Management of *Clostridium difficile* Infection: Thinking Inside and Outside the Box

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Treatment of *Clostridium difficile* infection (CDI) has relied on 2 antimicrobial agents, metronidazole and vancomycin, since the recognition of this disease entity. While effective, these “inside the box” approaches to CDI management have the disadvantage of further microbial disruption of the host indigenous microflora. “Outside the box” therapies use non-antimicrobial approaches to management and are theoretically less prone to causing recurrent CDI episodes. Recent advances in understanding of “inside the box” approaches include appreciation of the decreased efficacy of metronidazole overall and the superior efficacy of vancomycin for treatment of severe CDI, as well as a new agent under development, fidaxomicin, which appears to be equal in efficacy to vancomycin but with less risk of subsequent CDI recurrences. Several “outside the box” approaches have also entered clinical development, including use of monoclonal antibodies, active vaccination, luminal toxin binders, and nontoxigenic *C. difficile*. These reports provide optimism that more-effective management of CDI is forthcoming.

Antimicrobials have been the agents of choice for treatment of *Clostridium difficile* infection (CDI) for >30 years, primarily metronidazole and vancomycin. Antimicrobials have been highly successful and are likely to continue to play a major role in the treatment of patients who already have CDI. However, there remain several areas of CDI treatment that are suboptimal—namely, the management of fulminant or complicated severe CDI and management of recurrent CDI.

Patients with fulminant disease frequently experience failure to respond to medical management with antimicrobials and either die or require subtotal colectomy as a life-saving measure. Antimicrobial treatment is thought to be, at least in part, responsible for frequent CDI recurrences, presumably as a result of the unintended effects on the normal gastrointestinal microbiota that leave patients vulnerable to relapse or reinfection. As a result, a number of non-antimicrobial management approaches have been proposed and under development, some of which have entered clinical trials. In addition, new antimicrobial treatments designed to improve response and to avoid damage to the microbiota are also under clinical development.

The prevention and treatment of CDI may include infection control measures, antimicrobial stewardship, restoration of the protective microbiota, and increased immunity to *C. difficile* toxins, in addition to antimicrobial treatment agents (Figure 1). It is our purpose to review the progress in antimicrobial treatment, the “inside the box” approach to CDI management, as well as to review non-antimicrobial “outside the box” strategies for CDI management that are in trials involving humans or that are currently available for treatment.

**ANTIMICROBIAL “INSIDE THE BOX” CDI MANAGEMENT**

Shortly after the infectious cause of pseudomembranous colitis was recognized, oral vancomycin and metronidazole were demonstrated as effective treatments, although vancomycin is the only agent that has received US Food and Drug Administration (FDA) approval for this indication (Table 1). Early prospective, randomized trials concluded that metronidazole was not inferior to vancomycin, with initial cure rates >90% [1, 2]. Recurrent infections, however, occurred at substantial rates for both agents, and avoiding this complication remains a major unmet need in CDI management. Decreased response rates and slower responses for metronidazole have been noted since 2004 [3–7]. A microbiological and clinical observational study
Figure 1. Schematic of current hospital epidemiology and management strategies to prevent and treat *Clostridium difficile* infection (CDI). Current methods being employed are designated by white arrows (items 1–3). Future prevention and treatment strategies are designated by gray arrows (items 4–6).

showed that patients treated with metronidazole were less likely to have resolution of diarrhea and were more likely to have *C. difficile* detected in feces at day 5, compared with those treated with vancomycin [8]. In 2007, Zar et al [9], in a randomized, prospective clinical study, showed that vancomycin was superior to metronidazole for treatment of severe CDI. Other, older agents that have been evaluated as treatment for CDI include bacitracin, teicoplanin, and fusidic acid [10–12]. These drugs, however, either offer no advantage over metronidazole and vancomycin or have been unavailable to clinicians in the United States.

Rifaximin has FDA approval for indications other than CDI but has also been used to treat CDI. Rifaximin was compared with vancomycin in a small study (20 patients) that showed comparable cure rates [13]. Recently, rifaximin has been used as an adjunct agent to treat patients with multiple CDI recurrences. We used a 2-week course of rifaximin (usually at 400 mg twice a day) immediately following the last course of vancomycin treatment (the “rifaximin chaser”) for a group of patients who had a mean of 6 recurrences within 1–2 weeks after stopping treatment for the previous episode [14]. With this approach, there were no additional recurrences in 11 (79%) of 14 patients who had recurrent CDI [15]. Garey et al [16] took a different approach and used rifaximin to treat the symptomatic CDI episode and continued rifaximin for a total of 4 weeks (at 400 mg 3 times a day for 2 weeks, followed by 200 mg 3 times a day for 2 weeks). Five of the 6 rifaximin-treated patients had no additional episodes. One caution in using rifaximin is the increasing recognition of clinical *C. difficile* isolates with high-level resistance (minimum inhibitory concentration [MIC] values, >256 µg/mL), including isolates from 2 of the 3 patients whose “rifaximin chaser” protocol failed [14, 15]. Widespread dissemination of these strains or prior CDI treatment with rifaximin may limit its efficacy. Another rifamycin, rifampin, has been used successfully when coadministered with vancomycin in a small group of patients with recurrent CDI [17]. A more recent randomized study of rifampin coadministered with metronidazole for treatment of a primary episode of CDI demonstrated no benefit from the addition of rifampin [7]. Treatment with metronidazole plus rifampin and treatment with metronidazole alone had similar cure rates (63% vs 65%) and recurrence rates (42% vs 38%). There were more deaths in the group given metronidazole plus rifampin, although only 1 of the 6 deaths was attributed to CDI.

Nitazoxanide, FDA approved for treatment of giardiasis and cryptosporidiosis, interferes with the anaerobic metabolism of some bacteria, as well as protozoa, and has been compared to both metronidazole and vancomycin for the treatment of CDI. Musher et al [18] compared nitazoxanide at 2 durations to metronidazole in a prospective, randomized, double-blinded study involving 142 patients with CDI. Nitazoxanide (at 500 mg twice a day for 10 days or 500 mg twice a day for 7 days) was at least as effective as metronidazole (at 250 mg 4 times a day for 10 days), with similar response rates (90% vs 82%) and recurrence rates (18% vs 23%). Nitazoxanide was also effective in patients who had failed to respond to metronidazole or who had recurrence after successful treatment with metronidazole [19]. In a smaller randomized comparison of nitazoxanide with vancomycin (involving 50 patients), results were similar, but because of the small sample size, noninferiority could not be established [20].

Intravenous tigecycline has been reported as an effective treatment for severe CDI in one small series (4 patients) and one case report [21, 22]. Tigecycline was used as adjunctive treatment for these patients who had failed to respond to metronidazole and vancomycin, or, in one case, tigecycline was
used as primary treatment. Fecal concentrations of tigecycline may be higher than those of metronidazole when given intravenously, and establishment of tigecycline efficacy in well-designed trials would be a welcome advance in treatment of severe CDI.

Among the experimental agents being developed for treatment of CDI, fidaxomicin has the most clinical data. It is a member of a new class of macrocyclic antibiotics that targets bacterial RNA polymerase, shows no cross-resistance with other antibiotics, and is highly active against *Clostridium difficile* and other clostridia. A phase 2 dose-ranging study of fidaxomicin for treatment of CDI showed low plasma concentrations but fecal concentrations that exceeded the MIC<sub>90</sub> by 2000–10,000-fold [23]. Sequential, semiquantitative stool cultures for a cohort of patients treated with fidaxomicin, compared with a control group treated with vancomycin, showed that both drugs decreased *C. difficile* counts from a mean (± standard deviation) of 7.0 ± 2.4 log<sub>10</sub> colony-forming units (cfu)/g at the start of treatment to <10<sup>2</sup> cfu/g in the majority of patients after 10 days of treatment [24]. In contrast, there was no decrease in *Bacteroides* group counts with fidaxomicin treatment, whereas the *Bacteroides* group counts decreased by almost 3 logs in patients treated with vancomycin (Figure 2). These data demonstrate that fidaxomicin is less active against one element of the normal microbiota and may help to explain the lower CDI recurrence rates after fidaxomicin treatment.

Two large, phase 3, randomized, placebo-controlled, double-blinded trials comparing fidaxomicin with vancomycin have been completed, including >1000 patients with CDI from North America and Europe. Interim analysis of the phase 3 studies showed that fidaxomicin is not inferior to vancomycin for the primary end point of response to therapy and has a significantly lower recurrence rate [25]. Other preliminary findings suggest that concomitant antibiotic administration for systemic infection increases the recurrence rate [26] and that infection with the epidemic BI/NAP1/027 strain significantly lowers the initial cure rates regardless of treatment agent [27]. Lower cure rates were also predicted by advanced patient age, low serum albumin level, and elevated white blood cell count or fever [28].

Ramoplanin is a glycolipodepsipeptide that is active against vancomycin-resistant enterococci as well as *C. difficile*. Ramoplanin was shown to inhibit *C. difficile* spore formation in vitro in a chemostat model of the human gut and in vivo in clindamycin-treated hamsters, compared with vancomycin [29]. Preliminary results of a phase 2 clinical study that compared ramoplanin at 2 doses and oral vancomycin showed comparable cure rates for ramoplanin at 200 mg twice a day, ramoplanin at 400 mg twice a day, and vancomycin at 125 mg 4 times a day (79%, 86%, and 86%, respectively), although the study was small (<30 patients per group) [30]. At the end of treatment, ramoplanin-treated patients were less likely to have enterococci recovered from their stools, and those with positive cultures had enterococci counts that were several logs lower than those of the vancomycin-treated patients. Recurrence rates were similar for the 3 treatment groups (22.7%, 20%, and 20%, respectively).

**NON-ANTIMICROBIAL “OUTSIDE THE BOX” CDI MANAGEMENT**

Non-antimicrobial, or “outside the box,” approaches to CDI treatment and prevention can be divided into 3 groups: intraluminal toxin binders or neutralizers, biotherapeutics to restore the protective microbiota, and antibodies (active and passive) to improve CDI immunity (Table 1). The attraction of these approaches is that they all avoid the continued suppression of normal bacterial microbiota that occurs with antimicrobial management.
Intraluminal toxin neutralization. Toxin-binding agents, such as the anion-exchange resins cholestyramine and colestipol, were initially thought to be beneficial in CDI management, but a placebo-controlled clinical trial of colestipol showed no advantage over placebo, and in the hamster model, cholestyramine also proved ineffective [31, 32]. A toxin binder using oligosaccharide sequences to bind toxin A was attached to an inert support system (SYNSORB) and was evaluated in animals, with some success; it was also tried for patients, but the study never reached formal publication of results before being abandoned [33]. Because there was no attempt to bind toxin B, it is not clear how successful this approach would have been. One toxin-binding agent, the high molecular weight (>400 kDa) anionic polymer tolevamer, showed promise in hamster studies and in a phase 2 trial versus vancomycin [34, 35]. Toxin neutralization for a variety of C. difficile isolates, including BI/NAP1/027 epidemic strains, was demonstrated in vitro [36]. Unfortunately, in 2 phase 3 trials, tolevamer was markedly inferior to both metronidazole and vancomycin in treatment response, although it did demonstrate a significantly decreased recurrence rate in patients who responded [37, 38].

It is unclear why tolevamer failed in these studies, but its in vitro affinity for toxin A was much higher than that for toxin B, and an essential role for toxin B in CDI pathogenesis has been reported [36, 39]. In the human gut model of CDI, tolevamer was not effective in neutralizing C. difficile toxins, consistent with the results of the phase 3 clinical trials [40].

Intraluminal toxin binding has also been attempted with antibodies to toxins A and B generated by vaccination of animals, harvesting of antibodies from milk or eggs, and oral administration of antibodies to treat or prevent CDI by neutralization of toxins in the gut lumen [41–49]. Immunization of cows and chickens with toxin A and toxin B proteins has yielded antibodies in milk and eggs that, when administered orally to hamsters, protected against CDI [41, 42, 46]. Bovine-derived immunoglobulin G (IgG) has been found to be degraded by acid conditions and by transit through the human gastrointestinal tract; however, toxin neutralizing activity was detectable in human feces and ileal fluid [44, 45]. Successful human prevention and treatment trials of bovine- and chicken-derived IgG antibodies have not been reported, possibly because of difficulty in delivering an effective neutralizing dose of IgG to the colon.

More recently, whey protein in immunized cow’s milk, which contains high levels of secretory IgA, has been used clinically for CDI recurrence prevention and CDI primary treatment [46, 48, 49]. Results remain inconclusive, because the CDI recurrence prevention trial (2 weeks of daily anti–C. difficile whey protein after standard CDI therapy) resulted in a recurrence rate of 10% (11 of 109 episodes of CDI) but was open label and uncontrolled [49]. A primary treatment trial, which was randomized and double blinded, compared C. difficile immune whey at 200 mL 3 times a day with metronidazole at 400 mg 3 times a day for 14 days. There was initial treatment response in 20 (100%) of 20 patients treated with metronidazole and 16 (89%) of 18 patients treated with C. difficile immune whey. The rate of sustained response at 70 days was 55% with metronidazole and 16 (89%) of 18 patients treated with C. difficile immune whey treatment (6 recurrences). The study was interrupted because of bankruptcy of the sponsor, but C. difficile immune whey was as effective as metronidazole in this small study.[48]

Biotherapeutic agents. Although not likely to be as effective as primary therapy, biotherapeutic agents (live microor-
ganisms) may be beneficial in restoring microbiom protection against CDI following disruption by antimicrobial therapy. Although so-called probiotic approaches to prevention and treatment of CDI have been largely inconclusive or disappointing to date (and are not covered in this review), fecal transplants have been highly effective at preventing additional CDI episodes in patients with multiple recurrences of CDI [47, 50–52]. The success of fecal transplants in preventing additional recurrence of CDI supports the concept that there are microorganisms in feces that can reestablish protection against subsequent CDI. Identification of the specific protective organism(s) has not been successful, but a synthetic mixture of bacteria was successful in reestablishing protection against additional CDI recurrence in humans in one uncontrolled report [53]. A prospective, randomized trial of fecal transplants for recurrent CDI is underway in the Netherlands [47].

It has been known for >25 years that nontoxigenic C. difficile strains occur naturally and, when given to hamsters during or after antibiotic treatment, are able to harmlessly colonize the gut and prevent subsequent infection challenge with toxigenic strains of C. difficile [54–56]. It has also been shown in patients that natural asymptomatic colonization with C. difficile (toxigenic or nontoxigenic strains) is associated with decreased risk of CDI [57]. The use of nontoxigenic C. difficile to prevent primary or recurrent CDI is an attractive strategy because it can be administered orally as spores that pass through the gastric acid barrier readily, and if as effective in humans as in hamsters, will provide protection within 1–2 days. Specific strains of nontoxigenic C. difficile may be more efficient and durable as colonizing organisms; however, the mechanism by which nontoxigenic C. difficile is able to prevent colonization by toxigenic strains has not been elucidated [56]. Human safety trials of nontoxigenic C. difficile were completed in early 2010, and trials involving patients are expected to begin in late 2010.

**Antibody approaches.** Active and passive immunity against C. difficile toxins may be the ultimate protection strategy against CDI. Both passive immunity with monoclonal antibodies and active vaccines are currently undergoing clinical trials involving humans. The use of 2 intravenously administered monoclonal antibodies directed against toxins A and B when added to standard CDI treatment with vancomycin or metronidazole in a phase 2 clinical trial involving 200 patients decreased the CDI recurrence rate in patients who received the monoclonal antibodies to 7%, compared with 25% in the placebo group (P < .001) [58]. In treated patients with >1 prior CDI episode, recurrence rates were decreased to 7%, compared with 38% for the placebo group (P = .006), and in treated patients with the BI/NAP1/027 strain, recurrence was 8%, compared with 32% for the placebo group (P = .06). Hamster studies had confirmed the superiority of 2 monoclonal antibodies directed against both toxin A and toxin B, compared with 1 monoclonal antibody alone, and this was further confirmed in a small trial involving humans that used only the CDA-1 anti–toxin A antibody that resulted in no significant difference in recurrence with the single monoclonal antibody (5 [17%] of 29), compared with placebo (3 [18%] of 17) [59, 60].

The only currently available antibody treatment for CDI is pooled intravenous immunoglobulin (IVIG), for which only retrospective clinical evaluation for treatment of severe and recurrent CDI has been published [61–64]. IVIG was initially reported as effective for immunoglobulin-deficient children with chronic recurrent CDI [65]. IVIG preparations contain neutralizing levels of IgG antibody to toxin A and toxin B [66]. No conclusive evidence of benefit for IVIG has been demonstrated in retrospective analyses of its use for treatment of recurrent CDI, nor has an effective dose been established (range, 125–400 mg/kg in single or repeated doses) [63, 64]. For severe or fulminant CDI, one retrospective study compared 18 patients who received IVIG (dose range, 200–300 mg/kg) with a group of patients with similarly severe CDI who did not receive IVIG and found no difference in mortality, colectomy rate, or length of stay; the conclusion was that the use of IVIG for severe CDI is unsubstantiated [62]. A larger and more recent uncontrolled, retrospective series of 21 patients with severe CDI treated with widely varying doses of IVIG demonstrated survival in only 43% of patients [61]. Thus, to date, there are insufficient data to support use of IVIG for either recurrent or severe CDI [67].

Active rather than passive immunization is an attractive goal for effective and durable protection against CDI. Vaccines have been focused on developing immunity to the C. difficile toxins, on the basis of animal studies and human natural antibody levels that indicate protection against clinical illness correlates with serum IgG antibody to toxins A and B, while having no apparent effect on gastrointestinal colonization [68–70]. This observation is consistent with the high rates of stool colonization with toxigenic C. difficile among patients in the hospital environment who remain asymptomatic [57, 71]. In humans, preliminary trials of a parenteral vaccine containing toxoids A and B have shown that the product is safe and induces vigorous serum antibody responses in healthy adults [72, 73]. Among 3 patients given the vaccine who required continuous vancomycin to manage recurrent CDI, all 3 were able to discontinue vancomycin, and large increases in levels of serum IgG antibodies to toxin A (3–4-fold) and toxin B (20–52-fold) were found in 2 of the patients [74]. A phase 2 trial is currently ongoing to evaluate injectable C. difficile toxoid vaccine for prevention of CDI recurrence. Major questions regarding the immune response to vaccine in elderly populations, the magnitude and duration of vaccine protection, and the selection of an appropriate at-risk population for vaccination remain to be answered.
CONCLUSIONS

Antimicrobials remain the mainstay of CDI treatment, despite their limitations. Future management of CDI (both prevention and treatment) is likely to incorporate a variety of antimicrobial and non-antimicrobial complimentary approaches, depending on the successful demonstration of their effectiveness in randomized clinical trials and the individual needs of patients.

Acknowledgments

Financial support. US Department of Veterans Affairs Research Service (to D.N.G. and S.J.).

Potential conflicts of interest. D.N.G. has served as a consultant for Astellas, Viropharma, Optimter, Cepheid, Merck, Theradoc, Cubist, Acotelon, and Medicines Co.; he holds research grants from Merck, Viropharma, Optimter, Cepheid, Sanofi-Pasteur, and GOJO; and he holds patents for treatment of CDI licensed to Viropharma. S.J. has served as a consultant for Astellas, Bio-K+, Viropharma, and Optimter.

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