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Journal

Expert Review of Gastroenterology & Hepatology, 10(2)

ISSN

1747-4124

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Publication Date

2016-02-01

DOI

10.1586/17474124.2016.1109444

Peer reviewed



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To cite this article: David Padua & Charalabos Pothoulakis (2016) Novel approaches to treating Clostridium difficile-associated colitis, Expert Review of Gastroenterology & Hepatology, 10:2, 193-204, DOI: [10.1586/17474124.2016.1109444](https://doi.org/10.1586/17474124.2016.1109444)

To link to this article: <http://dx.doi.org/10.1586/17474124.2016.1109444>



Published online: 07 Dec 2015.



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REVIEW

Novel approaches to treating *Clostridium difficile*-associated colitis

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ABSTRACT

Clostridium difficile is being recognized as a growing threat to many health-care systems. Epidemiology data shows that infection rates are soaring and the disease burden is increasing. Despite the efficacy of standard treatments, it is becoming evident that novel therapeutics will be required to tackle this disease. These new treatments aim to enhance the intestinal microbial barrier, activate the immune system and neutralize the toxins that mediate this disease. Many of these therapies are still in the beginning stages of investigation, however, in the next few years, more clinical data will become available to help implement many of these exciting new therapeutic approaches.

ARTICLE HISTORY

Received 3 August 2015
Accepted 14 October 2015

KEYWORDS

Clostridium difficile; novel therapeutics; fecal microbial transplant; vaccine therapy; probiotics

Introduction

Along with methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci*, *Clostridium difficile* is among the leaders of nosocomial infections and infectious colitis in hospitals in the United States.[1] In a recent study of health-care-associated infections, *C. difficile* was found to be the most commonly reported pathogen resulting in 12.1% of all health-care-associated infections.[2] *C. difficile* infected close to half a million people in 2011 and was found to quadruple the cost of hospitalizations and increased annual expenditures by approximately \$1.5 billion.[3,4] Given the magnitude and the scope of the problem, new therapies are required to tackle this ever-growing threat.

C. difficile is an anaerobic Gram-positive, toxin-producing bacillus that is typically transmitted via the fecal-oral route and infects the large bowel. The spores are found on inanimate objects and are resistant to typical decontaminants such as heat, acid and antibiotics for long periods of time without losing viability.[5] The pathogenesis of *C. difficile* infection (CDI) is closely linked to the release of two protein exotoxins that can disrupt the actin cytoskeleton resulting in cell death of colonic epithelial cells. The net result is a loss of intestinal barrier function, diarrhea and development of colitis. In most cases, *C. difficile* colonization is prevented by the barrier properties of the endogenous fecal microbiota. Weakening of this microbial barrier by antibiotics is a major risk factor for disease. Antibiotic-associated diarrhea occurs in about 10–28% of patients receiving

antibiotics, of which *C. difficile* accounts for 20% of these cases.[6,7]

The presenting symptoms of CDI can be variable but typically a person can develop diarrhea with abdominal pain and can progress to severe and fatal complications such as toxic megacolon, bowel perforation, renal failure, systemic inflammation syndrome, and sepsis.[8] CDI can be detected via nucleic acid amplification tests such as PCR for *C. difficile* toxins or glutamate dehydrogenase screening test with subsequent toxin A and B enzyme immunoassay testing.[9] Severe infections with *C. difficile* have been associated with infection-related mortality of 5% and an all-cause mortality of up to 15–20%.[3,10] The traditional risk factors for *C. difficile* infected hospitalized patients include age greater than 65, recent hospitalizations, residing in long-term care facilities, and recent antibiotic exposure such as clindamycin, cephalosporins and penicillins.[4,11]

Up to 3% of healthy adults may be asymptotically colonized with *C. difficile*. However, in the health-care setting, this percentage is dramatically increased. Elderly patients for example are especially at risk of acquiring *C. difficile* with as many as 10% colonized on hospital admission.[12] Within the first week, up to 20% of hospitalized patients acquire *C. difficile* and by 4 weeks, 50% of all in-house patients are colonized.[13,14] Once infected, the risk of *C. difficile* recurrence after treatment ranges from 20% after an initial episode to 60% after multiple prior recurrences.[15,16] Recurrence can be due to re-exposure to or reactivation

of spores in patients who have an impaired immune response to infection and weakened barrier function of the colonic microbiota.[17]

In 2011, studies identified 453,000 cases of *C. difficile* infection with up to one quarter of the infected patients being community-acquired.[18] In the past two decades, epidemiological data from the United States have shown a two- to four-fold increase in the incidence of CDI, particularly in the elderly.[19–22] The incidence of *C. difficile* in hospitalized patients has risen from 31 cases per 100,000 patients in 1996 to 84 cases per 100,000 in 2004.[23] Studies in the pediatric population show that the incidence in children has also increased up to 12-fold in the last twenty years.[24,25] Importantly, mortality rates have also risen from 1.5% of cases in 1997 to 6.9% in 2004 and account for up to 14,000 deaths per year in the United States.[26] *C. difficile* infections with high mortality and morbidity have also been reported in Canada, Asia, Australia and Central America making this a truly international problem.[27]

C. difficile 027/BI/NAP1 strain

The high prevalence of CDI is in part driven by community-based outbreaks, which affect patients initially thought to be low risk, and by the emergence of the *C. difficile* strain BI/NAP1/027.[28] *C. difficile* BI/NAP1/027 was originally found in the 1980s, however, it was not until the early 2000s during an outbreak in Quebec, Canada that this strain was identified as an epidemic strain.[29] This outbreak was associated with a mortality rate as high as 16%.[30] During this same period, several US hospitals across six states reported similar outbreaks by the *C. difficile* strain BI/NAP1/027.[26]

Guideline-based therapy

Several agencies have produced comprehensive guidelines on the treatment and management of CDI, including the Infectious Diseases Society of America (IDSA), the European Society of Clinical Microbiology and Infectious Diseases and the American College of Gastroenterology.[8,9,31] These groups typically stratify *C. difficile* infections into mild-to-moderate, severe disease, severe complicated disease and recurrent CDI.[9] According to the IDSA, mild and moderate disease consists of diarrhea as the sole symptom without features of severe disease described below. Severe *C. difficile* infections are defined by hypoalbuminemia with a serum albumin less than 3 g/dl and either an elevated white blood cell count of greater than 15,000 cells/mm³ or abdominal pain without criteria of complicated

disease. Complicated infections are defined by hospitalizations requiring stays in the intensive care unit, hypotension with or without vasopressor support, fever greater than 38.5°C, ileus, significant abdominal distension, mental status decline, white blood cell count greater than 35,000 cells/mm³ or less than 2000 cells/mm³, elevated serum lactate levels, or any evidence of end-organ damage.[8]

The pillars for CDI treatment since the 1970s have been metronidazole and oral vancomycin. Despite their extensive use in *C. difficile* management, clinically relevant resistance to either drug has yet to be reported.[4] Metronidazole is a nitroimidazole prodrug that is taken up and reduced by bacterial cells where it binds covalently to DNA to inhibit nucleic acid synthesis.[32] Vancomycin is a glycopeptide that inhibits the synthesis of peptidoglycans necessary for bacterial cell wall formation.[33] According to the IDSA guidelines, patients with the first infection or recurrent episode of mild to moderate disease should be treated with metronidazole 500 mg orally three times per day for 10 days in the absence of contraindications.[8] Vancomycin 125 mg orally four times per day for 10 days is recommended for the treatment of severe CDI and, in combination with intravenous metronidazole, for severe with complicated infection.[8,34] Studies have shown that vancomycin is superior to metronidazole in the setting of severe infections. In one analysis, patients with mild CDI have a cure rate of 90% with metronidazole and 98% with vancomycin. The cure rates for severe disease, however, are much lower with metronidazole at 76% while vancomycin maintained a cure rate at 97%.[35]

One important consideration is a surgical evaluation for patients with complicated CDI. Surgical intervention should be considered in patients with hypotension requiring vasopressors, sepsis, end-organ damage, mental status changes, and white blood cell count greater than 50,000 cells/mm³ or complicated infection with failure to improve on medical therapy after 5 days. However, emergency colectomy for fulminant CDI can be associated with mortality as high as 80%.[36]

Given the concerns regarding the NAP1/BI/027 strain, studies have focused on its potential resistance to standard therapies. However, there is no evidence that the NAP1/BI/027 strain is more resistant to metronidazole than less virulent strains or historic isolates. Importantly, the relatively low fecal concentrations with metronidazole therapy could be clinically relevant and therefore continued surveillance for metronidazole resistance has been recommended.

Despite the effective therapies, recurrence of *C. difficile* continues to pose a significant problem. The

guidelines recommend treating first recurrences with the same antibiotic that was used for the initial therapy. Of note, vancomycin is recommended for repeated treatments due to the risk of neuropathy from continued metronidazole use. The optimal treatment regimen for severe recurrences is vancomycin 125 mg four times daily for 10 days, even if the initial episode had been treated with metronidazole. In the cases where the initial episode was treated with vancomycin, a tapered and pulsed regimen of vancomycin should be used. Unfortunately, there have been no randomized controlled trials studying extended vancomycin use and the recommendations are a result of expert opinion.[9] The efficacy of recurrent treatment is approximately 50% when using either metronidazole or vancomycin for 10 to 14 days.[37,38] A second or later recurrences are typically treated with tapered and pulsed vancomycin regimen.[8] Recently, certain guidelines suggest that if there is a third recurrence after a pulsed vancomycin regimen, fecal microbiota transplant (FMT) should be considered.[9]

Novel approaches to therapy

Given the rise in *C. difficile* infections across the world, new and effective therapies are required. These challenges are being met by many new therapeutic options (Figure 1). In the past 30 years, only two drugs have been approved for treating *C. difficile* infections, vancomycin and fidaxomicin. To date, however, there are 57 open clinical trials investigating treatments for CDI

according to the NIH website clinicaltrials.gov. These trials are testing a range of therapeutics from antibiotics, fecal microbial transplants, passive immunization agents, vaccines, and antibiotic inactivation agents.[39] As the treatment options expand, new hope is given that the morbidity and mortality associated with *C. difficile* infections can be reduced. Figure 1 shows the various sites of actions for new therapeutics that will be described below.

Antibiotic therapy

Aside from metronidazole and vancomycin, multiple antibiotics have been found to have activity against *C. difficile* including rifaximin, nitazoxanide, ramoplanin, teicoplanin, and tigecycline. Tigecycline is a novel analog of minocycline and can broadly target Gram-negative and Gram-positive organisms. Several case reports have suggested tigecycline as a rescue strategy for severe *C. difficile* infection when vancomycin and metronidazole have failed.[9] Preclinical and clinical evidence shows that rifaximin is effective against *C. difficile* but was similar to vancomycin in terms of diarrheal resolution.[40,41] While in vitro studies show that it is effective against most strains of *C. difficile*, several strains did show evidence of resistance.[42] Rifaximin is currently not recommended as a monotherapy but has been suggested for recurrent infections after treating with vancomycin.[43,44] Nitazoxanide is an antiparasitic drug that it possesses activity against *C. difficile*, and has been shown to be as effective as vancomycin and

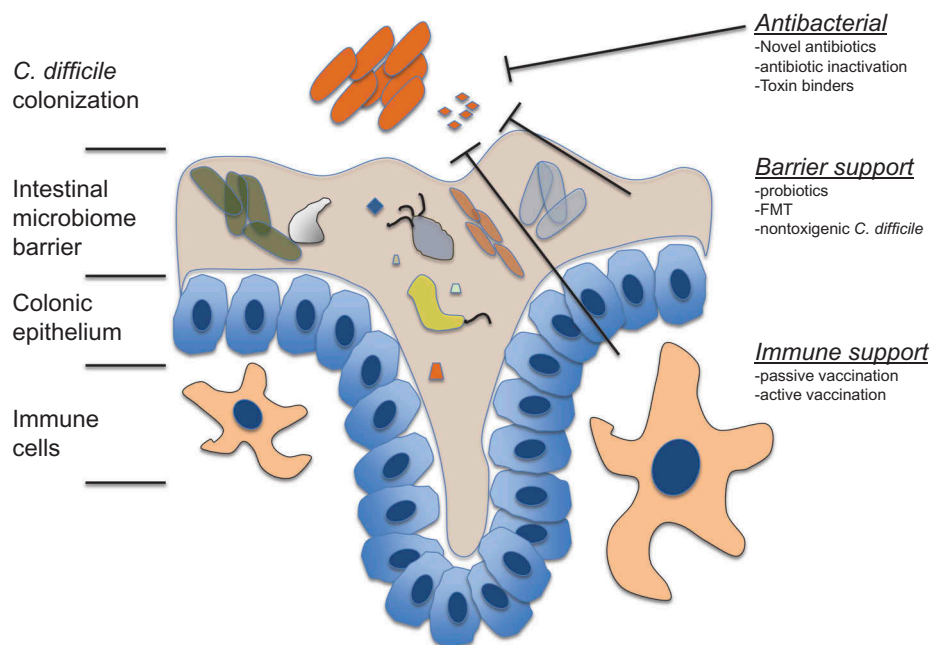


Figure 1. Mechanistic overview of the novel *C. difficile* therapeutic approaches.

metronidazole.[45,46] However, in each of these cases, because of limited data, cost, and adverse-event profile, the routine use of these antibiotics has not been recommended except in rare cases where the standard therapy yields unacceptable side effects or in intractable recurrent infections.[4]

Fidaxomicin

In 2011, fidaxomicin became the second drug to be approved by the Food and Drug Administration (FDA) for the treatment of *C. difficile* infection. Initially discovered in 1975, it is a poorly absorbed, bactericidal, macrolide displaying a narrow spectrum of activity against *C. difficile* and a few other Gram-positive anaerobes. The mechanism of action is through its inhibitory effects on the RNA polymerase of bacteria but recent results indicate additional anti-inflammatory effects in the intestine exposed to *C. difficile* toxin A.[39,47] Importantly, fidaxomicin has little or no systemic absorption after oral administration making it an ideal therapy for gut bacterial infections.[48] Given the narrow spectrum of activity, fidaxomicin is thought to spare certain species of the endogenous microbiota such as *Bacteroides* and *Bifidobacterium* and lower the selection pressure for the rise of harmful bacteria.[49]

Phase 3 clinical trials have shown that fidaxomicin cure rates were comparable with vancomycin rates. The risk of recurrence was markedly improved for the fidaxomicin group at 15% as compared with 25% among those receiving vancomycin.[50] This dramatic reduction in recurrence rates was met with great enthusiasm for this medication. However, further analysis revealed that the subset of patients infected with the BI/NAP1/027 strain (approximately 38% of patients) did not experience any reduction in recurrence. The strain-specific variability needs to be further explored in prospective studies. To date, there are no minimal inhibitory concentration differences between BI/NAP1/027 strains and non-BI/NAP1/027 strains and both vancomycin and fidaxomicin have similar spectra of activity against Gram-positive bacteria. In addition to its strain-specific activity, broad use of fidaxomicin has been hampered by high pricing. A ten-day course of metronidazole, vancomycin and fidaxomicin pills cost approximately \$22, \$680 and \$2800, respectively.[9] The majority of cost-effectiveness studies show that the price of fidaxomicin is not economical even when taking into account the reductions in recurrences of nonhypervirulent strains. It is estimated that fidaxomicin would need to be priced at less than half of its current price to be thought of as a cost-effective

strategy.[51–53] In the setting of complicated or fulminant disease, fidaxomicin does not appear to have a firm role. While its application is for recurrent disease or treatment of initial infections with a high risk of recurrence, further studies are needed to identify the optimal use of fidaxomicin.[54]

Cadazolid and surotomycin

Given the limitations of the currently available antibiotics used for CDI therapy, new antibiotics are being developed to treat this infection. Two antibiotics, cadazolid and surotomycin, are currently under development and may be potential future antibiotics to consider. Cadazolid is a quinolonyl-oxazolidinone inhibitor of protein synthesis that can inhibit DNA synthesis.[55] Studies on cadazolid have shown efficacy both *in vitro* and *in vivo*. It has been shown to be more bactericidal than vancomycin and can strongly inhibit de novo toxin A and B formation. Animal studies in hamsters and mice showed that cadazolid confers protection from diarrhea and death with a similar potency as vancomycin.[55] Recently, a Phase 2 trial was performed to evaluate the efficacy and safety of cadazolid in *C. difficile* patients. Patients treated with cadazolid were found to have cure rates ranging from 68–80% depending on the dose of the drug. Additionally, cadazolid was found to have a lower recurrence rate when compared with vancomycin.[56] Cadazolid is currently being tested in a Phase 3 clinical trial to compare the efficacy of cadazolid with vancomycin with patients with mild-moderate or severe *C. difficile*. Surotomycin, is a cyclic lipopolypeptide that can induce cytoplasmic membrane depolarization.[57,58] It has bactericidal properties and is able to efficiently kill both growing and non-growing *C. difficile* organisms and will undergo Phase 3 trials.[59] The future use of these antibiotics, however, will depend on their performance in these trials. Of particular interest will be their effects on the hypervirulent forms of *C. difficile* and whether they can affect recurrence rates.

Antibiotic inactivation

One of the cornerstones of *C. difficile* management is improved antibiotic stewardship to reduce unnecessary exposure to antibiotics that can predispose patients to developing CDI. A new strategy to address this problem has been to limit the antibiotics-induced alterations in the gut microbiome leading to colonization and expansion of harmful bacteria. Two new medications, SYN004 and DAV132, aim to sequester and neutralize the effects of antibiotics in the gut.[60,61] SYN004 is entering

Phase 1 clinical trials and is an oral formulation of a beta-lactamase that digests intravenously administered beta-lactam antibiotics. DAV132 has completed Phase 1 trials and is an activated charcoal sorbent coated with a pH-dependent enteric polymer that neutralizes antibiotic residues in the colon thereby preventing antibiotics to significantly alter the gut microbiome.[39] While it is still too early to tell if this strategy can impact the rates of CDI, it is certainly a tantalizing approach to curbing some of the more dangerous aspects of broad spectrum antibiotic therapy.

Toxin binders

Since the microbial disturbances are often attributed to antibiotic therapy, nonantibiotic treatments strategies have been investigated for CDI. One strategy involves the use of polymers to bind and thereby inactivate the toxins from *C. difficile*. However, randomized clinical trial data has shown that nonabsorbable anionic polymers, including colestipol and cholestyramine, were not effective in CDI.[54] Studies have also looked at tolevamer, a novel polystyrene binder of *C. difficile* toxins in a Phase 2, multicenter, randomized, double-blinded trial involving patients with mild to moderately severe CDI. The primary endpoint was time to resolution of diarrhea, defined as the two consecutive days with hard or formed stools or less than two loose or watery stools. The results indicated that patients treated with 6 g/day of tolevamer achieved their primary endpoint with an 83% response rate, which was comparable to the 91% response rate achieved by vancomycin.[62] In 2014, a larger multinational trial involving 563 patients treated with tolevamer, 289 receiving metronidazole, and 266 patients treated with vancomycin, indicated that tolevamer was inferior to both metronidazole and vancomycin. However, for the patients that did respond to tolevamer, the recurrence rates over the 30 days were remarkably lower (4.5%) than the recurrence rates for either metronidazole (23.0%) or vancomycin (20.6%). The authors suggest that treatment with this toxin binder might result in lower recurrence rates due to less disruption of the gut microflora. Of note, the authors caution that these conclusions may be due to a selection bias of patients responding to tolevamer being more likely to have milder disease.[63] Despite its clinical inferiority, the increasing rates of *C. difficile* infection highlight the need for multipronged approaches to treat this infection. Although the available clinical evidence does not support toxin-binding agents as a monotherapy for CDI, they could be studied as adjunct therapy following standard antibiotic treatment with the aim of reducing recurrence.

Intestinal microbiome barrier support

Accumulating evidence suggests that the gut microbiome plays a multifaceted role in disease pathogenesis. The barrier function established by the microbiome is thought to be one of the first lines of defense against toxigenic *C. difficile* infections. Disruptions in this barrier by brief exposures to antibiotics can lead to a decline in microbial diversity and predispose a patient to colonization and subsequent infection by *C. difficile*. Eliminating the offending antibiotic agent is an effective method to help restore the balance of the intestinal microbiome but this recovery may take twelve weeks or longer.[4]

Probiotics

Probiotic therapy aims to reestablish a more protective gut microbiome that would prevent the colonization of offensive bacteria, including *C. difficile*. However, the challenge lies in identifying which particular microbial agent could protect against infections. Probiotics are live microorganisms consisting of nonpathogenic yeast and bacteria given to patients with the aim of balancing the microbial contents of the gut altered by infections.[64] An early study from 1989 looked at preventing recurrence of *C. difficile* infection by administering the yeast, *Saccharomyces boulardii*, to 13 patients with recurring *C. difficile* cytotoxin-positive diarrhea. They were treated with 10 days of vancomycin and a 30-day course of *S. boulardii*. Of the thirteen patients, eleven did not have further recurrences of *C. difficile*. [65] Further studies investigating the role of probiotics for treatment of recurrent disease were performed. In one analysis, 124 patients with *C. difficile*-associated disease were randomized to receive four weeks of the nonpathogenic yeast *S. boulardii* or placebo in addition to treatment with standard antibiotics. This study found that administration of *S. boulardii* yeast resulted in a statistically significant relative risk reduction of 0.43 when compared to placebo. The efficacy of *S. boulardii* treatment, however, was only significant in patients with recurrent disease and not in patients with initial infections.[66] Subsequently, a 2006 meta-analysis of probiotics for the treatment of antibiotic-associated diarrhea evaluated twenty-five randomized control trials and found that *S. boulardii*, *Lactobacillus rhamnosus* GG, and probiotic mixtures significantly reduced the development of antibiotic-associated diarrhea. Additionally, this meta-analysis found that only *S. boulardii* was effective for preventing subsequent recurrences of *C. difficile* infection,[67] confirming strong preclinical evidence for a role of this

probiotic in experimental CDI with effects directed against the bacteria, its toxins as well as the host and the host's microbiome.[68,69] More recently, a Cochrane analysis from 2013 looked at a number of randomized controlled trials investigating probiotics for prevention of primary *C. difficile*-associated diarrhea or *C. difficile* infection in patients taking antibiotics. In total, 23 trials with 4213 participants were included in the analysis. This analysis showed that probiotic therapy reduced *C. difficile*-associated diarrhea by 64%, with the incidence of CDAD in the probiotic group at 2% and the incidence in control group at 5.5%.[70] Of note, while there was significant decrease in CDAD, the rates of *C. difficile* colonization did not show a statistically significant effect. The authors of this analysis hypothesized that probiotics may be effective in preventing the symptoms of the infection but may not be able to prevent the colonization of this bacteria.

Fecal microbial therapy

There has been a recent interest in the fecal microbial transplantation as a strategy to re-diversify a patient's microbiome and treat *C. difficile* infections. FMT was first reported in the modern clinical literature in 1958.[71] However, FMT has only recently emerged as a nonantibiotic based therapy for treating recurrent CDI. In 2013, the first randomized clinical trial testing FMT for recurrent infection was interrupted early given the impressive results. In this trial, the researchers compared a high dose vancomycin regimen followed by FMT through a nasoduodenal tube to a full course of vancomycin or a vancomycin regimen with bowel lavage alone. The primary endpoint at 10 weeks was the resolution of diarrhea without relapse. Of 16 patients in the FMT group, a surprising 13 (81%) patients had resolution of diarrhea after the first infusion. The other three patients received a second infusion of a microbial transplant derived from a different donor. Two of these three additional patients had resolution of the infection for an overall success rate of 94%. In the vancomycin group, *C. difficile* infection resolution was seen in 4 of 13 patients (31%) receiving vancomycin alone and in 3 of 13 patients (23%) receiving vancomycin with bowel lavage. Importantly, there were no significant differences in adverse events among the three groups other than mild diarrhea and abdominal cramping in the FMT group on the day of infusion.[72] A systematic review published in 2011 of 27 studies and case reports looked at 317 patients with recurrent *C. difficile* infections treated with FMT found that FMT has an overall success rate of 92%, with 89% of patients responding after a single treatment. In these studies, 35% of patients

received the microbial transplant via enema, 23% by the nasogastric route, and 19% by colonoscopy, with a response rate of 95, 76 and 89%, respectively.[73,74]

The challenges of this treatment option, however, include the procurement, storage and delivery of fecal microbial material. Additionally, many centers have issues with insurance coverage and logistics related to administering this therapy. Typically, an FMT donor is a close family member or trusted friend. Previous studies have shown that that microbiota from unrelated donors can be efficiently engrafted into patients. There is no evidence that genetic similarities between the donor and recipient affects the clinical outcome.[75] However, the definition of a 'normal' FMT donor is yet to be determined and the long-term effects of fecal transplantation remain to be elucidated. Typically, this procedure costs around \$1300, most of which goes towards screening potential donors to prevent the spread of communicable.[26] To date, there is at least one company (Rebiotix) and one nonprofit organization (OpenBiome) providing clinicians with filtered and frozen stool for FMT at a low cost.[39] OpenBiome offers three formulations of microbiota preparations: a lower delivery preparation that is 250 mL fecal microbiota preparation for delivery via a colonoscope or enema, an upper delivery preparation that is a 30 mL fecal solution to be offered via an enteric naso-gastric tube, and lastly a capsule preparation that is available under OpenBiome's compassionate care program for research purposes only. FMT is an exciting new therapeutic option, however, there is very limited randomized controlled clinical trial evidence to support its use. New studies are needed to confirm these claims and currently there are eighteen open clinical trials further assessing its role in *C. difficile* management. As more data is collected, FMT's role in treatment will be clarified.

Nontoxigenic *C. difficile* strategy

Numerous antibiotics have been shown to disrupt the gut microbiome and predispose patients to colonization by *C. difficile*. One hospital based study found that 46% of patients with *C. difficile* were colonized with nontoxigenic strains and this colonization was associated with lower infection rates when compared to noncolonized patients.[76] These results suggest that a less virulent form of the bacteria could occupy the residential niche of the more toxigenic *C. difficile* bacteria and theoretically prevent toxigenic *C. difficile* overgrowth. Preclinical trials in hamsters later showed that colonization with nontoxigenic *C. difficile* could, in fact, prevent the development of infections with the

toxigenic strains.[77] Viropharma Inc, developed a nontoxigenic *C. difficile* strain named VP20621 with the aim of administering it to prevent toxigenic *C. difficile* infections. The company previously completed a randomized-controlled safety trial looking at the colonization effectiveness wherein healthy controls were given single or multiple doses of VP20621 suspension containing 10^4 , 10^6 or 10^8 spores. This initial study showed that the volunteers were successfully colonized with the nontoxigenic form of the bacteria without any adverse side effects.[78] Recently, a Phase 2 randomized, double-blinded, placebo-controlled trial was completed looking at patients with either the first CDI episode or first recurrence. The patients were treated with varying doses of VP20621 and the primary endpoint was assessing safety and tolerability within seven days of treatment. As a secondary endpoint, recurrence rates were assessed through week six. Interestingly, patients treated with VP20621 had a significantly lower rate of recurrence (14 of 125 patients, 11%) versus placebo treated patients (13 of 43 patients, 30%).[79] Colonization by the nontoxigenic strain appeared to eliminate the toxigenic *C. difficile* strain, which the authors hypothesized could reduce the risk of transmission in high-risk environments such as hospitals and long-term care facilities. The authors theorize that this therapy could provide transient protection and therefore enable more time for other delayed onset strategies, such as vaccines, to become effective. However, one of the concerns with the use of nontoxigenic strains is the potential of acquiring toxin genes from toxigenic strains *in vivo*. Laboratory studies have shown that the pathogenicity locus could be transferred and that a functional toxin B could be produced.[80] These *in vitro* transfers highlight the risks of transferring the pathogenicity locus and converting nontoxigenic into toxigenic strains.[79] As with many of these experimental therapies, further studies are needed to assess whether these risks might limit broad implementation of these therapeutics.

Immune support

Passive immunization

Moving from therapies that provide antibiotic support or improve the intestinal barrier, researchers have also focused on enhancing the body's natural defenses against infection. Passive immunization involves delivering antibodies to nonimmune individuals with the aim of boosting the endogenous defenses. This form of immunization via oral or parental administration of

antitoxin antibodies was shown to protect animals from lethal doses of *C. difficile* spores.[81] Medarex, Inc and MassBiologics have developed human monoclonal antibodies against *C. difficile* toxins A and B and tested human subjects previously treated for *C. difficile* infection in a Phase 2, randomized, double-blind, placebo-controlled study. The main outcome was recurrence at 84 days after the administration of the antibodies. Two hundred patients were enrolled and the rate of recurrence was significantly lower in the group treated with the antibody therapy (7 versus 25%). The recurrence rates for the epidemic strain BI/NAP1/027 was also lower in the treatment group compared to control (8 versus 32%).[82] A Merck Phase 3 trial (MODIFY I) looking at human monoclonal antibodies against toxins A and B has been completed with the results pending (NCT01241552).

The mechanism of action for these monoclonal antibodies has yet to be determined. The toxins are secreted into the lumen and exert toxic effects regionally. Circulating antibodies do not typically cross the intact intestinal barrier. Studies from animal models, however, have shown that antibodies are present in the infected lumen compared to healthy intestines. It was hypothesized that the toxin-mediated disruption of the intestine resulted in a porous barrier enabling para-cellular transport of the antibodies into the luminal space and allowing them to block toxin mediated destruction. Indeed, Koon et al showed that monoclonal antibodies against toxin A and B can prevent the molecular activation of important inflammatory signaling cascades such as NF- κ B and MAP-kinase pathways in both human colonocytes and peripheral blood mononuclear cells following *C. difficile* toxins exposure.[83] The mean half-life for the circulating anti-TcdA and anti-TcdB antibodies is 26 and 22 days, respectively. Given this limited time, these antibodies can offer transient boosts of circulating antitoxin IgG and serve as an important adjunct to treating recurrent infections.[82]

Active immunization

While passive immunizations can offer temporary aid and may prevent recurrent disease, research groups and companies are looking toward active vaccines to help stem the spread of *C. difficile* as well reduce the severity of infection and reduce recurrent disease. The first candidate vaccine against *C. difficile* tested in humans is a toxoid-based vaccine developed by Sanofi Pasteur and requiring at least three parenterally administered doses. This vaccine has been tested in six

Phase 1 trials with more than 200 volunteers.[84] The Phase 1 trials concluded that the vaccine was safe and immunogenic with no vaccine-related adverse events reported. However, seroconversion was less common in the elderly, who are the target population of this vaccine, compared with those less than 55 years of age. Currently, a Phase 3 trial (NCT01887912) is recruiting adults aged 50 and older at risk for *C. difficile* infection. The aim of this trial is to assess the efficacy of the vaccine in preventing primary infections in patients having received at least one injection in a three-year post vaccination time frame. The completion date for this trial is expected to be in 2017. In addition to Sanofi Pasteur, both Pfizer and Valneva pharmaceutical companies have also initiated Phase 1 trials testing their vaccines against toxoid A and B.[81] Hopefully injectable antitoxin vaccines could provide more durable protection against *C. difficile*, however, these therapies also come with the caveat that their efficacy will require weeks to months to mount an antibody response especially in the elderly. Vaccine administration regimens of three injections spaced at 0, 7, and 30 days make them impractical for use in a public health setting requiring a rapid response but rather could be more useful in long-term prevention of disease.

In addition to generating vaccines against TcdA and TcdB, research labs are studying additional components of *C. difficile* pathogenicity. Various adherence factors such as S-layer proteins, glycoproteins and flagellar proteins have been studied as potential vaccine candidates. The flagellar proteins are involved in a diverse set of functions such as adherence, toxin production, and biofilm formation. It has been theorized that vaccine generation against the TcdA, TcdB and a flagellar protein may offer a therapeutic advantage and prevent bacterial colonization. Several studies have shown that the flagellar proteins can be immunogenic. Interestingly, during the course of infection, anti-flagellar responses may play a role in preventing colonization. Glycoproteins present on both spores and cells are also interesting vaccine targets. One example is lipoteichoic acid present on most *C. difficile* strains. Animal studies involving vaccines raised against lipoteichoic acid were able to offer partial protection against lethal bacterial challenge.[81,85]

Expert commentary

Metronidazole and vancomycin have been the mainstays of *C. difficile* therapy for over thirty years. For the majority of cases, these reliable antibiotics have been

able to treat and effectively eradicate the bacteria. However, the rise in community-acquired infections has expanded the reach of *C. difficile* beyond the standard set of patients. Additionally, as the population ages, more patients will be exposed to health-care settings such as long-term care facilities and hospitals and by extension to *C. difficile*. Finally, strains such as the hypervirulent BI/NAP1/027 strain have challenged the ability of our therapies to effectively tackle this infection. As consequence, incidence rates are likely to continue increasing along with the associated morbidity and mortality. Of particular concern are the recurrent and resistant to therapy infections where patients have reached the limits of traditional treatment regimens yet continue to exhibit signs of infection. The challenge lies in developing new treatment paradigms to stem this problem.

Broadly characterized, the new strategies outlined above aim to find new antibiotic molecules, inhibit the intestinal effects of *C. difficile* toxins or neutralize these toxins before they reach the colonic epithelium, bolster the intestinal microbiome barrier, and activate the endogenous immune system (Figure 1). While some of these strategies show promising results, their application in clinical practice will need to be further investigated. In particular, studies should focus on tailoring these treatments to specific subpopulations including patients with recurrent disease, high-risk exposures, and those with hypervirulent strains. Cost-effective analyses will also be critical to their adoption into standard of care. As an example, one of the main limitations in implementing fidaxomicin therapy has been the high cost of treatment. Future treatments will need to be superior both in efficacy and overall cost.

An important component of the challenges that we face in treating CDI relates to the major gaps in our knowledge related to the complicated pathophysiology of this disease. Major questions related to CDI pathogenesis remain to be addressed, including the specific role the human microbiome plays in enabling primary infection following antibiotic use as well as following effective therapy of the primary infection. Why some patients develop symptoms following colonization with *C. difficile*, while others remain asymptomatic? What is the role, if any, of the microbial communities in the development of an immune response to *C. difficile* toxins? Clearly, a more complete understanding of the pathophysiology of this infection will enable development of better therapies for this complicated disease.

Five-year view

As more clinical data are accumulated in the next few years, the treatment algorithms will continue to be refined and expanded to include these new therapies. One of the more promising directions in clinical investigation is the intestinal microbiome barrier with the idea of bolstering the gut microbiome resulting in several exciting therapeutic options. Fecal microbial transplants, chief among these, have shown impressive response rates and significantly reduce recurrence rates. Future clinical studies should shed light on the specific contexts in which it may be most effective. It is yet to be determined if this strategy should be implemented as first line therapy or reserved for recurrent, difficult to treat disease. To date, the exact mechanism of action for FMT has not been worked out. Additionally, the current strategy for fecal transplantation is far from standardized and leaves much room for variability and potential caveats for future treatment. It is theorized that FMT is able to recolonize the gut with beneficial bacteria, however, the identity of these microbes is unknown. The future of this field will be in identifying the protective bacteria and engineering specific microbiome cocktails to treat patients. As sequencing technology becomes more efficient and less expensive, it is hoped that the specific beneficial microbial components can be identified and developed into a standardized therapy for enhancing the intestinal microbiome barrier.

In addition to advances in microbial therapy, the data regarding vaccinations will be released in the coming few years. Vaccinations offer an exciting and

potentially game-changing therapy against *C. difficile*. If an effective vaccine can be identified, it would go a long way in prevention and reducing overall morbidity. It is possible that high-risk groups such as the older nosocomial populations, residents of long-term care facilities or patients undergoing immunosuppressive therapy may be the optimal target groups for vaccination. Lastly, new antibiotics such as cadazolid and surotomycin as well as toxin binders like tolevamer offer additional treatment avenues that may serve as either primary or adjuvant therapy. The preclinical trials for the antibiotics and the toxin binders are encouraging; however, detailed clinical studies as well as more treatment experience will be needed to identify the appropriate use of these therapies. The treatment approaches discussed here offer new avenues for treatment and it is the aim of these therapies to reduce the morbidity and mortality of this infection.

Financial & competing interests disclosure

D Padua is supported by the National Institute of Health GI T32 Training Grant (T32 DK07180-40). C Pothoulakis is supported by National Institute of Health grants (R01 DK060729, R01 DK047343, 1P50 DK64539); CURE: DDRC P30 DK 41301; the Blinder Research Foundation for Crohn's Disease; and the Eli and Edythe Broad Chair. C Pothoulakis has received research grants from Biocodex Inc, Cubist Pharmaceuticals and Merck Pharmaceuticals. The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Key issues

- *Clostridium difficile* is one of the leaders of nosocomial infections and the primary cause of infectious colitis in hospitals in the United States.
- In the past two decades, there has been a two- to fourfold increase in the incidence of *C. difficile* infection, particularly in the elderly patient population.
- The rise in *C. difficile* infections is partly due to increased exposure of patients in health-care settings, increased community-acquired infections and new hypervirulent strains such as BI/NAP1/027.
- Metronidazole and vancomycin have been the mainstays of *C. difficile* therapy for over 30 years.
- New therapeutic strategies aim to find new antibiotic molecules, inhibit the intestinal effects of *C. difficile* toxins or neutralize toxins before they reach the colonic epithelium; bolster the intestinal microbiome barrier; and activate the endogenous immune system.
- Fecal microbial transplant is a new therapeutic option currently being investigated by ongoing clinical trials designed to assess its role in *C. difficile* management.
- Antitoxin vaccines could offer a more durable protection against *C. difficile* and provide protection in a select high-risk populations.

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