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The potential for emerging therapeutic options for *Clostridium difficile* infection

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Abbreviations: NGS, next generation sequencing; CDI, *Clostridium difficile* infection; PMC, pseudomembranous colitis; PaLoc, pathogenicity locus; RNA, ribonucleic acid; RBS, ribosome binding site; R027, ribotype 027; CdtLoc, binary toxin locus; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; FMT, faecal microbiota transplantation; IDSA, Infectious Diseases Society of America; MIC, minimum inhibitory concentration; SHEA, Society for Healthcare Epidemiology of America; GIT, gastrointestinal tract; M21V, methionine to valine substitution at residue 21; DPC, Dairy Products Collection; ATCC, American Type Culture Collection; NVB, Novacta Biosystems Ltd; V15F, valine to phenylalanine substitution at residue 15; HIV, human immunodeficiency virus; IgG, immunoglobulin G; ETEC, enterotoxigenic *E. coli*; LTA, lipoteichoic acid; RBD; receptor binding domain; DNA, deoxyribonucleic acid; FDA, Food and Drug Administration.

Clostridium difficile is mainly a nosocomial pathogen and is a significant cause of antibiotic-associated diarrhea. It is also implicated in the majority of cases of pseudomembranous colitis. Recently, advancements in next generation sequencing technology (NGS) have highlighted the extent of damage to the gut microbiota caused by broad-spectrum antibiotics, often resulting in *C. difficile* infection (CDI). Currently the treatment of choice for CDI involves the use of metronidazole and vancomycin. However, recurrence and relapse of CDI, even after rounds of metronidazole/vancomycin administration is a problem that must be addressed. The efficacy of alternative antibiotics such as fidaxomicin, rifaximin, nitazoxanide, ramoplanin and tigecycline, as well as faecal microbiota transplantation has been assessed and some have yielded positive outcomes against *C. difficile*. Some bacteriocins have also shown promising effects against *C. difficile* in recent years. In light of this, the potential for emerging treatment options and efficacy of anti-*C. difficile* vaccines are discussed in this review.

Introduction

Clostridium difficile is a Gram-positive anaerobic sporeformer and is the etiological agent responsible for *C. difficile*-associated diarrhea. *C. difficile* was initially considered a harmless commensal of the gastrointestinal tract of infants, when originally isolated in 1935 but its role in nosocomial diarrhea and pseudomembranous colitis (PMC) was appreciated only in the 1970s.[1,2] The development of antibiotics for the treatment of infectious diseases in the 20th century has been a significant accomplishment. However, it is ironic that antibiotics, and in particular broad spectrum antibiotics, are the main etiological agents of one of the most notorious nosocomial infections, CDI. CDI has significant financial implications, and an estimated €5000–15000 is spent per CDI case in England and approximately €3 billion per year in the EU in total. The corresponding figure is about €2–4 billion per year in the US.[3,4] The majority of *C. difficile* strains produce toxin A and toxin B, which are responsible for the clinical manifestation of the disease. The recent emergence and widespread dissemination of 'hypervirulent' outbreak-associated *C. difficile* strains have caused problems for clinical practitioners. Furthermore, the ongoing problem of CDI recurrence post-antibiotic therapy, caused by perturbations of the gut microbiota, has encouraged scientists to seek alternative therapeutic options. Perhaps the most potent defense against CDI is the maintenance/restoration of a fully intact gut microbiota, providing protection through a complex process referred to as 'colonization resistance.'

This review focuses on the successes and failures of current and emerging treatment options for CDI. In particular, we focus on antibiotics and adjunctive therapeutic options which have the potential to replace the current standard metronidazole and/or vancomycin therapy. In this regard, recent *in vivo* studies and clinical trials conducted with alternative and/or adjunct anti-*C. difficile* therapeutic options are discussed.

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Roles of *C. difficile* toxin genes and disease

Until recently, investigating the role of *C. difficile* genes had been problematic. However, advancements in targeted mutagenesis systems have helped this cause.[5-7] A 19.6 kb pathogenicity locus (PaLoc) encodes the genes (*tcdA* and *tcdB*) for toxins A and B (TcdA and TcdB respectively), along with genes for putative positive and negative regulators of toxin expression (*tcdR* and *tcdC* respectively), as well as *tcdE*, encoding a putative holin protein (**Fig. 1A**).[8,9] TcdR functions as an alternative RNA

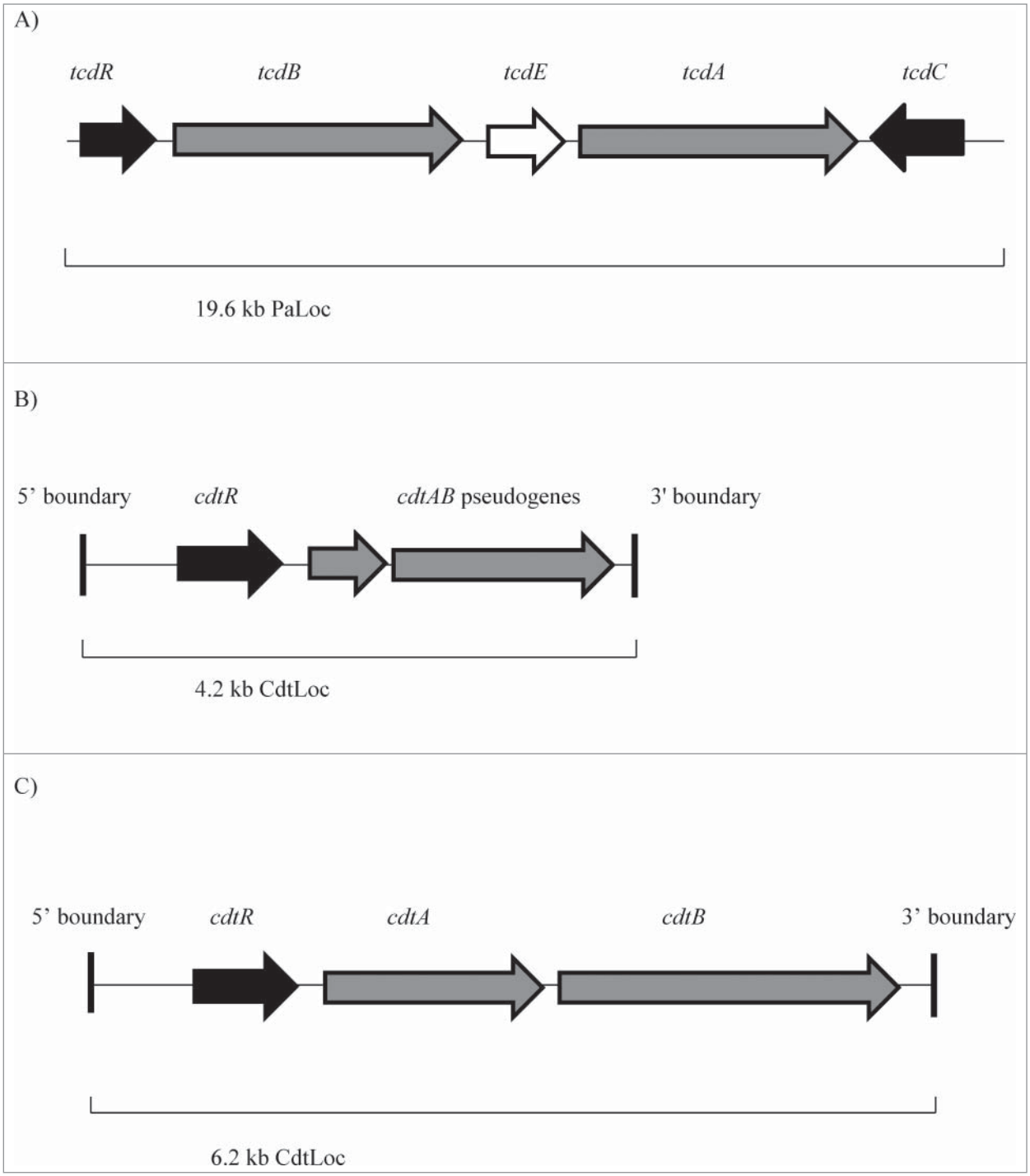

Figure 1. (**A**) *Clostridium difficile* pathogenicity locus (PaLoc). Schematic organization of the *C. difficile* PaLoc, which is 19.6 kb in length. *tcdA* and *tcdB* (shaded in gray) are the 2 genes encoding the 2 large *C. difficile* toxins, TcdA and TcdB respectively. *tcdR* (shaded in black) encodes a positive regulator of transcription, whereas *tcdC* (shaded in black) encodes a putative negative regulator/modulatory protein. *tcdE* (shaded in white) encodes a holin protein. Adapted from J Dupuy et al.[8] J Med Microbiol 2008; 57: 685–90 and Carter et al.[5] J Bacteriol 2007; 189: 7290–7301. (**B**) Binary toxin locus (CdtLoc) from *C. difficile* 630. Schematic organization of the binary toxin locus (4.2 kb in length) from the binary toxin-negative *C. difficile* 630 strain. The CDT binary toxin-encoding pseudogenes, *cdtAB*, are shaded in gray. The response regulator gene, *cdtR* is shaded in black. The highly conserved 5′ and 3′ boundaries are also indicated. Adapted from Carter et al.[5] J Bacteriol 2007; 189: 7290–7301. (**C**) Binary toxin locus (CdtLoc) from *C. difficile* QCD-32g58. Schematic organization of the binary toxin locus (6.2 kb in length) from the binary toxin-positive *C. difficile* QCD-32g58 strain. The CDT binary toxin-encoding genes *cdtA* and *cdtB*, are shaded in gray. The response regulator gene, *cdtR,* is shaded in black. The highly conserved 5′ and 3′ boundaries are also indicated. Adapted from Carter et al.[5] J Bacteriol 2007; 189: 7290–7301.

polymerase sigma factor, and thus behaves as a positive regulator of toxin gene expression.[10] In contrast, TcdC was initially thought to serve as a negative regulator of toxin production, destabilising the TcdR-holoenzyme, thus hindering transcription of the PaLoc.[8] However, in recent times, it has been demonstrated that transcription levels of the genes in the PaLoc and consequent total toxin production barely differs between a wild type *C. difficile* 630 Δ*erm* strain and its *tcdC* mutant, suggesting that TcdC may not be a key regulator of toxin expression in the strain.[11] It is noteworthy that earlier studies investigating the role of TcdC were *in vitro* investigations.[12,13] The *in vivo* mechanisms of this protein had remained largely unclear however. A recent *in vivo* study has now indicated that TcdC may not actually play a key role in *C. difficile* virulence.[14] Furthermore, in another recent study, it was noted that there was no decrease in *tcdC* expression levels during stationary phase of growth, implying that TcdC may serve a modulatory function instead of a previously-hypothesized repressive function.[15] Polymorphisms in the *tcdR-tcdB* intergenic region as well as in the *tcdR* ribosome binding site (RBS) in the 'non-hypervirulent' VPI 10463 strain (which still produces high levels of toxins) likely results in increased translation of TcdR, consequently leading to read-through transcription of the toxin genes. Such polymorphisms may account for the increased levels of toxins produced by some isolates.[15] In addition, the study found that epidemic-associated strains sporulated at an earlier stage and produced a greater number of spores than other non-epidemic-associated isolates. Thus, increased sporulation rates along with high level toxin production may explain the outbreak-associated nature of such 'hypervirulent strains.'[15] In an *in vitro* study, Vohra & Poxton noted that outbreak-associated R027 strains produced higher amounts of toxins in the logarithmic and stationary growth phases, compared to other ribotypes.[16] Moreover, epidemic-associated strains were found to produce more toxins and a greater number of spores relative to R012.[16] It was also particularly noteworthy that *tcdC* expression levels were not attenuated during stationary growth phase, as was previously thought, lending credence to the novel hypothesis that TcdC has a modulatory effect on toxin production, instead of a repressive one. In addition, an elevated level of expression of *tcdE* in R027 strains highlights its involvement in the release of the toxins. Therefore, it is hypothesized that a combination of factors, including greater toxin production, increased spore formation and increased expression of holin proteins may contribute to the epidemic-associated traits of certain strains.[16] Interestingly however, using isogenic strains of *C. difficile*, Carter et al. showed that a naturally occurring mutation in *tcdC* is responsible for the hypervirulence of epidemic *C. difficile* isolates.[12] Thus, the precise mechanisms of action of TcdC *in vivo* have yet to be definitively ascertained.

For a long time, the elucidation of the precise functions of TcdA and TcdB using a genetic approach was hampered by a lack of tools to isolate *C. difficile* isogenic toxin gene mutants. However, Lyras and co-workers as well as Kuehne and coworkers facilitated the study of isogenic mutants.[17,18] Syrian golden hamsters were infected with either toxin A^- mutants, toxin B^- mutants or wild type strains in a study.[17] Infection with the wild type strain resulted in the death of 90% of hamsters included in the study. Infection with toxin A^- mutants resulted in death of 94% of hamsters, indicating that toxin A is not crucial for disease. Interestingly, infection with toxin B^- strains only caused disease in 22% of hamsters, highlighting that toxin B was in fact the main virulence factor rather than toxin A, contrary to previous assumptions. More significantly, the study helped to explain the pathogenicity caused by toxin A^-B^+ isolates in clinical settings.[17]

Some *C. difficile* strains also produce an additional toxin called binary toxin (CDT). Importantly, the genes encoding binary toxin, designated *cdtA* and *cdtB* are not part of the PaLoc but are nevertheless found on the chromosome as part of the binary toxin locus (CdtLoc) (**Fig. 1B**).[5,19-21] CdtR is a response regulator encoded by CdtLoc and controls binary toxin production.[5] Isolates which do not produce binary toxin, such as *C. difficile* CD37, have a conserved 68 bp sequence in place of the CdtLoc.[5] The binary toxin-negative *C. difficile* 630 strain consists of fused *cdtAB* pseudogenes as part of a 4.2 kb CdtLoc, whereas binary toxin-positive isolates such as *C. difficile* QCD-32g58 consist of a 6.2 kb CdtLoc, composed of the 2 binary toxin genes *cdtA* and *cdtB*, in addition to the regulatory gene, *cdtR* (**Fig. 1B and C**).[5]

Therapeutic Options

Current treatment of CDI and antibiotics

The current treatment modalities for CDI involve the immediate discontinuation of antibiotics given to the patient for other diseases, and commencement of metronidazole and vancomycin administration post-haste. The rates of metronidazole treatment failure are significantly higher in patients who are still on other antibiotics, due to continued perturbations of the competing gut microbiota.[21] One study suggested that metronidazole and vancomycin were equally effective for mild cases of CDI, with treatment success rates of 90% and 98% respectively.[22] However, for more severe cases, vancomycin was the treatment of choice, as success rates for metronidazole and vancomycin were 76% and 97% respectively, though recurrence rates were similar i.e. 15% of metronidazole-treated patients, compared to 14% for vancomycin.[22] In this regard, it must be noted that slightly different success rates are reported for metronidazole and vancomycin against *C. difficile*, depending on a variety of factors, such as type of study conducted, sample size and geographical location. High failure rates for metronidazole, due to the emergence of the outbreak-associated R027 strains and also a rise in the number of elderly patients in hospitals affected by CDI who are already being treated with other antibiotics, have also been reported.[23,24] The treatment for non-epidemic and epidemic *C. difficile* strains appears to be similar, with metronidazole as the primary treatment choice for mild-moderate cases of CDI, followed by vancomycin for more severe CDI, according to the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines. Fidaxomicin has already been proven to have promising efficacy as a therapeutic for CDI. Other antibiotics such as ramoplanin, tigecycline and the rifamycin group of antibiotics

have shown potent activity against *C. difficile* and research is ongoing regarding their clinical efficacy against CDI. Fidaxomicin and faecal microbiota transplantation (FMT) is also strongly advocated by ESCMID for recurrent CDI cases.[25,26]

For mild-moderate CDI, 500 mg of oral metronidazole 3 times a day for 10–14 d is recommended, whereas 125 mg of oral vancomycin 4 times a day for the same duration is indicated for more severe cases of CDI.[27] Oral vancomycin, supplemented with intravenous metronidazole if necessary, is recommended for severe CDI by the IDSA. Although oral administration of metronidazole or vancomycin is optimal, metronidazole can also be administered intravenously as it is capable of reaching the intestinal lumen via diffusion across the inflamed colon. A prospective study conducted by Wenisch et al. demonstrated that oral metronidazole (7.4% mortality) was more effective against *C. difficile* than intravenous metronidazole (38.1% mortality).[28] Vancomycin may also be introduced intracolonically or as a retention enema. A dose of 100 mg of the glycopeptide teicoplanin twice a day in addition to metronidazole or vancomycin is recommended by the ESCMID for CDI.[29] However, fusidic acid and bacitracin do not seem to be as effective as glycopeptides or metronidazole and thus are contraindicated by ESCMID. Fusidic acid, oral bacitracin and teicoplanin are not recommended in the USA for CDI.[29]

Alternative vancomycin dosing strategies

Though vancomycin and metronidazole have been used for the treatment of CDI for the last 3 decades, recurrence and relapse of disease still remains a serious problem. About 50% of cases of recurrence of disease are due to relapse, whereby the original *C. difficile* strain that was culpable for the infection causes symptoms again, due to spores surviving the CDI treatment.[30] Alternative dosing regimens for vancomycin have been investigated to circumvent the problem of recurrent CDI. Pulsed vancomycin dosing, which involves short intermittent administration of vancomycin, as well as tapered dosing of vancomycin have proved successful. In a retrospective study conducted by McFarland et al. with a total of 163 recurrent CDI patients, a subset received vancomycin and a smaller subset received tapered doses of vancomycin, whereby vancomycin was decreased incrementally from 500 mg-3 g daily to 125–750 mg daily, over 3 weeks.[31] A 31% recurrence rate was noted for this tapered dose strategy compared to recurrence rates of 43–54% with conventional vancomycin dosing for 10 d. Pulsed vancomycin administration of 125–500 mg per day every 2–3 d for 27 d resulted in a 14% recurrence rate.[31] Although no randomized controlled trials have assessed such dosing regimens, the IDSA and the SHEA recommend the use of pulsed or tapered vancomycin dosing for second or third recurrences of CDI.[27] In cases of extremely severe *C. difficile* colitis, intracolonic vancomycin administration may have the potential to be used as an adjunct, to ensure higher concentrations of the drug in the colon.[32,33] This may involve the use of an intracolonic bolus with an intravenous solution of vancomycin. A rectal retention enema such as 500 mg of vancomycin in 1 L of saline solution may be another option. Kim et al. reported a 70% success rate in a recent trial whereas Apisarnthanarak et al. approximated a success rate of 57–75% for intracolonic vancomycin administration in a review of relevant case series.[32,33]

Fidaxomicin

Fidaxomicin is an oral macrocyclic antibiotic, produced by *Dactylosporangium aurantiacum*, targeted against *C. difficile*.[34] One of the main advantages of fidaxomicin is that it is tailored specifically toward *C. difficile*, with little impact on the commensal gut microbiota and has been shown to inhibit spore formation and toxin production in *C. difficile*.[25,36] This narrow spectrum of action is hugely beneficial as it permits quick restoration of the commensal gut microbiota in CDI patients, and thus decreases the risk of recurrence of disease due to overgrowth of *C. difficile*.[25,36] Another advantage is that it sustains a certain level of antibacterial activity for a more prolonged period, compared to metronidazole and vancomycin.[34] Therefore, it is capable of inhibiting *C. difficile* at concentrations lower than the MIC.

A number of *in vitro* and *in vivo* studies have highlighted the potential of fidaxomicin in combating *C. difficile*. Fidaxomicin MICs against 114 *C. difficile* isolates were reported to be between 0.008–0.125 μg/ml in a recent study by Rashid et al.[37] Fidaxomicin was shown to inhibit *C. difficile* toxin gene expression and consequent toxin production by measuring total mRNA and protein in another study.[38]. With respect to the propensity for fidaxomicin resistance development, isolates with attenuated sensitivity to the antibiotic displayed mutations in the *rpoB* gene as well as the gene CD22120 (a *marR* homolog) in an *in vitro* study.[39] Encouragingly, since fidaxomicin does not exhibit activity against Gram negatives, the possibility for development of resistance among other enteric bacteria is low.[40] Fidaxomicin was shown to interact in a synergistic manner with rifamycins in a study, while at the same time, having a reduced likelihood of resistance development.[41] It was also encouraging that no cross resistance with rifamycins was apparent in the *in vitro* study.[41]

With respect to *in vivo* studies, Koon et al. noted that the administration of 20 μM fidaxomicin or 120 μM of OP-1118 (a primary metabolite of fidaxomicin) reduced the level of enteritis caused by TcdA in a model of the mouse ileum.[42] Among the most notable histological differences between administration of fidaxomicin and the control group was the decreased cell rounding of colonic CCD-18Co fibroblasts as a result of fidaxomicin and OP-1118. Thus, fidaxomicin may exert anti-inflammatory effects in addition to its anti-*C. difficile* activity. A study by Chilton et al. showed that fidaxomicin as a first line treatment option was effective at treating CDI in a human gut model, irresponsive to metronidazole or vancomycin.[43] It was shown that administration of 200 mg/L fidaxomicin 2 times per day decreased the total viable count of *C. difficile* as well as the toxin titres below the minimum detection levels, 2 and 4 d after administration respectively. Fidaxomicin also prevented spore recrudescence and had negligible effects on the gut microbiota.[43] In addition, fidaxomicin was still detected 21 d after administration in the gut models. The results from the study indicated that fidaxomicin fared better

than metronidazole and vancomycin in treating primary and secondary CDI.[43]

It is promising to note that fidaxomicin has potent antimicrobial activity against outbreak-associated R027 strains as well as non-epidemic-associated strains.[44-46] Phase I clinical trials conducted by Shue et al. reported low plasma concentrations of fidaxomicin of less than or equal to 5 ng/ml, with a concomitant near 100% recovery of fidaxomicin and its metabolite in faeces.[47] These findings were in accordance with more recent Phase II and Phase III trials which reported that the concentrations of fidaxomicin in faeces were 2000–10000-fold higher than the MIC_{90} value against *C. difficile*. Some other clinical trials with CDI patients have shown that fidaxomicin caused fewer recurrences and thus, is indicated for mild, moderate, severe and recurrent CDI.[48,49] A Phase II trial by Louie and co-workers assessed the efficacy of several doses of fidaxomicin in treating CDI.[50] Response rates of 71%, 80% and 94% were found for patients treated with 100 mg, 200 mg and 400 mg of fidaxomicin respectively. Four patients from the group receiving either 100 mg or 200 mg of fidaxomicin failed therapy, representing an 8.9% treatment failure rate whereas 2 out of 41 patients in the study exhibited recurrence of disease a month after treatment.[50] In 2 phase III clinical trials with 1105 CDI patients, treatment with fidaxomicin resulted in comparable initial response rates to vancomycin.[51,45] Also, the group of patients treated with fidaxomicin had a 47% lower recurrence rate compared to vancomycin.[51] In patients with recurrent CDI, 35.5% of 128 patients receiving vancomycin had a further recurrence whereas only 19.7% had another recurrence when treated with fidaxomicin, as reported in 2 studies.[44-46] Although lower recurrence rates have been observed for fidaxomicin compared to vancomycin, the recurrence rates are broadly similar for R027 strains.[25,44-46] By using a whole-genome sequencing approach, Eyre et al. showed that fidaxomicin was more effective than vancomycin in preventing relapse of CDI in recent phase III trials.[52]

Rifamycin antibiotics

The rifamycin subgroup of antibiotics, which include rifaximin, rifampin, rifalazil and others display potent anti-*C. difficile* activity *in vitro*.[53,54] However, only a handful of reports regarding their clinical use are available.[55] In one instance it was reported that 7 patients recovered from CDI with 3 d of rifampin treatment. The dose was 300–600 mg rifampin administered every 12 hours in combination with vancomycin.[56] Another study evaluated the effects of metronidazole and rifampin combination therapy, but no beneficial effects were noted compared to metronidazole treatment alone.[57] Rifaximin, another rifamycin antibiotic, also shows strong antimicrobial activity against *C. difficile in vitro*.[53] The rates of *C. difficile* resistance to rifaximin are significantly lower compared to rifampin.[55] In an *in vitro* study, O'Connor and coworkers found that 14 out of 80 *C. difficile* isolates tested in the study were resistant to rifaximin, whereas rates of metronidazole resistance among *C. difficile* strains have been reported to vary from 15–35%.[58,59]

A clinical trial conducted by Johnson et al. showed that 7 of 8 patients administered rifaximin following vancomycin displayed no recurrence of CDI.[60] Rifaximin was effective in eradicating symptoms of diarrhea in 3 liver transplant patients, within 36–48 hours of administration.[54] A success rate of 64% for rifaximin was reported by Patrick-Basu and co-workers in 25 patients who failed metronidazole treatment.[61] A study conducted in a hospital in Houston in the period between May 2007 and September 2011 showed that a rifamycin-resistant strain of *C. difficile* was found in 49 of 283 (17.3%) patients with CDI, which compared with rates of 34% and 35% rifamycin-resistance in hospital-acquired and community-acquired cases respectively.[62] Obuch-Woszczatyński et al.[63] documented an outbreak caused by a rifampicin-resistant *C. difficile* R046 isolate in tuberculosis patients in Poland while Carman et al.[64] also reported the isolation of a *C. difficile* strain resistant to the rifamycin group of antibiotics from a patient being treated with rifaximin for recurrent CDI. The strain was isolated within 32 hours of rifaximin administration and resistance was attributed to single point mutations within the *rpoB* gene.[64]

Nitazoxanide

Nitazoxanide is a nitrothiazole benzamide which displays potent antimicrobial activity against intestinal parasites and gastrointestinal tract (GIT) pathogens including *C. difficile*.[65] Musher et al. conducted a randomized, prospective double blind study with hospitalised patients with *C. difficile* colitis. Patients included in the study were those who had primary CDI (a minimum of 3 unformed stools per day), with symptoms such as abdominal pain, fever or leukocytosis and an enzyme immunoassay indicating CDI.[66] 89.5% of CDI patients responded to nitazoxanide therapy, which was better than the 82.4% response rate for metronidazole, after a week of therapy in the trial. Furthermore, the sustained response to nitazoxanide a month after therapy was comparable to metronidazole rates.[66] The same authors further investigated the efficacy of nitazoxanide in treating CDI patients who had failed metronidazole treatment. Initially, a 74% response rate for nitazoxanide was noted with this patient group. However, subsequent recurrence of disease resulted in a final cure rate of 54% for nitazoxanide in treating CDI patients not responding to metronidazole.[67] Another recent prospective double blind randomized study in 2009, also conducted by Musher et al. compared the efficacy of 10 d of nitazoxanide therapy versus 10 d of vancomycin therapy with 50 CDI patients. Patients included in the study were those who had confirmed positive tests for *C. difficile* toxins, had more than 3 unformed stools within a 24 hour period and presented with at least one of the following: abdominal pain, fever or leukocytosis.[68] Response rates of 77% for nitazoxanide and 74% for vancomycin were noted initially. Initial response rates in the study were defined as the absence of any CDI symptoms between days 11–13.[68] Among the patient group who completed nitazoxanide and vancomycin therapy, 94% and 87% final response rates were noted respectively. One patient treated with nitazoxanide and 2 patients treated with vancomycin displayed relapse of disease. Although a

small sample size was used in the study, acknowledged by the investigators, the findings of the study led to the conclusion that nitazoxanide was comparable to vancomycin in terms of treating CDI.[68] A separate case report of a patient with severe recurrent CDI, failing metronidazole and vancomycin therapy successfully treated with 2 weeks of oral nitazoxanide followed by 2 weeks of nitazoxanide and tapered vancomycin administration was also recently documented.[69]

Tigecycline

Tigecycline, a derivative of minocycline, is a drug which undergoes very little metabolism, resulting in a large percentage of the active compound being excreted in the faeces.[70] In a study by Baines et al. using a human gut model, tigecycline prevented the growth of *C. difficile* and consequent toxin production.[71] Similar observations were made by Jump et al. studying tigecycline using a mouse model of infection.[72] A few case reports have described the success of tigecycline in combination with other antibiotics, in treating CDI in patients failing conventional metronidazole and vancomycin therapy[73,74] while another study has highlighted the success of tigecycline on its own in resolving CDI.[70] However, tigecycline has been shown to be a risk factor for CDI in a murine model by causing changes in the composition of the gut microbiota, more specifically by eliciting significant reductions in *Bacteroidetes* numbers and increases in *Proteobacteria* numbers.[75] Such disruptions of the gut microbiota were shown to predispose to CDI.[75] One report however stated that tigecycline administration for as long as 14 d still failed to treat a case of CDI.[76] Despite a handful of case reports highlighting the success of tigecycline and rifaximin, the guidelines drafted by the SHEA/IDSA in 2010 do not include tigecycline, rifaximin or linezolid as part of CDI therapeutic options.[27]

Ramoplanin

Ramoplanin is a lipoglycodepsipeptide antibiotic which was developed as an oral agent for use in patients colonized with vancomycin-resistant enterococci but also exhibits potent anti-*C. difficile* activity mediated through the inhibition of cell wall synthesis.[77,78] Using a hamster model of *C. difficile*-induced colitis, Jabes and coworkers reported that ramoplanin was a better treatment choice than vancomycin, while in a separate study, Freeman and co-workers found the efficacy of ramoplanin to be comparable to vancomycin in hamster models of infection.[79,77] The study by Freeman et al. showed that administration of ramoplanin resulted in the resolution of symptoms in a hamster model of CDI and a reduction in toxin titer in an *in vitro* gut model.[77] The study also showed the superior efficacy of ramoplanin over vancomycin against *C. difficile* spores, as spores were recovered less often from the ramoplanin-treated hamsters, compared to vancomycin-treated hamsters.[77] Doses of 200–400 mg of ramoplanin administered twice a day for 10 d were effective and comparable to vancomycin for the treatment of CDI, according to a phase II trial.[80] Although ramoplanin is not yet used to treat CDI, it may eventually become an alternative antibiotic of choice due to its potent anti-*C. difficile* activity.

Bacteriocins against *C. difficile*

Bacteriocins are ribosomally synthesized antimicrobial peptides with either narrow spectrum or broad spectrum activity against other bacteria.[81] To date, the activity of a few bacteriocins has been assessed against *C. difficile*. Bacteriocins, due to their ribosomally-synthesized nature, can also be the subject of bioengineering strategies to find derivatives with ameliorated bioactivity against specific bacterial targets, such as *C. difficile*. Furthermore, some probiotic strains have the ability to produce bacteriocins *in situ*. Since bacteriocins are currently not used clinically against *C. difficile*, the development of resistance among target *C. difficile* strains has not been a problem thus far. When considering the use of bacteriocins as an alternative/adjunctive therapeutic option for CDI, the mode of delivery of the bacteriocin to the colon must be carefully evaluated. Encapsulation of the bacteriocin may be a means to overcome proteases. It must be noted that the anti-*C. difficile* activities of the bacteriocins described herein are predominantly based on *in vitro* studies and the *in vivo* efficacies of the majority of these bacteriocins have yet to be determined.

Thuricin CD

Thuricin CD is a recently discovered bacteriocin with potent narrow spectrum activity against *C. difficile*.[82] The main advantage of thuricin CD is that its antimicrobial activity is largely restricted to *C. difficile* and has little or no impact on other commensal gut microbes. This was demonstrated using a model of the human distal colon and a high-throughput sequencing approach which revealed that thuricin CD had minimal impact on the numbers of *Firmicutes*, *Bacteroidetes* and *Proteobacteria*, compared to vancomycin and metronidazole which elicited a decrease in *Firmicutes* and *Bacteroidetes* numbers, concomitant with an increase in *Proteobacteria* numbers.(**Fig. 2**)[82]

Nisin and lacticin 3147

Nisin is a member of the lantibiotic family of bacteriocins with broad spectrum antimicrobial activity against a range of Gram-positive bacteria, including antibiotic-resistant bacteria and food pathogens.[83] Studies by Field et al. showed that a bioengineered derivative of nisin A, designated M21V, displayed more potent antimicrobial activity against a variety of Gram-positive pathogens, including *C. difficile* R027, compared to wild type nisin A.[83] Lacticin 3147 is a 2-peptide lantibiotic produced by *Lactococcus lactis* DPC 3147.[84] Unlike thuricin CD, it has a broad spectrum of antimicrobial activity against Gram-positive pathogens. Lacticin 3147 seems to trigger a rapid lysis of log phase *C. difficile* cells, measured by quantifying the release of acetate kinase. Addition of high lacticin 3147 concentrations of 6 μg/ml results in the decrease of *C. difficile* ATCC 43593 cell numbers from 10^6 cfu/ml to zero in 2 hours. Subsequent studies with lacticin 3147 using a model of the human distal colon showed that it caused a decrease in *Firmicutes* numbers with a concomitant increase in *Proteobacteria* numbers, similar to the effects seen with vancomycin and metronidazole.[82] (**Fig. 3**)

Actagardine and NVB302

Actagardine A is a 19-amino acid lantibiotic with potent antimicrobial activity against Gram-positive bacteria, including *C. difficile*. A bioengineered V15F derivative of actagardine A exhibited lower minimum inhibitory concentration (MIC) values against the same *C. difficile* targets compared to the wild type actagardine.[85] NVB302 is a semi-synthetic type B lantibiotic, derived from actagardine and is effective against *C. difficile*. Crowther et al. conducted a recent study investigating the efficacy of NVB302 compared to vancomycin in treating CDI employing an *in vitro* gut model.[86] The gut microbiota count as well as *C. difficile* viable counts and spores were enumerated following NVB302 and vancomycin administration and a decrease in viable *C. difficile* counts with vancomycin and NVB302 administration was noted. NVB302 performed better than vancomycin as cytotoxin levels were undetectable 7 d subsequent to NVB302 administration, compared to undetectable cytotoxin levels 5 d after vancomycin instillation. *C. difficile* spores did not germinate following either vancomycin or NVB302 instillation.[86]

LFF571 (GE2270 derivative)

GE2270 is a thiopeptide bacteriocin which inhibits translation in bacteria.[87] LFF571 is a semi-synthetic derivative of the thiopeptide GE2270, developed by Novartis, which displays antimicrobial activity against a range of Gram-positive bacteria, including *C. difficile*.[88] The *in vivo* activity of LFF571 against *C. difficile* was compared with vancomycin in a study with Golden Syrian hamster models.[89] LFF571 was administered orally 24 hours post-infection. Doses of 0.2, 1, 2, 5 or 10 mg/kg of LFF571 were used. Administration of 5 mg/kg LFF571 resulted in a 71% initial response rate, whereby 5 out of the 7 hamsters survived after 21 days, while 37.5% of animals survived 21 d when treated with 20 mg/kg vancomycin. In terms of recurrence rates, LFF571 once again fared better than vancomycin. Only 2.2% of hamsters had recurrence at the conclusion of treatment with 5 mg/kg LFF571, whereas 37.8% of hamsters which survived at the termination of treatment with 20 mg/kg vancomycin, experienced recurrence.[89]

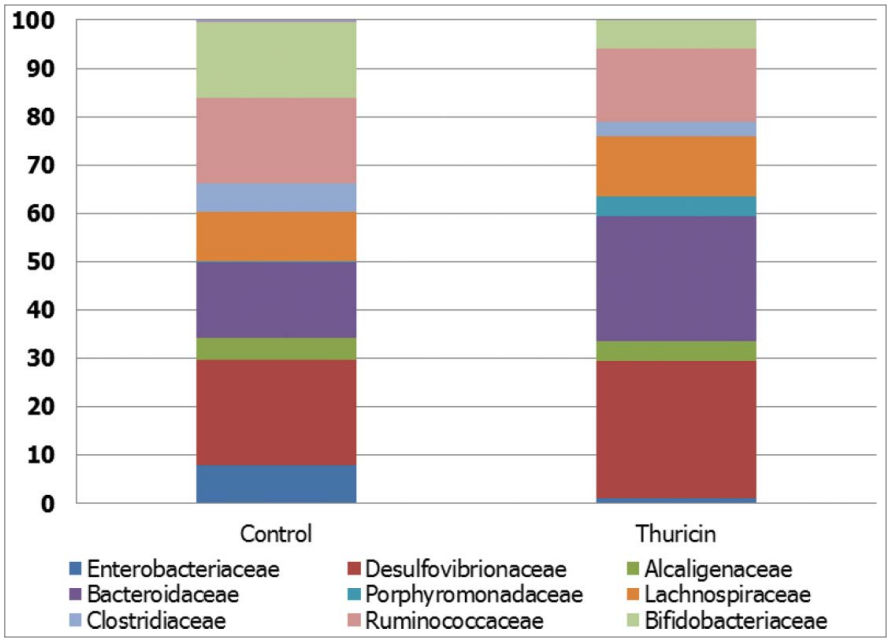

Figure 2. Narrow spectrum antimicrobial effects of thuricin CD. The effect of thuricin CD (90 μM) on family-level taxonomic distribution of the microbial communities present in model of the distal colon, expressed as percentage of total assignable sequences. Redrawn from Rea et al.[82] Proc Natl Acad Sci U S A 2011; 108: 4639–44. © Proceedings of the National Academy of Sciences of the United States of America. Reproduced by permission of Kay McLaughlin. Permission to reuse must be obtained from the rightsholder.

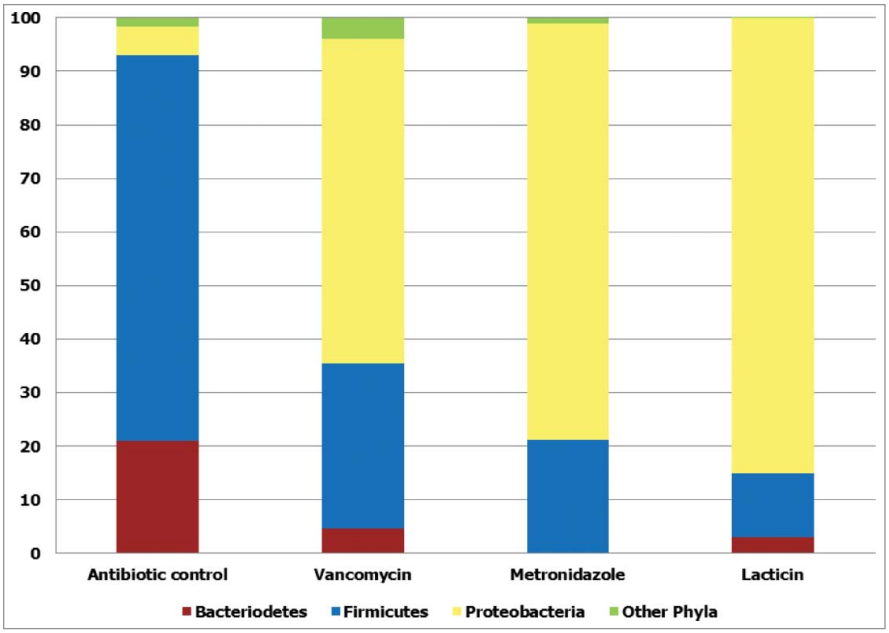

Figure 3. Broad spectrum antimicrobial effects of vancomycin, metronidazole and the bacteriocin lacticin 3147. The effects of the broad spectrum antimicrobials vancomycin, metronidazole and lacticin 3147 on phylum level diversity of gut communities in a model of the distal colon, expressed as percentage of total population of assignable tags. Other phyla: Actinobacteria, Spirochaetes, Lentisphaerae, and Tenericutes. Redrawn from Rea et al.[82] Proc Natl Acad Sci U S A 2011; 108: 4639–44. © Proceedings of the National Academy of Sciences of the United States of America. Reproduced by permission of Kay McLaughlin. Permission to reuse must be obtained from the rightsholder.

Recently, a randomized double blind trial was conducted investigating the efficacy and safety of LFF571 in healthy volunteers.[90] Encouragingly, no serious side effects of LFF571 were noted among 56 volunteers. LFF571 largely remained in the gut with very low concentrations noted in serum (the highest concentration being 3.2 ng/ml in serum in one volunteer). Moreover, LFF571 was tolerated equally well irrespective of single or multiple doses in healthy volunteers participating in the study.[90]

A summary of MIC values of antibiotics being investigated to replace metronidazole/vancomycin as well as various bacteriocins against *C. difficile*, as reported in published studies, is included in **Table 1**.

Faecal Microbiota Transplantation

Since the main risk factor for acquiring CDI appears to be the perturbation of the gut microbiota due to broad spectrum antibiotics, and subsequent overgrowth of *C. difficile*, the restoration of the intestinal microbiota via faecal microbiota transplantation (FMT) seems like an appropriate therapeutic option. FMT is the process of introducing faeces from a healthy donor, in a liquid suspension, into the GIT of a patient.[101] Typically, patients considered for FMT are those who have confirmed *C. difficile* colitis and have had at least 2 relapses following antibiotic therapy. Stool donors are screened for HIV-1, HIV-2, hepatitis A, B, C and faecal samples tested for bacterial/parasitic pathogens such as *Salmonella*, *C. difficile*, *S. aureus.*[101,102] The majority of cases of FMT occur through the rectum, but nasogastric, nasoduodenal, nasojejunal instillations are common as well. 250 mg of vancomycin every 8 hours for 4 d and 2 20 mg doses of omeprazole per day for 4 d are administered to the transplant recipient to decrease *C. difficile* numbers and allow the introduced bacteria to colonize by decreasing acid production in the stomach and consequently elevating the pH, in the event of nasogastric instillations.[103-105]

The treatment success rate of FMT for recurrent CDI has been reported to be approximately 90% in a study involving 18 subjects, while a response rate of 100% was reported when 12 recurrent CDI patients were treated with FMT in a separate study.[101,106] Over the last 5 years, investigators who have used FMT to treat recurrent cases of CDI have reported success rates ranging from 86–100%.[102,106-108] The first clinical trial comparing FMT against vancomycin was recently conducted by van Nood et al. and an 81% success rate for faecal transplantation after the first infusion (13/16 cases resolved) was significantly better than the 31% success rate found for vancomycin alone.[109] A mix of 33 different bacteria from healthy stool samples was effective in treating CDI in 2 patients, in another recent study.[110] Most recently, a retrospective assessment of 31 patients treated with either FMT or rectal bacteriotherapy (RBT) was conducted by Emanuelsson et al.[111] The results indicated that overall out of 31 recurrent CDI patients, 74% were treated successfully, defined by a continued lack of symptoms and diarrhea within 3 d of treatment. 70% of patients treated with FMT responded successfully, while the corresponding rates for RBT were 88%.[111]

FMT can lead to alterations in the composition of the gut microbiota. A rise in *Bacteroides, Faecalibacterium* and *Roseburia* numbers with a concomitant decrease in *Enterobacteriaeceae* in *C. difficile* patients who are treated with FMT is common.[107] Hamilton et al. also noted a rise in *Bacteroidetes* and *Firmicutes* numbers in 3 patients treated with FMT.[108] An increase in *Bacteroidaceae, Porphyromonadaceae* and *Rikenellaceae* families of the *Bacteroidetes* phylum, accompanied by an increase in *Ruminococcaceae* and *Lachnospiraceae* families of the *Firmicutes* phylum following FMT were noted in a study and it has been hypothesized that the presence of such families is associated with gastrointestinal health.[108] Fuentes et al. studied the composition, diversity and changes that take place in the faecal microbiota as a result of FMT by utilizing a phylogenetic microarray platform.[112] Compositional analysis of the gut microbiota from faecal samples of 9 recurrent CDI patients was performed and data were analyzed before and after

Table 1. Summary of minimum inhibitory concentration values (MIC) of anti-*C. difficile* antibiotics and bacteriocins. *In vitro* MICs of alternative antibiotics to metronidazole and vancomycin as well as as *in vitro* MICs of bacteriocins and bioengineered derivatives thereof against *C. difficile* strains as reported in the literature

Antibiotic	Description	Mode of action	MIC range against *C. difficile*(μg/ml)	References
Fidaxomicin	Macrocyclic antibiotic	Inhibition of RNA polymerase	0.008–0.25 (MIC_{90})	37,91-93
Rifaximin	Rifamycin group	Inhibition of RNA polymerase and transcription	0.0075–0.015 (MIC_{50} and MIC_{90})	53
Rifalazil	Rifamycin group	Inhibition of RNA polymerase and transcription	0.004–0.03	53,94
Nitazoxanide	Nitrothiazole benzamide	Interferes with pyruvate:ferredoxin oxidoreductase	0.03–1.0	53,95
Tigecycline	Minocycline derivative	Protein synthesis inhibitor	0.03–0.25 (MIC_{90})	53,96,97
Ramoplanin	Lipoglycodepsipeptide	Inhibition of cell wall synthesis	0.03–0.5 (MIC_{90})	98
Bacteriocin	Description	Mode of action	MIC range against *C. difficile*(μg/ml)	References
Thuricin CD	Sactibiotic	Unknown	0.7–2.8	99
NisinA	Lantibiotic	Inhibition of cell wall synthesis and pore formation	8.38	83
NisinV (M21V)	Bioengineered derivative of NisinA	Inhibition of cell wall synthesis and pore formation	4.19	83
Lacticin 3147	Lantibiotic	Inhibition of cell wall synthesis and pore formation	3.6(MIC_{50})	84
Actagardine A	Lantibiotic	Inhibition of cell wall synthesis	1.5–12	85,99
Actagardine V15F	Bioengineered derivative of Actagardine A	Inhibition of cell wall synthesis	2–4	85
LFF571	Thiopeptide derivative	Inhibition of translation by binding elongation factor Tu	0.5–2.0	100

faecal infusion, as well as 10 weeks after therapy. The predominant change in the composition of the gut microbiota went from a low-diversity state composed mainly of Proteobacteria and Bacilli to a more diverse state, predominantly composed of *Clostridium* and *Bacteroides* groups as well as butyrate producers.[112] The findings of these types of studies could form the basis for the identification of biomarkers which can predict recurrence or resolution of CDI and could help us understand an optimal microbiota composition for targeted therapeutic strategies. FMT has been shown to normalize the composition of faecal bile acid in recurrent CDI cases.[113] Faecal samples prior to FMT consisted mainly of primary bile acids and bile salts with extremely low concentrations of secondary bile acids. However, faecal samples post-FMT had an abundance of secondary bile acids, similar to donor samples from non-CDI volunteers.[113] Thus, FMT has a major role in restoring faecal microbiota composition as well as metabolic composition. Recurrent CDI patients are unable to metabolize primary bile acids to secondary bile acids.[113]

Animal trials can also be invaluable in terms of providing information and optimizing successful FMT procedures. Six different bacteria were used to cure *C. difficile* R027-infected mice, in a recent *in vivo* trial.[114] During CDI resolution, 4 out of the 6 bacterial strains managed to colonize the mice while several other commensals also proliferated, increasing the microbial diversity in doing so. Despite the promising *in vivo* and clinical trial outcomes, there has been a general reluctance in resorting to FMT among both patients and doctors due to the unattractive nature of the procedure, as well as the extensive screening of donor samples for pathogens that is required prior to transplantation.[115] Banking of frozen stool samples which have already been screened for the presence of pathogens, may be a means of expediting the processes involved in performing FMT for CDI cases.

Vaccines for CDI

Earlier vaccine studies

The development of vaccines for CDI has been ongoing for the last 20 years. Torres et al. found that a *C. difficile* culture filtrate inactivated with formalin was effective in hamsters.[116] An inactivated *C. difficile* toxoid administered intramuscularly or via the rectum also conferred protection to hamsters.[117] It was found that an adjuvanated toxoid B vaccine allowed colonization of toxin A^-B^+ *C. difficile* strains to occur in hamsters but prevented disease from occurring in a more recent trial.[118] A study by Sougioultzis and coworkers reported the effects of an 8-week immunization course with a toxoid vaccine on 3 recurrent CDI patients.[119] The authors found significantly elevated levels of serum IgG anti-toxin A and anti-toxin B in 2 of the 3 patients. All 3 patients recovered from recurrent CDI, even after vancomycin treatment was ceased, implying that the injectable toxoid vaccine was effective.[119]

Sanofi pasteur toxoid vaccine

More recently, a toxoid vaccine has been developed by the Sanofi Pasteur Institute and its development has been fast-tracked by the US FDA since 2010. This Sanofi Pasteur vaccine consists of formalin-inactivated toxins A and B from *C. difficile* VPI 10463. The primary target group for this Sanofi vaccine is elderly patients who may be immunocompromised and/or hospitalised patients on antibiotics, who are at a greater risk of acquiring CDI. Phase I and Phase II clinical trials with the Sanofi vaccine have already been conducted and results of the Phase II trial are intended to be published shortly (unpublished data of Phase II trial (H-030–012) presented at the 114th General Meeting of the American Society for Microbiology, 19 May 2014, Boston, USA). In 2013, a Phase III clinical trial with a vaccine called *Cdiffense* was launched by Sanofi, recruiting approximately 15000 volunteers as part of a randomized observer-blind multinational trial, with a total duration of approximately 4.5 y. The aim of this *Cdiffense* trial, currently ongoing, is to assess the immunogenicity and safety of the toxoid vaccine. Anosova et al. assessed the immunogenicity and protective capabilities of the Sanofi Pasteur toxoid vaccine in a hamster model of CDI.[120] The hamsters showed an increase an IgG titres in response to the vaccine. There is a positive correlation between the levels of antibody-toxin binding and neutralisation titres and consequent protection against *C. difficile*.

Recombinant vaccines

Karczewski et al. investigated the potential of a recombinant toxin fragment vaccine, composed of 2 separate fragments of TcdB, against *C. difficile*.[121] When such recombinant fragments of TcdB were administered to Golden Syrian hamsters, in combination with TcdA, the animals displayed significant IgG responses and strong neutralising antibody titres. Significantly, the recombinant TcdB vaccines investigated in the study were also effective against subsequent challenge with *C. difficile* spores.[121] Spencer et al. also noted in their study that recombinant fragments from TcdA and TcdB provide protection to hamsters against *C. difficile*.[122] Importantly, however, a greater immune response is mounted when the binding domain of TcdB is replaced with the glucosyltransferase domain of the toxin.[122]

An injectable subunit recombinant protein vaccine for immunogenicity and safety in volunteers was tested in 2011.[123] This recombinant vaccine is made up of truncated versions of *C. difficile* toxins A and B, designated IC84. Intercell IC84v is composed of recombinant fusion proteins of the binding regions of toxins A and B. Both alum-adjuvanated and non-adjuvanated recombinant vaccines were immunogenic in volunteers under 65 y of age.[123]

Ghose et al. assessed the immunogenic potential of a recombinant fusion protein vaccine consisting of the *Salmonella enterica serovar Typhimuriu*m flagellin (FliC) subunit D1 fused to the non-toxic domains of TcdA and TcdB.[124] The authors observed that anti-TcdA and anti-TcdB IgG titres were elevated in sera of mice. The outcome of the study proved that a recombinant protein-based vaccine which targets the receptor binding motifs of TcdA and TcdB, when adjuvanated with *S. enterica serovar Typhimurium* flagellin (FliC) subunit D1, stimulates a quick high-level immune response in a mouse model of infection, provided that the mouse is challenged with the same strain of

C. difficile from which the antigens were obtained.[124] Such findings could have implications for the development of 'designer' recombinant vaccines, using antigens from the most common *C. difficile* outbreak strains. A plasmid-based approach to produce a recombinant toxoid in a $TcdA^{-}TcdB^{-}$ strain of *C. difficile* has also been utilised.[125] As expected, the TcdA and TcdB toxoids expressed in this plasmid system were significantly less toxic to human IMR-90 cells. Such formalin-inactivated antigens elicited a protective effect in hamster models of CDI.[125]

Wang et al. developed a chimeric toxin vaccine, consisting of immunogenic epitopes from both TcdA and TcdB.[126] In essence, the receptor binding domain of TcdA replaced that of TcdB and the resulting chimeric toxin displayed strong immunogenic potential. The chimera cTxAB was generated by deleting the glucosyltransferase domains of both TcdA and TcdB and was found to be equally effective against laboratory and outbreak-associated strains.[126] Thus, a blueprint for design of recombinant toxin chimeras may serve a useful purpose in anti-*C. difficile* vaccine development. Bertolo et al. created a dual vaccine consisting of PSII from *C. difficile* fused to the LTB enterotoxin from enterotoxigenic *E. coli* (ETEC).[127] Such a vaccine could play an important role in preventing diarrhea from *C. difficile* as well as ETEC.

Vaccines based on the polysaccharide glycans and glycoconjugate vaccines

Other investigators have studied the immunogenicity of vaccines based on polysaccharide glycans found on the surface of *C. difficile* cells, namely PSI, PSII, PSIII.[128-132] Adamo et al. noted that mice, hamsters, as well as farm animals had elevated levels of anti-PSII antibodies in response to vaccines containing the polysaccharide.[129,132] Interestingly, due to exposure to *C. difficile*, humans also carry anti-PSII IgA and IgG antibodies. Such antibodies could have great potential in targeting PSII on the outer surface of *C. difficile* cells. Jiao et al. further studied the importance of *C. difficile* PSI polysaccharide as a basis for the development of anti-*C. difficile* vaccines.[128] It is apparent that while PSII is found on the cell surface of biofilms of *C. difficile*, PSI and PSIII are expressed stochastically. The pentasaccharide moiety of PSI, chemically synthesized and fused to an exotoxin B subunit could be an effective vaccine with 2 separate antigens to mount an immune response. Importantly in the study, anti-PSI IgG antibodies were found in sera of horses in response to both the native PSI polysaccharide, as well as the chemically synthesized non-phosphorylated PSI repeating block, thus highlighting the potential for such an approach in vaccine development.[128] Novel chemical synthesis protocols to generate large quantities of the pentasaccharide repeating unit and oligosaccharide structures were optimised by Martin et al. to overcome problems with low-level expression of natural PSI.[133] Using mouse models, the authors demonstrated that a chemically synthesized PSI repeating unit CRM197 conjugate elicited an immune response. The use of glycan microarrays helped to identify the minimal immunogenic epitopes such as the repeating PSI-pentasaccharide repeating unit, as a basis for vaccine development.[133]

As PSII is expressed at a higher level by the majority of *C. difficile* ribotypes in comparison to PSI and PSIII, it has a greater potential to form the basis for a *C. difficile* vaccine.[134] Romano et al. studied effectiveness of recombinant toxin A and B fragments fused to PSII glycoconjugates.[134] While anti-PSII IgG levels were stimulated in response to both TcdA_B2 and TcdB_GT, the TcdB_GT induced a higher level of IgG. This increase in IgG titres was encouraging as it proves that glycoconjugate vaccines in combination with different *C. difficile* antigens can be effective as potential vaccines.[134]

Cox et al. investigated the role of a lipoteichoic acid-based glycoconjugate as a possible antigen to evoke an immune response.[135] Antibodies in response to the conserved lipoteichoic acid (LTA) glycoconjugate were stimulated in mouse and rabbit models. The authors optimised an amino functionality as the conjugation point. Overall, the study highlighted that the surface of *C. difficile* cells contains highly conserved LTA polymers, which has the potential to act as an antigen to evoke an immune response.[135] Oberli et al. chemically synthesized an oligosaccharide-conjugate vaccine composed of the PSII hapten of a *C. difficile* R027 strain.[136] The authors found that immunized mice displayed specific IgG antibodies in serum in response to the synthetic hexasaccharide and diphtheria toxoid variant CRM(197) conjugate vaccine. The IgG antibodies were mounted specifically against the glycan repeating subunit of the conjugate vaccine.[136]

DNA-based and other types of vaccines

Baliban et al. investigated the effect of cloning the receptor binding domain (RBD) of TcdA and TcdB on protection against *C. difficile* and vaccination with such DNA sequences elicited high levels of anti-RBD antibodies in mouse models.[137] In another study, a gene which encoded the receptor binding domain of TcdA was synthesized and designed for expression in human cells.[138] Vaccination of mice with this DNA vaccine stimulated high concentrations of antibodies to be produced and prevented death when inoculated with TcdA.[138]

A DNA vaccine expressing the glucosyltransferase domain of TcdB has also been developed and the authors found that only a fraction of *C. difficile* toxin fragments, which included an N-terminal glucosyltransferase domain of TcdB and the C-terminal receptor binding domain of TcdA evoked antibody responses as measured by cytotoxicity assays and/or prevention of death using mouse models.[139] Importantly, the antibodies generated in response to the N-terminus of the TcdB DNA vaccine provided a greater degree of protection, when used in conjunction with anti-TcdA antibodies in a mouse model of infection.[139] Seregin et al. developed an adenovirus-based vaccine against *C. difficile* and the investigators observed that the vaccine induced significant and rapid humoral and cellular responses (T-cells) in mouse models.[140] This immune response was sufficient to protect mice from subsequent *C. difficile* challenge. Furthermore, IgG antibodies specific to TcdA in plasma were notable 3 d after vaccination in the immunized mice. In addition to these findings, the authors discovered the main immune-dominant T cell epitopes in TcdA.[140] Thus, such an adenovirus-based vaccine also has the potential to be used for CDI.

Conclusions

The overuse of broad spectrum antibiotics has led to numerous *C. difficile* outbreaks, especially in North America, Canada and Europe over the last 2 decades. The advent of next-generation sequencing technology in recent years has helped to emphasize the extent of damage caused to the gut microbiota due to broad spectrum antibiotics. The perturbation of the gut microbiota as a result of antibiotics removes the most potent defense against opportunistic pathogens such as *C. difficile* i.e., the presence of a fully intact gut microbiota. This often leads to a continuous cycle of CDI and recurrence, as further treatment with broad spectrum antibiotics inhibits the restoration of the commensal gut microbiota, leading to acquisition of CDI again. Thus, it is clear that there is an urgent need to develop alternative/adjunctive therapeutic options to metronidazole and vancomycin in order to circumvent this ongoing problem of recurrence of disease. Fidaxomicin has already been proved to have promising efficacy as a therapeutic for CDI. Other antibiotics such as ramoplanin, nitazoxanide, tigecycline and the rifamycin group have shown potent *in vitro* activity and some promising *in vivo* results against *C. difficile* and research is ongoing regarding their clinical efficacy against CDI. Development of these alternative antibiotics is crucial as the overuse of the current antibiotics metronidazole and vancomycin may lead to development of resistance among *C. difficile* targets. Furthermore, there is an inherent risk with the overuse of vancomycin with respect to the spread of vancomycin-resistant enterococci in hospital environments.

The restoration of the commensal gut microbiota using FMT has also shown encouraging results in recent years. FMT has tremendous potential in this regard, as it directly tackles the root cause of the problem i.e. dysbiosis caused by broad spectrum antibiotics, resolved by restoring the commensal microbiota via faecal transplantation. Perhaps the most elusive therapeutic option over the years to arrest the cyclic pattern of relapse and recurrence of CDI, however, has been a narrow spectrum antimicrobial with potent anti-*C. difficile* activity and lack of activity against the commensal gut microbiota. It may be the case that a narrow spectrum antimicrobial targeted against *C. difficile* and/or restoration of the gut microbiota via FMT may eventually prove to be the most effective treatment regimens for CDI. The importance of mounting an effective immune response as a result of *C. difficile* vaccines must not be overlooked either. Overall, due to the vast number of studies investigating anti-*C. difficile* therapeutic options in recent years, scientists are closer than ever at finally tackling this notorious infection.

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