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Role of the Intestinal Microbiota in Resistance to Colonization by *Clostridium difficile*

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Abstract

Antibiotic-associated infection with the bacterial pathogen *Clostridium difficile* is a major cause of morbidity and increased healthcare costs. *C difficile* infection (CDI) follows disruption of the indigenous gut microbiota by antibiotics. Antibiotics create an environment within the intestine that promotes *C difficile* spore germination, vegetative growth, and toxin production, leading to epithelial damage and colitis. Studies of patients with CDI and animal models have shown that the indigenous microbiota can inhibit expansion and persistence of *C difficile*. Although the specific mechanisms of these processes are not known, they are likely to interfere with key aspects of the pathogen's physiology, including spore germination and competitive growth. Increasing our understanding of how the intestinal microbiota manage *C difficile* could lead to better means of controlling this important nosocomial pathogen.

Keywords

Colonization resistance; *C difficile*; microbiome; microbial ecology; antibiotics

Introduction

The fulfillment of Koch's postulates for *C difficile* in 1977 was one of the first formal recognitions of a microbiome-related disease. Although *C difficile* itself fit into the mold of a typical bacterial pathogen, *C difficile* infection (CDI) was unique in that previous antibiotic treatment of the patient was necessary for development of the full disease phenotype. We review *C difficile* as a pathogenic organism and discuss the basic

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epidemiologic features CDI, as well as its molecular pathogenesis. Research into the developing story of how *C difficile* interacts with the indigenous intestinal microbiota has provided important insights into the role of the microbiome in human health and disease.

C difficile and Antibiotic-associated Colitis

C difficile is a Gram-positive, anaerobic, spore-forming bacterium that was first isolated from the feces of healthy infants[1]. Interestingly, the organism appears to be highly prevalent in infants, who rarely demonstrate any clinical signs of infection with fully virulent strains[2, 3]. As the child and subsequently the microbiome matures, *C difficile* is no longer readily detected; in the healthy adult population, asymptomatic colonization with this organism is considered to be a rare event[3, 4]. Rates of colonization are increased primarily among adults with frequent healthcare-associated contact and patients in chronic-care facilities[4].

The pathogenesis of CDI involves production of 2 members of the family of large clostridial toxins: TcdA and TcdB, which are products of genes located with a pathogenicity locus. [5–7] In *C difficile*, these toxins are large proteins with glycosyltransferase activities targeting the host GTPases Rho, Rac, and Cdc42, which regulate actin[8, 9]. Administration of purified toxin in model systems, including ligated ileal loops, is sufficient to replicate the intestinal damage that is characteristic of CDI[10]. Furthermore, there is evidence that these toxins have systemic effects on the host[11]. Although TcdA and TcdB are the most well-studied virulence factors of *C difficile*, other putative virulence factors have also been described[12]. A subset of *C difficile* strains produce an additional toxin, often referred to as binary toxin, that is related to iota toxin from *C perfringens*[13, 14]. Recent interest in binary toxin is likely related to the fact that the recent epidemic strains of *C difficile*, exemplified by the NAP1/027/BI strain, often produce this putative virulence factor[15].

The epidemiology of CDI has been characterized by increases in incidence and severity that began around the year 2000[16, 17]. It was recently estimated that CDI is the most common health care-associated infection, producing estimated annual hospital costs of more than $3 billion [18]. The increase in virulence has been associated with the increasing isolation of epidemic strains related to NAP1/027/BI[15, 19]. There has been debate as to whether epidemic strains have enhanced virulence properties that result in more severe disease or if these strains simply cause more cases of CDI without causing worse patient outcomes [20]. However, the overall increase in incidence and severity of CDI in the past decade has spurred research into *C difficile* pathogenesis. During the same period of time, the growing interest in the role of the indigenous microbiota in human health and disease, exemplified by projects such as the National Institutes of Health's Human Microbiome Project[21, 22] and the European MetaHIT consortium,[23] has produced a scientific environment well-suited for the study of CDI. There is now much interest in the interactions between this pathogen in the intestinal microbiome.

The Microbiota Affects *C difficile* Invasion

The concept that the indigenous gut microbiota mediates some form of resistance against colonization by bacterial pathogens was proposed long before *C difficile* was associated with

antibiotic-associated pseudomembranous colitis[24]. Productive infection of animals with enteric bacterial pathogens often requires pre-exposure to antibiotics [25–27]. However, until recently, the specific mechanisms by which the indigenous microbiome could prevent colonization by pathogenic organisms were not of widespread scientific interest. The requirement for antibiotic treatment before infection with a pathogen was simply regarded as a technical hurdle to overcome in order to investigate specific interactions between the pathogen in the host. However, increasing interest in the role of the indigenous microbiome in maintaining intestinal homeostasis has focused attention on its interactions with pathogens. The development of tractable animal models for studying CDI and the concurrent application of experimental microbial ecology to host-associated bacterial communities has led to multiple reports of how they influence the development of CDI and its complications. Work in experimental models of CDI and with clinical material from patients has indicated that *C difficile*, the intestinal microbiota, and other host factors form a complex system; specific alterations to this system can promote pathogenesis of CDI (Figure 1).

Studies in animals and humans have demonstrated that antibiotics have profound and, in some cases, long-lasting effects on the community structure of the gut microbiota[28, 29]. Antibiotics affect not only the overall size of the gut bacterial community, but also the composition of the community (they alter proportions of specific bacterial species). However, these effects do not necessarily occur together[30]. These effects on community structure are presumed to alter functions of the community,[31] and for purposes of this discussion, might reduce resistance to CDI.

The hamster model of CDI was initially used to study interactions among *C. difficile*, the microbiota, and host tissues[32], but mouse models are increasing in use. In the hamster model of CDI, clindamycin is the standard antibiotic used to generate a susceptible state[33]. A recent study followed the effect of clindamycin on the gut microbiota of hamsters associated with experimental CDI[34]. In the mouse models of disease, a variety of antibiotics have been shown to lead to loss of resistance to *C difficile* colonization[35–38]. Additionally, specific effects of antibiotics on the mouse gut microbiome have been described, allowing researchers to investigate how specific members of the microbiome mediate resistance to colonization[39, 40].

Recent interest in mouse models of CDI began with a 2008 report in which Chen et al. used a cocktail of 5 antibiotics, followed by a single dose of clindamycin, to render mice susceptible to colonization by *C difficile* and subsequent development of a severe colitis[35]. A subsequent study by a separate group, using this same model, associated susceptibility with a loss of the normal members of the cecal community (mainly members of the family Lachnospiraceae) and a relative increase in the abundance of Enterobacteriaceae[37]. Furthermore, the severity of disease was related to the relative dynamics of these bacterial families, with more severe outcomes associated with a failure of Lachnospiraceae to return and a continued dominance with Enterobacteriaceae. Formal tests of the relative roles of these organisms in mediating colonization resistance were accomplished by monocolonization of germ-free mice with murine representatives of each of these groups of bacteria[39]. Colonization with a murine *E coli* isolate had no effect on the ability of *C*

difficile to colonize the intestine and induce colitis, whereas a Lachnospiraceae isolate significantly reduced the levels of colonization by *C difficile* and the severity of colitis.

Then, Lawley et al. demonstrated that administration of clindamycin permitted long-term, non-fatal colonization of mice with *C difficile* [40]. They identified 6 phylogenetically diverse species of bacteria that when administered together could restore colonization resistance and prevent chronic carriage of *C difficile*. It is interesting to note that clindamycin has not been reported to disrupt resistance against *C difficile* colonization in all genetically identical mice. Two studies reported that clindamycin can overcome colonization resistance in C57BL/6 mice[38, 40], whereas a third group only achieved transient (2–3 day) colonization of the same strain of mice with *C difficile* following an identical dose of clindamycin[37]. Baseline differences in microbiota of mice from different colonies might cause these differences in results. Comparative microbiome analysis of these different groups of mice could provide additional information about the roles of specific groups of bacteria in resistance to colonization with *C difficile*.

Experimental infection of humans with *C difficile* is not ethical, but studies of the microbiomes of patients with naturally occurring *C difficile* infection have provided information that correlates with findings from animal studies. Antibiotics alter the structure of microbial communities[29], and fecal samples from patients who developed CDI after antibiotic therapy have decreased diversity and other changes in the microbiota, compared to patients who did not develop CDI[34]. The intestinal microbiome of patients with recurrent disease has lower diversity than patients without CDI or those whose CDI was successfully treated and did not recur [41]. Furthermore, patients with primary or recurrent symptomatic CDI have fecal microbiomes that differ in composition from patients with asymptomatic colonization by *C difficile*[4]. This observation makes it clear why fecal transplantation is so successful in the treatment of recalcitrant CDI (see FMT review by Alex Khoruts and Elaine Petrof in this same issue).

Microbiome Structure and Function in Resistance to *C difficile* Colonization

Although disruption of the healthy fecal microbiota can create an ecologic environment in which *C difficile* can flourish, little is understood about the changes in the environmental and microbial communities that occur in the intestine. Host and microbial community factors are directly involved in *C difficile* invasion of a dysbiotic intestinal community. *C difficile* selects specific environments for expansion and colonization, which appear to occur via complementary mechanisms. Development of CDI may involve altered bile acid metabolism and competition among microbes for nutrients, although other mechanisms by which the indigenous microbiota resists colonization by pathogenic bacteria exist [42].

Bile Salts in *C difficile* Spore Germination and Vegetative Growth

Germination of *C difficile* spores can be stimulated by a complex mixture of bile salts. The mechanisms by which individual bile salts and amino acids affect spore germination have only recently been elucidated [43–46]. Interestingly, the primary bile acids cholate and chenodeoxycholate have different effects on the spore germination process. The conjugated and deconjugated forms of cholate, along with the amino acid glycine, function together to

promote germination of *C difficile* spores in vitro, whereas chenodeoxycholate is a potent inhibitor of germination [44, 45]. Importantly, physiologically relevant levels of these bile acids are sufficient to affect germination in vitro. Chenodeoxycholate competitively inhibits germination at a concentration ~10-fold lower than the stimulatory concentration of cholate. So, under normal physiological conditions, chenodeoxycholate suppresses *C difficile* invasion in vivo.

Cholate and chenodeoxycholate are metabolized into the secondary bile acids deoxycholate and lithocholate, respectively. In line with the effects of primary bile acids on spore germination, deoxycholate stimulates germination whereas lithocholate is a potent inhibitor. However, deoxycholate is toxic to vegetative *C difficile*; germination in the presence of this compound leads to quick death of bacteria. Therefore, an environment in which cholate levels are markedly higher than these other primary and secondary bile acids would support *C difficile* germination and expansion.

Administration of antibiotics causes a large shift in the bile acid pool in the cecum of mice, which can disrupt this balance and lead to spore germination (see Figure 2). [47, 48]. Cholate is not detectable in the cecum of mice with a normal microbiota, but antibiotics increase its levels while levels of secondary bile acids remain low. So, *C difficile* spores are not expected to germinate until they are induced to do so by changes in concentrations of bile acids in the intestine.

Until this past year, only in vitro studies had shown that bile acids were able to impact the germination of *C difficile* spores. Recently, researchers have shown that bile acid derivatives that inhibit spore germination in vitro reduce the ability of *C difficile* to colonize the intestines of mice[52]. Furthermore, in an elegant genetic screen, Sorg et al. identified a putative protease (CspC) that is required for spore germination in vitro [53] and could be a long-sought after spore germination receptor. *cspC* mutants of *C difficile* cannot germinate in the presence of cholate and glycine, and are unable to colonize and cause disease in hamsters. Therefore, the ability to sense bile is critical for *C difficile* colonization of the intestine. These findings support a role for bile metabolism in *C difficile* colonization of the intestine.

Ecologic Niche Expansion: Availability of Food Sources

The intestinal microbiota can also impact colonization by pathogens such as *C difficile* by providing competition for resources. Antibiotic disruption of the intestinal microbiota alters sources of nutrients in at least 2 ways. First, by reducing the diversity of the intestinal microbiota, antibiotics decrease competition for limited resources and free up previously unavailable ecological niches. Second, bacterial cell lysis releases carbon sources that the remaining community can consume. Despite the large number of *C difficile* genomes that have been sequenced, there have been relatively few studies of the metabolic networks used by *C difficile* to colonize the intestine. In vitro studies have shown that *C difficile* can grow on simple sugars, amino acids, peptides, and complex carbohydrates [54–57].

Wilson and Perini used continuous flow cultures to investigate whether a complex microbiota suppresses *C difficile* infection by competing for available resources [58]. In

growth medium formulated from feces of germ-free mice, cultivation of the cecal microbiota from hamsters gave rise to a community that not only resisted *C difficile* invasion but could also displace an already established *C difficile* infection. When hamster cecal communities were grown on a rich synthetic medium, they lost their ability to resist *C difficile*, although they were still diverse. To investigate what components of the germ-free mouse fecal medium promoted expansion of intestinal bacteria that suppresses *C difficile*, the authors added back individual carbon sources that were major components of this medium. Interestingly, they found that components of mucin (sialic acids, N-acetylglucosamine, and N-acetylneuraminic acid, which are abundant in the fecal medium) fully restored the ability to suppress *C difficile* to communities cultured in rich medium.

In support of these findings, *C difficile* was recently reported to use host-derived sialic acids to proliferate in the intestines of mice [59]. *C difficile* was found to be able to break down sialic acids and use them as a carbon source; mutants *C difficile* that lacked this activity had a 4-fold reduction in colony-forming units/g of feces. Although these results show that *C difficile* can metabolize sialic acids to reach high densities in the intestine, additional factors are likely to be required for expansion of *C difficile* in the intestine.

Rolf Freter proposed a chemostat model for pathogen colonization, based on observations made while studying the persistence of individual microbes in a complex microbiota [60]. This model might explain how *C difficile* persists in the intestine after antibiotic treatment and how competing microbes could displace *C difficile* during bacteriotherapy treatment [61]. For example, if 2 organisms are in direct competition for a limited nutrient, the organism that can most efficiently use this nutrient will outcompete the other. This model also predicts that when one organism already fully occupies an intestinal niche, it is difficult for other organisms to invade.

In support of this idea, colonization of hamsters with a non-toxigenic strain of *C difficile* strain before with a toxigenic strain can completely prevent *C difficile*-associated colitis [62, 63]. These findings indicate that the non-toxigenic strain occupies a niche cannot be taken over by a new strain. In a prospective study, patients who were followed until they developed CDI were found to have reduced proportions of the family Clostridiales Incertae Sedis XI just prior to infection, compared with individuals who did not develop CDI[64]. *C difficile* is a member of the family Clostridiales Incertae Sensu XI—loss of the Clostridiales Incertae Sedis XI could create a niche that *C difficile* is able to occupy. However, due to other alterations in the intestinal microbiota of these patients, we cannot conclude that loss of these microbes is directly responsible for the subsequent *C difficile* colonization.

Therapeutic Interventions with Defined Microbial Communities

Administration of defined microbes, usually single-strain probiotics, has had only limited success in ameliorating the symptoms of CDI. This is not surprising given recent animal and human studies associating large-scale alterations in microbial communities with antibiotic use and CDI. FMT is becoming more accepted for the treatment of recurrent CDI (reviewed by Khoruts and Vercoe, this issue). Despite its success, the undefined and complex nature of

stool makes it difficult to envision widespread FMT therapy for individuals other than those with severe recurrent CDI.

Defined communities of microbes have been shown to be effective against recurrent CDI in patients and animal models. Interestingly, the 3 defined communities that are effective against recurrent CDI are remarkably simple, compared to the diversity of the intestinal microbiota. In addition, each community shares few organisms at the genus level, indicating there will be many combinations that can suppress CDI. Tvede and Rask-Madsen reported that a cocktail of 10 facultative aerobes and anaerobes was effective against recurrent CDI in 5 patients[65]. They noted that all of the patients were deficient in Bacteroides before the cocktail was administered, and then afterward the genus was detected in fecal samples from the patients. Petrof et al. reported using a 33-strain combination of microbes cultured from human fecal samples to treat 2 patients with recurrent CDI [66]. The strains were selected based primarily on their antibiotic susceptibility and safety, while the researchers attempted to generate as much taxonomic diversity as possible. Both patients recovered from CDI, although it is not clear this was a direct result of population of the intestine with the defined microbes or an indirect result of stimulation of the indigenous microbiota. Sequencing analysis of microbial communities from fecal samples of the patients indicated that the 33 strains administered to the patients were gradually lost.

Although Petrof et al. only included 2 patients in their trial, the concept that the indigenous microbiota can be altered through addition of microbes was supported by the effects of a 6 species community of microbes administered to a mouse model of CDI [40]. Interestingly, none of the 6 strains appeared to be able to colonize the mice over long time periods, but a single administration of these microbes increased the microbial diversity in the intestines of mice. It therefore appears that the defined communities might act by stimulating a suppressed indigenous microbiota.

Conclusions

The ability of FMT to cure recurrent CDI in up to 95% of patients provides support for strategies aimed at restoring the diversity of the intestinal microbiota. The efficacy of fecal communities from a variety of donors with diverse microbiotas, indicates that many microbial community structures will be able to prevent or treat CDI. For most cases, the fecal material for transplantation spends a significant amount of time being prepared under aerobic conditions, thus it is likely that a substantial proportion of the microbes being administered in FMTs do not remain viable. Studies are underway to understand how feces,, as well as defined microbial communities, are able to interfere with CDI. It is also critical to understand not only how these communities suppress *C difficile* invasion, but how the implantation of new communities may affect other aspects of human health. Studies have linked the intestinal microbiota with diseases such as diabetes, colon cancer, obesity, and atherosclerosis. If we use the microbiota to treat disease, we may inadvertently increase the risk of others. Continued research into the roles of the intestinal microbiome in health and disease might lead to ways to manipulate this community for benefit while minimizing unintended consequences.

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References Cited

1. Hall IC, O'Toole E. Intestinal flora in new-born infants with the description of a new pathogic anaerobe, *Bacillus difficilis*. Am J Dis Child. 1935; 49:390–402.
2. Thompson CM Jr, Gilligan PH, Fisher MC, et al. Clostridium difficile cytotoxin in a pediatric population. Am J Dis Child. 1983; 137:271–4. [PubMed: 6823926]
3. Rousseau C, Poilane I, De Pontual L, et al. Clostridium difficile carriage in healthy infants in the community: a potential reservoir for pathogenic strains. Clin Infect Dis. 2012; 55:1209–15. [PubMed: 22843784]
4. Rea MC, O'Sullivan O, Shanahan F, et al. Clostridium difficile carriage in elderly subjects and associated changes in the intestinal microbiota. J Clin Microbiol. 2012; 50:867–75. [PubMed: 22162545]
5. Voth DE, Ballard JD. *Clostridium difficile* Toxins: Mechanism of Action and Role in Disease. Clin Microbiol Rev. 2005; 18:247–63. [PubMed: 15831824]
6. Pruitt RN, Lacy DB. Toward a structural understanding of Clostridium difficile toxins A and B. Front Cell Infect Microbiol. 2012; 2:28. [PubMed: 22919620]
7. Shen A. Clostridium difficile toxins: mediators of inflammation. J Innate Immun. 2012; 4:149–58. [PubMed: 22237401]
8. Hecht G, Pothoulakis C, LaMont JT, et al. Clostridium difficile toxin A perturbs cytoskeletal structure and tight junction permeability of cultured human intestinal epithelial monolayers. J Clin Invest. 1988; 82:1516–24. [PubMed: 3141478]
9. Just I, Fritz G, Aktories K, et al. Clostridium difficile toxin B acts on the GTP-binding protein Rho. J Biol Chem. 1994; 269:10706–12. [PubMed: 8144660]
10. Kelly CP, Becker S, Linevsky JK, et al. Neutrophil recruitment in *Clostridium difficile* toxin A enteritis in the rabbit. J Clin Invest. 1994; 93:1257–65. [PubMed: 7907603]
11. Hamm EE, Voth DE, Ballard JD. Identification of Clostridium difficile toxin B cardiotoxicity using a zebrafish embryo model of intoxication. Proc Natl Acad Sci U S A. 2006; 103:14176–81. [PubMed: 16966605]
12. Vedantam G, Clark A, Chu M, et al. Clostridium difficile infection: Toxins and non-toxin virulence factors, and their contributions to disease establishment and host response. Gut Microbes. 2012:3. [PubMed: 23249777]
13. Rupnik M, Grabnar M, Geric B. Binary toxin producing Clostridium difficile strains. Anaerobe. 2003; 9:289–94. [PubMed: 16887714]
14. Stiles BG, Wigelsworth DJ, Popoff MR, et al. Clostridial binary toxins: iota and C2 family portraits. Front Cell Infect Microbiol. 2011; 1:11. [PubMed: 22919577]
15. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. N Engl J Med. 2005; 353:2433–41. [PubMed: 16322603]
16. Pepin J, Valiquette L, Alary ME, et al. *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. CMAJ. 2004; 171:466–72. [PubMed: 15337727]
17. Freeman J, Bauer MP, Baines SD, et al. The changing epidemiology of Clostridium difficile infections. Clin Microbiol Rev. 2010; 23:529–49. [PubMed: 20610822]
18. Dubberke E. *Clostridium difficile* infection: the scope of the problem. J Hosp Med. 2012; 7 (Suppl 3):S1–4. [PubMed: 22407993]
19. Goorhuis A, Bakker D, Corver J, et al. Emergence of Clostridium difficile infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. Clin Infect Dis. 2008; 47:1162–70. [PubMed: 18808358]
20. Walk ST, Micic D, Jain R, et al. Clostridium difficile ribotype does not predict severe infection. Clin Infect Dis. 2012

21. Proctor LM. The Human Microbiome Project in 2011 and beyond. Cell Host Microbe. 2011; 10:287–91. [PubMed: 22018227]
22. Human Microbiome Project C. A framework for human microbiome research. Nature. 2012; 486:215–21. [PubMed: 22699610]
23. Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. Nature. 2011; 473:174–80. [PubMed: 21508958]
24. 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]
25. Bohnhoff M, Drake BL, Miller CP. Effect of streptomycin on susceptibility of intestinal tract to experimental Salmonella infection. Proc Soc Exp Biol Med. 1954; 86:132–7. [PubMed: 13177610]
26. Wadolkowski EA, Laux DC, Cohen PS. Colonization of the streptomycin-treated mouse large intestine by a human fecal Escherichia coli strain: role of growth in mucus. Infect Immun. 1988; 56:1030–5. [PubMed: 3281898]
27. Wadolkowski EA, Burris JA, O'Brien AD. Mouse model for colonization and disease caused by enterohemorrhagic Escherichia coli O157:H7. Infect Immun. 1990; 58:2438–45. [PubMed: 2196227]
28. Antonopoulos DA, Huse SM, Morrison HG, et al. Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. Infect Immun. 2009; 77:2367–75. [PubMed: 19307217]
29. Dethlefsen L, Huse S, Sogin ML, et al. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 2008; 6:e280. [PubMed: 19018661]
30. Robinson CJ, Young VB. Antibiotic administration alters the community structure of the gastrointestinal micobiota. Gut Microbes. 2010; 1:279–284. [PubMed: 20953272]
31. Robinson CJ, Bohannan BJ, Young VB. From structure to function: the ecology of host-associated microbial communities. Microbiol Mol Biol Rev. 2010; 74:453–76. [PubMed: 20805407]
32. Bartlett JG, Onderdonk AB, Cisneros RL, et al. Clindamycin-associated colitis due to a toxin-producing species of *Clostridium* in hamsters. J Infect Dis. 1977; 136:701–5. [PubMed: 915343]
33. Lusk RH, Fekety R, Silva J, et al. Clindamycin-induced enterocolitis in hamsters. J Infect Dis. 1978; 137:464–75. [PubMed: 649990]
34. Peterfreund GL, Vandivier LE, Sinha R, et al. Succession in the gut microbiome following antibiotic and antibody therapies for Clostridium difficile. PLoS One. 2012; 7:e46966. [PubMed: 23071679]
35. Chen X, Katchar K, Goldsmith JD, et al. A mouse model of *Clostridium difficile*-associated disease. Gastroenterology. 2008; 135:1984–92. [PubMed: 18848941]
36. Theriot CM, Koumpouras CC, Carlson PE, et al. Cefoperazone-treated mice as an experimental platform to assess differential virulence of Clostridium difficile strains. Gut Microbes. 2011; 2 [in press].
37. Reeves AE, Theriot CM, Bergin IL, et al. The interplay between microbiome dynamics and pathogen dynamics in a murine model of *Clostridium difficile* Infection. Gut Microbes. 2011; 2:145–58. [PubMed: 21804357]
38. Buffie CG, Jarchum I, Equinda M, et al. Profound Alterations of Intestinal Microbiota following a Single Dose of Clindamycin Results in Sustained Susceptibility to Clostridium difficile-Induced Colitis. Infect Immun. 2012; 80:62–73. [PubMed: 22006564]
39. Reeves AE, Koenigsknecht MJ, Bergin IL, et al. Suppression of Clostridium difficile in the Gastrointestinal Tract of Germ-Free Mice Inoculated with a Murine Lachnospiraceae Isolate. Infect Immun. 2012
40. Lawley TD, Clare S, Walker AW, et al. Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing Clostridium difficile disease in mice. PLoS Pathog. 2012; 8:e1002995. [PubMed: 23133377]
41. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile*-associated diarrhea. J Infect Dis. 2008; 197:435–8. [PubMed: 18199029]

42. Britton RA, Young VB. Interaction between the intestinal microbiota and host in Clostridium difficile colonization resistance. Trends Microbiol. 2012
43. Howerton A, Ramirez N, Abel-Santos E. Mapping interactions between germinants and Clostridium difficile spores. J Bacteriol. 2011; 193:274–82. [PubMed: 20971909]
44. Sorg JA, Sonenshein AL. Bile salts and glycine as co-germinants for Clostridium difficile spores. J Bacteriol. 2008
45. Sorg JA, Sonenshein AL. Chenodeoxycholate is an inhibitor of Clostridium difficile spore germination. J Bacteriol. 2009; 191:1115–7. [PubMed: 19060152]
46. Wilson KH. Efficiency of various bile salt preparations for stimulation of *Clostridium difficile* spore germination. J Clin Microbiol. 1983; 18:1017–9. [PubMed: 6630458]
47. Antunes LC, Finlay BB. A comparative analysis of the effect of antibiotic treatment and enteric infection on intestinal homeostasis. Gut Microbes. 2011; 2:105–8. [PubMed: 21637027]
48. Giel JL, Sorg JA, Sonenshein AL, et al. Metabolism of bile salts in mice influences spore germination in Clostridium difficile. PLoS One. 2010; 5:e8740. [PubMed: 20090901]
49. Krag E, Phillips SF. Active and passive bile acid absorption in man. Perfusion studies of the ileum and jejunum. J Clin Invest. 1974; 53:1686–94. [PubMed: 4830231]
50. Mekhjian HS, Phillips SF, Hofmann AF. Colonic absorption of unconjugated bile acids: perfusion studies in man. Dig Dis Sci. 1979; 24:545–50. [PubMed: 456241]
51. Schiff ER, Small NC, Dietschy JM. Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat. J Clin Invest. 1972; 51:1351–62. [PubMed: 5024036]
52. Howerton A, Patra M, Abel-Santos E. A new strategy for the prevention of Clostridium difficile infection. J Infect Dis. 2013; 207:1498–504. [PubMed: 23420906]
53. Francis MB, Allen CA, Shrestha R, et al. Bile acid recognition by the Clostridium difficile germinant receptor, CspC, is important for establishing infection. PLoS Pathog. 2013; 9:e1003356. [PubMed: 23675301]
54. Bouillaut L, Self WT, Sonenshein AL. Proline-dependent regulation of Clostridium difficile Stickland metabolism. J Bacteriol. 2013; 195:844–54. [PubMed: 23222730]
55. Jackson S, Calos M, Myers A, et al. Analysis of proline reduction in the nosocomial pathogen Clostridium difficile. J Bacteriol. 2006; 188:8487–95. [PubMed: 17041035]
56. Karasawa T, Ikoma S, Yamakawa K, et al. A defined growth medium for Clostridium difficile. Microbiology. 1995; 141 (Pt 2):371–5. [PubMed: 7704267]
57. Nakamura S, Nakashio S, Yamakawa K, et al. Carbohydrate fermentation by Clostridium difficile. Microbiol Immunol. 1982; 26:107–11. [PubMed: 6806571]
58. Wilson KH, Perini F. Role of competition for nutrients in suppression of Clostridium difficile by the colonic microflora. Infect Immun. 1988; 56:2610–4. [PubMed: 3417352]
59. Ng KM, Ferreyra JA, Higginbottom SK, et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature. 2013; 502:96–9. [PubMed: 23995682]
60. Freter R, Brickner H, Botney M, et al. Mechanisms that control bacterial populations in continuous-flow culture models of mouse large intestinal flora. Infect Immun. 1983; 39:676–85. [PubMed: 6339388]
61. Wilson KH. The microecology of *Clostridium difficile*. Clin Infect Dis. 1993; 16 (Suppl 4):S214–8. [PubMed: 8324122]
62. Merrigan M, Sambol S, Johnson S, et al. Susceptibility of hamsters to human pathogenic Clostridium difficile strain B1 following clindamycin, ampicillin or ceftriaxone administration. Anaerobe. 2003; 9:91–5. [PubMed: 16887694]
63. Sambol SP, Merrigan MM, Tang JK, et al. Colonization for the prevention of Clostridium difficile disease in hamsters. J Infect Dis. 2002; 186:1781–9. [PubMed: 12447764]
64. Vincent C, Stephens DA, Loo VG, et al. Reductions in intestinal Clostridiales precede the development of nosocomial *Clostridium difficile* infection. Microbiome. 2013:1. [PubMed: 24467924]
65. Tvede M, Rask-Madsen J. Bacteriotherapy for chronic relapsing *Clostridium difficile* diarrhoea in six patients. Lancet. 1989; 1:1156–60. [PubMed: 2566734]

66. Petrof EO, Gloor GB, Vanner SJ, et al. Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: 'RePOOPulating' the gut. Microbiome. 2013:1. [PubMed: 24467924]

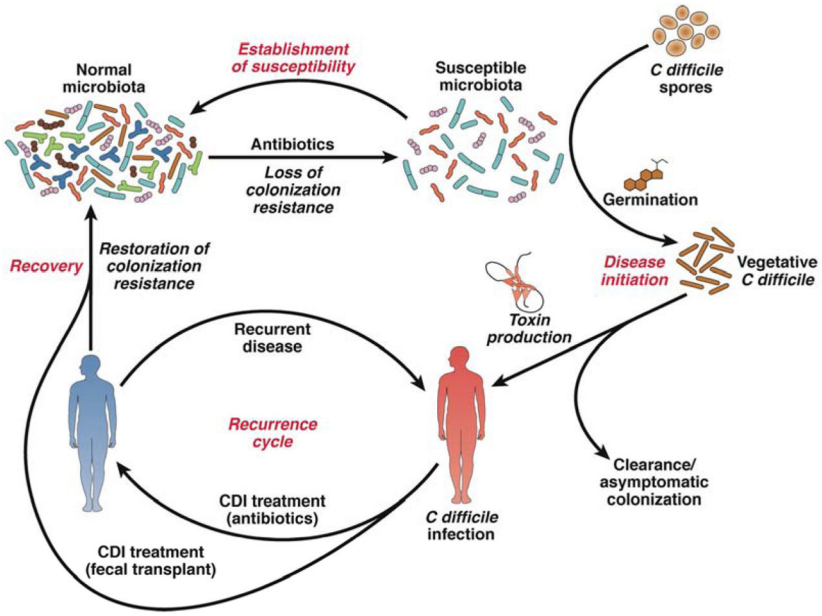

Figure 1. Cycle of CDI

Antibiotic administration alters the indigenous intestinal microbiota, producing an environment that permits germination of *C difficile* spores and expansion of the pathogen. *C difficile* produces toxins that cause colitis and other symptoms. Antibiotics directed against *C difficile* can decrease the load of the pathogen and toxin production. Returning the microbiota to a state of colonization resistance cures CDI. However, if the microbiota is unable to restore resistance to colonization by *C difficile* patients have recurring CDI. In certain cases, repeat courses of anti-*C difficile* antibiotic therapy can eradicate the pathogen. In other cases, therapeutic restoration of a diverse microbiota via FMT is required to overcome CDI.

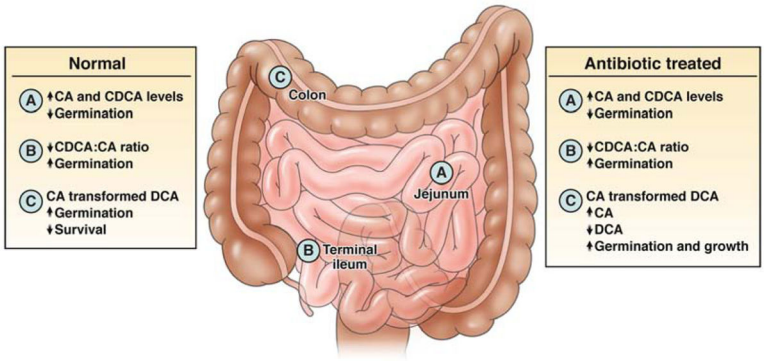

Figure 2. Bile Acids Affect the Ability of C difficile to Colonize the Intestinal Tract

In the healthy microbiota, levels of cholate (CA) and chenodeoxycholate (CDCA) are high in the small intestine, and chenodeoxycholate inhibits germination. Because cholate is less well absorbed than chenodeoxycholate in the terminal ileum, the net effect is an increased ratio of cholate: chenodeoxycholate, resulting in increased spore germination [49–51]. As vegetative *C difficile* cells move into the colon, they are exposed to the toxic effects of deoxycholate (DCA), further preventing *C difficile* from gaining a foothold in the colon. When the microbiota is disrupted with antibiotics bacterial transformation of primary bile acids (such as CA to DCA) can be greatly suppressed. In this case CA levels remain high while bacterial transformation of CA to DCA does not occur. This results in increased CA mediated spore germination and a loss of inhibitory effects of deoxycholate on C difficile growth. The net effect is the expansion of this pathogen will readily occur in the colon.