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Future Novel Therapeutic Agents for *Clostridium difficile* Infection

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Abstract

Importance of the field—*Clostridium difficile* is the most important definable cause of healthcare acquired diarrhea. The increasing incidence and mortality associated with this enteric pathogen and the significant rate of treatment failures and recurrences with current antibiotics emphasize the need for discovery of new and improved therapeutic and preventative agents.

What the reader will gain—We review upcoming novel therapeutic agents and the clinical evidence to support their efficacy in treating *C. difficile* infection. We also provide an extensive comparison of antimicrobial susceptibilities of *C. difficile* based on *in vitro* susceptibilities published in the literature.

Areas covered in this review—This review was conducted by thorough examination of the public sources, including journals and scientific meeting abstracts, up to February 2009.

Take home message—A number of new therapeutic agents are in development and being tested in clinical trials. However, high costs and concerns for resistance may limit the use of these antimicrobials for the treatment of *C. difficile* infection. Passive and active immunotherapy may have important future roles as therapeutic and preventative strategies for *C. difficile* infection.

Introduction

Clostridium difficile disease (CDI) is one of the most important nosocomial infections and the most common definable cause of antibiotic-associated diarrhea. The incidence of CDI is rapidly increasing in the US, with recent rates tripling from 2000 to 2005 to at least 61 cases per 100,000 US population annually.^{1, 2} Severe *C. difficile* (CD) colitis is more commonly being recognized, with complications including toxic megacolon, colonic perforation, colectomy, and septic shock occurring more frequently.³ Current treatment modalities are suboptimal, with up to 20% of treated patients failing to respond to antibiotics and relapses occurring in up to 25% additional cases after initial clinical resolution. The frequency of refractory and recurrent disease appears to be increasing. Up to 30% of patients who acquire this infection will not survive despite conventional therapy with antibiotics or surgical

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intervention for more severe cases.^{4, 5} Mortality rates continue to rise and increased by 35% each year between 1999 and 2004 in the United States.⁶ Direct attributable mortality has been reported to be as high as 6.9%, with CDI contributing to an additional 7.5% mortality.⁷ Health-related costs for afflicted persons are significant and are in excess of \$4,000 per case.⁸ A new epidemic hypervirulent strain, restriction-endonuclease analysis group BI/ North American PFGE type 1/PCR ribotype 027 (BI/NAP1/027), may be contributing to these increases in morbidity and mortality.⁸

In the past, comparisons between metronidazole and vancomycin demonstrated similar clinical efficacy with treatment response rates above 80%.⁹ Concerns for the promotion of vancomycin resistance, particularly with vancomycin resistant enterococci (VRE) and staphylococci (VISA and VRSA), and the high cost of this medication led experts such as the Centers for Disease Control and Prevention and the Infectious Diseases Society of America (IDSA) to recommend metronidazole as the first-line therapy for CDI.^{10, 11} Unfortunately, metronidazole has been associated with rising rates of treatment failures and CDI recurrence.¹² Increasing metronidazole resistance among CD isolates may be contributing to these clinical failures.¹³ Recently, vancomycin was shown to be more effective than metronidazole for severe CDI.¹⁴ However, significant treatment failures have been reported with vancomycin as well for the treatment of CDI.^{12, 15}

More evidence-based guidance is needed in the management of recurrent CDI. Current recommendations for recurrent CDI therapy, particularly for patients with two or more recurrent episodes, vary according to different experts because clinical evidence is lacking. Treatment recommendations include re-administering after the first recurrence the initially administered antibiotic (metronidazole or vancomycin),¹⁶ tapered or pulsed dosing of vancomycin,¹⁷ rifaximin “chasers,”¹⁸ probiotics including fecal transplantation,¹⁹ and passive immunotherapy.²⁰ Many of these treatments are eventually offered to patients with persistently recurrent CDI, in a desperate attempt to break the cycle of debilitating recurrence.

Fulminant or severe CDI is another important aspect of CDI for which the optimal treatment strategy is not well-established. Current IDSA recommendations include administration of vancomycin 125 mg orally or via nasogastric tube every 6 hours and metronidazole 500 mg intravenously every 6 hours. Vancomycin by rectal enema every 4–12 hours should be considered and surgical, infectious disease, and gastroenterology services consulted.¹¹ However, it is unclear how effective these antibiotic regimens are for severe CDI. Colonic vancomycin concentrations may be inadequate with impaired gastrointestinal motility in critically ill CDI patients or those suffering complications of ileus or toxic megacolon. Intracolonic delivery of vancomycin by rectal enema may not be sufficient when the transverse or ascending colon is involved.²¹

These important deficiencies in our current understanding regarding the optimal management of CDI, in addition to fears of emerging antibiotic resistance, the increasing rate of clinical failure and recurrence with metronidazole and vancomycin for CDI, and rising CDI mortality rates, underscore the pressing need for new and improved therapeutic options. We review upcoming novel therapeutic agents for CDI and the clinical evidence supporting their use as treatment for CDI, and discuss the use of passive immunotherapy for CDI. We also provide an extensive comparison of *C. difficile* *in vitro* susceptibilities to these antimicrobial agents after a review of the literature (Table 1).

Fidaxomicin (FDX; OPT-80)

Fidaxomicin is a poorly absorbed bactericidal macrocycle with specific activity against anaerobic gram-positive bacteria including *Clostridium* sp., such as *C. difficile* and *C.*

perfringens. This drug is also active against staphylococci and enterococci, but has poor activity against anaerobic, facultative, or aerobic gram-negative bacilli or streptococci.^{22, 23} *In vitro* studies have demonstrated potent fidaxomicin activity against *C. difficile*, with an MIC₉₀ ranging from 0.0078 to 0.5 µg/mL²⁴ (Table 1). The antimicrobial's narrow spectrum of activity is believed to be advantageous, as it may minimize alteration of the native intestinal microbiome. Successful maintenance of the normal microflora, such as *Bacteroides* sp., has been shown with 10 days of fidaxomicin (400 mg per day), while *Bacteroides* sp. counts significantly decreased by ~ 3 log-fold with 10 days of oral vancomycin compared to baseline counts ($p=0.03$). Normal intestinal microflora may serve an important protective role, providing "colonization resistance" and preventing CDI recurrences.²⁵

With approximately 93% of the fidaxomicin metabolite, OPT-1118, unabsorbed from the gastrointestinal tract,²³ this medication appears to be well-tolerated and safe. Although fidaxomicin inhibits bacterial RNA polymerase-mediated transcription similar to rifaximin,²² the development of resistance or cross-resistance to other antimicrobials such as the rifamycins is felt to be unlikely.^{26, 27} Serial passage and spontaneous resistance frequency experiments have demonstrated a low propensity for CD to develop resistance. Fidaxomicin appears to have a different mechanism of action from the rifamycins because this drug was shown to be synergistic with rifampin against CD.²⁷

In a phase II open-label, randomized, dose-ranging clinical trial, fidaxomicin was evaluated for treatment of primary or first relapse episodes of mild-moderate CDI (≥ 3 unformed stools/day or ≥ 6 unformed stools within 36 hours with a positive CD toxin assay)²⁸ (Table 2). Severe disease with >12 diarrheal stools/day, ileus, severe abdominal tenderness, white blood cell count $> 30 \times 10^9$ /liter, toxic megacolon, or evidence of life-threatening CDI; patients unlikely to survive the study period; and individuals who had > 1 CDI recurrence were excluded. Subjects were randomized to receive 10 days of 50 mg ($n=14$), 100 mg ($n=15$), or 200 mg ($n=16$) of fidaxomicin twice daily and were followed for 6 weeks to monitor for relapse or recurrence. Clinical cure was defined as the passage of ≤ 2 semi-formed or formed stools per day during the 10 day treatment period without the receipt of any additional CDI medication. Time to CDI resolution was defined as the time from the first medication dose to the first day of sustained improvement during the 10-day treatment period (only 1–2 formed stools passed within a 24-hour period). Clinical cure rates were 71%, 80%, and 94% among the three treatment groups, corresponding with increasing dosages ($p=NS$). Overall, two (5%) patients suffered recurrent CDI. One recurrent CDI patient had received 100 mg per day, and another had received 400 mg per day of fidaxomicin. The median times to clinical CDI resolution were 5.5 days, 3.5 days, and 3.0 days for the 100 mg, 200 mg, and 400 mg per day dosages ($p=NS$). The study medication was well-tolerated with no reported adverse events attributed to fidaxomicin. This lack of adverse events was consistent with low systemic drug absorption. The highest detectable plasma drug concentration was 84.9 ng/ml after 10 days of the highest dose of study medication. Minimal systemic absorption allowed the drug to achieve high fecal concentrations after 10 days, with mean fecal concentrations ($\pm$ standard deviation) reaching 256 ± 136 µg/g and $1,433 \pm 975$ µg/g in the 100 mg per day and 400 mg per day treatment groups, respectively. Fecal fidaxomicin concentrations after 10 days of 400 mg per day of medication were greater than 10,000-fold the MIC₉₀ (0.125 µg/ml) for *C. difficile*. Investigators concluded from this dose-ranging clinical trial that the higher fidaxomicin dose of 400mg per day may be most effective for providing clinical cure and the most rapid time to symptomatic resolution.

C. difficile isolates from the phase II clinical trial were characterized by restriction endonuclease analysis (REA).²⁹ 42% of CD isolates were identified as the epidemic REA BI

type, with a similar distribution among the three different fidaxomicin dosing groups. No difference in antibiotic susceptibility to fidaxomicin was noted for BI and non-BI strains ($MIC_{90} = 0.125 \mu\text{g/mL}$ for both strain types). However, the MIC_{90} was two dilutions higher for BI isolates for both metronidazole and vancomycin ($2 \mu\text{g/mL}$ for both antibiotics) compared to non-BI isolates ($0.5 \mu\text{g/mL}$ for both antibiotics). This decrease in antibiotic susceptibility was significantly different only for metronidazole ($p=0.01$). The overall clinical cure rate was lower for fidaxomicin subjects infected with BI strains (79%) compared to those infected with non-BI strains (95%), although this difference was not statistically significant. The study's small sample size may have prevented the detection of a significant difference in treatment response for subjects infected with different CD types. Eradication of CD counts from patients' stools after 10 days of the different fidaxomicin doses was compared to stools from 8 CDI patients receiving vancomycin.²⁵ Similar reduction in CD counts were shown among the different FDX doses and with vancomycin.

A prospective, randomized, multicenter phase III clinical trial in North America comparing fidaxomicin to oral vancomycin for the treatment of CDI was recently completed^{30, 31} (Table 2). Subjects were randomized to either 10 days of fidaxomicin 200 mg twice daily ($n=265$) or vancomycin 125 mg four times daily ($n=283$). Cure rates were similar for fidaxomicin (92%) and vancomycin (90%) ($p=0.90$). However, there were significantly less recurrences with fidaxomicin (13%) than vancomycin (24%) ($p=0.004$). Interestingly, CD isolates were typed in this study, and it was noted that patients infected with epidemic BI strains had lower treatment response rates, irrespective of which antibiotic they received, compared to patients infected with non-epidemic CD isolates. Rates of recurrence with the BI strains were similar to recurrence with non-BI isolates.

Nitazoxanide

Nitazoxanide is a thiazolide compound with broad antimicrobial properties, with activity against enteric parasites, bacteria, and viruses. It is currently approved by the FDA for the treatment of diarrhea caused by *Giardia lamblia* and *Cryptosporidium parvum* in children.^{32–34} Nitazoxanide and its metabolite, tizoxanide, are active against *C. difficile* in *in vitro* studies (Table 1). The antimicrobial activity of this drug is related to the inhibition of anaerobic metabolism.³⁵ Approximately 66% of tizoxanide is nonabsorbed and is excreted in stool.³⁶

Two prospective, randomized, double-blind clinical trials have demonstrated similar clinical efficacy with nitazoxanide for CDI compared to metronidazole³⁵ and vancomycin³⁷ (Table 2). Clinically unstable patients requiring care in the intensive care unit were excluded in these studies. No serious adverse events were attributed to nitazoxanide in either trial. Nitazoxanide 500 mg twice daily for 7 days ($n=40$) and 10 days ($n=36$) were compared to metronidazole 250 mg four times daily for 10 days ($n=34$) for CDI. No significant difference in the primary endpoint, clinical resolution after 7 days of treatment, (95% CI, -7.0 to 25.5) was demonstrated between the two antibiotics. However, there was a trend for a better response rate for 7 days (90.0%) and 10 days (88.9%) of nitazoxanide compared to metronidazole (82.4%). Subjects were followed for 31 days after enrollment and evaluated for CDI recurrence. A greater proportion of 7-day (65.8%) and 10-day (74.3%) nitazoxanide subjects achieved a sustained clinical response compared to metronidazole subjects (57.6%), although the difference was not significant ($p=0.34$). No significant difference in the median time to symptomatic resolution for the two antibiotics was observed ($p=0.2$). The majority of CD isolates (85.7%) identified in this study were non-epidemic strains.³⁵

Ten days of nitazoxanide 500 mg twice daily ($n=22$) was also compared to vancomycin 125 mg four times daily ($n=27$) for the treatment of CDI.³⁷ Unfortunately, too few patients were

enrolled to demonstrate noninferiority of nitazoxanide compared to vancomycin as CDI therapy. The study was terminated early due to slow recruitment. However, results were promising, with similar response rates for nitazoxanide (77%) compared to vancomycin (74%) (95% CI, -18% to 30%). The time to clinical resolution and the proportion of patients who remained relapse-free over the 31-day follow-up period (nitazoxanide, 73%, vs. vancomycin, 67%; 95% CI, -22% to 32%) were also similar for the two antibiotics.

The higher cost of nitazoxanide will likely prohibit use of this medication as first-line therapy for CDI (Table 3), unless future clinical trials demonstrate clinical efficacy superior to metronidazole and vancomycin. A potential role for nitazoxanide may be as salvage therapy for refractory or recurrent CDI.³⁸ Nitazoxanide was evaluated as salvage therapy for CDI in a small, open-label, uncontrolled clinical trial.³⁹ CDI patients who failed metronidazole treatment, with persistent symptoms or ≥ 2 more relapses despite therapy were enrolled. Nineteen of 35 (54%) patients with refractory or recurrent CDI responded to 10 days of nitazoxanide, with no relapses over 60 days of follow-up. Four additional patients eventually experienced clinical resolution with repeated nitazoxanide treatment. Treatment failure in this cohort did not appear to be related to antibiotic resistance or infection with the epidemic BI/NAP-1 strain, which was identified in only a minority of cases (15%). Further studies are needed to evaluate the clinical efficacy of nitazoxanide, its effect on the intestinal microbiome, and the risk of development of bacterial resistance to this drug.

Rifaximin

Rifaximin is a minimally absorbed, bactericidal rifamycin derivative, currently approved in the US for the treatment of travelers' diarrhea secondary to noninvasive *Escherichia coli*.⁴⁰ Rifaximin inhibits bacterial transcription and protein synthesis by binding to RpoB, the beta-subunit of the bacterial RNA polymerase.⁴¹ Less than 0.4% of orally administered rifaximin is absorbed systemically,^{40,42} leading to high fecal concentrations of approximately 8000 $\mu\text{g/g}$ and an excellent safety profile and few drug interactions.⁴⁰ These properties make rifaximin an ideal antimicrobial for gastrointestinal infections. Although rifaximin is similar to fidaxomicin with its mechanism of action and low systemic absorption, rifaximin is bactericidal against a broader spectrum of enteric pathogens, including gram-positive, gram-negative, aerobic and anaerobic bacteria. However, rifaximin also appears to produce minimal alterations in the intestinal microflora. After 2 weeks of rifaximin (600 mg/day), only a 1 log reduction in intestinal coliforms per gram of stool was noted, which may be advantageous in CDI treatment.⁴³

Limited clinical data exists supporting the use of rifaximin for CDI. One small, randomized, open-label trial compared 10 days of rifaximin 200 mg three times daily ($n=10$) to vancomycin 500 mg twice daily ($n=10$)⁴⁴ (Table 2). Clinical response rates were similar with 9 of 10 rifaximin patients and 10 of 10 vancomycin patients successfully treated. There was no significant difference in the time to clinical resolution between rifaximin (4.9 ± 2.38 days) versus vancomycin (3.8 ± 1.48 days). CD toxin production was detected for a shorter duration with vancomycin (4.8 ± 1.48 d) than rifaximin (8.1 ± 1.79 d) ($p < 0.005$). However, the clinical significance of this finding is unclear because persistent toxin production does not appear to correlate with clinical response, and repeat toxin testing is not recommended once CDI has been diagnosed.^{11, 45}

Rifaximin has been used for both the prevention and treatment of recurrent CDI in two small case series. Johnson et al. reported administering a rifaximin "chaser" to 8 patients with unremitting recurrent CDI.¹⁸ Each patient had previously suffered 4–8 CDI episodes and had received 79–372 days of antibiotic therapy for CDI. Rifaximin 400–800 mg was given daily for 2 weeks immediately following successful treatment with vancomycin, in hopes of

interrupting the cycle of recurrence. Seven of 8 patients experienced no further CDI episodes with follow-up ranging from 51–431 days. One patient suffered diarrhea afterwards, which was treated with a second course of rifaximin and subsequently had no further recurrences during 9 months of follow-up. A CD isolate was cultured from stool prior to the 1st rifaximin course for this patient and was shown to have an MIC of 0.0078 µg/mL, but a second isolate identified after the 2nd course of antibiotics had an MIC >256 µg/mL.

We reported a second case series describing rifaximin use for the treatment of 6 patients with recurrent CDI.⁴⁶ These patients had fewer recurrences (1–4 episodes) and received less antibiotics (7–43 days) prior to rifaximin initiation compared to the previous study. Two patients had received nitazoxanide but subsequently developed a recurrent CDI episode. All six patients were treated with rifaximin while they were symptomatic with CDI. Five patients were given a tapered regimen of rifaximin 400 mg three times daily for 14 days followed by 200 mg three times daily for 14 days. All 5 patients achieved complete CDI resolution, with an average ($\pm$ standard deviation) time to resolution of 8 ± 5 days, with no recurrences during follow-up of 54 – 398 days. One patient suffered persistent diarrhea despite 36 days of treatment with rifaximin 400 mg three times daily. This patient had received 6 days rifaximin in combination with conventional metronidazole therapy for a previous CDI episode. The patient continued to pass CD toxin-positive diarrhea, despite 4 separate fecal cultures negative for CD, until expiring from cardiorespiratory failure secondary to an unrelated comorbidity.

Despite rifaximin's excellent *in vitro* activity against *C. difficile* (the second lowest weighted mean MIC₉₀ in Table 1), resistance to rifaximin should be monitored and may limit this antibiotic's future utility in treating CDI. Significant *C. difficile* resistance to rifampin, another rifamycin, has been demonstrated with *in vitro* susceptibility testing, particularly among epidemic BI/NAP1 CD strains.^{47–49} In one institution, approximately 37% of CD isolates were found to be resistant to rifampin; 97% of these resistant CD isolates were typed as the BI/NAP1 epidemic clone.⁴⁸ Mutations in the *rpoB* gene, which encodes for the β -subunit of *C. difficile* RNA polymerase, the target of rifamycins, may confer resistance to both rifampin and rifaximin. Rifampin episolometer test (E-test) strips have been used as a surrogate assay for rifaximin susceptibility because rifaximin E-test strips are currently not available. Agar dilution testing for *C. difficile*, currently considered as the most accurate method for antibiotic susceptibility, is labor-intensive, and most laboratories are not capable of conducting this method of testing. In one study, rifampin MICs by E-test were consistent with rifaximin MICs by agar dilution for 14 resistant CD isolates identified.⁴⁷ However, we have shown in another study that CD susceptibility to rifampin may not correlate with susceptibility to rifaximin. Among 12 CD isolates resistant to rifampin by E-test, only 2 of these isolates were resistant to rifaximin by agar dilution.⁵⁰ The concordance for antibiotic resistance between these two drugs needs to be further evaluated. Studies are also needed to clarify whether these *in vitro* susceptibility tests and genetic mutations are clinically meaningful, given the very high rifaximin concentrations achieved in the gastrointestinal tract.

Ramoplanin

Ramoplanin is a natural glycopeptide which inhibits the bacterial transglycosylase in peptidoglycan synthesis. This drug is bactericidal for gram-positive bacteria such as enterococci and anaerobes such as CD. This drug is not appropriate for parenteral administration due to instability in the blood stream and poor tolerability, but has ideal properties as a nonabsorbable oral antibiotic including high fecal concentrations.⁵¹ Ramoplanin has excellent *in vitro* activity against *C. difficile* with a weighted mean MIC₉₀

of 0.399 (Table 1). Ramoplanin was compared to vancomycin in both a hamster and *in vitro* gut model.⁵² The two antibiotics produced similarly rapid symptomatic resolution (by day 4) in ill hamsters suffering clindamycin-induced CDI. Both antibiotics caused a rapid decline in CD toxin production in the *in vitro* gut model. However, in both models, there was greater eradication of CD spores and suppression of spore formation with ramoplanin than vancomycin. The authors suggest that greater spore reduction may lead to decreased CD relapse rates with ramoplanin.

Although this cell-wall inhibiting antibiotic is similar to the glycopeptide, vancomycin, cross-resistance between the two antimicrobials is unlikely because the target sites for ramoplanin and the glycopeptides differ.^{51, 53} Ramoplanin has been shown to retain excellent activity against CD isolates (MIC₉₀ = 0.25 µg/mL) with reduced susceptibility to vancomycin (MIC 8 µg/mL) and resistance to metronidazole (MIC 64 µg/mL).⁵⁴ Ramoplanin also has excellent activity against vancomycin-resistant enterococci (VRE), and phase III clinical trials are being conducted using ramoplanin to decontaminate VRE colonization of the gastrointestinal tract.^{55, 56} The drug is also currently in Phase III clinical trials as a therapeutic agent for CDI.⁵¹

Tigecycline

Tigecycline is a glycylcycline antibiotic, a derivative of minocycline, with activity against gram-positive and gram-negative facultative and obligate anaerobes. This antimicrobial has been approved in the US for the treatment of complicated skin and soft-tissue infections, complicated intra-abdominal infections, and recently for community-acquired pneumonia.⁵⁷ Tigecycline appears active against CD with low MIC₉₀ values, ranging from 0.06 to 0.25^{49, 58} (Table 1). Fecal concentrations of this antibiotic in formed stool are much higher than the MIC₉₀, with studies reporting a median of 5.6 µg/mL (range 3.0–14.1 µg/mL), and may be even higher with colitis, as seen with metronidazole.^{59, 60}

Tigecycline has been reported to be effective for severe, refractory CDI in a small case series of 4 patients.⁶¹ These patients were all severely ill with CDI and suffered complications of hypovolemic shock. One patient developed an ileus, while the other three patients had copious diarrhea (>8 diarrheal stools per day). Colonic inflammation was evident in 3 patients by the passage of hemorrhagic stools (n=2) or the detection of pseudomembranes (n=2). Three of these severe CDI cases were refractory, despite prolonged courses of oral vancomycin and intravenous metronidazole. One severe CDI patient received tigecycline as the primary treatment for the CDI. All four patients experienced clinical resolution within 7 days after the initiation of tigecycline. No relapses were identified within 3 months of follow-up.

Antibiotic therapy for severe CDI in critically ill patients with impaired gastrointestinal motility or with complications of ileus or toxic megacolon is currently inadequate. In these severe CDI patients, the combination of intravenous metronidazole and oral vancomycin is recommended because there is currently no alternative effective agent. Tigecycline is administered intravenously like metronidazole, but achieves higher fecal concentrations than metronidazole and exhibits greater *in vitro* activity against *C. difficile*.^{59, 60} (Table 1). Well-designed clinical trials are needed to evaluate whether tigecycline may be more effective than either of these antibiotics in critically ill patients with severe CDI.

Rifalazil

Rifalazil is another poorly absorbed rifamycin-derivative with a broad spectrum of activity against gram-positive and gram-negative bacteria and mycobacteria. As a rifamycin, rifalazil is unique with its long half-life (t_{1/2} = 100 hours),⁶² high intracellular concentrations, and

absence of cytochrome P450 interactions. *In vitro* susceptibility testing has shown this drug to be the most active antibiotic against CD, with MIC₉₀ reported to be as low as 0.004 µg/mL⁶³ (Table 1). In a clindamycin-induced hamster model of CDI, oral administration of rifalazil 20 mg/kg daily was compared to vancomycin 50 mg/kg daily both as a preventative and as a therapeutic agent. Both antibiotics were effective in preventing and treating CDI during their 5-day administration. However, once vancomycin was discontinued, all hamsters in the prophylaxis group and 65% of hamsters in the treatment group died, while none of the hamsters in either the rifalazil prophylaxis ($p<0.05$) or the treatment group ($p<0.001$) died for up to 34 days post-infection with CD. This study suggested that rifalazil, with its long half-life, may be more effective in preventing CDI relapse than vancomycin, although clinical studies are needed to confirm similar effects in human subjects.⁶³

Future human clinical trials with rifalazil for CDI will need to proceed with caution. The development of mild-moderate adverse events in human clinical studies with mycobacterial disease, although reversible, prevented further clinical trials for this infection. The most common reported adverse events included headache, myalgia, back pain, dizziness, and fever.⁶⁴ Reports of resistance may also prohibit widespread use of rifalazil for CDI.⁴⁹ The RNA polymerase binding site is shared among rifamycin derivatives, and *rpoB* mutations may lead to resistance among the entire rifamycin class.⁶⁵

Probiotics

Probiotics are broadly defined as live microorganisms which are beneficial to the host.⁶⁶ The popularity and widespread use of probiotics by the general public is remarkable given the limited amount of scientific evidence supporting their clinical efficacy, particularly for the treatment and prophylaxis of CDI. A number of protective mechanisms have been proposed for probiotics as therapeutic and preventative agents for CDI including restoration of “colonization resistance” impaired by antibiotic effects on the normal host microbiome⁶⁷; prevention of disruption of the integrity of the colonic epithelium which might lead to secondary bacterial invasion⁶⁸; inhibition of CD or CD toxin adhesion to the host colonic epithelia⁶⁹; and promotion of the host immune response including the upregulation of anti-toxin antibody production.⁷⁰ Unfortunately, there are many challenges with the evaluation of probiotic clinical efficacy and their potential utility as therapeutic agents. Lack of stringent regulation of the manufacture and labeling of probiotics has been shown to result in significant inaccuracies in the reporting of probiotic contents, including probiotic concentrations, viability, and identification of the actual probiotic strains contained.^{71–73} The optimal dose of probiotics for CDI is unknown, with possibly different probiotic concentrations necessary for different probiotic strains. In addition, although adverse events are relatively uncommon with probiotic use, *S. boulardii* fungemia^{74, 75} and lactobacillus bacteremia^{76, 77} and hepatic abscess have been reported.⁷⁸

Only one well-designed prospective study has demonstrated as a primary outcome that probiotics are clinically effective for CDI. McFarland et al. conducted a randomized, double-blind, placebo-controlled trial with *Saccharomyces boulardii* in combination with conventional antibiotics (metronidazole, vancomycin, or both) for patients suffering primary or recurrent CDI.⁷⁹ Subjects received either 3×10^{10} colony-forming units (cfu) of *S. boulardii* (n=67) or placebo (n=57) daily for 4 weeks, while antibiotics were administered according to the treating physician’s discretion. Significantly fewer patients receiving *S. boulardii* experienced CDI recurrence (26%) compared to placebo (45%; $p=0.05$). However, a subanalysis revealed this protective effect was only observed in patients with a history of previous CDI. No significant difference in CDI recurrence was noted in patients enrolled with a primary CDI episode who were treated with adjunctive probiotics compared to placebo. Study limitations include the lack of distinction between CD colonization versus

infection in this study, since a positive CD culture alone without a toxin assay was sufficient to be considered as CDI, and the lack of random assignment of CD antibiotics.⁸⁰ However, the empiric use of antibiotics, regularly prescribed to patients suspected of CDI by physicians, makes randomization of anti-CD antibiotic therapy difficult to achieve.

S. boulardii was also examined as adjunctive treatment for recurrent CDI by the same research group in another randomized, double-blind, placebo-controlled trial.⁸¹ Subjects received either 1 g of *S. boulardii* daily (n=90) or placebo (n=78) for 4 weeks in addition to antibiotics chosen by their physician. This trial failed to demonstrate a significant decrease in CDI recurrence with *S. boulardii* compared to placebo. However, in a subanalysis, among patients receiving high dose vancomycin (2 grams/day), those given the probiotic (n=18) had a lower CDI recurrence rate than subjects receiving placebo (n=14) (17% versus 50%, respectively; $p=0.05$). No beneficial probiotic effects were observed for patients receiving low-dose vancomycin (500 mg/day) or metronidazole 1 g/day). No explanation was given by the study authors for the initiation of the study drug on day 7 of antibiotic therapy.⁸⁰

Other well-designed CDI trials failed to show a significant decrease in CDI recurrence with *Lactobacillus plantarum* (n=20)⁸² or *Lactobacillus rhamnosus* GG (n=15)⁸³ as adjunctive therapy, but were significantly under-powered to detect any difference compared to placebo. One randomized, double-blind, placebo-controlled primary prophylaxis trial demonstrated no significant difference in the rate of CDI when patients were given either 2×10^{10} cfu of *L. acidophilus* and *Bifidobacterium* for 20 days (n=69) or placebo (n=69) within 72 hours of antibiotic initiation (3% vs. 7%, respectively, $p=0.4$). The authors acknowledge that this study was also underpowered, leading to difficulty in interpreting the results.⁸⁴ A number of clinical trials using different probiotic strains have evaluated primary prevention of CDI as a secondary outcome with mixed results. Some studies have shown a significant reduction in CDI rates,^{85, 86} while others have reported no difference in CDI rates compared to placebo.^{87, 88} Well-designed clinical trials with sufficient power are needed to further investigate the potential benefits of probiotics for CDI, especially given the current widespread, indiscriminate use of probiotics for many patients receiving antibiotics.

Passive Immunotherapy

The host humoral immune response is an important determinant of susceptibility to CDI. Kyne et al. demonstrated that patients colonized with CD, who produced low levels of serum anti-toxin A immunoglobulins (IgGs), were at much greater risk of developing diarrhea (OR = 48, 95% CI, 3.4 – 678) than colonized patients with more robust antibody responses.⁸⁹ Serum antibodies to toxin A also appear to be important for prevention of recurrent CDI, with significantly greater risk of recurrent diarrhea associated with lower serum anti-toxin A IgG concentrations (OR 48, 95% CI 3.5 – 663).⁷⁰

Medarex, Inc. and Massachusetts Biological Laboratories have developed human monoclonal antibodies (IgG1κ) to CD toxins A and B. Intravenous infusion of monoclonal antibodies to toxin A has been shown to be well-tolerated, with no serious adverse events, in an open-label dose escalation study (n=30). Potentially related mild-moderate adverse events included transient blood pressure changes, headache, nausea, diarrhea, abdominal discomfort, and nasal congestion. The median terminal elimination half-life ($t_{1/2}$) ranged from 22.9 to 30.3 days. No human anti- monoclonal antibodies were detected.⁹⁰

A phase II randomized, double-blind, placebo-controlled trial evaluated human monoclonal antibodies against CD toxin A for the prevention of CDI recurrence.⁹¹ This multi-center trial enrolled CDI patients receiving standard care with metronidazole or vancomycin. Subjects received either a single infusion of 10 mg/kg of monoclonal antibody (n=29) or placebo (n=17). Patients were subsequently followed for 56 days after administration of the study

medication. There was no significant difference in the CDI recurrence rate between the antibody (17.2%) vs. the placebo group (17.7%) ($p=1.0$). When individuals with CDI recurrence were compared with those with only a single CDI episode, lower concentrations of both neutralizing anti-toxin A and anti-toxin B antibodies and infection with epidemic REA BI type CD strains were significantly associated with recurrent disease. This trial was subsequently terminated early, and a new clinical trial evaluating the combination of anti-toxin A and B monoclonal antibodies for CDI was initiated. No serious adverse events were reported with the study antibody infusion.

The second phase II randomized, double-blind, placebo-controlled trial compared intravenous administration of combined human monoclonal antibodies against CD toxins A and B ($n=101$) versus placebo ($n=99$) for secondary prevention of CDI.²⁰ The investigators enrolled CDI patients who were being treated with metronidazole or vancomycin. The recurrence rate was significantly lower for the antibody group (7%) compared to the placebo group (25%) ($p<0.001$). When subjects were stratified by history of >1 previous CDI episode, treatment of the initial CDI episode with metronidazole, or outpatient status, the recurrence rates remained significantly lower for the antibody group than the placebo group. There was a trend for less frequent recurrence in the treatment group versus the placebo group, when patients were treated with vancomycin for the initial CDI episode ($p=0.08$) or infected with the epidemic BI/NAP1/027 strains ($p=0.06$). It should be noted that all patients in the antibody group who experienced recurrence were inpatients, who in general were older and had more severe underlying illness than outpatients at enrollment. Intravenous immunoglobulins had little impact as an adjunctive therapeutic agent for the initial CDI episode, for which patients were receiving antibiotics at enrollment. No significant difference in the severity of the CDI episode, the duration of illness, length of hospitalization, or in the number of treatment failures between the two groups was noted. The immunoglobulin infusion was well-tolerated, with adverse events reported with either similar or less frequency than the placebo. The human monoclonal antibodies were not immunogenic and did not lead to production of human anti-human antibodies.

Despite the 72% relative risk reduction in CDI recurrence attributed to the monoclonal antibodies, the anticipated high cost of these immunoglobulins will likely prohibit their use as a routine preventative measure. It was hoped that these monoclonal antibodies might have a role as adjunctive therapy for severe CDI, but the immunoglobulin infusion failed to improve clinical outcomes in CDI patients. However, critically ill patients and individuals with severe CDI colitis requiring surgery were excluded in this study. Further studies will need to investigate whether immunoglobulins provide any benefit for severe CDI.

Expert Opinion

Current conventional anti-*C. difficile* antibiotics, including metronidazole and vancomycin, are suboptimal, with significant rates of treatment failures and recurrences. Worsening clinical outcomes and increasing mortality, detection of metronidazole resistance, and rising prevalence of refractory and recurrent cases demand the search for more effective treatment and preventative strategies. A number of new antibiotics are being studied as potential treatment for CDI. Fidaxomicin is a promising novel macrocycle, which appears to have excellent activity against CD, with minimal alteration of the native intestinal microflora given its narrow spectrum of activity. This antibiotic has the most clinical evidence supporting its use. Although it appears to have similar clinical efficacy to vancomycin, the reduced recurrence rate associated with fidaxomicin may lead to its consideration as a first-line agent if costs are not prohibitive. Nitazoxanide has been shown to be noninferior to metronidazole and to have potentially similar efficacy to vancomycin for CDI. Unfortunately, the high cost of nitazoxanide will likely prohibit its use as a primary

treatment for CDI, although it may have a role as salvage therapy for refractory or recurrent CDI. There is much more limited clinical data to support the use of other antimicrobials including rifaximin, ramoplanin, tigecycline, and rifalazil. Although the rifamycins are theoretically an attractive gastrointestinal-selective agent for CDI, resistance associated with mutations in the RNA polymerase encoding gene may render this class of antimicrobials ineffective. Further study with rifaximin is needed to see if its use leads to resistant CD strains. Tigecycline may be useful as an alternative parenteral antibiotic for CDI with severe disease or impaired gastrointestinal motility. Probiotics are currently widely used by physicians for prevention of antibiotic-associated diarrhea including CDI, but little evidence exists supporting their clinical efficacy. Infusion with human monoclonal antibodies against toxins A and B appear to be protective against secondary CDI episodes. However, the anticipated high costs associated with this passive immunotherapy will likely prevent immunoglobulins from serving as a primary preventative agent. These immunoglobulins may be beneficial for those patients with refractory or recurrent CDI who have failed alternative therapies. Active immunotherapy with a *C. difficile* vaccine may be more attractive in terms of efficacy, availability, and affordability as a preventative strategy and is currently being evaluated in phase II clinical trials for secondary prevention.⁹²

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Article Highlights Box

- Fidaxomicin, a novel macrocycle, appears in a limited study to be as effective as vancomycin for treating CDI and may be more effective for preventing CDI recurrences.
- Nitazoxanide is similar in efficacy to metronidazole and possibly vancomycin as CDI therapy.
- Clinical data supporting the use of antimicrobials, including rifaximin, ramoplanin, tigecycline, and rifalazil, and the use of probiotics for CDI is limited and warrants further study.
- Protection against secondary CDI episodes with passive immunotherapy with human monoclonal antibodies against toxins A and B is promising but costs may influence their indications for use.

Table 1

Comparison of Weighted Average Minimum Inhibitory Concentrations (MICs) for Antibiotics Active Against *Clostridium difficile* (CD)

Antibiotic	No. CD isolates Evaluated	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	Range (µg/ml)
Rifalazil ^{63, 93}	141	0.006	0.024	0.0019 to >16
Rifaximin ^{93, 94}	273	0.021	0.081	0.0038 to >32
Tigecycline ^{93, 95, 96}	225	0.083	0.160	0.016 to 8
Nitazoxanide ^{93, 97}	125	0.083	0.170	0.03 to 1
Fidaxomicin ^{22, 26, 29, 93, 98, 99}	453	0.193	0.365	<0.008 to 2
Ramoplanin ^{54, 93, 100, 101}	305	0.309	0.399	0.03 to 1
Vancomycin ^{13, 22, 26, 29, 93, 97–104}	1407	0.688	1.354	0.016 to 4
Metronidazole ^{13, 22, 26, 29, 93, 95, 97–105}	1474	0.306	1.500	0.016 to >32

Note: Over 1500 strains from published studies were assessed to determine the weighted means for the MIC₅₀ and MIC₉₀ values for each antibiotic. Mean MIC values were weighted by the number of strains reported tested in the *in vitro* susceptibility studies.

Table 2Randomized Clinical Trials for Antibiotic Treatment of *Clostridium difficile* Disease

Study design	N	Treatment	Treatment Response	P-Value	Recurrence Rate	P-Value
Double blind ¹⁴ (Mild CDI)	41	Metronidazole 250 mg q6h	98%	0.36	8%	0.67
	40	Vancomycin 125 mg q6h	90%		5%	
(Severe CDI)	38	Metronidazole 250 mg q6h	76%	0.02	21%	0.30
	31	Vancomycin 125 mg q6h	97%		10%	
Open label ^{28, 29}	14	Fidaxomicin 50 mg q12h	71%	0.27	8%	0.75
	15	Fidaxomicin 100 mg q12h	80%		0%	
	16	Fidaxomicin 200 mg q12h	94%		6%	
Double blind ^{106, 107}	265	Fidaxomicin 200 mg q12h	92%	0.9	24%	0.004
	283	Vancomycin 125 mg q6h	90%		13%	
Double blind ³⁵	40	Nitazoxanide 500 mg q12h (7 days)	90%	0.59	23%	0.53
	36	Nitazoxanide 500 mg q12h (10 days)	89%		14%	
	34	Metronidazole 250 mg q6h	82%		24%	
Double blind ³⁷	22	Nitazoxanide 500 mg q12h (10 days)	77%	1.00	5%	1.00
	27	Vancomycin 125 mg q6h	74%		7%	
Open label ⁴⁴	10	Rifaximin 200 mg q8h	90%	1.00	Unknown	
	10	Vancomycin 500 mg q12h	100%		Unknown	

Table 3Relative Costs of Antibiotics for *C. difficile* Therapy

Antibiotic	Dose	Form *	Price Per Unit	Price for 10 day course
Metronidazole	250 mg	PO	\$4.33	\$173.20 (q6h)
Metronidazole	500 mg	PO	\$7.28	\$218.40 (q8h)
Metronidazole	500 mg	IV	\$9.42	\$282.60 (q8h)
Vancomycin	125 mg	PO	\$24.31	\$972.40 (q6h)
Nitazoxanide	500 mg	PO	\$22.76	\$455.20 (q12h)
Rifaximin	400 mg	PO	\$12.68	\$253.60 (q12h) \$380.40 (q8h)
Tigecycline	100 mg	IV	\$147.00	\$1,543.50 (100mg × 1, then 50 q12h)
Tigecycline	50 mg	IV	\$73.50	

Note: Prices reflect average wholesale prices from the RED BOOK™.

* PO, oral; IV, intravenous