in the identification of *C. difficile* as the main causative agent ([11, 36]). Koch's postulates were first fulfilled on a rodent model using Syrian hamsters ([10]). Hamsters treated with clindamycin and subsequently challenged with *C. difficile* developed CDI manifested as a fatal colitis with pseudomembrane formation ([11]). Many other antibiotics were subsequently demonstrated to predispose to CDI, with the highest risk associated with clindamycin, cephalosporin, penicillin, and fluoroquinolones ([41, 90]).

Since *C. difficile* was first identified, a large area of research has centered on elucidating the role of the clostridial toxins in the colitis that characterizes CDI. The *C. difficile* toxins TcdA and TcdB mediate disease, and their genes are encoded on the 19.6-kb pathogenicity locus (*PaLoc*). The toxins are monoglycosyltransferases that catalyze the transfer of glucose onto the Rho family of GTPases, leading to disruption of the actin cytoskeleton and the barrier function of the epithelium, cell death, and apoptosis ([125]). Other genes housed on the *PaLoc* include a putative holin (*tcdE*) and two regulators of toxin production (*tcdC*, a negative regulator, and *tcdR*, an activator/sigma factor) ([61, 75, 76, 125]). Within the last decade there have been outbreaks worldwide due to an epidemic strain, BI/NAP1/027, that is attributed with increased toxin production and ability to sporulate, which could aid in transmission ([79, 82, 114, 127]). Epidemic strains contain virulence factor toxins TcdA and TcdB, produce increased toxin, have deletions in *tcdC* (which functions to repress toxin production), have a binary toxin, and are more resistant to fluoroquinolones in vitro ([9, 114, 127]).

The strong association between CDI and antibiotic treatment has prompted investigation of how the indigenous intestinal microbiota normally keep the pathogen in check. Early on it was proposed that antibiotic administration alters both the structure and the function of the resident microbiota, reducing colonization resistance against *C. difficile* and other enteric pathogens ([124, 131]). Potential mechanisms for mediation of colonization resistance by members of the gut microbiota include competition for nutrients, taking up physical space, production of bacteriocins, and the host response ([5, 94, 133]). Multiple studies over the last 70 years have demonstrated that colonization resistance could be overcome by administering antibiotics in small animal models ([17, 18, 42, 106, 121]). The reduction of intestinal microbiota seen in mice correlated to a decrease in colonization resistance, with an increase in colonization of opportunistic pathogens *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* ([121]). Colonization resistance can be restored by a bacterial population composed primarily of anaerobes before antibiotic therapy and after recovery ([121, 130]). Multiple studies describe how antibiotics alter the gut microbiota, decreasing colonization resistance against pathogens, including *Salmonella* sp., *Citrobacter* sp., *E. coli*, and *C. difficile* ([55, 73, 96, 116]). The vast majority of studies have not determined exactly how antibiotics cause these effects on the microbial community. However, recent advances in our ability to characterize the structure and function of complex microbial communities have renewed interest in studying the exact mechanisms by which this occurs.

STRUCTURE AND FUNCTION OF THE INDIGENOUS GUT MICROBIOTA

The human microbiome is the complex community of microorganisms that inhabits niches in and on the body. It is composed of an estimated 10^{14} cells, which is an order of magnitude higher than the number of host cells found in the human body ([101]). Gut microbiota constitute 70% of the human microbiome, with the highest numbers inhabiting the distal gastrointestinal tract, where there is an estimated density of 1012 organisms per gram (dry weight) of feces ([35, 122, 137]). Early studies of the gut microbiota utilizing culture-based techniques estimated that this community contains ~400–500 distinct species ([85, 137]). After the advent of culture-independent techniques to profile the gut microbiota, studies have estimated that there are at least 1,800 genera and between 15,000 and 36,000 species of bacteria in the human gut ([39]). A majority of the gut community (90–99%) in healthy humans and animals comprises two dominant phyla, *Firmicutes* and *Bacteroidetes*, and a smaller portion is made up of *Proteobacteria, Actinobacteria, Verrucomicrobia*, and *Cyanobacteria* ([35, 72]).

The recognition that the microbiome could play a critical role in human health and disease has led to efforts to characterize the structure and ultimately the function of this symbiotic community. During the last decade, a global movement directed by the International Human Microbiome Consortium in conjunction with the National Institute of Health's Human Microbiome Project (HMP) began to catalog the bacterial populations residing on and in the human body ([52, 93]). Studies from these initiatives helped define the microbiota of healthy humans in different body sites (gut, skin, and vagina) and revealed unique microbiota populations associated with each site. ([53]). The microbial diversity within the healthy human population was affected by diet, environment, host genetics, and early exposure to microbes ([53]). Studies like this, focusing on the community structure of the gut microbiota, suggest that there is no single healthy microbiome structure, although this is still controversial ([32]). There is also evidence in both humans and other primates that the structure of the gut microbiota can be classified as distinct types or enterotypes ([64, 84, 134]). However, these initial efforts focusing on microbiome structure could investigate only indirectly the function of these different structures and how they related to health and disease.

The gut microbiota provide many benefits to the host. They confer colonization resistance, shape the host immune response, and participate in key metabolic transformations ([5, 63, 121]). A great deal of effort has been devoted to studying the most abundant phyla in the gut, *Bacteroidetes* and *Firmicutes*, under the assumption that the organisms contributing the greatest biomass are more likely to play a key role in bacterial metabolism ([45, 113]). Taking advantage of the availability of genetic tools to study the *Bacteroidetes*, many groups have demonstrated their ability to break down host glycans and nondigestible carbohydrates—specifically, resistant starches and plant cell wall polysaccharides ([37, 67, 108]). *Firmicutes*, especially members of the *Clostridium* genus, are known for their ability to degrade polysaccharides and ferment amino acids ([30, 37]). *Firmicutes* make up more than 50–70% of the colonic bacterial community—specifically, members of the *Lachnospiraceae* and *Ruminococcaceae* families ([38])—but our lack of genetic tools has hindered our knowledge of this phylum's mechanistic functions. Development of genetic tools for members of the

Firmicutes will increase our understanding of how this group contributes to the metabolic profile of the gut.

Anaerobic microorganisms in the gut play a major role in fermenting complex carbohydrates and amino acids into short chain fatty acids (SCFAs), which are important for intestinal health ([74, 120]). It is estimated that each day 20 to 60 grams of undigested carbohydrates reach the colon, where they are available for microbial fermentation ([29, 105]). SCFAs, specifically butyrate, play an important role in regulating host gene expression, inflammation, differentiation, and apoptosis ([23, 43, 107]). Additionally, members of the gut microbiota are important for amino acid and protein digestion, although this has not been investigated extensively ([30]).

Members of the gut microbiota also play a pivotal role in lipid or bile acid metabolism ([97]). Bile acids are synthesized from cholesterol by hepatic enzymes, and they modulate lipoprotein, glucose, drug, and energy metabolism ([27, 97]). Many spore-forming, anaerobic members of the *Clostridium* genus are able to perform enzymatic reactions on conjugated bile acids, including deconjugation and 7α-dehydroxylation ([27, 83, 97, 129]). Once synthesized in the gallbladder, primary bile acids (unconjugated and conjugated) travel through the small intestine, where 95% of bile is absorbed in the terminal ileum, and through the hepatic system ([97]). The small amount of bile acids that reach the large intestine are further biotransformed by members of the gut microbiota via deconjugation and dehydroxylation into secondary bile acids, including deoxycholate (DCA), lithocholate (LCA), and ursodeoxycholate (UDCA) (Figure 1*a*) ([83, 97]).

EFFECTS OF ANTIBIOTICS ON THE STRUCTURE AND FUNCTION OF THE GUT MICROBIOTA

Antibiotics have profound effects on the structure and function of the gut microbiota. Dethlefsen & Relman ([31]) demonstrated that a five-day antibiotic course of ciprofloxacin significantly decreased richness and diversity of the gut bacterial community structure in three patients. Recovery of the bacterial community structure, to pre-antibiotic levels, was seen in only one patient four weeks after antibiotic treatment; others did not recover until six months after antibiotics ([31]). Correspondingly, mouse models have shown similar long-term disturbances to the gut microbiota after antibiotics: An antibiotic cocktail of amoxicillin, metronidazole, and bismuth (AMB) significantly altered the gut microbiota by decreasing bacterial diversity ([3]). Antibiotic-treated animals had a shift in gut microbial community structure, with an increase in *Proteobacteria* and a decrease in the *Bacteroidetes* and *Firmicutes* populations ([3]). Complete recovery time of the gut microbiota structure to baseline levels differed among animals based on treatments, with some mice recovering two weeks after stopping AMB and others recovering six months after stopping broader-spectrum cefoperazone treatment.

In 2004, Young & Schmidt ([136]) demonstrated that administration of amoxicillin–clavulanic acid was associated with development of antibiotic-associated diarrhea (AAD). Administration of amoxicillin–clavulanic acid resulted in diarrhea that was not due to *C. difficile*. Antibiotic administration significantly reduced populations of members of the

Clostridiaceae family, which includes many of the butyrate-producing bacteria that are essential for colonic health ([136]). After a recovery period without antibiotics, *Clostridiaceae* returned to pre-antibiotic levels. Although this study did not directly assess the functional significance of the structure changes in the microbiome resulting from antibiotic administration, the authors and others speculated that accumulation of undigested carbohydrates in the colon resulted in osmotic diarrhea ([14, 136]).

To directly measure alterations in the function of the gut after antibiotics, researchers have moved beyond 16S rRNA gene sequencing surveys and are starting to apply other technologies, including genomics, transcriptomics, proteomics, and metabolomics. As noted above, metagenomic and metatranscriptomic analyses provide a catalog of potential metabolic functions of the microbiome; however, they do not directly measure function. Measuring proteins produced by the gut microbiota is a better measurement of function, although there are limitations in identifying uncharacterized proteins and evaluating enzyme activity. Currently, the function of the gut microbiota is most easily measured by defining the gut metabolome, the collection of host- and microbe-derived small molecules. The gut metabolome has direct links to phenotype and function of the gut microbiome ([1, 62]). Antibiotics and murine models have been exploited to better understand how the metabolic activities of the gut microbiota, in conjunction with host metabolism, shape the overall intestinal metabolome.

Because antibiotics alter the gut microbial community structure, this in turn significantly alters the gut metabolome ([4, 135, 138]). Streptomycin alters 87% of the fecal metabolome in mice, specifically those important for sugar, amino acid, fatty acid, steroid, bile acid, and eicosanoid metabolism ([4]). Bile acid metabolism was significantly altered, with increases in glycocholate, taurocholate (TCA), and taurochenodeoxycholate and decreases in chenodeoxycholate and cholate ([4]). Similarly, gentamycin and ceftriaxone decrease monosaccharides (glucose, fucose, xylose, and galactose) and SCFAs and increase oligosaccharides (sucrose, cellobiose, raffinose, and stachyose) as well as alter amino acid and bile acid metabolism ([138]). From these metabolomic studies and others, it is becoming evident that antibiotics not only alter the structure of the gut microbiota, but also alter gut bacterial metabolism, specifically bile acid, carbohydrate, and amino acid metabolism ([100, 135, 139]).

ALTERATIONS IN GUT MICROBIAL COMMUNITY STRUCTURE AND *C. DIFFICILE* INFECTION

There are many animal models used to study CDI ([16, 69]); however, the mouse and hamster models have been most often leveraged to study the dynamics between antibiotics, the gut microbiota, and colonization of *C. difficile*. Clindamycin-treated Syrian hamsters are susceptible to CDI, and susceptibility was associated with a significant reduction in *Bacteroidetes* and *Firmicutes* and increased *Proteobacteria* in the gut ([92]). Early studies, shortly after Koch's postulates were fulfilled in clindamycin-treated hamsters, found that mice treated with clindamycin alone did not succumb to CDI; however, germfree mice lacking gut microbiota were susceptible ([89]). In 2008 Chen et al. ([26]) reported a *C. difficile*

mouse model that approximates human CDI: An oral antibiotic cocktail (gentamycin, kanamycin, colistin, metronidazole, and vancomycin) is followed by an intraperitoneal injection of clindamycin to render mice susceptible to experimental CDI. Reeves et al. ([96]) used this model and others ([22]) used clindamycin alone to demonstrate that antibiotic pretreatment alters the gut microbiota: Overall bacterial diversity decreases, the relative abundance of *Firmicutes* and *Bacteroidetes* decreases, and *Proteobacteria* from the family *Enterobacteriaceae* increases. Mice treated with the broad-spectrum third generation cephalosporin, cefoperazone, also succumbed to CDI and had long-lasting changes to the gut microbiota, with a significant decrease in bacterial diversity, and a predominance of bacteria from the *Firmicutes* phylum—specifically, members of the *Lactobacillaceae* family ([96, 119]). Another study looked at the effects of tigecycline, a broad-spectrum glycylcycline that is being considered for treatment of patients with recurrent CDI because of its potent in vitro activity against *C. difficile* ([12]). Tigecycline treatment increased the levels of bacteria from the phyla *Verrucomicrobia* and *Proteobacteria*, with large losses in *Bacteroidetes* species resulting in susceptibility to *C. difficile*. Taken together, these studies indicate that susceptibility to CDI after antibiotics in murine models is associated with a decrease in bacterial diversity, an increase in *Proteobacteria*, and a decrease in *Bacteroidetes*. It is important to note, however, that no single microbiota structure confers susceptibility to *C. difficile* colonization. This implies that an examination of microbial community structure alone may not be sufficient to determine the mechanisms by which the gut microbiota can mediate colonization resistance.

To help determine the role of specific members of the gut microbiome in colonization resistance, investigators have introduced specific bacteria into antibiotic-treated or germfree mice. One group was able to reestablish colonization resistance against *C. difficile* with a bacterial consortium made up of *Staphylococcus, Enterococcus, Lactobacillus, Anaerostipes, Bacteroidetes*, and *Enterorhabdus* ([68]). Others have had some success with monoassociating germfree mice with bacteria, and partially restoring colonization resistance against *C. difficile* with nontoxigenic *C. difficile, E. coli, Bifidobacterium bifidum*, and members of the *Lachnospiraceae* ([28, 86, 95]). Again, because no single organism or community type appears to mediate colonization resistance independently, this implies that multiple organisms and communities may share functions critical for the establishment of colonization resistance.

Human studies looking at the interaction between antibiotics, the gut microbiota, and CDI are more limited. Most human studies have used the fecal microbiota as representative of the intestinal microbiota because samples are readily available and can be collected noninvasively. It should be noted that there is controversy over how representative the fecal community is to the more proximal sections of the intestine. In 2008, Chang et al. reported that patients with recurrent CDI had decreased bacterial diversity of the fecal microbiota, suggesting this could be a factor in colonization resistance ([25]). These results agree with a more recent study by Antharam et al., in 2013, who compared fecal microbiota of healthy subjects (n = 40) and subjects with AAD (n = 36) or CDI (n = 39) ([2]). A decrease in bacterial diversity, and species richness, was seen in the fecal microbiota of AAD and CDI patients compared to healthy controls. There was a decrease in butyrate-producing *Ruminococcaceae* and *Lachnospiraceae*, and from Clostridia clusters IV and XIVa. Similar

to mice, CDI patients had increased *Enterococcus, Veillonella, Lactobacillus*, and bacteria from the *Gammaproteobacteria* class ([2]). Similarly, another study, using a statistical modeling approach, found the loss of *Bacteroides, Lachnospiraceae*, and *Ruminococcaceae* was associated with CDI in humans ([103]).

More recent work has studied fecal microbiota transplantation (FMT) for recurrent CDI. Patients with severe CDI refractory to traditional antibiotic treatment have had success with FMT, which restores colon homeostasis by reintroducing bacteria from healthy donor stool ([59, 123]). Although the success rate for FMT for patients with recurrent CDI is greater than 90%, the mechanism behind this treatment is unknown ([66]). Investigators have examined how FMT changes the structure of the fecal microbiota in patients with recurrent CDI. *Bacteroidetes* increased and *Proteobacteria* decreased in fecal microbiota following successful transplantation ([47, 104]). Moreover, the metagenomic potential of the bacterial communities were defined, and amino acid transport systems were overrepresented in samples prior to FMT ([104]).

Structural changes to the gut microbiota in humans do resemble those in mice that are susceptible to CDI. Similar to what was observed in mouse models of CDI, there is not a single structure that correlates with susceptibility to CDI; accordingly, multiple community structures exhibit colonization resistance to *C. difficile*. This has prompted examination of the functional changes that antibiotics cause in the intestinal microbiota. Here, we focus on the intersection between alterations in the gut metabolome and the physiology of *C. difficile*.

ANTIBIOTIC-INDUCED ALTERATIONS IN GUT MICROBIAL METABOLISM AND RELATIONSHIP TO *C. DIFFICILE* PHYSIOLOGY

To correlate observed antibiotic-associated decreases in bacterial diversity and shifts in the most abundant bacteria with changes in colonization resistance, our group and others have examined how antibiotic administration alters intestinal bacterial metabolism. We assumed that an understanding of the metabolic requirements for *C. difficile* germination, growth, and toxin production in vivo could provide mechanistic understanding of how this organism is able to gain a foothold in the gut following antibiotic alteration of the gut environment.

We examined structural and metabolomic changes induced by antibiotics in the gut and correlated them with susceptibility to CDI in a mouse model. We again noted that multiple structures of the murine gut microbiota result in resistance to CDI. Despite these structural differences, we noted that the metabolic profiles associated with these different communities were similar, suggesting that multiple structures have redundant functions and that structure alone does not dictate functionality of the gut ([119]). Susceptibility to CDI was associated with an increase in the primary bile acid TCA, a germinant of *C. difficile* spores, and increases in amino acids, simple sugars, and sugar alcohols—growth substrates for *C. difficile* vegetative cells ([119]). Alterations in the gut metabolome after antibiotics allow for *C. difficile* spore germination and outgrowth of vegetative cells, suggesting that alterations in gut bacterial metabolism are important for colonization resistance against *C. difficile* (Figure 1*b*). To put these results into context, we now discuss how products from bacterial

metabolism are able to affect key aspects of *C. difficile* physiology and pathogenesis, including spore germination, vegetative outgrowth, and toxin production.

Bile Acid Metabolism

Members of the large intestinal microbiota play an important role in the biotransformation of primary bile acids into secondary bile acids ([97]). *C. difficile* spores require a germinant—specifically, bile acids—to switch from a dormant state to an actively growing vegetative cell ([110, 132]). Initial work on the relationship between bile acids and *C. difficile* spore germination dates back to 1982 in vitro studies by Wilson et al. ([132]), who found that media supplemented with sodium taurocholate increased the recovery of spores. Subsequent work by Sorg & Sonenshein ([112]) demonstrated that, in vitro, bile acids made by the host can both inhibit and support *C. difficile* spore germination and outgrowth. For maximal spore germination, both glycine and TCA are needed, whereas chenodeoxycholate (CDCA) inhibits both spore germination and outgrowth of vegetative cells ([110]). Most important, the secondary bile acid DCA stimulates spore germination but inhibits growth of *C. difficile*, which has been suggested as a mechanism of colonization resistance ([110, 111]). Clinical strains of *C. difficile* can utilize a wide spectrum of bile acids for spore germination, whereas others are specific to TCA ([24, 48]).

Although there have been many in vitro studies looking at the requirements for spore germination, there are fewer ex vivo and in vivo studies. Filtered gut content from antibiotic-treated mice is able to stimulate growth of *C. difficile* spores ([44]). This was also seen in the small intestine of non-antibiotic-treated mice ([44]). Spore germination occurs before and after antibiotics primarily in the ileum of mice, and outgrowth of *C. difficile* is localized to the large intestine, only after antibiotics ([65]). Bile acids present in the murine ileum after antibiotics cholate and taurochenodeoxycholate (TCDCA) are able to support germination of *C. difficile* spores at physiological concentrations ([65]). Antibiotics alter the bacterial community that is capable of deconjugation and dehydroxylation of primary bile acids in the gut, resulting in decreased secondary bile acids and increased primary and conjugated bile acids (Figure 1*b*) ([117, 119, 126]).

The ability of specific bile acids to either enhance or inhibit *C. difficile* spore germination and vegetative cell outgrowth will be important for reestablishing colonization resistance against *C. difficile*. This concept was first suggested by Sorg & Sonenshein ([112]) in 2010, and more recent data support this hypothesis ([21, 109, 128]). Multiple studies have shown that restoration of secondary bile acids by members of the gut microbiota helps restore colonization resistance against *C. difficile* in humans, and to some extent in mice ([21, 119, 128]). Going forward we need to understand how the gut microbiota alter bile acids in vivo and how this in turn alters *C. difficile* germination and colonization. Collecting relevant samples from the human gastrointestinal tract continues to be challenging, because spore germination and colonization occur upstream of the feces, which is the most commonly used analyte to measure gut microbiota structure.

Bacterial cocktails to replenish the level of secondary bile acids in the large intestine are a potential therapeutic approach to restore colonization resistance against *C. difficile*. Ability to control the amount of secondary bile acid produced is an important consideration, as too

much DCA increases the risk of colon cancer ([15]). Because bile acids are important for regulating the physiology of the host, a careful balance is necessary to ensure that both the host and the gut microbiota remain healthy. Delivery of bacteria and targeted enzyme therapies will need to be explored in animal models before they are used to treat humans. It would be convenient if bile acids were the sole mechanism for colonization resistance against *C. difficile* in the gut, but other less-studied factors may contribute to colonization resistance, including competition for nutrients.

Fermentation of Carbohydrates and Amino Acids

Members of the gut microbiota have different metabolic requirements and are able to compete for a variety of nutrients. *C. difficile* requires amino acids (cysteine, isoleucine, leucine, proline, tryptophan, and valine) and vitamins (biotin, pantothenate, and pyridoxine) to grow ([20], [56]). Also, *C. difficile* is able to ferment carbohydrates including fructose, glucose, mannitol, mannose melezitose, sorbitol, and sialic acids ([87], [102]). In 1988, Wilson & Perini ([133]) first highlighted how the colonic microbiota were able to compete for nutrients, including glucose, *N*-acetylglucosamine, and sialic acids, against *C. difficile*, resulting in suppression of growth. Since then few studies have looked at the role of competitive exclusion by members of the gut microbiota. Epidemic strains have shown an increased competitive fitness and expanded nutrient utilization profile compared with nonepidemic strains ([98], [102]). Other *Clostridium* species with overlapping metabolic requirements compete with *C. difficile* for nutrients, suppressing its growth ([70], [86]).

In germfree mice monoassociated with *Bacteroides thetaiotaomicron*, sialidases from *B. thetaiotaomicron* released host-sugar sialic acids from the mucus, providing *C. difficile* nutrients for enhanced growth ([88]). This finding is interesting, although it may be specific to the germfree background, because all other identified microbiota structures resulting in susceptibility to *C. difficile* are associated with a loss of *Bacteroidetes*. Competition for nutrients is more complex in the gut of a conventional mouse, with a complex gut microbiota; thus, it is critical to determine how the bacteria in a community are able to suppress *C. difficile*.

Antibiotic treatment alters anaerobic fermentation of carbohydrates in the gut, resulting in decreased SCFA production ([50], [51]). Only a handful of studies have looked at the level of SCFAs with respect to colonization resistance against *C. difficile* in the gut. Pigs on a high-fiber diet produced more SCFAs, especially butyrate, which correlated with decreased pH and was able to inhibit *C. difficile* colonization ([77]). Similarly, SCFA concentrations in the hamster cecum (specifically, butyrate) were able to inhibit growth of *C. difficile* in vitro ([99]). Antibiotics alter the gut microbiota, decreasing the level of SCFAs present in the gut, resulting in the ability of *C. difficile* to colonize ([119]). The loss of SCFAs in the gut is hypothesized to increase pH, but this has not been extensively studied in relation to how SCFAs can alter *C. difficile* colonization. There is some evidence that proton-pump inhibitor (PPI) usage is associated with increased risk of CDI, although a large-scale study has found no association between PPIs and recurrent CDI in patients ([40]).

C. difficile toxin expression is regulated by many nutrients found in the gut, including butyrate ([58]). *C. difficile* toxin is induced during stationary phase growth, when nutrients

become limited, and is affected by amino acids, butyrate, butanol, glucose, and other carbon sources. More specifically, proline, cysteine, butanol, or glucose represses *C. difficile* toxin when supplemented in media ([34], [54], [57], [58]). However, butyrate supplemented in growth media induces *C. difficile* toxin, suggesting a relationship between *C. difficile* metabolism and virulence factors ([19], [58]). Understanding what metabolites are present in the gastrointestinal tract before and throughout CDI will be important for modulating virulence.

CONCLUDING REMARKS

There is and will continue to be great interest in studying the gut microbiota in the context of different disease states. Many diseases are associated with alterations in the gut microbiome, including CDI, diabetes, obesity, cancer, and metabolic syndrome; however, few studies have demonstrated a mechanistic link. Strategies including metabolomic analysis will allow us to move beyond structural characterization and associations toward a determination of causation via the functions that the microbial communities carry out. We have done more than determine the structure of microbial communities in the context of CDI; we have defined the chemical environment and identified key metabolites that *C. difficile* can utilize for germination and growth, which leads to disease. This approach provides a mechanistic understanding of how this organism is able to gain a foothold in the gut following antibiotic alteration of the gut environment. By applying new strategies to define the many functions of the gut microbiota, we are closing in on the causes—which will ultimately lead us to better preventive and therapeutic interventions against infectious diseases.

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LITERATURE CITED

1. Aldridge BB, Rhee KY. Microbial metabolomics: innovation, application, insight. Curr. Opin. Microbiol. 2014; 19:90–96. [PubMed: 25016173]
2. Antharam VC, Li EC, Ishmael A, Sharma A, Mai V, et al. Intestinal dysbiosis and depletion of butyrogenic bacteria in *Clostridium difficile* infection and nosocomial diarrhea. J. Clin. Microbiol. 2013; 51:2884–2892. [PubMed: 23804381]
3. Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML, Young VB. Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. Infect. Immun. 2009; 77:2367–2375. [PubMed: 19307217]
4. Antunes LC, Han J, Ferreira RB, Lolic P, Borchers CH, Finlay BB. The effect of antibiotic treatment on the intestinal metabolome. Antimicrob. Agents Chemother. 2011; 55:1494, 1503. [PubMed: 21282433]
5. Artis D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. Nat. Rev. Immunol. 2008; 8:411–420. [PubMed: 18469830]
6. Bartlett JG. Antibiotic-associated colitis. Dis. Mon. 1984; 30:1–54. [PubMed: 6391879]

7. Bartlett JG. Management of *Clostridium difficile* infection and other antibiotic-associated diarrhoeas. Eur. J. Gastroenterol. Hepatol. 1996; 8:1054–1061. [PubMed: 8944365]
8. Bartlett JG. Clinical practice: antibiotic-associated diarrhea. N. Engl. J. Med. 2002; 346:334–339. [PubMed: 11821511]
9. Bartlett JG. Narrative review: the new epidemic of *Clostridium difficile*-associated enteric disease. Ann. Intern. Med. 2006; 145:758–764. [PubMed: 17116920]
10. Bartlett JG, Chang TW, Gurwith M, Gorbach SL, Onderdonk AB. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. N. Engl. J. Med. 1978; 298:531–534. [PubMed: 625309]
11. Bartlett, JG.; Onderdonk, AB.; Cisneros, RL.; Kasper, DL. Clindamycin-associated colitis due to a toxin-producing species of *Clostridium* in hamsters. In: Bartlett, JG.; Onderdonk, AB.; Cisneros, RL.; Kasper, DL., editors. J. Infect. Dis. Vol. 190. 2004. p. 202-209.Commentary
12. Bassis CM, Theriot CM, Young VB. Alteration of the murine gastrointestinal microbiota by tigecycline leads to increased susceptibility to *Clostridium difficile* infection. Antimicrob. Agents Chemother. 2014; 58:2767–2774. [PubMed: 24590475]
13. Bassis, CM.; Young, VB.; Schmidt, TM. Methods for characterizing microbial communities associated with the human body. In: Fredricks, DN., editor. The Human Microbiota: How Microbial Communities Affect Health and Disease. Hoboken, NJ: Wiley; 2013. p. 51-74.
14. Beaugerie L, Petit JC. Microbial-gut interactions in health and disease: antibiotic-associated diarrhoea. Best Pract. Res. Clin. Gastroenterol. 2004; 18:337–352. [PubMed: 15123074]
15. Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. Mutat. Res. 2005; 589:47–65. [PubMed: 15652226]
16. Best EL, Freeman J, Wilcox MH. Models for the study of *Clostridium difficile* infection. Gut. Microbes. 2012; 3:145–167. [PubMed: 22555466]
17. Bohnhoff M, Drake BL, Miller CP. Effect of streptomycin on susceptibility of intestinal tract to experimental *Salmonella* infection. Proc. Soc. Exp. Biol. Med. 1954; 86:132–137. [PubMed: 13177610]
18. Bohnhoff M, Miller CP. Enhanced susceptibility to *Salmonella* infection in streptomycin-treated mice. J. Infect. Dis. 1962; 111:117–127. [PubMed: 13968487]
19. Bouillaut L, Dubois T, Sonenshein AL, Dupuy B. Integration of metabolism and virulence in *Clostridium difficile*. Res. Microbiol. 2014; 166:375–383. [PubMed: 25445566]
20. Bouillaut L, Self WT, Sonenshein AL. Proline-dependent regulation of *Clostridium difficile* Stickland metabolism. J. Bacteriol. 2013; 195:844–854. [PubMed: 23222730]
21. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, et al. Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. Nature. 2014; 517:205–208. [PubMed: 25337874]
22. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat. Rev. Immunol. 2013; 13:790–801. [PubMed: 24096337]
23. Canani RB, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J. Gastroenterol. 2011; 17:1519–1528. [PubMed: 21472114]
24. Carlson PE Jr, Walk ST, Bourgis AE, Liu MW, Kopliku F, et al. The relationship between phenotype, ribotype, and clinical disease in human *Clostridium difficile* isolates. Anaerobe. 2013; 24:109–116. [PubMed: 23608205]
25. Chang JY, Antonopoulos DA, Kalra A, Tonelli A, Khalife WT, et al. Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile*-associated diarrhea. J. Infect. Dis. 2008; 197:435–438. [PubMed: 18199029]
26. Chen X, Katchar K, Goldsmith JD, Nanthakumar N, Cheknis A, et al. A mouse model of *Clostridium difficile*-associated disease. Gastroenterology. 2008; 135:1984–1992. [PubMed: 18848941]
27. Chiang JY. Bile acids: regulation of synthesis. J. Lipid Res. 2009; 50:1955–1966. [PubMed: 19346330]

28. Corthier G, Dubos F, Raibaud P. Modulation of cytotoxin production by *Clostridium difficile* in the intestinal tracts of gnotobiotic mice inoculated with various human intestinal bacteria. Appl. Environ. Microbiol. 1985; 49:250–252. [PubMed: 3977313]
29. Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. J. Appl. Bacteriol. 1991; 70:443–459. [PubMed: 1938669]
30. Dai ZL, Wu G, Zhu WY. Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. Front. Biosci. 2011; 16:1768–1786.
31. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. PNAS. 2011; 108(Suppl. 1):4554–4561. [PubMed: 20847294]
32. Ding T, Schloss PD. Dynamics and associations of microbial community types across the human body. Nature. 2014; 509:357–360. [PubMed: 24739969]
33. Dubberke ER, Olsen MA. Burden of *Clostridium difficile* on the healthcare system. Clin. Infect. Dis. 2012; 55:S88–S92. [PubMed: 22752870]
34. Dupuy B, Sonenshein AL. Regulated transcription of *Clostridium difficile* toxin genes. Mol. Microbiol. 1998; 27:107–120. [PubMed: 9466260]
35. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, et al. Diversity of the human intestinal microbial flora. Science. 2005; 308:1635–1638. [PubMed: 15831718]
36. Fekety R, Silva J, Toshniwal R, Allo M, Armstrong J, et al. Antibiotic-associated colitis: effects of antibiotics on *Clostridium difficile* and the disease in hamsters. Rev. Infect. Dis. 1979; 1:386–397. [PubMed: 549190]
37. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. Gut. Microbes. 2012; 3:289–306. [PubMed: 22572875]
38. Frank DN, Pace NR. Gastrointestinal microbiology enters the metagenomics era. Curr. Opin. Gastroenterol. 2008; 24:4–10. [PubMed: 18043225]
39. Frank DN, St. Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. PNAS. 2007; 104:13780–13785. [PubMed: 17699621]
40. Freedberg DE, Salmasian H, Friedman C, Abrams JA. Proton pump inhibitors and risk for recurrent *Clostridium difficile* infection among inpatients. Am. J. Gastroenterol. 2013; 108:1794–1801. [PubMed: 24060760]
41. Freeman J, Wilcox MH. Antibiotics and *Clostridium difficile*. Microbes Infect. 1999; 1:377–384. [PubMed: 10602670]
42. Freter R. The fatal enteric cholera infection in the guinea pig, achieved by inhibition of normal enteric flora. J. Infect. Dis. 1955; 97:57–65. [PubMed: 13242854]
43. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013; 504:446–450. [PubMed: 24226770]
44. Giel JL, Sorg JA, Sonenshein AL, Zhu J. Metabolism of bile salts in mice influences spore germination in *Clostridium difficile*. PLOS ONE. 2010; 5:e8740. [PubMed: 20090901]
45. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, et al. Metagenomic analysis of the human distal gut microbiome. Science. 2006; 312:1355–1359. [PubMed: 16741115]
46. Hall JC, O'Toole E. Intestinal flora in new-born infants with a description of a new pathogenic anaerobe, *Bacillus difficilis*. Am. J. Dis. Child. 1935; 49:390–402.
47. Hamilton MJ, Weingarden AR, Unno T, Khoruts A, Sadowsky MJ. High-throughput DNA sequence analysis reveals stable engraftment of gut microbiota following transplantation of previously frozen fecal bacteria. Gut Microbes. 2013; 4:125–135. [PubMed: 23333862]
48. Heeg D, Burns DA, Cartman ST, Minton NP. Spores of *Clostridium difficile* clinical isolates display a diverse germination response to bile salts. PLOS ONE. 2012; 7:e32381. [PubMed: 22384234]
49. Hogenauer C, Hammer HF, Krejs GJ, Reisinger EC. Mechanisms and management of antibiotic-associated diarrhea. Clin. Infect. Dis. 1998; 27:702–710. [PubMed: 9798020]

50. Hove H, Tvede M, Mortensen PB. Antibiotic-associated diarrhoea, *Clostridium difficile* and short-chain fatty acids. Scand. J. Gastroenterol. 1996; 31:688–693. [PubMed: 8819219]
51. Hoverstad T, Carlstedt-Duke B, Lingaas E, Midtvedt T, Norin KE, et al. Influence of ampicillin, clindamycin, and metronidazole on faecal excretion of short-chain fatty acids in healthy subjects. Scand. J. Gastroenterol. 1986; 21:621–626. [PubMed: 3749800]
52. Hum. Microbiome Proj. C. A framework for human microbiome research. Nature. 2012; 486:215–221. [PubMed: 22699610]
53. Hum. Microbiome Proj. C. Structure, function and diversity of the healthy human microbiome. Nature. 2012; 486:207–214. [PubMed: 22699609]
54. Ikeda D, Karasawa T, Yamakawa K, Tanaka R, Namiki M, Nakamura S. Effect of isoleucine on toxin production by *Clostridium difficile* in a defined medium. Zentralbl. Bakteriol. 1998; 287:375–386. [PubMed: 9638867]
55. Kamada N, Kim YG, Sham HP, Vallance BA, Puente JL, et al. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. Science. 2012; 336:1325–1329. [PubMed: 22582016]
56. Karasawa T, Ikoma S, Yamakawa K, Nakamura S. A defined growth medium for *Clostridium difficile*. Microbiology. 1995; 141(Part 2):371–375. [PubMed: 7704267]
57. Karasawa T, Maegawa T, Nojiri T, Yamakawa K, Nakamura S. Effect of arginine on toxin production by *Clostridium difficile* in defined medium. Microbiol. Immunol. 1997; 41:581–585. [PubMed: 9310936]
58. Karlsson S, Lindberg A, Norin E, Burman LG, Akerlund T. Toxins, butyric acid, and other short-chain fatty acids are coordinately expressed and down-regulated by cysteine in *Clostridium difficile*. Infect. Immun. 2000; 68:5881–5888. [PubMed: 10992498]
59. Kelly CP. Current strategies for management of initial *Clostridium difficile* infection. J. Hosp. Med. 2012; 7(Suppl. 3):S5–S10. [PubMed: 22407996]
60. Kelly CP, LaMont JT. *Clostridium difficile* infection. Annu. Rev. Med. 1998; 49:375–390. [PubMed: 9509270]
61. Kelly CP, LaMont JT. *Clostridium difficile*—more difficult than ever. N. Engl. J. Med. 2008; 359:1932–1940. [PubMed: 18971494]
62. Kinross J, Li JV, Muirhead LJ, Nicholson J. Nutritional modulation of the metabonome: applications of metabolic phenotyping in translational nutritional research. Curr. Opin. Gastroenterol. 2014; 30:196–207. [PubMed: 24468802]
63. Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and disease. Genome Med. 2011; 3:14. [PubMed: 21392406]
64. Knights D, Ward TL, McKinlay CE, Miller H, Gonzalez A, et al. Rethinking “enterotypes”. Cell Host Microbe. 2014; 16:433–437. [PubMed: 25299329]
65. Koenigsknecht MJ, Theriot CM, Bergin IL, Schumacher CA, Schloss PD, Young VB. Dynamics and establishment of *Clostridium difficile* infection in the murine gastrointestinal tract. Infect. Immun. 2014; 83:934–941. [PubMed: 25534943]
66. Koenigsknecht MJ, Young VB. Faecal microbiota transplantation for the treatment of recurrent *Clostridium difficile* infection: current promise and future needs. Curr. Opin. Gastroenterol. 2013; 29:628–632. [PubMed: 24100717]
67. Larsbrink J, Rogers TE, Hemsworth GR, McKee LS, Tauzin AS, et al. A discrete genetic locus confers xyloglucan metabolism in select human gut *Bacteroidetes*. Nature. 2014; 506:498–502. [PubMed: 24463512]
68. Lawley TD, Clare S, Walker AW, Stares MD, Connor TR, et al. Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing *Clostridium difficile* disease in mice. PLOS Pathogens. 2012; 8:e1002995. [PubMed: 23133377]
69. Lawley TD, Young VB. Murine models to study *Clostridium difficile* infection and transmission. Anaerobe. 2013; 24:94–97. [PubMed: 24076318]
70. Leatham MP, Banerjee S, Autieri SM, Mercado-Lubo R, Conway T, Cohen PS. Precolonized human commensal *Escherichia coli* strains serve as a barrier to *E. coli* O157:H7 growth in the streptomycin-treated mouse intestine. Infect. Immun. 2009; 77:2876–2886. [PubMed: 19364832]

71. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, et al. Burden of *Clostridium difficile* infection in the United States. N. Engl. J. Med. 2015; 372:825–834. [PubMed: 25714160]
72. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006; 124:837–848. [PubMed: 16497592]
73. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of *Enterobacteriaceae*. Cell Host Microbe. 2007; 2:119–129. [PubMed: 18005726]
74. Macfarlane GT, Macfarlane S. Bacteria, colonic fermentation, and gastrointestinal health. J AOAC Int. 2012; 95:50–60. [PubMed: 22468341]
75. Mani N, Dupuy B. Regulation of toxin synthesis in *Clostridium difficile* by an alternative RNA polymerase sigma factor. PNAS. 2001; 98:5844–5849. [PubMed: 11320220]
76. Matamouros S, England P, Dupuy B. *Clostridium difficile* toxin expression is inhibited by the novel regulator TcdC. Mol. Microbiol. 2007; 64:1274–1288. [PubMed: 17542920]
77. May T, Mackie RI, Fahey GC Jr, Cremin JC, Garleb KA. Effect of fiber source on short-chain fatty acid production and on the growth and toxin production by *Clostridium difficile*. Scand. J. Gastroenterol. 1994; 29:916–922. [PubMed: 7839098]
78. McCollum DL, Rodriguez JM. Detection, treatment, and prevention of *Clostridium difficile* infection. Clin. Gastroenterol. Hepatol. 2012; 10:581–592. [PubMed: 22433924]
79. McDonald LC, Killgore GE, Thompson A, Owens RC Jr, Kazakova SV, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. N. Engl. J. Med. 2005; 353:2433–2441. [PubMed: 16322603]
80. McFarland LV. Antibiotic-associated diarrhea: epidemiology, trends and treatment. Future Microbiol. 2008; 3:563–578. [PubMed: 18811240]
81. McFee RB, Abdelsayed GG. Clostridium difficile. Dis. Mon. 2009; 55:439–470. [PubMed: 19540996]
82. Merrigan M, Venugopal A, Mallozzi M, Roxas B, Viswanathan VK, et al. Human hypervirulent *Clostridium difficile* strains exhibit increased sporulation as well as robust toxin production. J. Bacteriol. 2010; 192:4904–4911. [PubMed: 20675495]
83. Midtvedt T. Microbial bile acid transformation. Am. J. Clin. Nutr. 1974; 27:1341–1347. [PubMed: 4217103]
84. Moeller AH, Peeters M, Ayouba A, Ngole EM, Esteban A, et al. Stability of the gorilla microbiome despite simian immunodeficiency virus infection. Mol. Ecol. 2015; 24:690–697. [PubMed: 25545295]
85. Moore WE, Holdeman LV. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Appl. Microbiol. 1974; 27:961–979. [PubMed: 4598229]
86. Nagaro KJ, Phillips ST, Cheknis AK, Sambol SP, Zukowski WE, et al. Nontoxigenic *Clostridium difficile* protects hamsters against challenge with historic and epidemic strains of toxigenic BI/NAP1/027 *C. difficile*. Antimicrob. Agents Chemother. 2013; 57:5266–5270. [PubMed: 23939887]
87. Nakamura S, Nakashio S, Yamakawa K, Tanabe N, Nishida S. Carbohydrate fermentation by *Clostridium difficile*. Microbiol. Immunol. 1982; 26:107–111. [PubMed: 6806571]
88. Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature. 2013; 502:96–99. [PubMed: 23995682]
89. Onderdonk AB, Cisneros RL, Bartlett JG. *Clostridium difficile* in gnotobiotic mice. Infect. Immun. 1980; 28:277–282. [PubMed: 7380566]
90. Owens RC Jr, Donskey CJ, Gaynes RP, Loo VG, Muto CA. Antimicrobial-associated risk factors for *Clostridium difficile* infection. Clin. Infect. Dis. 2008; 46(Suppl. 1):S19–S31. [PubMed: 18177218]
91. Pepin J, Saheb N, Coulombe MA, Alary ME, Corriveau MP, et al. Emergence of fluoroquinolones as the predominant risk factor for *Clostridium difficile*-associated diarrhea: a cohort study during an epidemic in Quebec. Clin. Infect. Dis. 2005; 41:1254–1260. [PubMed: 16206099]

92. Peterfreund GL, Vandivier LE, Sinha R, Marozsan AJ, Olson WC, et al. Succession in the gut microbiome following antibiotic and antibody therapies for *Clostridium difficile*. PLOS ONE. 2012; 7:e46966. [PubMed: 23071679]

93. Proctor LM. The Human Microbiome Project in 2011 and beyond. Cell Host Microbe. 2011; 10:287–291. [PubMed: 22018227]

94. Rea MC, Sit CS, Clayton E, O'Connor PM, Whittal RM, et al. Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against *Clostridium difficile*. PNAS. 2010; 107:9352–9357. [PubMed: 20435915]

95. Reeves AE, Koenigsknecht MJ, Bergin IL, Young VB. Suppression of *Clostridium difficile* in the gastrointestinal tracts of germfree mice inoculated with a murine isolate from the family *Lachnospiraceae*. Infect. Immun. 2012; 80:3786–3794. [PubMed: 22890996]

96. Reeves AE, Theriot CM, Bergin IL, Huffnagle GB, Schloss PD, Young VB. The interplay between microbiome dynamics and pathogen dynamics in a murine model of *Clostridium difficile* infection. Gut Microbes. 2011; 2:145–158. [PubMed: 21804357]

97. Ridlon JM, Kang D-J, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J. Lipid Res. 2006; 47:241–259. [PubMed: 16299351]

98. Robinson CD, Auchtung JM, Collins J, Britton RA. Epidemic *Clostridium difficile* strains demonstrate increased competitive fitness compared to nonepidemic isolates. Infect. Immun. 2014; 82:2815–2825. [PubMed: 24733099]

99. Rolfe RD. Role of volatile fatty acids in colonization resistance to *Clostridium difficile*. Infect. Immun. 1984; 45:185–191. [PubMed: 6735467]

100. Romick-Rosendale LE, Goodpaster AM, Hanwright PJ, Patel NB, Wheeler ET, et al. NMR-based metabonomics analysis of mouse urine and fecal extracts following oral treatment with the broad-spectrum antibiotic enrofloxacin (Baytril). Magn. Reson. Chem. 2009; 47(Suppl. 1):S36–S46. [PubMed: 19768747]

101. Savage DC. Microbial ecology of the gastrointestinal tract. Annu. Rev. Microbiol. 1977; 31:107–133. [PubMed: 334036]

102. Scaria J, Chen JW, Useh N, He H, McDonough SP, et al. Comparative nutritional and chemical phenome of *Clostridium difficile* isolates determined using phenotype microarrays. Int. J. Infect. Dis. 2014; 27:20–25. [PubMed: 25130165]

103. Schubert AM, Rogers MA, Ring C, Mogle J, Petrosino JP, et al. Microbiome data distinguish patients with *Clostridium difficile* infection and non-*C. difficile*-associated diarrhea from healthy controls. mBio. 2014; 5:e01021–14. [PubMed: 24803517]

104. Seekatz AM, Aas J, Gessert CE, Rubin TA, Saman DM, et al. Recovery of the gut microbiome following fecal microbiota transplantation. mBio. 2014; 5:e00893–e00814. [PubMed: 24939885]

105. Silvester KR, Englyst HN, Cummings JH. Ileal recovery of starch from whole diets containing resistant starch measured in vitro and fermentation of ileal effluent. Am. J. Clin. Nutr. 1995; 62:403–411. [PubMed: 7625349]

106. Smith DG, Robinson HJ. The influence of streptomycin and streptothricin on the intestinal flora of mice. J. Bacteriol. 1945; 50:613–621.

107. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013; 341:569–573. [PubMed: 23828891]

108. Sonnenburg JL, Xu J, Leip DD, Chen CH, Westover BP, et al. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science. 2005; 307:1955–1959. [PubMed: 15790854]

109. Sorg JA. Microbial bile acid metabolic clusters: the bouncers at the bar. Cell Host Microbe. 2014; 16:551–552. [PubMed: 25525784]

110. Sorg JA, Sonenshein AL. bile salts and glycine as cogerminants for *Clostridium difficile* spores. J. Bacteriol. 2008; 190:2505–2512. [PubMed: 18245298]

111. Sorg JA, Sonenshein AL. Chenodeoxycholate is an inhibitor of *Clostridium difficile* spore germination. J. Bacteriol. 2009; 191:1115–1117. [PubMed: 19060152]

112. Sorg JA, Sonenshein AL. Inhibiting the initiation of *Clostridium difficile* spore germination using analogs of chenodeoxycholic acid, a bile acid. J. Bacteriol. 2010; 192:4983–4990. [PubMed: 20675492]

113. Sridharan GV, Choi K, Klemashevich C, Wu C, Prabakaran D, et al. Prediction and quantification of bioactive microbiota metabolites in the mouse gut. Nat. Commun. 2014; 5:5492. [PubMed: 25411059]
114. Stabler RA, He M, Dawson L, Martin M, Valiente E, et al. Comparative genome and phenotypic analysis of *Clostridium difficile* 027 strains provides insight into the evolution of a hypervirulent bacterium. Genome Biol. 2009; 10:R102. [PubMed: 19781061]
115. Stanley JD, Bartlett JG, Dart BW IV, Ashcraft JH. *Clostridium difficile* infection. Curr. Probl. Surg. 2013; 50:302–337. [PubMed: 23764494]
116. Stecher B, Macpherson AJ, Hapfelmeier S, Kremer M, Stallmach T, Hardt WD. Comparison of *Salmonella enterica* serovar Typhimurium colitis in germfree mice and mice pretreated with streptomycin. Infect. Immun. 2005; 73:3228–3241. [PubMed: 15908347]
117. Swann JR, Want EJ, Geier FM, Spagou K, Wilson ID, et al. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. PNAS. 2011; 108(Suppl. 1):4523–4530. [PubMed: 20837534]
118. Tedesco FJ, Barton RW, Alpers DH. Clindamycin-associated colitis: a prospective study. Ann. Intern. Med. 1974; 81:429–433. [PubMed: 4412460]
119. Theriot CM, Koenigsknecht MJ, Carlson PE Jr, Hatton GE, Nelson AM, et al. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to *Clostridium difficile* infection. Nat. Commun. 2014; 5:3114. [PubMed: 24445449]
120. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012; 489:242–249. [PubMed: 22972297]
121. van der Waaij D, Berghuis-de Vries JM, Lekkerkerk L-van der Wees. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. J. Hyg. 1971; 69:405–411. [PubMed: 4999450]
122. Van Houte J, Gibbons RJ. Studies of the cultivable flora of normal human feces. Antonie Van Leeuwenhoek. 1966; 32:212–222. [PubMed: 5296851]
123. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. N. Engl. J. Med. 2013; 368:407–415. [PubMed: 23323867]
124. Vollaard EJ, Clasener HA. Colonization resistance. Antimicrob. Agents Chemother. 1994; 38:409–414. [PubMed: 8203832]
125. Voth DE, Ballard JD. *Clostridium difficile* toxins: mechanism of action and role in disease. Clin. Microbiol. Rev. 2005; 18:247–263. [PubMed: 15831824]
126. Vrieze A, Out C, Fuentes S, Jonker L, Reuling I, et al. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. J. Hepatol. 2014; 60:824–831. [PubMed: 24316517]
127. Warny M, Pepin J, Fang A, Killgore G, Thompson A, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. Lancet. 2005; 366:1079–1084. [PubMed: 16182895]
128. Weingarden AR, Chen C, Bobr A, Yao D, Lu Y, et al. Microbiota transplantation restores normal fecal bile acid composition in recurrent *Clostridium difficile* infection. Am. J. Physiol. Gastrointest. Liver Physiol. 2014; 306:G310–G319. [PubMed: 24284963]
129. Wells JE, Hylemon PB. Identification and characterization of a bile acid 7α-dehydroxylation operon in *Clostridium* sp. strain TO-931, a highly active 7α-dehydroxylating strain isolated from human feces. Appl. Environ. Microbiol. 2000; 66:1107–1113. [PubMed: 10698778]
130. Wensinck F, Ruseler-van Embden JG. The intestinal flora of colonization-resistant mice. J. Hyg. 1971; 69:413–421. [PubMed: 4937854]
131. Wilson KH. The microecology of *Clostridium difficile*. Clin. Infect. Dis. 1993; 16(Suppl. 4):S214–S218. [PubMed: 8324122]
132. Wilson KH, Kennedy MJ, Fekety FR. Use of sodium taurocholate to enhance spore recovery on a medium selective for *Clostridium difficile*. J. Clin. Microbiol. 1982; 15:443–446. [PubMed: 7076817]
133. Wilson KH, Perini F. Role of competition for nutrients in suppression of *Clostridium difficile* by the colonic microflora. Infect. Immun. 1988; 56:2610–2614. [PubMed: 3417352]

134. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011; 334:105–108. [PubMed: 21885731]

135. Yap IK, Li JV, Saric J, Martin FP, Davies H, et al. Metabonomic and microbiological analysis of the dynamic effect of vancomycin-induced gut microbiota modification in the mouse. J. Proteome Res. 2008; 7:3718–3728. [PubMed: 18698804]

136. Young VB, Schmidt TM. Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. J. Clin. Microbiol. 2004; 42:1203–1206. [PubMed: 15004076]

137. Young VB, Schmidt TM. Overview of the gastrointestinal microbiota. Adv. Exp. Med. Biol. 2008; 635:29–40. [PubMed: 18841701]

138. Zhao Y, Wu J, Li JV, Zhou NY, Tang H, Wang Y. Gut microbiota composition modifies fecal metabolic profiles in mice. J. Proteome Res. 2013; 12:2987–2999. [PubMed: 23631562]

139. Zheng X, Xie G, Zhao A, Zhao L, Yao C, et al. The footprints of gut microbial-mammalian co-metabolism. J. Proteome Res. 2011; 10:5512–5522. [PubMed: 21970572]

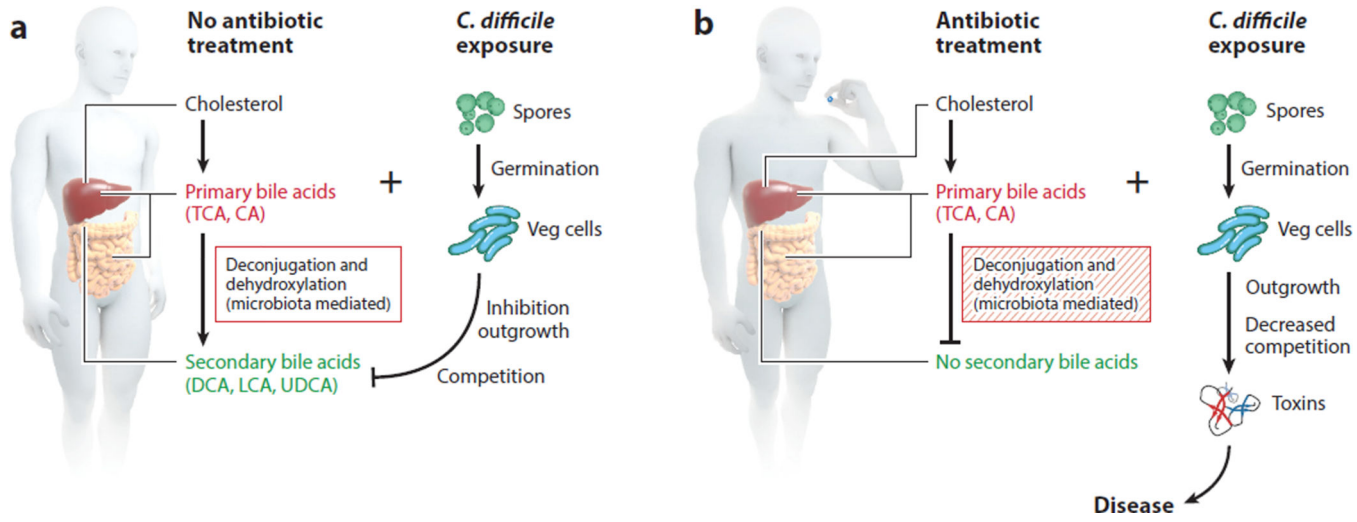

Figure 1.

Antibiotic-induced alterations in gut microbial metabolism decrease colonization resistance against *C. difficile*. (*a*) Bile acids are synthesized from cholesterol by hepatic enzymes. Once synthesized in the gallbladder, primary bile acids (e.g., conjugated, TCA, and unconjugated, CA) travel through the small intestine, where 95% of bile is absorbed in the terminal ileum and through the hepatic system. The small amount of bile acids that reaches the large intestine is further biotransformed by members of the gut microbiota via deconjugation and dehydroxylation into secondary bile acids, including DCA, LCA, and UDCA. *C. difficile* spores can use primary bile acids TCA and CA in the ileum for germination from a spore to an actively growing vegetative cell. The presence of secondary bile acids and competition from other members of the indigenous gut microbiota are able to inhibit *C. difficile* outgrowth and colonization in the large intestine. (*b*) Antibiotic treatment alters the gut microbiota structure, specifically decreasing bacteria that are able to deconjugate and dehydroxylate primary bile acids into secondary bile acids, as shown in the striped red box. The loss of secondary bile acid metabolism and competition from the gut microbiota allow for *C. difficile* outgrowth, toxin production, and disease. Abbreviations: CA, cholate; DCA, deoxycholate; LCA, lithocholate; TCA, taurocholate; and UDCA, ursodeoxycholate.