Clostridium difficile is a major cause of infection worldwide and is associated with increasing morbidity and mortality in vulnerable patient populations. Metronidazole and oral vancomycin are the currently recommended therapies for the treatment of C. difficile infection (CDI) but are associated with unacceptably high rates of disease recurrence. Novel therapies for the treatment of CDI and prevention of recurrent CDI are urgently needed. Important developments in the treatment of CDI are currently underway and include: novel antibacterial agents with narrower antimicrobial spectra of activity, manipulation of the gut microbiota and enhancement of the host antibody immune response.

Keywords: BI/NAP1/ribotype 027 strain • Clostridium difficile infection • fecal microbiota transplant • fidaxomicin • human microbiome

Background

Clostridium difficile is a major cause of infectious colitis, particularly in hospitalized patients and patients residing in long-term care facilities. In the last decade, C. difficile has become an increasingly problematic pathogen. Increased virulence, transmissibility and ineffective control measures have allowed for the emergence of C. difficile as a major cause of infection worldwide [1]. Historical treatments for C. difficile infection (CDI) are associated with unacceptably high rates of treatment failure and disease recurrence. The changing epidemiology of C. difficile, pitfalls of current therapeutic options, recent clinical trial evidence regarding new uses of old drugs for treatment of CDI, and emerging novel therapies for CDI will be reviewed in this article.

■ Epidemiology of C. difficile infection

The clinical and molecular epidemiology of C. difficile changed dramatically in the early 2000s. Large, multi-institutional outbreaks of CDI were observed in regions of Canada [2], the USA [3,4] and Europe [5,6]. These epidemics were associated with increased disease severity, refractory and increasingly recurrent symptoms, and more frequent complications including death [2,4,7-11]. Furthermore, the incidence of CDI increased significantly among otherwise healthy individuals in community settings who previously would not have been considered at risk for the disease [12].

Subsequent analyses confirmed the emergence of a hypervirulent strain of C. difficile that was responsible for the majority of clinical cases identified in the North American outbreaks [3,7]. This strain is referred to as BI/NAP1/027 based on its restriction endonuclease analysis, pulsed field gel electrophoresis and PCR ribotyping, respectively [3]. The BI/NAP1/027 strain differs from endemic strains of C. difficile in several ways. First, the BI/NAP1/027 strain has a mutation in the tcdC gene, a gene that normally downregulates the production of toxins A and B [3,7].
In one in vitro study, the BI/NAP1/027 isolates produced 16-fold more toxin A and 23-fold more toxin B compared with a control strain [3]. In addition, the BI/NAP1/027 strain produces a binary toxin that was previously uncommon among C. difficile isolates [3]. It is presumed that increased toxin production, at least in part, contributes to the increased disease severity associated with the outbreak strain [7]. Additionally, BI/NAP1/027 isolates are resistant to fluoroquinolones [3]. Finally, the BI/NAP1/027 strain is associated with increased sporulation in vitro [14]. Thus, increased toxin production, sporulation and reduced antimicrobial susceptibility all likely contribute to the observed increased pathogenesis and transmissibility associated with this hypervirulent strain of C. difficile.

In Europe, the prevalence of CDI caused by the BI/NAP1/027 strain has decreased since the initial outbreaks in the mid-2000s [6,15]. However, other epidemiologically important strains have increased considerably since 2005, including ribotype 078, which is associated with severe diarrhea and attributable mortality similar to ribotype 027 [16].

**Pathogenesis of C. difficile infection**

Specific host factors promote primary and recurrent CDI. C. difficile spores are transmitted from human to human via fecal–oral transmission. C. difficile spores are relatively acid-resistant and therefore pass through the stomach to the intestine where they subsequently germinate. The human colon is naturally colonized by a diverse population of symbiotic microorganisms [17]. In normal hosts, this indigenous population of bacteria inhibits colonization by C. difficile. As a result, C. difficile is a part of the normal indigenous flora of only a small minority of humans [18]. However, antibiotic administration induces a change in gut microorganisms that allows for subsequent expansion and colonization of the colon by C. difficile [18]. An individual’s ability to reconstitute normal intestinal microbiota following initial antimicrobial therapy for CDI is important to prevent subsequent disease recurrence [19]. Thus, ideal therapeutic agents for CDI would specifically target C. difficile and would minimize perturbation of the indigenous gut microbiota.

**Antimicrobial therapies for C. difficile infection**

- **Current recommended antibacterial agents & their pitfalls**

Metronidazole and oral vancomycin are the current recommended first-line therapies for CDI [20]. Few studies have directly compared oral vancomycin and metronidazole for the treatment of CDI. A double-blind trial of oral vancomycin versus metronidazole for CDI was conducted from 1994 to 2002 to specifically evaluate the comparative efficacy of oral vancomycin and metronidazole for treating CDI based on disease severity [21]. Oral vancomycin was associated with greater cure rates than metronidazole in patients with severe disease (97 vs 76%; p = 0.02), defined as either having endoscopic evidence of pseudomembranous colitis or having two or more of the following criteria: age >60, temperature >38.3°C, white blood cell count >15,000 cells/µl or albumin <2.5 mg/dl within 48 h of enrollment [21]. Conversely, there was no significant difference in clinical cure rates between oral vancomycin and metronidazole in patients who did not meet criteria for severe disease [21].

Based on the results of the above investigation, the 2010 treatment guidelines published by the Society for Healthcare Epidemiology of America and the Infectious Diseases Society of America recommend oral metronidazole for the treatment of mild-to-moderate CDI and oral vancomycin at a dose of 125 mg every 6 h for treatment of severe CDI, where severe CDI is defined as a white blood cell count of >15,000 cells/µl and serum creatinine >1.5-times the patient’s baseline [20]. For patients who meet criteria for complicated infection, defined as hypotension, shock, ileus or megacolon, the guidelines recommend using oral vancomycin at a dose of 500 mg every 6 h plus intravenous metronidazole as initial treatment [20]. The recommendations for the higher dose of vancomycin and coadministration of intravenous metronidazole are based on expert opinion only, and are not supported by clinical trial data.

Despite the current treatment recommendations, it is well known that both metronidazole and oral vancomycin are associated with unacceptably high recurrence rates in some patients. Prior studies demonstrated that recipients of either drug experienced recurrent CDI 5–30% of the time following completion of an initial course of therapy [22]. A retrospective study of 2042 patients diagnosed with CDI at a Canadian tertiary referral center found that the rate of 60-day recurrence of CDI after initial treatment with metronidazole increased significantly from 2003 to 2004 compared with a baseline period from 1991 to 2002 (47.2 vs 20.8%; p < 0.001) [23]. Additionally, the emergence of reduced in vitro susceptibility of some clinical C. difficile isolates to metronidazole has been demonstrated [24].

More effective therapies are needed for treatment of patients with recurrent CDI and those who are at risk for recurrent CDI. Ideal antimicrobial agents would effectively inhibit C. difficile while minimizing the disturbance of protective bystander gut microorganisms. Both the repurposing of antibiotics currently approved for non-CDI indications and the development of novel agents for the treatment of CDI have been actively explored and are discussed in further detail in the
sections that follow. Comparison of the mechanism of action, spectrum of activity, and stage of investigation for each of the antimicrobial agents discussed is presented in Table 1.

- **Rifaximin**
  Rifaximin is a semisynthetic antibiotic derived from rifamycin that, like vancomycin, is poorly absorbed from the gastrointestinal tract. Rifaximin is approved by the US FDA for treatment of traveler’s diarrhea and hepatic encephalopathy [25]. Anecdotal reports indicated that treatment with rifaximin after completion of a course of metronidazole or oral vancomycin was associated with interruption of CDI in patients with recurrent CDI [26–28]. This treatment strategy became known as the rifaximin ‘chaser,’ whereby rifaximin was given for a 2–4 week period following completion of other anti- *C. difficile* therapy in selected patients who had demonstrated prior recurrence of CDI.

The efficacy of a rifaximin chaser regimen was evaluated in a single-center, randomized, double-blind, placebo controlled trial [29]. In this evaluation, 68 adult inpatients were randomized to receive either rifaximin 400 mg three-times daily or placebo for 20 days

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**Table 1. Comparison of antimicrobial agents approved or evaluated for treatment of *Clostridium difficile* infection.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Mechanism of action</th>
<th>Spectrum of activity</th>
<th>Regulatory status</th>
<th>Comments</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>Nitroimidazole</td>
<td>Produces cytotoxic intermediates</td>
<td>Gram-positive and -negative anaerobes</td>
<td>US FDA approved for a non-CDI indication</td>
<td>Recommended first-line treatment for patients with primary episode mild-moderate CDI [20]</td>
<td>[71]</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Glycopeptide</td>
<td>Inhibits bacterial cell wall synthesis</td>
<td>Gram-positive organisms; minimal systemic absorption</td>
<td>FDA approved for CDI</td>
<td>Recommended first-line treatment for patients with severe CDI [20]</td>
<td>[46]</td>
</tr>
<tr>
<td>Fidaxomicin</td>
<td>Macro cyclic</td>
<td>Inhibits bacterial RNA synthesis</td>
<td>Gram-positive anaerobes; spares <em>Bacteroides</em> spp.; minimal systemic absorption</td>
<td>FDA approved for CDI</td>
<td>Noninferior to oral vancomycin; associated with decreased recurrence of CDI within 28 days in Phase III trials [40,41]</td>
<td>[34,36–38]</td>
</tr>
<tr>
<td>Rifaximin</td>
<td>Rifamycin</td>
<td>Inhibits bacterial RNA synthesis</td>
<td>Broad-spectrum; minimal systemic absorption</td>
<td>FDA approved for a non-CDI indication</td>
<td>Anecdotal evidence for use as a ‘chaser’ following standard treatment for CDI; not supported by RCT data [29]</td>
<td>[72]</td>
</tr>
<tr>
<td>Nitazoxanide</td>
<td>Nitrothiazolidine</td>
<td>Inhibits anaerobic metabolism</td>
<td>Anaerobes, parasites</td>
<td>FDA approved for a non-CDI indication</td>
<td>Similar in efficacy to metronidazole and vancomycin; RCTs underpowered to demonstrate noninferiority [32,33]</td>
<td>[30]</td>
</tr>
<tr>
<td>Act-0179811</td>
<td>Quinolonyl-oxazolidinone</td>
<td>Inhibits protein synthesis</td>
<td>Undefined</td>
<td>Under investigation</td>
<td>Phase III investigation</td>
<td>[45]</td>
</tr>
<tr>
<td>LFF571</td>
<td>Thiopeptide</td>
<td>Inhibits protein synthesis</td>
<td>Gram-positive anaerobes</td>
<td>Under investigation</td>
<td>Phase II investigation</td>
<td>[24,46]</td>
</tr>
<tr>
<td>Ramoplanin</td>
<td>Lipoglycodepsipeptide</td>
<td>Inhibits bacterial cell wall synthesis</td>
<td>Gram-positive organisms; minimal systemic absorption</td>
<td>Under investigation</td>
<td>Phase III investigation</td>
<td>[48]</td>
</tr>
<tr>
<td>CB-183,315</td>
<td>Lipopeptide</td>
<td>Depolarizes cell membrane</td>
<td>Gram-positive organisms, anaerobes; reduced <em>in vitro</em> activity against <em>Bacteroides</em> spp., <em>Enterobacteriaceae</em></td>
<td>Under investigation</td>
<td>Phase III investigation</td>
<td>[50,51]</td>
</tr>
</tbody>
</table>

CDI: *Clostridium difficile* infection; RCT: Randomized, controlled trial.
following the completion of a 10–14 day course of metronidazole or vancomycin for treatment of CDI. All patients had ≥ three unformed bowel movements per day for at least 2 days, or more than six unformed stools in 1 day and had positive stool testing for the \textit{C. difficile} toxin. Patients were excluded from the study if they had evidence of severe infection, had experienced > one recurrence or were receiving concurrent antidiarrheal or probiotic agents.

The primary study end point was recurrent diarrhea, including recurrent CDI and self-reported non-CDI diarrhea, within 3 months following the completion of the rifaximin course. Overall, 24 out of 68 (35%) patients experienced the primary end point of recurrent diarrhea. Recurrent diarrhea occurred less frequently in patients who received rifaximin versus those who received placebo (17 out of 35 vs 7 out of 33; \( p = 0.018 \)). There was a trend toward decreased recurrence of CDI in patients who received rifaximin versus those who received placebo (31 vs 15%; \( p = 0.11 \)), but the study was underpowered to detect a significant difference between the two groups.

While there is anecdotal evidence to support its use, a rifaximin chaser regimen cannot be routinely recommended for treatment of recurrent CDI on the basis of the limited evidence from this single-center, small study. Furthermore, the emergence of resistance to rifaximin following treatment has been observed in clinical isolates of \textit{C. difficile} \cite{31}, and the implications of resistance on the use of rifaximin in clinical practice are not fully understood at this time.

\begin{itemize}
\item **Nitazoxanide**
\end{itemize}

Nitazoxanide is a nitrothiazolide antibiotic that is approved for treatment of intestinal infections caused by Cryptosporidium and \textit{Giardia} \cite{30}. In addition to having activity against protozoa and helminths, nitazoxanide also exhibits \textit{in vitro} activity against certain anaerobic bacteria including \textit{Bacteroides} spp., \textit{Clostridium} spp. and \textit{Helicobacter pylori}. Nitazoxanide works by inhibiting anaerobic metabolism \cite{30} and has demonstrated good activity against \textit{C. difficile} \textit{in vitro} \cite{31}.

Nitazoxanide has been evaluated as a treatment for CDI in prospective, randomized trials. In the first investigation, nitazoxanide was compared with metronidazole for the treatment of CDI \cite{32}. In this study, 142 hospitalized patients with CDI were randomized to treatment with 10 days of metronidazole therapy versus 7 or 10 days of nitazoxanide. Patients with severe disease requiring intensive care or vasopressor support were excluded from participation in this study. The primary study end point was resolution of symptoms at the end of 7 days of therapy. Ultimately, the response at 7 days was similar in patients who received nitazoxanide compared with those who received metronidazole (68 out of 76 [90%] vs 28 out of 34 [82%]; difference 7.1%; 95% CI: -7.0–25.5%) \cite{32}.

In another study, nitazoxanide was compared with vancomycin for treatment of CDI \cite{33}. Hospitalized patients were eligible for this study if they had clinical evidence of CDI and a positive stool test for the \textit{C. difficile} toxin. Patients who required intensive care, had a history of more than one recurrence of CDI in the last 6 months, or had another explanation for diarrhea were excluded from participation in this study. In total, 49 study participants were randomized to receive a 10-day course of either nitazoxanide or oral vancomycin; 41% of the study population met criteria for severe disease as defined by Zar et al. (i.e., two or more of the following criteria: age >60, temperature >38.3°C, white blood cell count >15,000 cells/µl or albumin <2.5 mg/dl within 48 h of enrollment) \cite{21}.

The primary end point was resolution of all clinical signs and symptoms at 3 days after completion of therapy. A secondary end point was recurrent disease within 31 days of treatment initiation. The primary response rates were similar for patients who received nitazoxanide versus those who received vancomycin (17 out of 22 [77%] vs 20 out of 27 [74%]; difference 3%; 95% CI: -24–28%). Two patients in the vancomycin treatment group and one patient in the nitazoxanide treatment group experienced recurrent CDI within 31 days of treatment initiation.

Thus, in two small, randomized, double-blind, controlled trials, nitazoxanide was found to be similar in efficacy to metronidazole and vancomycin, respectively. Both studies involved relatively few patients and were underpowered to demonstrate non-inferiority of nitazoxanide. Furthermore, follow up was limited in the second study to 1 month after treatment initiation. Thus, it is unclear based on the available data which, if any, patient populations would benefit from treatment with nitazoxanide over other anti-\textit{C. difficile} regimens.

\begin{itemize}
\item **Fidaxomicin**
\end{itemize}

Fidaxomicin is a macrocyclic antibiotic that is the first new antibiotic to be approved specifically for the treatment of CDI. Fidaxomicin acts by inhibiting bacterial RNA polymerase \cite{34}. Fidaxomicin exhibits minimal systemic absorption and achieves high concentrations in the stool \cite{35}. Fidaxomicin has a narrower spectrum of antimicrobial activity compared with metronidazole and oral vancomycin, which makes it desirable for the treatment of CDI. In particular, fidaxomicin has excellent activity against \textit{C. difficile} and \textit{Clostridium perfringens}, moderate activity against other Gram-positive organisms, and very little activity against \textit{Bacteroides} spp. and enteric Gram-negative organisms \cite{36–38}. Finally,
Fidaxomicin has been shown to inhibit sporulation of *C. difficile* in vitro [39].

Fidaxomicin was shown to be noninferior to vancomycin for the clinical cure of CDI in two randomized, double-blind, Phase III clinical trials [40,41]. One of the studies was conducted in sites only within North America [41] and the other included patients from sites in North America and Europe [40]. In both trials, patients were considered for study inclusion if they were ≥16 years old and presented with CDI. For these trials, CDI was defined as having more than three unformed bowel movements within 24 h and positive stool testing for *C. difficile* toxin A and/or B. Participants were excluded from the study if they had experienced more than one prior episode of CDI in the preceding 3-month period, if they had evidence of life-threatening or fulminant disease, defined as white blood cell count >30,000 cells/μl, temperature >40°C, systolic blood pressure <90 mmHg, shock, peritoneal signs, severe dehydration or toxic megacolon, at the time of randomization; if they had received more than four doses or 24 h of treatment with vancomycin or metronidazole; or if they had had any other drug with activity against *C. difficile* prior to the time of study enrollment [40,41]. Study participants were randomized to treatment with either oral vancomycin 125 mg every 6 h or oral fidaxomicin 200 mg every 12 h. Randomization was stratified by study site as well as by primary infection versus first recurrence.

The primary end point was clinical cure, defined as resolution of diarrhea (≤ three unformed bowel movements over two consecutive days) without any need for further therapy for CDI as assessed 2 days after the end of the treatment period. Participants who achieved the primary end point were followed for an additional 28 days after the completion of therapy. The secondary end point was clinical recurrence, defined as return of more than three unformed bowel movements in a 24-h period, positive stool testing for *C. difficile* toxin A and/or B, and the need for retreatment for CDI.

The study populations were similar in each trial. The median age of combined participants from the two trials was 64 years. Study participants had a median of six unformed stools per day and 37% of enrolled subjects had severe CDI, defined as ≥ ten unformed stools per day or white blood cell count >15,000/μl. The majority (63%) of patients in both studies were inpatients. Overall, 16% of participants presented with a first recurrence of CDI [42].

In the international study, 87.7% of patients who received fidaxomicin achieved clinical cure versus 86.7% of patients receiving vancomycin in the modified intention to treat analysis [40]. The results of the North American trial were similar: 88.2% of patients receiving fidaxomicin and 85.8% of patients receiving vancomycin reached clinical cure in the modified intention to treat analysis [41].

In both studies, treatment with fidaxomicin was associated with decreased rate of recurrence of CDI at 28 days after the completion of therapy in patients who initially responded to therapy. In the international study, 12.7% of fidaxomicin recipients experienced recurrence versus 26.9% of vancomycin recipients (p = 0.0002) [40]. In the North American study, 15.4% of fidaxomicin and 25.3% of vancomycin recipients experienced recurrence (p = 0.005) [41]. Subgroup analysis demonstrated that the difference in recurrence rate was greater in patients who were infected with a non-BI/NAP1/027 strain (9.2% of fidaxomicin recipients vs 27.4% of vancomycin recipients; p = 0.0003) [40]. Among patients infected with a BI/NAP1/027 strain, 22.2% who received fidaxomicin experienced clinical relapse compared with 38.0% who received vancomycin (p = 0.079) [40].

Additional subanalyses of the combined trial results demonstrated other important findings. There was no difference in clinical cure rates between the fidaxomicin and vancomycin treatment groups among patients who presented with a first recurrence of CDI (93.7 and 91.6%, respectively), but rates of recurrence were lower in the fidaxomicin treatment group versus the vancomycin treatment group (19.7 and 35.5%, respectively; p = 0.045 for per-protocol analysis) [45].

Thus, fidaxomicin is highly active against *C. difficile* and demonstrated clinical cure rates that were noninferior to vancomycin for patients presenting with primary CDI or a first recurrence of CDI. Patients who are at high-risk for development of recurrent CDI may benefit most from this new drug. Unfortunately, clinical trial data regarding the efficacy of fidaxomicin for patients who have had multiple recurrences of CDI are lacking. Additionally, the sickest patients were excluded from the clinical trial evaluations of fidaxomicin. Thus, the utility of fidaxomicin for treatment of life-threatening CDI or multiply recurrent CDI is unknown. In our experience, the expense of fidaxomicin precludes its routine use.

### Antimicrobial agents in development

Investigational Phase II and III clinical trials of several additional new drugs for the treatment of CDI are currently ongoing [101]. This is particularly encouraging, especially in the current era when many major pharmaceutical companies have abandoned antimicrobial development. Act-0179811, also known as cadazolid, is a quinolonyl–oxazolidinone antibiotic that contains both quinolone and oxazolidinone motifs and acts by inhibiting bacterial protein synthesis [44,45]. It has demonstrated good *in vitro* activity against *C. difficile* [45].
Cadazolid is currently undergoing Phase III evaluation. LFF571 is a novel semisynthetic thiopeptide antibiotic that also acts by inhibiting bacterial protein synthesis and exhibits selective activity against Gram-positive anaerobic organisms (24,46). LFF571 has demonstrated excellent activity against *C. difficile in vitro* (47) and is currently undergoing Phase II investigation. Ramoplanin is a lipoglycodepsipeptide antibiotic that acts by inhibiting bacterial cell wall synthesis. Ramoplanin demonstrates selective activity for Gram-positive organisms and is minimally absorbed from the GI tract (48). Ramoplanin has demonstrated *in vitro* activity against *C. difficile* (49) and is undergoing Phase III investigation. Finally, CB-183,315, also known as surotomycin, is a lipopeptide antibiotic that is structurally similar to daptomycin and acts by disrupting the integrity of the bacterial cell membrane. Surotomycin has selective activity against Gram-positive and anaerobic bacteria with relatively minimal activity against *Bacteroides* spp. and *Enterobacteriaceae* (50). Surotomycin has shown promise as a potential new therapy for CDI (51) and is undergoing Phase III investigation.

**Nonantimicrobial therapies for CDI: toxin-binding therapy**

■ **Tolevamer**

Tolevamer is a nonantimicrobial drug that was developed as a potential novel therapeutic agent for treatment of CDI. Tolevamer is a large polymer molecule that binds and neutralizes *C. difficile* toxins A and B (52). Thus, tolevamer’s theoretical treatment advantage is its potential to neutralize toxins and eliminate symptoms of disease without having any antimicrobial effect on bystander gut microorganisms (53).

In a Phase II clinical trial, varying daily doses of tolevamer were compared with standard dosing of vancomycin for treatment of patients with mild-to-moderately severe CDI (52). Patients were recruited from over 60 centers in the USA, the UK and Canada. Approximately three quarters of patients had primary CDI. A total of 67% of patients receiving 3 g of tolevamer daily, 83% of patients receiving 6 g of tolevamer daily and 91% of patients who received vancomycin achieved resolution of diarrhea (p = 0.02 for the comparison of 6 g daily tolevamer dose to vancomycin) (52). Additional subanalyses demonstrated that tolevamer was associated with a trend toward a decreased rate of recurrent CDI (52).

Two Phase III studies, however, failed to demonstrate comparable efficacy of tolevamer dosed 9 g daily to existing treatments for CDI (54,55). Thus, while tolevamer’s proposed mechanism of action is novel and appealing, tolevamer is not indicated for primary treatment of CDI based on the currently available evidence.

**Nonantimicrobial therapies for CDI: microbiota therapy**

As discussed previously, disturbance of the normal intestinal microbiota allows for colonization of the gut by *C. difficile* and expansion of the *C. difficile* population to exceed the threshold necessary to cause disease. Thus, many have sought to restore normal gastrointestinal microbiota through the use of probiotic supplements or stool replacement therapy.

■ **Probiotics for the treatment of CDI**

*Saccharomyces boulardii* is a nonpathogenic yeast that is not part of the normal human intestinal microbiota. A randomized, placebo-controlled trial of *S. boulardii* provided early evidence for the use of probiotic supplementation as adjunctive therapy for CDI (56). Patients were randomized to receive *S. boulardii* versus placebo in addition to standard therapy for CDI including metronidazole or vancomycin. Overall, patients who received *S. boulardii* were less likely than patients who received placebo to develop recurrent CDI within 28 days of completion of therapy (adjusted response rate [RR]: 0.43; 95% CI: 0.20–0.97). A total of 34.6% of patients with recurrent CDI who received *S. boulardii* experienced relapse compared with 64.7% of patients with recurrent CDI who received placebo (p = 0.04). On the other hand, *S. boulardii* did not significantly affect the rate of recurrence in patients presenting with primary CDI (19.3% for *S. boulardii* vs 24.2% for placebo; p = 0.86). Thus, *S. boulardii* was most effective in reducing risk of future episodes of CDI in patients presenting with a recurrence of CDI.

No additional randomized, placebo-controlled, prospective trials have provided sufficient additional evidence to support the routine use of probiotics as adjunctive therapy for CDI (57). Considerably more research has focused on the efficacy of probiotic supplements including *Lactobacillus* spp. and *S. boulardii* for the prevention of CDI in at-risk patient populations (58,59). Primary prevention of CDI is beyond the scope of this review, and so these data are not discussed in detail here.

■ **Fecal microbiota transplant**

Fecal microbiota transplantation (FMT) has been evaluated for the treatment of recurrent CDI. FMT involves the transfer of fecal material obtained from a healthy donor to the GI tract of a patient with CDI. Case reports of FMT in the USA date back to at least 1958 when a four-patient case series documenting its use for treatment of pseudomembranous colitis was published (60). More recently, a growing body of evidence supporting the efficacy of FMT for treatment of recurrent CDI has emerged. Many single-center retrospective case reports and case series have reported high cure rates with FMT.
after conventional therapies for CDI had failed. A systematic review of 317 patients from 27 different case series and case reports found that the overall cure rate for FMT was 92% [61]. However, there was no standardization of protocols for selection and screening of donors, preparation of fecal suspension, periprocedural antimicrobial therapy or delivery of fecal material.

A recent single-center, randomized, controlled trial evaluated the efficacy of FMT for treatment of CDI [62]. Enrolled study participants were randomized to receive one of three treatment regimens for recurrent CDI:

- 4–5 days of treatment with oral vancomycin, followed by bowel lavage and instillation of donor feces;
- 14 days of treatment with oral vancomycin followed by bowel lavage; or
- A 14 day course of oral vancomycin dosed 500 mg every 6 h without bowel lavage.

Study eligibility criteria included age ≥18 years, life expectancy of ≥3 months, CDI that was defined as ≥ three unformed bowel movements per day for at least two consecutive days plus a positive stool test for C. difficile toxin, and a history of prior CDI for which ≥10 days of appropriately dosed metronidazole or vancomycin was received. Patients were excluded from the study if any of the following conditions were present: recent chemotherapy, HIV infection with CD4 count <240, pregnancy, prolonged high-dose corticosteroid treatment, concurrent use of antibiotics for indication other than CDI and need for ICU and/or vasopressors.

Patients randomized to receive FMT were treated with vancomycin 500 mg orally four times a day for 4–5 days. Subsequently, patients in the FMT arm underwent bowel lavage with 4 l of hyperosmotic solution. The following day, a suspension of donor feces was instilled via nasoduodenal tube. Donor feces were obtained from healthy donors that were unrelated to the case patients. All donors were screened for potentially transmissible infectious agents, including parasites, C. difficile, enteric bacterial pathogens, HIV, hepatitis A, B and C, Cytomegalovirus, Epstein–Barr virus, Treponema pallidum, Strongyloides stercolis and Entamoeba histolytica. Feces were collected on the day of transplantation and were diluted in 500 ml of sterile saline. The resultant suspension was filtered and the supernatant liquid was then infused into the recipient patients via a nasoduodenal tube.

An interim analysis was performed using data from 42 patients recruited for the study over a 28-month enrollment period. In total, 16 patients received FMT, 13 received vancomycin alone and 13 received vancomycin plus bowel lavage. Patients recruited for the study were elderly (mean ages in each treatment group were 73, 66 and 69, respectively) and had a median of three prior recurrences of CDI. Approximately a third of trial patients were inpatients at the time of recruitment for the study. Only four patients were found to have the hypervirulent strain, ribotype 027, although the strain type was missing for nine patients.

Patients were monitored for 10 weeks following FMT. The primary end point was clinical cure without relapse, defined as diarrhea with a positive stool test for C. difficile toxin, at the end of the follow-up period. Of the 16 patients who underwent FMT, 13 (81%) were cured following a single infusion of donor feces. Two of the remaining three patients experienced clinical cure following a second instillation of donor feces. Thus, the overall treatment efficacy including patients who received a second infusion of donor feces was 94%. Conversely, only four out of 13 patients (31%) in the vancomycin-only group and three out of 13 patients (23%) in the vancomycin plus bowel lavage group experienced clinical cure. Of the patients who failed vancomycin therapy, 18 elected to undergo off-protocol FMT; of these 18 patients, 11 were cured after one infusion and four were cured after two infusions for an overall cure rate of 83% in this subgroup.

The investigators analyzed 16S ribosomal RNA gene sequences from stool of the FMT recipients before and after the procedure to assess the phylogenetic diversity of intestinal microbiota. These results were compared with those of the donors. Nine FMT recipient patients’ feces were available for analysis. As predicted, the diversity of intestinal microbiota from patients prior to FMT was consistently and significantly less than that of the donor population. Within 2 weeks of FMT, the diversity of the recipients’ stool matched that of the recipients.

In summary, this was the first randomized, controlled, open-label trial of FMT for treatment of recurrent CDI. The findings demonstrated that FMT was highly effective for treatment of recurrent CDI in the study population. A randomized, controlled trial comparing the efficacy of FMT delivered via colonoscopy versus sham FMT procedure with reinfusion of patients’ own stool is currently enrolling study participants and will provide additional valuable information regarding the role of FMT for treatment of recurrent CDI [101].

**Synthetic stool transplant**

A synthetic stool substitute would theoretically confer the same benefit to patients as donor-derived FMT but would mitigate many of the logistical and aesthetic concerns related to transferring fecal material from one person to another. This therapeutic alternative is in the early phase of investigation [63]. A synthetic stool mixture has been created using cultured bacterial isolates from stool of a healthy donor. Data from an existing
metagenomic database were used to determine an appropriate relative proportion of bacterial isolates for inclusion in the synthetic product.

Two patients with recurrent CDI participated in a pilot study. The patients underwent stool lavage followed by instillation of the stool substitute into the right and transverse portions of the colon via colonoscopy. Both patients experienced resolution of their diarrhea following receipt of synthetic stool. Remarkably, both patients remained disease-free 24 and 26 weeks following treatment, despite each receiving multiple courses of antibiotic therapy for other indications [63]. While these observational data from this two-person case series are encouraging, further investigation of the safety and efficacy of a synthetic stool substitute as a treatment for recurrent CDI is warranted.

**Nonantimicrobial therapies for CDI: enhancing the immune response**
The immune system plays an active role in regulation of CDI. Individuals who are asymptomatic carriers of *C. difficile* have significantly higher levels of IgG antibodies against toxin A than individuals with symptomatic CDI [64]. Furthermore, a poor host IgG antibody response to toxin A during an initial CDI episode is a predictor of recurrence of CDI [65]. In light of these observed associations, there is ongoing research in the areas of immunomodulating therapies, including monoclonal antibodies and vaccines for the treatment and prevention of CDI.

- **Monoclonal antibody infusion for the prevention of recurrent *C. difficile* infection**
Human monoclonal antibodies against *C. difficile* toxins A and B have been developed and evaluated for the prevention of recurrent CDI. A randomized, placebo-controlled, double-blind Phase II investigation of a monoclonal antibody against *C. difficile* toxin A found that it was no better than placebo in preventing recurrence of CDI during a 56-day study period when given as a single infusion as an adjunct to standard therapy for CDI [66]. Five out of 29 (17.2%) patients who received monoclonal antibody and three of 17 (17.7%) patients who received placebo experienced recurrent CDI during the study follow-up period.

A second Phase II randomized, placebo-controlled trial evaluated the efficacy of combined monoclonal antibodies against *C. difficile* toxins A and B for the prevention of recurrent CDI [67]. In this trial, 200 patients were randomized to adjunctive treatment with the combined monoclonal antibodies versus placebo in addition to standard therapy for CDI. Nearly three-quarters of study participants were treated with metronidazole and approximately a third of enrolled patients had more than one prior episode of CDI. Patients were followed for a total of 8 weeks. At the end of the follow-up period, seven patients in the monoclonal antibody group and 25 patients in the placebo group had experienced relapse of CDI (p < 0.001). This reduction in recurrence was sustained in subgroup analyses. Only two out of 25 (8%) patients with the BI/NAP1/027 strain who received monoclonal antibody infusion experienced recurrence versus six out of 19 (32%) patients with the BI/NAP1/027 strain who received placebo (p = 0.06). Likewise, two out of the 29 (7%) patients with more than one recurrence of CDI at study entry who received monoclonal antibody infusion experienced subsequent recurrence versus 13 out of 32 (38%) patients with more than one recurrence of CDI at study entry who received placebo (p = 0.006).

In summary, infusion of combined monoclonal antibodies against *C. difficile* toxins A and B was apparently safe, well tolerated and effective for secondary prevention of CDI in Phase II investigation when used as an adjunct to standard therapy for CDI. A Phase III trial of three different monoclonal antibody formulations, antitoxin A, antitoxin B, and antitoxin A and B, is currently recruiting patients and will provide additional information regarding the safety and efficacy of monoclonal antibodies for the prevention of recurrent CDI [101].

- **C. difficile toxoid vaccine**
Several groups have investigated the development of vaccine for prevention of CDI in high-risk patients. A toxoid vaccine containing inactivated *C. difficile* toxins A and B has been developed for intramuscular injection and has demonstrated immunogenic potential in healthy individuals [68]. Furthermore, the vaccine was associated with interruption of recurrent CDI in three patients in an open-label pilot study [69]. Phase II trials evaluating the potential efficacy of a similar toxoid vaccine for the prevention of CDI in high-risk individuals are currently underway [70].

**Conclusion**
CDI causes a tremendous burden of healthcare-associated infections and is associated with significant attributable morbidity and patient deaths. CDI occurs when there is a disturbance of the indigenous intestinal microbiota and a suboptimal host immune response, allowing for the colonization and subsequent infection by pathogenic strains of *C. difficile*. Current recommended therapies for CDI are associated with unacceptably high rates of treatment failure and disease recurrence. Active investigation of many novel therapies for treatment of primary and recurrent CDI are currently ongoing. These novel therapies for CDI include narrower-spectrum antibacterial agents, stool replacement and other treatments to restore normal intestinal microbiota, and manipulation of the host.
Treat the subgroup(s) of patients for whom the benefits of stratification of treatment recommendations and identification of predictors to help clinicians assess an individual patient's risk had multiple recurrences of CDI is unclear. Prediction rules for treatment of patients who have had a first recurrent episode of CDI, the role of new drugs have been limited to patients with either a primary or first recurrent episode of CDI, the role of new drugs such as fidaxomicin for treatment of patients who have had multiple recurrences of CDI is unclear. Prediction rules to help clinicians assess an individual patient's risk of developing recurrent CDI might allow for further stratification of treatment recommendations and identify the subgroup(s) of patients for whom the benefits of initial treatment with fidaxomicin or other novel agents justify their excess cost.

Manipulation of the intestinal microbiota by FMT has demonstrated the greatest promise for the treatment of otherwise refractory cases of CDI. However, standardization of protocols regarding donor selection, screening and route of administration, as well as additional safety data are still needed. Achieving the same end goal via instillation of a synthetic stool substitute is inherently appealing and would alleviate many of the logistical and safety concerns surrounding FMT.

The development of more effective and targeted therapies for CDI is only one of the necessary components for controlling the *C. difficile* epidemic. In addition, research is ongoing regarding effective methods to prevent transmission and acquisition of CDI in healthcare settings, including enhanced methods of environmental cleaning, antimicrobial prophylaxis with *C. difficile*-active agents, the use of probiotic supplements including nontoxigenic *C. difficile*, and vaccination for the primary prevention of CDI.

### Executive summary

**Epidemiology & pathogenesis of *Clostridium difficile* infection**
- The epidemiology of *Clostridium difficile* infection (CDI) has changed significantly in the last 10–15 years.
- Hypervirulent strains of *C. difficile* including BI/NAP1/027 are associated with increased pathogenesis and transmissibility.
- In most normal hosts, indigenous intestinal microbiota inhibit gut colonization by *C. difficile*.
- Alteration of indigenous intestinal microbiota by antibiotic administration allows for colonization by *C. difficile* and subsequent disease.

**Antimicrobial & toxin-binding therapies for CDI**
- Metronidazole is recommended for first-line treatment of mild or moderate primary CDI, whereas oral vancomycin is currently recommended for first-line treatment of severe primary CDI.
- Both metronidazole and oral vancomycin are associated with high rates of CDI recurrence.
- Fidaxomicin is noninferior to oral vancomycin for the treatment of primary and first recurrent episodes of CDI.
- Fidaxomicin is associated with decreased risk of recurrent CDI in patients presenting with primary and first recurrent episodes of CDI compared with oral vancomycin.
- The Phase III clinical trials did not include patients with multiple recurrences or life-threatening CDI, and so the results cannot be generalized to these populations.
- A rifaximin ‘chaser’ given following completion of a standard treatment course for CDI is associated with decreased recurrence of diarrhea, but was not associated with a statistically-significant difference in recurrence of CDI in one underpowered clinical trial.
- Several antimicrobial agents with narrower spectra of activity and novel modes of action are undergoing Phase II and Phase III investigation.
- Tolevamer was developed as a novel nonantimicrobial treatment for CDI to minimize symptoms of CDI by binding and neutralizing toxin A and B. Phase III studies failed to show noninferiority of tolevamer to standard therapies for CDI.

**Nonantimicrobial therapies for CDI: microbiota therapy**
- Fecal microbiota transplant was superior to regimens including oral vancomycin plus bowel lavage or oral vancomycin therapy alone in a single-center, small, randomized, open-label clinical trial. Overall, 94% of patients achieved cure following one or two infusions of donor stool.
- Synthetic stool is undergoing evaluation for use in fecal microbiota transplant. The investigation is in the preliminary stages at this time, but shows promise for treatment of recurrent CDI.

**Nonantimicrobial therapies for CDI: enhancing the immune response**
- Infusion of human monoclonal antibodies against *C. difficile* toxins A and B was associated with decreased rate of relapse when administered in conjunction with standard treatment for primary or recurrent CDI in Phase II investigation. A Phase III trial to evaluate monoclonal antibodies for the prevention of recurrent CDI is currently underway.
- Toxoid vaccines are under active investigation for treatment and primary prevention of CDI in high-risk populations.
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References
Papers of special note have been highlighted as: ■ of interest
■ Describes the emergence of a novel, epidemic strain of Clostridium difficile characterized by unique restriction-endonuclease analysis, pulsed-field gel electrophoresis and toxinotyping.
■ This retrospective study highlights the changing epidemiology of C. difficile, with increasing refractory and recurrent disease following treatment with metronidazole.
Fidaxomicin versus vancomycin for the treatment of CDI.


This single-center study was the first randomized, open-label study to evaluate the efficacy of fecal microbiota transplant for the treatment of CDI. Fecal microbiota transplant was associated with significantly higher cure rates than treatment with oral vancomycin plus bowel lavage or treatment with oral vancomycin alone.


■ Website