Gastroenterology Report, 6(1), 2018, 21–28

doi: 10.1093/gastro/gox041
Advance Access Publication Date: 18 December 2017
Review

REVIEW

Treatment of recurrent *Clostridium difficile* colitis: a narrative review

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Abstract

Clostridium difficile is a gram-positive, spore-forming, obligate anaerobic bacillus that was originally isolated from the stool of a healthy neonate in 1935. In high-income countries, *C. difficile* is the most common cause of infectious diarrhoea in hospitalized patients. The incidence of *C. difficile* infection in the USA has increased markedly since 2000, with hospitalizations for *C. difficile* infections in non-pregnant adults doubling between 2000 and 2010. Between 20% and 35% of patients with *C. difficile* infection will fail initial antibiotic treatment and, of these, 40–60% will have a second recurrence. Recurrence of *C. difficile* infection after initial treatment causes substantial morbidity and is a major burden on health care systems. In this article, current treatments for recurrent *C. difficile* infection are reviewed and future directions explored. These include the use of antibiotics, probiotics, donor faecal transplants, anion resins, secondary bile acids or anti-toxin antibodies.

Key words: *Clostridium difficile*; recurrent infection; faecal microbiota transplant; anion-binding resins; monoclonal antibodies; secondary bile acid

Introduction

Clostridium difficile is a gram-positive, spore-forming, obligate anaerobic bacillus that was originally isolated from the stool of a healthy neonate in 1935 [1]. It was first identified as a major infectious cause of antibiotic-associated diarrhoea in 1978 [2]. In high-income countries, it is the most common cause of infectious diarrhoea in hospitalized patients [3,4]. The endospores from *C. difficile* are similar to those of *Bacillus anthracis* and *Clostridium perfringens*, in that they are impervious to desiccation, temperature fluxes, freezing, irradiation and many antiseptic solutions including alcohol-based hand gels and quaternary ammonium-based cleaning agents. *C. difficile* spores are spread by the faecal–oral route, hand-to-hand contact and also by air-borne environmental dispersal in hospital wards [5].

Epidemiology of *C. difficile* infection (CDI)

The incidence of *C. difficile* infection (CDI) in the USA has increased markedly since 2000, with hospitalizations for CDI in non-pregnant adults doubling between 2000 and 2010 [6]. Based on data from US death certificates, it is the leading cause of gastroenteritis-associated mortality, with estimated deaths of 14 000 in 2007 [7], 29 000 in 2011 [6] and 44 500 in 2014 [8]. Data from the Center for Disease Control and Prevention for 2011 showed an annual toll in US health care facilities that was estimated to be 453 000 cases, 83 000 recurrences and 15 000 deaths, with an estimated annual cost of approximately $US40 billion [9]. Excess health care costs due to CDI have been estimated at $US4.8 billion dollars for acute care facilities alone [10].

Most CDI cases present during or shortly after antimicrobial use [11–13], although the risk can persist for up to 90 days

Submitted: 21 September 2017; **Revised:** 17 October 2017; **Accepted:** 31 October 2017

[14,15]. Clindamycin, other macrolides, third-generation cephalosporins, penicillins and fluoroquinolones are the antibiotics most frequently associated with CDI [16]. Use of such broad-spectrum antibiotics leads to increased patient susceptibility to CDI infection and decreased 'herd immunity', particularly in health care facilities such as hospitals and nursing homes. *C. difficile* has been found to have a large number of mobile genetic elements within its genome, inserted during its phylogenetic evolution. Conjugative transposons and bacteriophages allow acquisition of antibiotic resistance through horizontal transfer from other genetically unrelated bacteria [17]. Antibiotic selection pressure provides antibiotic resistant *C. difficile* strains with a competitive advantage over the normal host intestinal microbiota. The three most common *C. difficile* North American Pulsed-field-type (NAP) strains found across 10 geographic regions in the USA in 2009–11 were NAP1/UK Ribotype (RT) 027 (28%), NAP4/RT014 (10.2%) and NAP11/RT106 (9.1%) [6]. The three most common strains in the Scottish CDI epidemic of 2007–08 were NAP1/RT027 (12.8%), NAP 11/RT106 (38.7%) and NAP2/001(24.5%), all three of which showed resistance to cefotaxime, clindamycin, erythromycin, moxifloxacin and levofloxacin, compared to other less virulent strains [18]. Overall, 3174 CDI cases were recorded between December 2007 and May 2008 at 38 Scottish hospitals, with 285 deaths and a mortality rate of 9% [18].

Hypervirulent *C. difficile* Ribotype 027 strain

The hypervirulent NAP1, PCR ribotype 027 strain is characterized by high-level fluoroquinolone resistance, efficient sporulation, enhanced cytotoxicity, markedly high toxin production [19,20] and a mortality rate three times higher than less virulent strains (such as the 001 or 014 ribotypes) [21,22]. This is related to *C. difficile* NAP1 acquiring binary toxin (CDT) production from *C. perfringens* and mutational loss of the toxin repressor gene tcdC, which is the regulator for *C. difficile* exotoxin A and B transcription and synthesis. Toxin A (TcdA) causes increased intestinal permeability and fluid secretion, and Toxin B (TcdB) is cytotoxic, causing colonic inflammation [15]. This occurs through toxin inactivation of host intestinal G-proteins of the Rho and ras families via glucosylation. NAP1 strains can synthesize 16 times more Toxin A and 23 times more Toxin B than less virulent strains, leading to increased cytotoxicity and disease severity [23]. Patients with NAP1 CDI are more likely to develop fulminant pseudomembranous colitis, toxic megacolon and multi-organ failure (MODS) and require emergency colectomy [22,24,25]. Systemic absorption of TcdB appears to be more important than TcdA in contributing to extra-intestinal damage, host pro-inflammatory responses and systemic toxaemia in severe CDI [26]. Patients infected with *C. difficile* strains producing binary toxin have a 60% greater mortality than those infected with binary toxin-deficient strains [23].

Initial CDI treatment

Current recommendations for treatment of initial CDI include oral metronidazole or vancomycin for 10–14 days for mild or moderate disease, as well as cessation of antibiotic therapy that may have predisposed to the infection. For severe infections, oral vancomycin (± IV metronidazole) or oral fidaxomicin have been recommended [16]. In patients with a paralytic ileus, colonic diversion or dilated colon, rectal vancomycin may be a useful alternative to oral administration [15].

Recurrent CDI

Between 20% and 35% of patients with CDI will fail initial antibiotic treatment [27–30] and, of these, 40–60% will have a second recurrence [31,32]. The majority of recurrences are due to relapses of CDI with the original strain rather than re-infection with a different strain [15]. Resistance to vancomycin or metronidazole is not considered a factor in recurrent CDI, but such antibiotics may contribute to continued intestinal dysbiota. Recurrent infection is more common in older patients (>65 years), females, Caucasian patients, those with current antibiotic use, concomitant use of proton pump inhibitors and more severe initial disease [6,33]. The presence of comorbidities, anti-neoplastic chemotherapy, inadequate IgG antibody response to Toxin A after initial episode, inflammatory bowel disease, organ transplantation, chronic kidney disease, hypogammaglobulinaemia, immunodeficiency and exposure to an infant carrier or infected adult have also been recognized as risk factors [34–37]. The contribution of proton pump inhibitors (PPIs) to CDI remains unclear. *C. difficile* spores are resistant to gastric acid, but vegetative forms are susceptible. In community-acquired CDI patients, PPI exposure was observed in 31% of patients with CDI, with no exposure to antibiotics [15]. There have been reports of increased CDI risk with PPIs [38]; however, other studies have reported no increase in risk following adjustment for co-existent conditions [39–41].

Health care costs in recurrent CDI

In a recent comprehensive analysis of hospital costs associated with recurrent CDI, Rodrigues *et al.* found that each recurrent CDI patient had an average of 4.4 stool tests for *C. difficile* toxin and received an average of 2.5 prescriptions for oral vancomycin. Most patients with recurrent CDI (84%) required hospitalization and 6% required urgent total colectomy. The total mean cost per patient was US$34 104, comprising hospitalization (68%), surgery (20%) and drug treatment (8%) costs. When applied to US national costs associated with rCDI, an annual cost of US$2.8 billion was extrapolated [42].

Treatment of recurrent CDI

Antibiotic therapy

The management of an initial CDI recurrence includes repeat administration of either oral metronidazole or vancomycin for 10–14 days. This achieves sustained cure rates in only 50% of patients [37,43]. The use of metronidazole is not recommended beyond the first CDI recurrence due to the risk of azole metabolite neurotoxicity [44]. Second recurrences may be treated by fidaxomicin or by a tapered, pulsed vancomycin regime [16]. Fidaxomicin is a poorly absorbed, orally administered macrolide antibiotic that is bactericidal towards *C. difficile* as compared to metronidazole and vancomycin, which are bacteriostatic [44]. Fidaxomicin has a narrower spectrum of antimicrobial activity than first-line antibiotic therapy, which results in less disruption of the normal gut flora. In a randomized–controlled trial (RCT), it had a similar cure rate but a significantly lower rate of recurrence than treatment with vancomycin (13% vs 24%), although this was in non-NAP Type 1 strains [45]. Fidaxomicin is considerably more expensive than vancomycin, and may have less activity against NAP1 CDI [44]. Rifaximin, another rifamycin, has also been tested in small case studies [46,47].

Stool transplant

The human gut has been estimated to harbour over 160 bacterial species and greater than 10^{14} individual bacteria, the majority of which exist within the colon [48]. Antibiotics diminish specific commensal species, which usually suppress the growth of gut pathogens, allowing uninhibited growth of pathogens such as *C. difficile* [49,50]. Butyrate-producing bacteria are depleted in CDI, including *Ruminococcaceae* and *Lachnospiraceae* families. Butyrate is a short-chain fatty acid (SCFA). SCFAs are important in host energy production, intestinal epithelial cell homeostasis, immune function and normal gut microbial growth [51]. Recurrent *C. difficile* has furthermore been associated with a reduced number of *Bacteroidetes* and *Firmicutes*, both dominant gut flora [52]. Re-implanting such strains via faecal transplantation from healthy individuals can restore the normal gut microbial biodiversity, community structure and metabolomic functional profiles. Faecal microbiota transplant (FMT) substantially increases the amounts of secondary bile acids and restores SCFA production by gut microbiota [51]. Mean cure rates in recurrent CDI of 91–96% can be achieved with FMT, indicating that donor faecal transplantation is effective in treating recurrent disease after initial antibiotic therapy [52–55]. Larger longitudinal studies are required to assess regulatory concerns and long-term adverse events in patients after FMT [56].

A variety of routes of administration of FMT have been reported. To date, the best mode of treatment is still being determined [57]. Postigo and Kim reported a pooled analysis that indicated a marginally improved response, although not statistically significant, for nasogastric administration of donor faeces over colonoscopic application (93% vs 85%) [58]. In a 2014 systematic review of different routes of FMT, diarrhoea resolution rates varied according to the site of infusion: 81% in the stomach; 86% in the duodenum/jejunum; 93% in the caecum/ascending colon; and 84% in the distal colon [59].

Case series have reported excellent resolution rates following rectal enema [57,60,61] or colonoscopic administration [62–67]. Colonoscopic administration allows direct application throughout the colon and also terminal ileum where *C. difficile* can be found. However, it involves either inpatient or outpatient use of endoscopy facilities and must be undertaken with caution to minimize the risk of perforation in an already diseased colon. In addition, efficacy of this form of administration may also depend on the protocol used to cleanse the colon prior to application. Bowel preparations similar to those used prior to colonoscopy may reduce the density of *C. difficile* organisms including the metabolically inactive spores [65].

Application via a nasogastric or nasojejunal feeding tube allows delivery to the small bowel, with subsequent passage downstream to the distal ileum and colon without pre-preparation of the bowel. van Nood *et al.*, in a landmark RCT, found a single nasoduodenal infusion of donor faeces was associated with a significantly higher rate of resolution of recurrent CDI compared to a vancomycin regime with or without bowel lavage (81% vs 23% and 31%) [52].

Oral preparations of frozen faecal microbial transplant capsules have also been studied with excellent results reported. Youngster *et al.* reported a sustained cure after a single oral administration of 30 frozen donor faecal capsules in 147/180 patients with recurrent or refractory CDI [68]. A second administration was successful in 17/26 patients who relapsed, resulting in an overall resolution of CDI diarrhoea in 91% of patients. Advantages of this method include outpatient administration of capsules and no requirement for instrumentation of the digestive tract. FMT is also more cost-effective in recurrent CDI than oral vancomycin or fidaxomicin [69–71].

In addition to FMT, research is continuing on a defined Microbial Ecosystem Therapeutic (MET-1). This uses a defined microbial population of 33 different bacterial species, prepared under laboratory conditions, which is then administered. MET has emerged due, in part, to the safety concerns in FMT of potential transfer of unidentified pathogens to the recipient and the logistics of screening suitable FMT donors. Petrof *et al.* reported that one MET preparation, derived from the faeces of a healthy human volunteer, was successfully used to cure patients with recurrent CDI [72]. Further research into MET-1 has suggested it may be effective as a mode of recurrent CDI prevention. MET-1 decreased both local and systemic inflammation and the overall amount of detectable intestinal TcdA in a mouse model. This occurred despite there being no decrease in the intestinal *C. difficile* burden in the mouse stool [73].

Probiotics

The altered composition of gut microbiota in the setting of *C. difficile* infection has raised interest in the potential role of probiotics. Treatment aims to re-colonize and restore the diversity of flora following the disruption due to antibiotic treatment and *C. difficile* overgrowth [74].

Probiotics may act through a number of mechanisms. These include temporary colonization, production of bactericidal acids and peptides, and competition with *C. difficile* for nutrients and epithelial adhesion. *Lactobacilli* have been shown to suppress growth of *C. difficile* in hamsters [75]. Probiotic bacteria produce lactic acid, which lowers digestive tract pH, as well as bacteriocins, both of which can inhibit growth of *C. difficile* [76]. They may disrupt the binding of *C. difficile* Toxins A and B to intestinal epithelial cells and stimulate host IgA anti-toxin production [15,77]. *Bifidobacterium longum* and *breve* have been shown to reduce the cytotoxic effect of *C. difficile* on the human intestinal epithelial cell line HT29 [78]. This was related to the specific reduction of TcdB levels, particularly by *B. longum*.

Some studies have suggested a benefit from probiotics in the treatment or prevention of *C. difficile* infection [79–81]. A three-strain combination of *Lactobacillus acidophilus*, *L. casei* and *L. rhamnosus* (Bio-K+) was used to prevent primary CDI in 45 000 adult patients given any antibiotic. Patients were monitored for over 10 years and a 39% decrease in the rate of CDI cases was found [56]. Currently, the most promising agents appear to be a combination of *L. acidophilus* and *L. casei*, other mixed species, *Saccharomyces boulardii* or *L. rhamnosus* [82].

The tropical yeast *S. boulardii* produces a specific protease which cleaves TcdA and may also inactivate TcdA receptors [77,83]. A RCT of recurrent CDI treatments showed *S. boulardii* (1 g/day for 28 days) in combination with high-dose oral vancomycin (2 g/day for 10 days) was effective in reducing the CDI recurrence rate to 16.7% as compared to high-dose vancomycin/placebo (50%, $p = 0.05$). It was not effective in combination with low-dose vancomycin or metronidazole, as *C. difficile* was not completely cleared in these patients [28,44].

Allen *et al.*, in a large RCT from 2013, showed that probiotics did not prevent *C. difficile* recurrence [84]. The 2016 Australasian Society of Infectious Diseases (ASID) [85] and the Society for Healthcare Epidemiology of America (SHEA) 2010 Guidelines [86] do not recommend probiotics be used as a preventative or adjunctive treatment in any *C. difficile* management algorithm. A recent meta-analysis, however, demonstrated its efficacy in

primary CDI prophylaxis in patients receiving systemic antibiotic treatment [87]. Hence, probiotics may be considered for this indication by future Infectious Diseases Society guidelines.

The use of probiotics for CDI prevention and FMT for recurrent CDI treatment has led to interest in intestinal small molecule inhibitors and bacteriocins. These are produced by commensal intestinal microbiota and can inhibit toxigenic *C. difficile*. Ebselen and methyl cholate are two small molecule inhibitors of TcdB [88]. Examples of bacteriocins include thuricin CD, nisin, lacticin 3147, actagardine, mutacin and diffocins [89]. Nisin and Lacticin 3147 are produced by *Lactococcus lactis* and have efficacy against *C. difficile* that is comparable or superior to vancomycin or metronidazole. Their application is limited by their undesirable effects on the commensal gut microbiome. Studies are proceeding of bioengineered bacteriocins, which have enhanced activity against *C. difficile* RT027 and fewer effects on normal gut flora [89].

Re-colonization with non-toxigenic *C. difficile* strains

Not all strains of *C. difficile* produce toxins, and thus re-colonization with non-toxigenic strains has been investigated as a potential treatment. Gerding *et al.* reported the results of a phase II randomized, double-blind, placebo-controlled trial that involved 168 patients [90]. They found a preparation containing the non-toxigenic *C. difficile* strain M3 (VP20621; NTCD-M3) was effective, well tolerated and appeared to be safe, with few adverse events reported. CDI recurrence occurred in only 11% of patients receiving the preparation compared to 30% taking the placebo. Furthermore, only 2% of patients who were successfully colonized with the NTCD-M3 strain suffered recurrence, compared to 31% receiving the placebo.

There are, however, potential areas of concern with this line of treatment—specifically, the occurrences of horizontal transfer of the pathogenicity locus (PaLoc) containing the genes encoding TcdA and TcdB between remnant toxigenic strains and introduced non-toxigenic strains. This has been proven experimentally by Brouwer *et al.* [91] and has also occurred in circulating *C. difficile* populations from a single geographic location [92]. Further research is therefore required into the circumstances whereby transfer of the PaLoc occurs before re-colonization with non-toxigenic *C. difficile* spores could be used as a viable treatment modality.

Primary bile acids and anion-binding resins

The use of anion-binding resins has not been shown to be superior to standard antibiotic treatment in CDI, but may have a role as an adjunctive therapy. Up to 80% of primary bile salts (e.g. taurocholate, glycocholate and cholate) excreted in the bile are converted to secondary bile salts (e.g. deoxycholate) by normal intestinal flora. For example, bacterial 7α-dehydroxylase converts taurocholate to deoxycholate. Taurocholate can also be converted to chenodeoxycholate by *Bacteroides* species with 12α-dehydroxylase activity [93]. Some primary bile salts such as cholate stimulate *C. difficile* spores to germinate in the small intestine and caecum, whilst the primary bile salt chenodeoxycholate inhibits spore germination and outgrowth of vegetative cells in the colon. Deoxycholate still allows spores to germinate but vegetative cells cannot grow. In broad-spectrum antibiotic-treated hosts, the reduction in normal bacterial flora results in lower levels of commensal 7α-dehydroxylase and higher concentrations of primary bile salts. This allows *C. difficile* spores to rapidly germinate and the resulting vegetative cells to grow and subsequently produce exotoxins [94].

Giel *et al.* compared the effects of intestinal and caecal extracts from untreated and antibiotic-treated mice and related this to cholestyramine administration. Caecal contents from the antibiotic-treated mice stimulated colony formation of *C. difficile* spores and exotoxin B levels by 10 000-fold after 24 hours [94]. Cholestyramine decreased the ability of taurocholate to germinate *C. difficile* spores by 200-fold. When treated with cholestyramine, there was a decrease in the ability of the intestinal extracts from the clindamycin-treated mice to stimulate colony formation [94]. Whilst cholestyramine resin is not effective as a primary therapy [95], potential exists for its adjunctive use as a primary bile acid sequestrant in human CDI [96]. One disadvantage of anion resins is that they also bind luminal vancomycin [95].

Tolevamer is an anionic, soluble polystyrene compound shown to sequester CDI Toxins A and B. It was associated with a lower CDI recurrence rate compared to oral vancomycin [97]. Oral vancomycin 500 mg/day was superior to 6 g/day and 3 g/day of oral Tolevamer in time for resolution of diarrhoea in moderate to severe CDI (2.0, 2.5 and 4.0 days, respectively) and in efficacy (91%, 83% and 67% respective resolution). A more recent study showed that Tolevamer was inferior to both metronidazole and vancomycin in analyses of both primary and recurrent CDI, concomitant use of antibiotics, CDI severity and infection with hypervirulent NAP1 CDI strain. However, in the small cohort of patients who did respond to Tolevamer, the CDI recurrence rate was 4.5%, which was significantly better than recurrence rates for metronidazole (23.0%) or vancomycin (20.6%) [98].

Synthetic bile salt analogues

Given the ability of taurocholate to bind and activate *C. difficile* spores, it has been possible to test taurocholate agonists and antagonists of *C. difficile* spore germination [99]. One of the analogues, CAmSA, was found to be a strong competitive inhibitor of taurocholate-mediated *C. difficile* spore germination. It was active at concentrations approximately 275-fold lower than taurocholate and was four times more active than the natural inhibitor chenodeoxycholate [99]. When tested in a mouse model, CAmSA was able to prophylactically prevent murine CDI caused by two different CD strains and could be titrated to ameliorate CDI signs in a dose-dependent manner [100]. This raises the possibility of its use in prophylaxis against CDI.

Secondary bile acid

Recent research has highlighted the importance of secondary bile acids in the pathogenesis and potential treatment of CDI. A loss in microbially derived intestinal secondary bile acids can lead to increased susceptibility for CDI, particularly in hypervirulent strains [101]. Weingarden *et al.* reported that there were no secondary bile acids (lithocholate, deoxycholate) and an abundance of primary bile acids (taurocholate, cholate, chenodeoxycholate) in the faeces of recurrent CDI patients prior to FMT [102]. After successful FMT, there were no faecal primary bile salts and the levels of secondary bile salts were restored to those of healthy persons.

It was shown that germination of spores was variable amongst *C. difficile* strains in response to primary bile acids, and was possibly related to expression of the spore germinant CspC. This is a *C. difficile* serine protease bile acid receptor that was

most distinct in the NAP7/RT078 hypervirulent livestock-derived CDI strain [102].

Weingarden *et al.* subsequently reported that ursodeoxycholic acid (UDCA) inhibited *both* spore germination and vegetative growth of all *C. difficile* strains they tested [103]. UDCA is produced by microbial conversion of lithocholate in the colon. They suggested that oral UDCA may be useful in patients who were not suitable for FMT (e.g. recurrent CDI pouch ileitis) or were refractory to antibiotic and FMT treatment.

Monoclonal antibodies

The use of systemic monoclonal antibodies has been reported to decrease the rate of *C. difficile* recurrence [30,104]. Bezlotoxumab has been shown by X-ray crystallography to block the binding of Toxin B to host cells, thereby negating its action [105]. The initial double-blind RCT using IV antibodies against both Toxins A and B found the overall rate of recurrence of *C. difficile* infection was lower amongst patients treated with monoclonal antibodies versus placebo (7% vs 25%; $p < 0.001$). The recurrence rate among patients with the epidemic BI/NAP1/027 strain was 8% for the antibody group and 32% for the placebo group ($p = 0.06$). In the patients with more than one previous episode of CDI, respective recurrence rates after monoclonal antibody treatment versus placebo were 7% and 38% ($p = 0.006$) [30].

The subsequent MODIFY I and II trials showed that single-dose intravenous Bezlotoxumab in conjunction with standard oral antibiotic treatment resulted in a significantly lower (38%) rate of recurrent CDI infection than with placebo and standard antibiotic treatment alone [104]. The rate of recurrent CDI was even lower with Bezlotoxumab (51%) in patients >65 years of age. The initial CDI cure rates of the Bezlotoxumab/antibiotic and placebo/antibiotic-treated groups were similar in pooled data (80%). This is related to the rapid effect of standard antibiotic treatment-lowering Toxin B levels in the initial CDI episode. The protective effect of Bezlotoxumab against recurrent CDI was sustained for 12 weeks after administration. A single dose of monoclonal antibody was used due to the long half life (19 days). There was no benefit found with Actoxumab (a monoclonal antibody directed against Toxin A) alone, nor did its combination with Bezlotoxumab increase the efficacy of treatment [104]. This is despite previous epidemiological evidence that showed generation of endogenous anti-Toxin A IgG antibodies was protective against recurrent CDI [34]. The relative protective effect of host IgG antitoxins against Toxins A or B may also be species-specific [104].

The results of MODIFY I and II prompted the US Food and Drug Administration (FDA) to approve Bezlotoxumab in 2016 for use as secondary prevention for patients at a high risk of CDI recurrence (prior history of CDI and >65 years) [9,104]. Further research is still required at this stage to identify which patients will benefit most from Bezlotoxumab. Additional cost analyses will also need to be undertaken before such treatment becomes more widely used. The efficacy of a tetravalent vaccine with antibodies to CDI binary toxin in addition to Toxins A and B is still being evaluated [106].

Hyperimmune bovine colostrum (HBC)

Colostrum is the initial milk produced by a lactating mammal following parturition. Bovine colostrum is known to be rich in immunoglobulins (particularly IgG), which are stable in the gastrointestinal tract. This can provide passive protection to the infant calf from environmentally acquired enteric pathogens such as rotavirus, *Salmonella enterica*, enterotoxigenic *Escherichia coli*, *Clostridium difficile*, *C. perfringens* and *Cryptospridium parvum*. By repeatedly inoculating a gestating cow with specific antigens, it is possible to stimulate the production of colostrum containing high concentrations of antigen-specific antibodies known as HBC. Recent research has investigated the potential of HBC to prevent or treat *C. difficile*. In 2015, Sponseller *et al.* demonstrated that HBC produced following inoculation of cows with recombinant mutants of Toxins A and B had the potential to be used in primary CDI treatment [107]. They developed a model of gnotobiotic piglets transplanted with normal human gut microbiota and then exposed to *C. difficile*. The piglets treated with non-immune colostrum developed symptoms of *C. difficile* colitis whereas those treated with HBC only suffered mild disease. More recently, a TcdB-specific HBC has been developed and investigated by Hutton *et al.* [108]. They demonstrated that administration of TcdB HBC alone or in combination with spore or vegetative cell-targeted colostrum prevented and treated CDI in mice and reduced recurrence by 67%. The production cost of colostrum IgG antibodies is less than intravenous monoclonal antibodies. This suggests that HBC may be a cost-effective future treatment for human enteric infections such as *C. difficile* colitis.

Bacteriophage therapy

C. difficile is known to produce biofilms, which consist of aggregates of cells embedded in a matrix of self-produced extracelllar polymetric substance (EPS) [109–111]. The matrix binds spores and vegetative cells and protects them from oxidative stress whilst enhancing their adhesion to abiotic surfaces [111]. This allows persistence and proliferation of *C. difficile*. Recent studies have investigated the therapeutic potential of bacteriophages (viruses that specifically infect bacteria). Nale *et al.*, in 2016, studied the application of a four-phage cocktail on *C. difficile* ribotype 014/020 biofilms [112]. They found the phages prevented biofilm formation and penetrated established biofilms leading to lysis, plaque formation and a reduction in bacterial viability and biomass *in vitro*. They also reported an enhanced effect when used as an adjunct to vancomycin. This was undertaken in an animal model, but the results are promising for bacteriophage administration to become a future prophylactic and therapeutic intervention in human CDI and recurrent CDI.

Conclusion

Recurrence of CDI after initial treatment causes substantial morbidity and is a major burden on health care systems. There is good evidence that FMT for recurrent CDI is both clinically and cost-effective in achieving a permanent cure. Probiotics are readily available and may assist in prevention of relapse of infection, but further research is required in their role in recurrent CDI. Whilst anion-binding resins may not be first-line treatment, they may be of use in an adjunctive setting. Monoclonal antibodies have proven preventative effect in CDI relapse, and have thus been approved by the FDA. Ongoing research is currently underway into secondary bile acid treatments (UDCA) and development of multivalent toxin vaccines. The emergence of treatments that re-establish intestinal microbiota homeostasis and enhance host immunocompetence is therefore of great importance in the future prevention and treatment of recurrent CDI.

Conflict of interest statement: none declared.

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