Breakthroughs in the treatment and prevention of *Clostridium difficile* infection

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Abstract | This Review summarizes the latest advances in the treatment and prevention of *Clostridium difficile* infection (CDI), which is now the most common health-care-associated infection in the USA. As traditional, standard CDI antibiotic therapies (metronidazole and vancomycin) are limited by their broad spectrum and further perturbation of the intestinal microbiota, which result in unacceptably high recurrence rates, novel therapeutic strategies for CDI are needed. Emerging CDI therapies are focused on limiting further perturbation of the intestinal microbiota and/or restoring the microbiota to its pre-morbid state, reducing colonization of the intestinal tract by toxigenic strains of *C. difficile* and bolstering the host immune response against *C. difficile* toxins. Fidaxomicin is associated with reduced CDI recurrences, and other emerging narrow-spectrum CDI antibiotic therapies might eventually demonstrate a similar benefit. Prevention of intestinal colonization of toxigenic strains of *C. difficile* can be achieved through restoration of the intestinal microbiota with faecal microbiota transplantation, as well as by colonizing the gut with nontoxigenic *C. difficile* strains. Finally, emerging immunological therapies, including monoclonal antibodies and vaccines against *C. difficile* toxins, might protect against CDI and subsequent CDI recurrences. The available clinical data for these emerging therapies, and their relative advantages and disadvantages, are described.

Over the past two decades, *Clostridium difficile* infection (CDI) has emerged as a global public health threat[1]. CDI is now the most common US health-care-associated infection[2]. Worldwide, CDI has emerged as an important cause of diarrhoeal illness in the community[1,3], including among those previously thought to be at low risk of CDI, such as healthy young adults[3,4] and children[5,6]. Changes in CDI epidemiology have been driven by the emergence of epidemic strains with novel virulence factors and antibiotic resistance, such as BI/NAP1/027 (REF. 1). According to data published in 2015, in the USA alone, *C. difficile* is responsible for nearly 500,000 incident infections and 30,000 deaths each year, and ~20% (83,000) of all CDIs recur[7]. Estimated annual health-care expenditures attributable to CDI range from US$1 billion[8] to >$4 billion[9]. In 2013, the US Centers for Disease Control and Prevention classified CDI among the most serious immediate antibiotic-resistant infectious "public health threats that require urgent and aggressive action" (REF. 8). This call to action has prompted increased investigation of CDI epidemiology, pathophysiology, treatment and prevention.

CDI pathophysiology is multifactorial and complex[1]. As a mature and highly variable intestinal microbiota confers *C. difficile* colonization resistance and protects against developing CDI, exposure to *C. difficile* spores alone is insufficient to cause CDI. Intestinal microbiota perturbation by antibiotics creates a permissible environment for *C. difficile* spores to germinate and for vegetative organisms to colonize the gastrointestinal tract and release toxins responsible for inducing CDI symptoms (BOX 1). Restoring the intestinal microbiota protects against CDI recurrences[1]. The host immune response is also important for modulating CDI[10]. For example, a lack of preformed *C. difficile* anti-toxin antibodies and/or failure to develop an adequate humoral immune response to *C. difficile* toxins contributes to the development of CDI[10]. Improved understanding of CDI pathophysiology has prompted reconsideration of CDI treatment and prevention strategies. Novel antibiotic, biotherapeutic and immunological therapies are promising emerging strategies for CDI treatment and prevention (FIG. 1). Rarely does an infection have such a diverse set of potential preventions and treatments as CDI at this time, some

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Key points

- The most commonly prescribed antibiotic therapies for *Clostridium difficile* infections (CDIs), namely metronidazole and vancomycin, have a broad antibiotic spectrum and are associated with unacceptably high CDI recurrence rates
- Emerging CDI therapies limit further perturbation of and/or restore the gut microbiota to its pre-morbid state, reduce colonization by toxigenic strains and bolster the host immune response against *C. difficile* toxins
- As emerging narrow-spectrum CDI antibiotic therapies have limited activity against several species of enteric commensal bacteria, these new antibiotics might result in less frequent CDI recurrences
- Faecal microbiota transplantation is highly efficacious for preventing CDI recurrences, but questions regarding the optimal route of administration and long-term risks remain
- Oral administration of spores from nontoxigenic *C. difficile* strains could prevent CDI by preventing intestinal colonization with toxigenic strains of *C. difficile*
- Emerging immunological therapies, including monoclonal antibodies and vaccines against *C. difficile* toxins, might protect against symptomatic CDI and subsequent CDI recurrences

of which are certain to modify the risk, incidence and severity of this infection in the future. This Review summarizes the rationale and the latest clinical data for the various commercially available and emerging therapeutic and preventive strategies for CDI.

Antibiotic therapies

Current standard therapies

Antibiotics, primarily metronidazole and vancomycin, have been the preferred CDI treatment modality for >30 years. In 2010, the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA)[11], jointly recommend treating adults with their first episode or first recurrence of mild or moderate CDI with metronidazole. Vancomycin orally (not systemically absorbed) is recommended for severe CDI, multiply recurrent CDI and CDI refractory to metronidazole. Combination therapy with metronidazole intravenously and vancomycin orally is recommended for severe, complicated CDI. Updated SHEA–IDSA CDI clinical practice guidelines are currently in development. The American College of Gastroenterology[12], the European Society of Clinical Microbiology and Infectious Diseases[13], and the American Academy of Pediatrics[14] have all published similar treatment recommendations. The established antibiotic options for CDI are reviewed in detail elsewhere[15]. Owing to the relative lack of comparative effectiveness studies for CDI treatment in children, the recommendations for the use of metronidazole and vancomycin are similar for adult and paediatric populations.

In one small trial of 150 adults with CDI[16], vancomycin was superior to metronidazole for severe CDI, but vancomycin and metronidazole were not statistically different for treatment of nonsevere CDI. A larger study of 1,118 adults with CDI analysed pooled data from two randomized controlled trials (RCTs) comparing tolevamer, an intraluminal *C. difficile* toxin-binding polymer, to metronidazole and to vancomycin[17]. CDI treatment failure was more frequent with tolevamer than either metronidazole or vancomycin in each individual trial. Multivariate analysis of pooled data from these two trials demonstrated that, compared with metronidazole, vancomycin was independently associated with higher clinical success (OR = 1.575, 95% CI 1.035–2.396, $P = 0.034$) for all CDI irrespective of severity.

Fidaxomicin

Fidaxomicin (OPT-80) is a novel macrocyclic antibiotic that inhibits bacterial nucleic acid synthesis[18]. The drug is only available in an oral formulation and systemic absorption is minimal[19]. Fidaxomicin has bactericidal activity against *C. difficile*, but reduced *in vitro*[20,21] and *in vivo*[22,23] activity against several enteric commensal bacterial species, particularly those thought to confer colonization resistance against *C. difficile* (that is, *Bacteroides* spp. other anaerobic Gram-negative bacilli and other *Clostridium* spp.).

Traditional CDI antibiotic therapies, including metronidazole and vancomycin, suppress the intestinal microbiota. This persistent dysbiosis is postulated, in part, to cause increased CDI recurrences[22,24]. Thus, microbiota-sparing antibiotics promote *C. difficile* colonization resistance and, at least in part, overcome this limitation. A phase III double-blind RCT conducted in North America compared 10-day courses of vancomycin (125 mg four times daily) and fidaxomicin (200 mg twice daily) in adults with their first or second CDI in the previous 90 days[25]. Clinical cures were similar, but fidaxomicin reduced recurrences in both the intention-to-treat ($n = 596$; 15% versus 25%, $P = 0.005$) and per-protocol analyses ($n = 548$; 13% versus 24%, $P = 0.004$). The rates and severity of treatment-related adverse events were similar. These findings were confirmed in a similarly designed phase III RCT conducted in North America and Europe[26]. Analysis of pooled data from these two RCTs demonstrated that patients with CDI caused by strain BI/NAP1/027 had lower cure rates (86.6%; 214 of 247) than those infected with non-BI/NAP1/027 strains (94.3%; 445 of 472; $P < 0.001$) and this statistically significant difference was observed for both fidaxomicin treatment ($P = 0.007$) and vancomycin treatment ($P = 0.02$). The *C. difficile* strain BI/NAP1/027 recurrence rates in each group were similar (31.3% [30 of 96] after vancomycin and 23.3% [21 of 90] after fidaxomicin; $P = 0.23$)[27]. In both phase III RCTs, concomitant antibiotic use negatively affected recurrence rates[25,26]. However, even among patients who had received concomitant antibiotic therapy, pooled data analysis demonstrated that fidaxomicin significantly reduced recurrences compared with vancomycin (17% versus 29%; $P = 0.048$)[28].

Fidaxomicin was FDA-approved for CDI in adults in May 2011 (REF. 18). Despite its narrow spectrum, recurrence rates with fidaxomicin are also unacceptably high (greater than 10%)[25]. Furthermore, widespread use of fidaxomicin is limited by its high cost, ranging US$1,680–3,360 for a 10-day course[29]. Despite the reduced CDI recurrence risk, fidaxomicin might not be cost-effective for general use[29,30]. However, fidaxomicin could be cost-effective in specific patient subgroups, including those with recurrent or severe CDI[31] and patients with CDI caused by non-NAP1/BI/027 strains[29].

Antibiotics in clinical development

Several antibiotics are in clinical development for CDI (TABLE 1). Ridinilazole (SMT19969), undergoing phase II clinical evaluation for CDI treatment, is a novel antibiotic that has potent *in vitro* activity against *C. difficile* and limited activity against Gram-negative (such as *Bacteroides fragilis*) and most Gram-positive intestinal flora (such as *C. ramosum*, *C. perfringens* and Bifidobacteria species, but retains potent activity against Clostridia cluster XIVa, a potentially important group for colonization protection against *C. difficile*)[32]. In a phase I trial of healthy adults, SMT19969 resulted in high faecal drug levels, low plasma drug levels and no reported serious adverse events[33]. Top-level preliminary results from a phase II RCT (CoDIFy) comparing ridinilazole (200 mg twice daily for 10 days) and vancomycin (125 mg four times daily for 10 days) for treatment of CDI in 100 adults have been described in a press release from the manufacturer[34]. The press release reports that ridinilazole was superior to vancomycin for sustained clinical cure (clinical cure with no recurrence within 30 days), 66.7% for ridinilazole compared to 42.4% for vancomycin ($P = 0.0004$).

Surotomycin (CB-183315) is a novel lipopeptide antibiotic that is structurally similar to daptomycin[35] and maintains bactericidal activity by disrupting the bacterial cell membrane. Surotomycin has reduced *in vitro* activity against intestinal commensal microorganisms that facilitate *C. difficile* colonization resistance[36]. In a completed phase II trial, CDI recurrence rates among 210 adults with CDI were 36%, 28%, and 17% within 28-day post-treatment with vancomycin 125 mg four times daily, surotomycin 125 mg twice daily, and surotomycin 250 mg twice daily, respectively[37]. Surotomycin is currently under investigation in adults and children with CDI in a phase III RCT (ClinicalTrials.gov identifiers NCT01597505 and NCT01598311)[38,39]. However, in an August 2015 US Securities and Exchange Commission filing, Merck (the current developer of surotomycin after purchase of Cubist) revealed it had received "unfavourable efficacy" data from a late-stage clinical trial testing surotomycin. This data resulted in an impairment charge of US$50 million[40]. Although no further details were available, this information could indicate that clinical development will be halted.

Cadazolid is a novel oxazolidinone antibiotic with bactericidal activity against *C. difficile*[41]. Structurally similar to linezolid, cadazolid inhibits protein synthesis and *C. difficile* sporulation and toxin production even in the absence of bacterial killing[42]. Cadazolid also contains a fluoroquinolone moiety, which confers low-level inhibition of DNA synthesis. Cadazolid retains excellent *in vitro* activity against *C. difficile* strains that are resistant to linezolid and/or moxifloxacin (for example, BI/NAP1/027), suggesting that cross-resistance is rare[42]. In a completed phase II RCT, 84 adults with first CDI or first recurrence of CDI were randomly assigned 1:1:1:1 to receive oral vancomycin 125 mg four times daily or cadazolid 250, 500 or 1,000 mg twice daily for 10 days[43]. Clinical cure rates of vancomycin and all three doses of cadazolid were similar. All three cadazolid doses resulted in lower recurrence rates than vancomycin (18–25% versus 50%), but this difference was not statistically significant in this small phase II trial. No evidence of resistance was found in isolates recovered before and after treatment with cadazolid[44].

Box 1 | **Symptoms, signs and features of CDI**[11,109]

Asymptomatic carriage
- No signs or symptoms of CDI

Mild or moderate CDI
- Diarrhoea (≥3 unformed stools in a 24 h period)
- Presence of mucus or occult blood in stool (haematochezia and/or melena are rare)
- Fever
- Abdominal cramping, discomfort or pain

Severe CDI
- Leukocytosis (>15,000 white blood cells/μL)
- Elevated serum creatinine levels (>1.5-fold higher than pre-morbid serum creatinine levels)
- Pseudomembranous colitis

Severe, complicated CDI
- Ileus (radiographical or clinical)
- Hypotension, shock or sepsis
- Toxic megacolon
- Abdominal perforation
- Requires intensive care transfer and/or admission for management of colitis
- Requires surgery for CDI-related complication (such as megacolon, perforation or refractory colitis)
- Death attributable to CDI

CDI, *Clostridium difficile* infection.

Biotherapeutics

Biotherapeutic approaches to prevent and treat CDI are garnering considerable interest. These efforts are driven largely by use of faecal microbiota transplantation (FMT) and oral FMT variations, which are emerging rapidly as treatments for multiply recurrent CDI (as in, more than one recurrence). Antibiotic disruption of the intestinal microbiota is the postulated mechanism that enables ingested *C. difficile* spores to vegetate, colonize the gut, produce toxins and cause clinical diarrhoea and colitis[24,45]. These microbiota disruptions are well-documented in adults using 16S ribosomal RNA gene amplification and have demonstrated the marked changes that result from antimicrobial exposure[22–24]. Patients with multiply recurrent CDI have markedly altered gut microbiota in terms of both bacterial diversity and numbers[24]. An alternative biotherapeutic approach does not entail gut microbiota replacement but rather focuses on the use of a nontoxigenic *C. difficile* strain to outcompete and replace the toxigenic strain, thus preventing further toxigenic colonization and CDI symptoms[46,47].

A wide variety of non-FMT probiotic preparations are undergoing clinical evaluations as CDI preventives. Multiple systematic reviews and meta-analyses

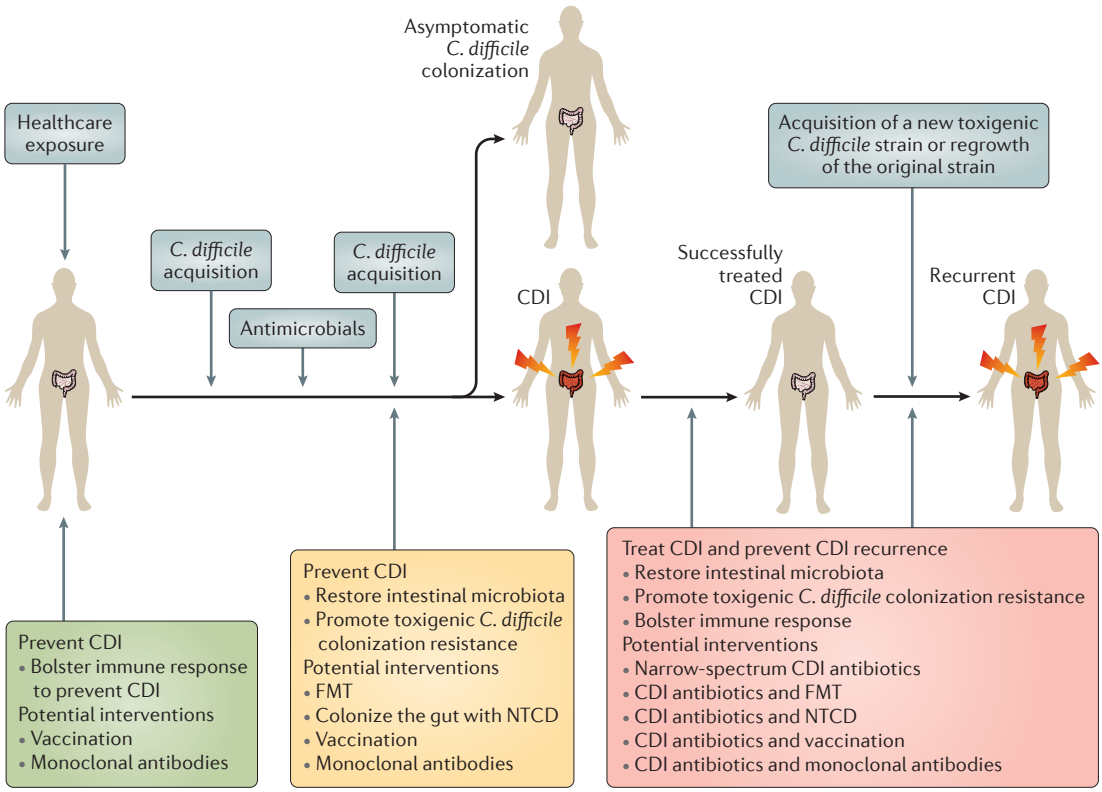

Figure 1 | **Emerging strategies for the prevention and treatment of CDI and subsequent recurrences.** CDI, *Clostridium difficile* infection; FMT, faecal microbiota transplantation; NTCD, nontoxigenic *Clostridium difficile*.

of probiotics suggest there might be some benefit for CDI prevention[48–52]. However, the largest double-blind, placebo-controlled RCT using lactobacilli and bifidobacteria as probiotics failed to show benefit[53]. In addition, available meta-analyses primarily include small studies and widely varied probiotic products. As probiotics constitute a review topic of their own, we do not include them here.

Faecal microbiota transplantation

The potential benefit of restoring colonization resistance was demonstrated by early studies of administration of faecal extracts or synthetic mixtures of faecal bacteria via enema or nasogastric tube[54,55]. Recurrent CDI episodes were interrupted by rectal instillation of a mixture of 10 faecal aerobic and anaerobic bacteria, which restored *Bacteroides* spp. to the recipient faecal flora[55]. Drekonja and colleagues[56,57] have summarized the very favourable published literature describing FMT for preventing CDI recurrences. Of note, to date, most FMT studies have been uncontrolled case series.

Van Nood *et al.*[58] performed an unblinded RCT comparing FMT (administered via nasoduodenal tube) to oral vancomycin (14 days) or vancomycin (14 days) plus gastrointestinal lavage in patients with 1–9 prior CDI recurrences. The FMT group received 4 days of vancomycin followed by bowel lavage before nasoduodenal FMT. The study only included 43 patients and was stopped early because of widely different response rates in the control and FMT arms. 13 of 16 (81%) patients in the FMT arm had sustained resolution of diarrhoea after the first faecal infusion compared with 4 of 13 (31%) patients who were treated with vancomycin and 3 of 13 (23%) who were treated with vancomycin plus bowel lavage ($P = 0.008$ and 0.003, respectively). Repeat FMT in patients who failed their first FMT resulted in success in 2 of 3 patients, raising FMT response rate to 94%.

Cammarota *et al.*[59] demonstrated similar efficacy of FMT in a RCT of 39 adults with recurrent CDI (median of three recurrences per patient). Of 20 patients receiving FMT via colonoscope, 13 (65%) were successful (no recurrences within 10 weeks of FMT) after a single FMT. Of the remaining seven patients, the first two patients who received FMT both had pseudomembranous colitis and relapsed within 1 week. They eventually were treated with vancomycin and died of sepsis. This finding resulted in a change of protocol to administer repeated FMT every 3 days (total of 2–4 FMTs per patient) to patients with pseudomembranous colitis (identified via colonoscopy) until this condition had resolved. Six of seven (86%) patients with pseudomembranous colitis received multiple infusions of donor faeces, and five of six (83%) patients with pseudomembranous colitis who received multiple donor faeces infusions were cured. Only five of 19 (26%) patients receiving vancomycin (10 days followed by pulsed dosing of 125–500 mg every 3 days for at least 3 weeks) had no further CDI recurrences within 10 weeks after completing the course of vancomycin. As patients in the

vancomycin group did not undergo colonoscopy, the proportion of those patients with pseudomembranous colitis is unknown. Although repeating FMT in patients who fail is frequently done, in both the van Nood *et al.*[58] and Cammarota *et al.*[59] studies, management of initial failures differed between the FMT and vancomycin groups. For example, vancomycin failures were not permitted a second treatment with vancomycin, whereas additional FMTs were permitted. Whether permitting additional courses of antibiotics in the vancomycin groups would have improved outcomes in those patients is unknown.

Unanswered questions remain about the preferred administration route for FMT, with enema, nasoduodenal, upper endoscopy and colonoscopy commonly used. Morbidity related to FMT administration is not trivial. For example, nasoduodenal tube insertion requires radiography to confirm placement and could result in abdominal perforation. Endoscopy and colonoscopy require sedation, which increases cost and potential for adverse events. Nasogastric and colonoscopic FMT administration have been previously compared, and similar effectiveness and safety were reported in this small study of 20 patients[60].

Owing to the difficulties in acquiring fresh stool from donors and the time and cost required for screening donors for pathogens, there is great interest in freezing stool from volunteers (paid or unpaid) until testing is complete[61]. Freezing does not seem to result in any loss of effectiveness[62], and frozen FMT recipients seem to develop a gut microbiota resembling that of the donor[63]. In a double-blind noninferiority trial of FMT in Canada, 232 adults with recurrent or refractory CDI were randomly assigned to receive either a frozen-thawed FMT product or fresh FMT product[62]. In the modified intention-to-treat population, the clinical resolution was 81 of 108 (75.0%) for the frozen FMT group and 78 of 111 (70.3%) for the fresh FMT group (difference, 4.7% (95% CI, –5.2% to infinity); $P<0.001$ for noninferiority)[62]. This finding was notable because of the logistical advantages of frozen FMT.

The RCT of nasogastric versus colonoscopic delivery of faecal microbiota transplants used frozen stool preparations in 20 patients (10 in each treatment arm) with a median of four (range 2–16) prior CDI relapses. Diarrhoea resolution was achieved in 14 patients (70%) after a single FMT (8 of 10 in the colonoscopy group and 6 of 10 in the nasogastric group)[60]. Thus, for the four RCTs of FMT, the response rate for one FMT was 199 of 275 (72.4%) patients, whereas for the 480 patients in 21 nonrandomized case-series studies who received FMT for recurrent CDI, 85% had CDI resolution without recurrence[57]. For indications other than recurrent CDI, such as refractory CDI and first episode of CDI, few FMT studies are reported and success rates were promising but highly variable[57,64].

In addition to commercially developed frozen FMT preparations available for purchase (for example, from OpenBiome), there is increasing interest in developing oral capsules to simplify administration[65,66]. Oral preparations have included thawed frozen FMT filtrates added to capsules just before administration, and fresh FMT filtrates added to capsules and freezing them before administration. Administration ranged from 6–22 (mean 10) capsules per day for 1 day to 15 capsules per day for 2 days[65,66]. Treated patients had a history of recurrent CDI, and response rates were 70%[66] and 68%[65], respectively, after one administration. Various lyophilization methods are also being applied to develop a dry capsule preparation that does not require frozen storage[67].

Ultimately, because of unknown risks of transplanting donor stool, the goal is to discover the essential bacteria in FMT that will restore colonization resistance and to prepare these microorganisms through fermentation under good manufacturing practice. In this regard, a large group of spore-forming bacteria has been isolated from faeces by treating with alcohol or heat to kill all vegetative cells. The isolated spores were administered orally. In two dosing cohorts, 1.5×10^9 and 1×10^8 colony-forming units, CDI recurrence was prevented in 13 of 15 (87%) and 10 of 11 (91%) patients, respectively[68]. These preliminary observations have not

Table 1 | **Antibiotic therapies currently in clinical development for CDI**

Antibiotic	Mechanism of action	Clinical status (ClinicalTrials.gov identifier)	Published clinical data
Surotomycin (CB-183315)	Disrupts bacterial cell membrane	Phase III NCT01597505 and NCT01598311	Phase II trial results: rates of CDI recurrence among 210 adults with CDI were 36%, 28% and 17% within 28 days post-treatment with vancomycin 125 mg four times daily, surotomycin 125 mg twice daily and surotomycin 250 mg twice daily, respectively[37]
Cadazolid	Protein synthesis inhibitor primarily Fluoroquinolone moiety also confers weak inhibition of DNA synthesis	Phase III NCT01983683 and NCT01987895	• Phase II trial results: clinical CDI cure rates among 84 adults receiving vancomycin or one of three different doses of cadazolid were similar • All three doses of cadazolid resulted in lower recurrence rates than vancomycin (18–25% versus 50%)[43]
Ridinilazole (SMT19969)	DNA synthesis inhibitor	Phase II NCT02092935	Phase I trial results: among healthy adults, SMT19969 resulted in high faecal drug levels, low plasma drug levels, and no reported serious adverse events[33]

CDI, *Clostridium difficile* infection.

yet been formally published but are available in abstract form. Furthermore, the preparation used required isolation from donor stool and the number of bacterial species was not disclosed.

Work in animal models also has identified several bacterial species that might be essential for restoring colonization resistance. A mixture of six bacterial species (*Staphylococcus warneri, Enterococcus hirae, Lactobacillus reuteri*, and three novel species, *Anaerostipes* spp. nov., *Bacteroidetes* spp. nov. and *Enterorhabdus* spp. nov.) restored colonization resistance in mice[69]. Also in mice, *Clostridium scindens*, a bile acid 7a-dehydroxylating intestinal bacterium, promoted resistance to CDI and, when administered, enhanced resistance to infection in a secondary bile-acid-dependent fashion[70]. Thus, identifying specific elements of the microbiota could optimize safety and efficacy of future biotherapeutic treatments and prevention strategies.

Screening donor stool and blood for infectious agents requires extensive and expensive methods that are well described[71]. Infectious complications attributed to FMT are rare; two patients developed norovirus gastroenteritis after FMT from asymptomatic donors[72], and one patient with Crohn's disease developed *Escherichia coli* bacteraemia after FMT[73]. Complications from FMT instillation include upper gastrointestinal bleeding after nasogastric tube insertion, colon perforation during colonoscopy[74] and fatal aspiration during sedation for colonoscopy[75]. Of perhaps more concern are the currently unknown and unintended long-term infectious and noninfectious consequences of FMT. For example, there are reports of a patient with ulcerative colitis who had a flare of previously quiescent colitis after FMT for recurrent CDI[76] and a patient who developed obesity after FMT[77]. Large RCTs with long-term follow-up will be required to identify the as yet unknown potential adverse events following FMT.

Nontoxigenic Clostridium difficile

An alternative biotherapeutic approach to FMT is oral administration of spores of a single M3 strain of nontoxigenic *C. difficile* (NTCD-M3)[46,47]. NTCD-M3 lacks genes for toxin production and has been found to asymptomatically colonize hospitalized patients[78]. This nontoxigenic strain prevents CDI in hamsters when challenged with toxigenic *C. difficile*[79,80]. NTCD-M3 was obtained from hospitalized patients, identified using restriction endonuclease analysis typing, and was selected for its high frequency of isolation from colonized inpatients. After establishing safety and ability to colonize volunteers age 60 years and older at doses ranging from 1×10^4 spores per day to 1×10^8 spores per day for 14 days, safety and efficacy were then investigated in patients who were treated with metronidazole or vancomycin for their first CDI episode or first CDI recurrence. The primary aim was to determine safety, and secondary end-points included colonization with NTCD-M3 and clinical prevention of CDI recurrence.

The double-blind RCT was performed in 168 patients who received either placebo or NTCD-M3 at doses of 1×10^4 spores per day for 7 days, 1×10^7 spores per day for 7 days or 1×10^7 spores per day for 14 days[46] (FIG. 2). Colonization with NTCD-M3, defined as detection in stool at any time following administration, occurred in 69% of patients and was higher after 1×10^7 spores per day versus 1×10^4 spores per day (72 of 84 (86%) versus 28 of 41 (68%), respectively; $P = 0.022$). CDI recurrence rate was 13 in 43 (30%) in patients receiving placebo and 14 of 125 (11%) in patients receiving NTCD-M3 ($P = 0.006$), with the lowest recurrence rate of 2 of 43 (5%) in patients receiving 1×10^7 spores per day for 7 days ($P = 0.010$ versus placebo). Reduced recurrence correlated with NTCD-M3 colonization (2 of 86 (2%) patients colonized with NTCD-M3 versus 12 of 39 (31%) patients not colonized with NTCD-M3; $P < 0.0001$). NTCD-M3 colonization was transient and all patients lost colonization by 22 weeks (FIG. 2). The mechanism by which CDI recurrence is prevented is unknown. NTCD-M3 has been speculated to outcompete and replace toxigenic *C. difficile* in patients and, once established, prevent further colonization by toxigenic strains. Although not proven, it is also postulated that decolonization with NTCD-M3 results from recovery of the gut microbiota to its pre-morbid state, which restores colonization resistance.

Immunological therapies

Much has been learned about the host immune response to *C. difficile* over the past 30 years (reviewed in detail elsewhere[10]). Levels of serum IgG antibodies against *C. difficile* toxins A and B are higher in patients with asymptomatic *C. difficile* colonization than adults who develop diarrhoea secondary to *C. difficile*[10]. These data suggest that humoral immunity to *C. difficile* toxins protects against developing CDI. In patients with CDI, development of measurable IgM and IgG against *C. difficile* toxin A protects against CDI recurrences[81]. This observation, suggesting the preferentially protective effect of antibodies against toxin A, is interesting because an experiment using a hamster model of CDI and genetic knockout models of the toxin genes suggests that toxin B is essential for virulence[82]. Nonetheless, the important observations of the host immune response to *C. difficile* suggested a role for passive and active immunization strategies for CDI prevention and treatment. As extensive animal data are available providing proof-of-concept for passive and active immunization strategies for CDI[83], several immunological therapies are in clinical development in humans.

Passive immunization: luminal antibodies

Passive immune protection of humans with CDI through oral administration of a whey protein concentrate (WPC) prepared from the colostrum of cows that had been immunized against *C. difficile* toxins has been studied (that is, hyperimmune bovine colostrum or HBC). HBC is an attractive therapeutic option because it contains anti-toxin antibodies that resist degradation when passing through the gastrointestinal tract[84,85]. Preclinical studies demonstrated that an anti-*C. difficile* WPC retains neutralizing activity in ileal fluid[85] and in stool[84] when given orally to healthy adult volunteers.

In a subsequent noncomparative clinical study[86], an anti-*C. difficile* WPC, in addition to either vancomycin or metronidazole, was administered to 101 adults with CDI. Only 10% of patients receiving WPC had a recurrence of CDI. The authors reported this finding as evidence of efficacy by comparing their results to the 20–25% recurrence documented in previous CDI epidemics in adults. A subsequent double-blind RCT comparing WPC and metronidazole in 38 adults with CDI[87], which was interrupted prematurely because of sponsor bankruptcy, demonstrated similar CDI recurrence rates in each group (55% in the metronidazole group versus 56% in the WPC group).

In a study published in 2015, HBC was assessed in a piglet model of CDI[88]. 24 h after inoculating 19 piglets with *C. difficile* spores, 10 piglets received HBC and nine control piglets received nonimmune colostrum. Most of the control piglets experienced progression of CDI severity, whereas CDI in piglets treated with HBC either remained mild or resolved. Bovine colostrum is currently publicly available as a dietary supplement and has demonstrated potential clinical benefit in other infectious and noninfectious conditions, such as cryptosporidiosis, rotavirus and failure to thrive[88–90]. Thus, HBC might emerge as a potential CDI treatment in the future.

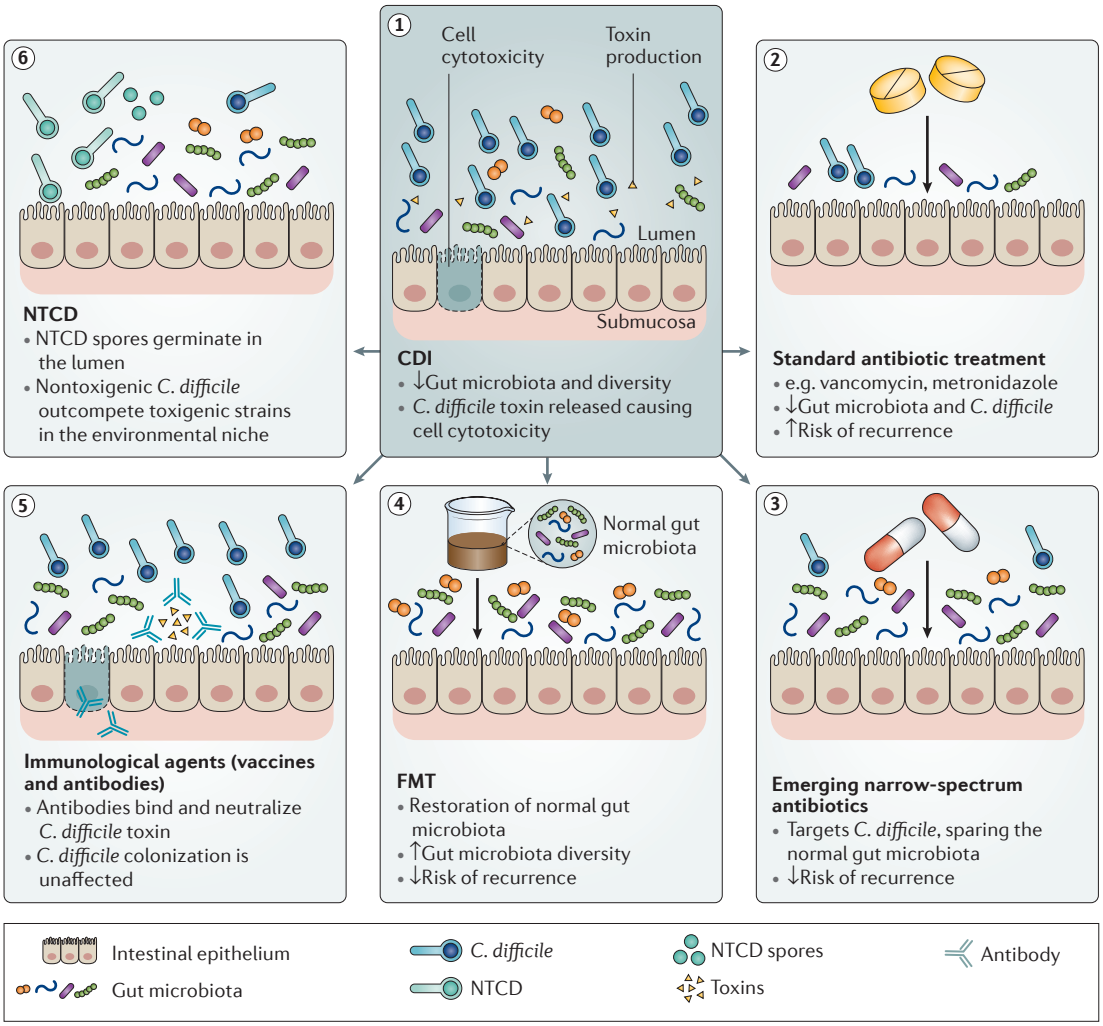

Figure 2 | **Underlying mechanisms for emerging treatment approaches for CDI.** After antibiotic exposure, depletion of the gut microbiota permits intestinal colonization by *C. difficile*; production of *C. difficile* toxin B leads to cytotoxicity of the mucosal epithelial barrier, resulting in acute diarrhoeal illness (1). Although standard CDI antibiotic therapies suppress *C. difficile*, they also further suppress the gut microbiota, increasing the risk of CDI recurrence (2). Emerging narrow-spectrum antibiotics suppress *C. difficile* whilst sparing the gut microbiota, potentially reducing the risk of CDI recurrence (3). FMT restores a more normal gut microbiota diversity, which promotes resistance to *C. difficile* colonization and reduces the risk of CDI recurrence (4). Anti-toxin antibodies, produced as a result of either passive immunization with monoclonal antibodies or vaccination, are released from the systemic circulation into the gut lumen, neutralize *C. difficile* toxins and prevent CDI (5). Spores of NTCD strain germinate in the gut; the vegetative NTCD microorganisms colonize the gut, outcompete toxigenic *C. difficile* and prevent CDI recurrence (6).
CDI, *Clostridium difficile* infection; FMT, faecal microbiota transplantation; NTCD, nontoxigenic *Clostridium difficile*.

Table 2 | **Vaccines currently in clinical development for CDI**

Vaccine product	Antigen	Formulation and schedule	Target population	Clinical status (ClinicalTrials.gov identifier)
Sanofi Pasteur *C. difficile* toxoid vaccine	Formalin-inactivated toxins A and B from VPI 10463	Intramuscular injection days 0, 7 and 30 Placebo comparator	Age >50 years	Phase III NCT01887912
Valneva Austria GmbH VLA84 *C. difficile* vaccine	Recombinant fusion protein of toxin A and B binding regions	+/– Aluminium adjuvant, intramuscular injection days 0, 7 and 28 Placebo comparator	Age 50–64 years Age >65 years	Phase II NCT02316470
Pfizer 3-dose *C. difficile* vaccine	Genetically modified and chemically treated recombinant vaccine	+/– Adjuvant, intramuscular injection days 1, 8 and 30 Placebo comparator	Age 50–85 years	Phase II NCT02117570 and NCT02561195

CDI, *Clostridium difficile* infection.

Passive immunization: intravenous antibodies

Intravenous immunoglobulin (IVIG) products contain neutralizing levels of IgG against *C. difficile* toxins A and B, albeit at varying levels[91]. The role of IVIG for CDI has only been assessed in several small uncontrolled, nonrandomized, retrospective studies[92]. Among 11 small case reports and case series of patients with CDI, 40 of 46 (87%) patients receiving a wide variety of IVIG dosing regimens had clinical resolution of diarrhoea and 14% had a recurrence of the infection[92]. However, because IVIG has not been assessed in a clinical trial at this time, convincing evidence of clinical efficacy of IVIG for CDI is lacking.

Although clinical benefit of IVIG has not been demonstrated, monoclonal antibodies against *C. difficile* toxins A and B might be beneficial. After success in a hamster model and confirming safety in healthy human volunteers, monoclonal antibodies against *C. difficile* toxins A (CDA1, also known as MK-3415 and actoxumab) and toxin B (CDB1, also known as MK-6072 and bezlotoxumab) were studied in a double-blind, placebo-controlled phase II RCT of 200 adults with CDI[93]. All participants received metronidazole or vancomycin, and study participants were randomly assigned to receive CDA1–CDB1 infusion or placebo. Compared with the placebo group, patients receiving CDA1–CDB1 had a lower recurrence rate within 12 weeks of enrollment (7% versus 25%, $P<0.001$), including those with CDI caused by *C. difficile* strain BI/NAP1/027 (8% versus 32%, $P=0.06$) and those with two or more previous CDI episodes before enrolment (7% versus 38%, $P=0.006$). No differences were observed between groups in time to diarrhoea resolution, hospitalization days and diarrhoea severity. Rates and severity of adverse events were similar. Subsequent animal work in the gnotobiotic piglet model indicated that CDB1 antibody alone was sufficient to protect against CDI, whereas CDA1 had no protective benefit and might have worsened disease[94].

Two phase III RCTs (MODIFY I and MODIFY II) of CDA1–CDB1 (now known as MK-3415A and actoxumab–bezlotoxumab) and the individual antibody components have been completed. In the MODIFY I trial[95], 1,452 adults receiving standard of care antibiotic therapy for primary or recurrent CDI were randomly assigned to receive either actoxumab alone, bezlotoxumab alone, both monoclonal antibodies, or placebo. CDI recurrence within 12 weeks was less frequent in patients receiving both monoclonal antibodies (15.9%, $P<0.0001$) or bezlotoxumab alone (17.4%, $P=0.0003$) compared with the placebo group (27.6%). Actoxumab resulted in a similar CDI recurrence rate as placebo (25.9%). Safety end-points were similar between the groups receiving both monoclonal antibodies, bezlotoxumab alone, or placebo, but serious adverse events and death were more common in those receiving actoxumab alone. In MODIFY II[96], 1,203 adults receiving standard of care antibiotic therapy for primary or recurrent CDI were randomly assigned to receive bezlotoxumab alone, both monoclonal antibodies, or placebo. CDI recurrence within 12 weeks was less frequent in patients receiving both monoclonal antibodies (14.9%, $P<0.0001$) or bezlotoxumab alone (15.7%, $P=0.0003$) compared with the placebo group (25.7%). The CDI recurrence rate between both monoclonal antibodies and bezlotoxumab alone were similar. Safety end-points were similar between the groups receiving both monoclonal antibodies, bezlotoxumab alone, or placebo. These studies suggest that a monoclonal antibody against toxin B is an efficacious adjunctive agent for reducing CDI recurrence.

Vaccines

Among approaches for CDI prevention, vaccines hold the only prospect for sustained long-term protection against future CDI episodes. Biotherapeutic approaches are subject to negation by repeated gut microbiota perturbation from further antibiotic use, and passive monoclonal antibodies will decline with time. Currently, three vaccine candidates are undergoing phase II and III clinical evaluation for CDI prevention (TABLE 2)[97]. One candidate toxoid vaccine being developed by Sanofi Pasteur has undergone multiple phase I trials, and recurrent CDI prevention was demonstrated in a phase II clinical trial in three patients[98,99]. The latest results of phase I testing of three adjuvanted doses (2, 10 and 50 μg) of this toxoid vaccine administered intramuscularly at 0, 28 and 56 days to healthy adult volunteers 18–55 years and >65 years were published in 2012 (REF. 100). In this study, seroconversion to toxin A

Table 3 | **Characteristics of potential interventions for prevention of CDI**

Intervention	Effectiveness in humans	Time to prevention onset	Duration of prevention	Use for primary CDI prevention	Use for recurrent CDI prevention	Projected cost
FMT or derivatives	Excellent for prevention of multiply recurrent CDI	Rapid (1–2 days)	Likely to be effective until further antibiotics are given	Untested	Yes	Low
Nontoxigenic *C. difficile*	Excellent for first and second CDI recurrence prevention	Rapid (1–2 days)	Effective for duration of colonization and thereafter until further antibiotics	Untested, but effective in animal models	Yes	Low
Monoclonal antibodies	Excellent for first and second CDI recurrence prevention	Very rapid (immediate)	Unknown, but not expected to persist beyond several half-lives	Untested	Yes	High
Injectable vaccine	Unknown, only 3 patients tested	Slow (weeks to months)	Unknown, but expected to be long	Yes	Unknown, depends upon time required for antibody response	Low
Oral vaccine	Unknown, no patients tested	Slow (weeks to months)	Unknown, but expected to be long	Yes	Unknown, depends upon time required for antibody response	Low

CDI, *Clostridium difficile* infection; FMT, faecal microbiota transplantation.

was more rapid and robust than for toxin B. Younger volunteers (18–55 years) had a more rapid antibody response than older volunteers, the expected target audience for the vaccine. Seroconversion to toxin B failed to occur in 75% of elderly (≥65 years) volunteers at the highest dose until day 70, 14 days after the last injection, indicating that the vaccine might not be effective for weeks to months. Additional data from a phase II double-blind multi-stage RCT were presented in abstract form. The first stage determined the appropriate vaccine formulation by comparing five groups: low dose, low dose plus aluminum salt adjuvant, high dose, high dose plus adjuvant, and placebo[101]. The high dose plus adjuvant was more immunogenic than the three other formulations. The second stage assessed various dosing schedules of the high-dose plus adjuvant formulation: days 0, 7 and 30 versus days 0, 7 and 180 versus days 0, 30 and 180 (REF. 102). Vaccine given on days 0, 7 and 30 resulted in the preferred immune response. Because high-dose plus adjuvant vaccine given at days 0, 7 and 30 was well-tolerated, the current phase III RCT (clinicaltrials.gov identifier NCT01887912 (REF. 103)) is assessing this dose, formulation and schedule (TABLE 2).

The vaccine being developed by Pfizer was initially prepared by introducing mutations in the enzymatic cytotoxicity domain of *C. difficile*[104]. However, residual low-level toxicity remained that required additional formalin inactivation. Valneva has developed a fusion protein containing the toxin A and B receptor binding domains that is protective in hamsters. Phase I human testing using an accelerated four-dose schedule on days 0, 7, 28 and 56 with or without aluminum as an adjuvant has been completed[105]. Both Pfizer and Valneva have completed phase I testing, but results are not yet available.

Although not yet in clinical trials, the prospect of developing an orally administered mucosal CDI vaccine is highly attractive. Among the possible vectors for such a vaccine are *Bacillus subtilis* spores, which have been engineered to express *C. difficile* toxin A and B repeat binding regions fused to CotB and CotC outer spore coat proteins of *B. subtilis* spores[106]. Interestingly, use of the *C. difficile* toxin A binding region alone was most effective in raising secretory IgA responses to both toxins A and B. When administered to hamsters orogastrically on days 0, 14, 35 and 57, 75% (6 of 8) of hamsters survived challenge with *C. difficile* strain 630, indicating that an oral vaccine can be effective in experimental models.

Numerous questions regarding *C. difficile* vaccine effectiveness remain. Vaccine candidates targeting toxins A and B might prevent clinical illness but are not expected to alter gastrointestinal tract colonization. Thus, the need to prevent *C. difficile* colonization has generated much interest in targeting nontoxin surface protein antigens to prevent colonization. Thus far, only modest protection by vaccines directed against surface proteins has been achieved in animal models[107]. As implied from oral vaccine studies, it is unclear whether single toxin antigens or both toxin A and B antigens are needed for an effective vaccine. In addition, binary toxin is present in epidemic strains such as *C. difficile* strain BI/NAP1/027, and the need for targeting this antigen in vaccines is unknown[108]. Other questions include the crossreactivity of antibodies to differing antigens among multiple *C. difficile* strains, the need for adjuvants and the specific adjuvants to use, demonstration of immune response in high-risk elderly individuals, the optimal subject age for immunization, and the determination of duration of protection in humans.

Conclusions

Multiple strategies for the treatment and prevention of CDI are under investigation. Each has specific advantages and disadvantages (TABLES 1,3). These approaches are not mutually exclusive, and it is likely that multiple methods will be used based on successful completion of human clinical trials (FIG. 1; TABLE 1). We look forward to these major improvements in CDI management.

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Author contributions

Both authors contributed equally to all aspects of this manuscript.

Competing interests statement

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FURTHER INFORMATION

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