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New and emerging therapies for *Clostridium difficile* infection

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Abstract (max 200 words)

Purpose of review: Clostridium difficile infection has attained high prominence given its prevalence and impacts on patients and healthcare institutions. Multiple new approaches to the prevention and treatment of CDI are undergoing clinical trials.

Recent findings: Bezlotoxumab is a monoclonal antibody against toxin B that has successfully completed phase three studies, demonstrating a significant reduction in recurrent CDI when given with standard of care antibiotics. Antibiotics under development include cadazolid and ridinilazole, while surotomycin has had disappointing phase 3 results. Multiple live biotherapeutics are being developed, including freeze thawed and encapsulated versions of faecal microbiota transplantation to improve the practicality of treating patients with recurrent CDI. Alternatives to faecal microbiota transplantation, that aim to improve safety, including a microbial suspension, RBX2660, and a complex spore formulation, SER-109, have progressed to phase 2 studies. A non-toxigenic *C. difficile* strain has also shown promise to prevent recurrent CDI. In addition, three *C. difficile* vaccines have progressed to phase 2/3 clinical trials.

Summary: The diverse approaches to treating and preventing CDI offer substantial promise that new treatment options will soon emerge, particular ones that reduce the risk of recurrences.

Introduction

In the US, *C. difficile* infection (CDI) occurs at a rate of 150 cases per 100,000 population per year creating a significant burden to patients, relatives and healthcare providers.[1] In modern healthcare, sophisticated medical interventions (e.g. organ transplantation, complex surgery and cancer treatments) come at the cost of an increased infection risk. In particular, antibiotic treatment causes dysbiosis of the host gut microflora and an increased risk of CDI. Complex health needs are unlikely to diminish, and so, together with an aging population, antibiotic stewardship programmes aimed at reducing the use of 'high-risk' antibiotic prescribing should reduce, but not eliminate, the risk of CDI. Strategies to reduce healthcare exposure to *C. difficile*, such as source isolation of CDI cases and enhanced environmental cleaning, have reduced CDI rates in some regions.[2] However, meticulous adherence to these interventions is unlikely to remove the risk of CDI entirely due the widespread presence of *C. difficile* in healthcare settings, the environment, livestock and (occasionally) food.[3-5] Therefore, there is an urgent need for effective CDI prevention and treatment strategies.

Currently, three antimicrobial agents provide the mainstay of CDI therapy; vancomycin, metronidazole and fidaxomicin.[6-7] Several novel antimicrobial agents are in development and, if successful, may increase the range of agents available to clinicians (Table 1). In addition, studies demonstrating the efficacy of faecal microbiota transplantation (FMT) for the treatment of recurrent CDI[8-9] have sparked interest in CDI interventions designed to target gut dysbiosis that typically involves reduced diversity.[10] These therapies may be particularly helpful for recurrent CDI, which occurs in approximately a quarter of patients following primary infection. Host gut antibody-mediated immunity is also known to be important to CDI pathogenicity,[11] and recent interventional trials have targeted this by using either pre-emptive vaccination strategies or passive antibodies (in addition to standard therapy). This review summarises emerging preventative and therapeutic approaches for CDI, with a brief description of each strategy and its stage of development.

Current CDI antibiotics

The usage of metronidazole and vancomycin for the treatment of CDI have been comprehensively described previously.[12] The most significant recent findings are from a pooled analysis of two phase III trials of tolevamer (a toxin binding polymer), which failed to demonstrate equivalence to standard treatment.[13] These data additionally showed that clinical success occurred significantly less often following metronidazole compared to vancomycin therapy (73% and 81%, respectively, p=0.02).[13] However, metronidazole continues as first-line CDI therapy in many countries due to low acquisition

cost and the perceived risk of VRE with vancomycin.[6-7] Oral vancomycin is often recommended for severe or complicated CDI.[6-7]

Fidaxomicin was added to the CDI treatment repertoire following FDA approval in 2011.[14] Fidaxomicin is an oral macrocyclic agent which inhibits RNA synthesis and demonstrates relatively little perturbation of the host microbiota[15] whilst achieving high faecal concentrations and reduced toxin and spore levels.[16-17] In two large phase III trials, fidaxomicin demonstrated non-inferiority to vancomycin and was associated with reduced CDI recurrence (15.4% after fidaxomicin vs. 25.3% after vancomycin, p=0.005).[18-19] Lower cure rates using fidaxomicin (or vancomyicn) were noted in BI/NAP1/ribotype 027 infections. Fidaxomicin is included in European guidelines on CDI treatment,[7] but usage has been limited by high drug acquisition cost. However, some studies suggest that the cost of this agent can be offset by reduced disease recurrence and shorter hospital admissions.[20,21] A recent before and after design study reported reduced mortality in some settings where fidaxomicin was the preferred CDI treatment agent.[21]

Novel antimicrobial therapy for CDI

The ideal antimicrobial agent for CDI would reduce vegetative *C. difficile* cells, toxins and spores in the host gut lumen without encouraging host microbial resistance (e.g. vancomycin resistant enterococci [VRE] or multi-resistant Gram negative bacilli [MRGNB]), perturbation of the host microbiota[11] or systemic adverse effects. It is enormously challenging for a single agent to meet all these criteria, but a number of promising new therapies are being investigated (summarised in Table 1).

Surotomycin is an oral lipopeptide antibiotic, which was developed semi-synthetically from daptomycin.[22-25] A randomised multi-centre double-blind non-inferiority controlled phase 2 trial compared surotomycin with vancomycin (n=210). This study demonstrated non-inferiority using 125 mg and 250 mg surotomycin doses when compared with oral vancomycin (92% and 87% vs. 89%, respectively).[26] Recurrence was lower in the surotomycin groups (28% [125mg group] and 17% [250mg group]) compared with vancomycin (36%) but the trial was too small to demonstrate significant outcome differences.[27] However, recent data from phase III trials (NCT01598311 and NCT01597505) comparing surotomycin with vancomycin have failed to demonstrate non-inferiority of surotomycin compared with vancomycin.[28] As such, it is doubtful that surotomycin will continue to be developed.

Cadazolid (Actelion) is a novel hybrid oxazolidinone-fluroquinolone antibiotic that inhibits *C. difficile* protein synthesis and, to a lesser extent, DNA synthesis, which is very poorly absorbed after oral twice daily administration, achieving high faecal concentrations but with relatively low impact on host microbiota.[29-31] A phase 2 double-blind, double-dummy, randomised study (n=84) showed that time to CDI symptom resolution was similar for cadazolid and vancomycin.[32] Cadazolid had a greater sustained response rate (primary cure without recurrence) compared with vancomycin (60%, 56%, 47% for 250mg, 500mg and 1000mg cadazolid, respectively, and 33% for vancomycin). However, this study had an unusually low response rate to vancomycin. A Phase III double-blind trial (NCT01987895) investigating non-inferiority to vancomycin in terms of clinical response and superiority in terms of sustained response is currently recruiting.

Ridinilazole (SMT19969) is a novel non-absorbable very narrow-spectrum antimicrobial[33] with minimal activity against host gut microbiota but good activity against selected clostridia including *C. difficile*.[34-37] Safety and tolerability has been demonstrated in healthy subjects and in a recently completed phase II randomised double-blind trial (CoDIFy).[38,39] CoDIFy was designed as a non-inferiority study and compared oral ridinilazole 200mg bd with oral vancomycin 125mg qds, both for 10 days; sustained clinical response rates were 67% and 42%, respectively (n=69 mITT population). Recurrence occurred in 14% of the ridinilazole group compared with 35% of the vancomycin group; this difference meant that ridinilazole achieved a sustained response rate of 66.7% versus 42.4% for vancomycin, which met pre-set statistical superiority criteria.[39]

***C. difficile* infection prophylaxis**

Ribaxamase (SYN-004, synthetic biologics) is a recombinant beta-lactamase which has been formulated as an oral accompaniment to beta-lactam antibiotics.[40-41] This agent degrades unmetabolised antibiotic in the host intestine in order to protect the gut microbiota from dysbiosis and is well tolerated.[42] Animal studies have demonstrated safety without interference with the systemic pharmacokinetics of ceftriaxone.[41] A phase 2 double-blind placebo-controlled study is ongoing and will examine safety and whether ribaxamase can reduce CDI risk.

Another novel approach to CDI prophylaxis is DAV132 (DaVolterra), which is an activated charcoal based product in an enteric coated pill.[43] This adsorbent product irreversibly captures antibiotics in the intestine whilst hopefully avoiding interruption of antibiotic absorption. DAV132 has been examined in a proof-of-concept study involving 18 healthy subjects. *In vitro* human gut model and *in vivo* hamster models also have positive findings but clinical efficacy data are awaited.[43]

Active *C. difficile* immunisation

Vaccination to boost host antibody-mediated immunity is a promising strategy to reduce CDI. A previous model of CDI vaccination demonstrated cost-effectiveness even with wide-ranging risks of CDI and varied vaccine efficacies and costs.[44] A CDI vaccine is therefore an appealing goal, with the potential for substantial clinical impact in healthcare institutions across the world.

Host immunity to CDI

C. difficile virulence is largely governed by two protein exotoxins, toxin A (TcdA) and toxin B (TcdB). The host immune response to these toxins likely influences the likelihood of disease, clinical severity and outcome of CDI.[10] A previous prospective study in 271 patients demonstrated higher serum IgG levels to *C. difficile* TcdA in patients with asymptomatic colonisation compared with those with CDI.[45] Furthermore, recurrent CDI has been associated with poor IgG and IgM responses supporting the hypothesis that reduced immune response leads to poor outcomes.[46]

Serum toxin-neutralising antibody can be measured to assess *C. difficile* vaccine efficacy. A good antibody response to TcdA seems most important, but recent evidence suggests that an effective vaccine requires neutralisation to both TcdA and TcdB.[47] Other antigens may also be important; antibodies to surface proteins are greater in *C. difficile* colonised versus infected patients.[48] Ideally, *C. difficile* vaccines could include targets that reduce primary colonisation as well as toxin neutralisation. A key issue for trials is which subjects will best benefit from vaccination.

Phase II/III C. difficile vaccines

Three vaccines that use *C. difficile* toxin targets have reached at least phase II trials. The most advanced is a formalin-inactivated toxoid-based vaccine developed by Sanofi Pasteur.[49] Six phase 1 trials have demonstrated it to be safe and well tolerated. Seroconversion to TcdA was stronger than TcdB (but took up to 70 days) and response was less common in elderly subjects (>65 years). Three doses were required to achieve an adequate neutralising-antibody response.[49-50] A randomised placebo-controlled two-stage phase II trial compared low versus high dose vaccine, both with and without an aluminium hydroxide (AlOH) adjuvant (n=455), and assessed schedules of administration (n=206). A 100 µg dose (with AlOH) had the best immunogenic profile and was chosen for future trials.[51] A phase III trial to assess the safety and efficacy of this vaccine in preventing primary CDI in at-risk subjects aged >50 years is ongoing (predicted completion in 2017; NCT01887912).

Another vaccine, developed by Pfizer, is also formalin-inactivated but has mutations in Tcd A and TcdB to reduce toxigenicity.[52] A phase I placebo-controlled, randomised, observer-blinded study demonstrated efficacy with three parenteral doses.[53] Phase II randomised observer-blinded studies, due to complete shortly, are comparing this vaccine with placebo in 65-85 year olds (NCT02117570 and NCT02561195). The primary outcomes include TcdA and TcdB neutralising antibody responses and an assessment of vaccine-related adverse effects.

C. difficile toxins are difficult to purify and may degrade over time, making them challenging vaccine candidate antigens. For this reason, a vaccine (VLA84, Valneva) containing recombinant toxin domains is being developed, and has satisfactorily completed an open-label phase 1 study assessing safety, immunogenicity and dose response in healthy subjects. A press release in 2015 stated that a phase II trial of 500 US/German volunteers demonstrated safety and good immunogenicity, but further data are not yet available.[54] A phase 3 programme is being planned. Oral vaccination is another possibility, potentially using a vector such as *Bacillus subtilis* spores; CDVAX uses this method but is only at an early stage of development.[55]

Passive *C. difficile* protection - monoclonal antibodies

Serum anti-toxin antibodies afford natural protection against recurrent CDI, but we do not understand what controls the production of these by some but not all individuals. There are no routinely available tests to measure anti-toxin antibodies (in either serum or faeces). Bezlotoxumab has been developed as a human monoclonal antibody that binds to and neutralizes *C. difficile* toxin B. It is given as a single infusion during standard of care CDI antibiotic therapy. In phase 3 clinical trials, bezlotoxumab was compared with actoxumab (a monoclonal antibody to toxin A), a combination of actoxumab and bezlotoxumab.[56] The primary endpoint was recurrent CDI, after initial clinical cure, within 12 weeks. Actoxumab was found to be ineffective at a planned interim analysis, and so was ceased. Bezlotoxumab had no effect on the initial clinical cure of CDI, but significantly reduced recurrence rates compared with placebo (17% vs. 27%, -10.0 95% CI -14.0,-6.0; $p \leq 0.0003$); actoxumab plus bezlotoxumab did not confer additional protection against recurrence. The 40% relative reduction in CDI recurrence achieved by bezlotoxumab was maintained across 4 vs 8 vs 12 weeks of follow up. Also, recurrence rate were lower in pre-defined subgroups with high risk of recurrent CDI and/or adverse outcome, including patients aged $\geq$65 years, those with CDI in the past 6 months, severe CDI, and the immunocompromised. The safety profile of bezlotoxumab was similar to that of placebo. In-patients treated with bezlotoxumab compared with placebo had fewer CDI-associated hospital readmissions (4.0% vs 9.6%, difference -5.7% [95% CI: -8.8, -2.7]) and all-

cause readmissions (23.2% vs. 26.9%, difference -3.7% [95% CI: -9.0, 1.5]) in the 30-days after discharge, in a post-hoc analysis.[57] Notably, bezlotoxumab was also associated with significant reductions in CDI-associated hospital readmissions in inpatients aged ≥65 years, with a history of ≥1 CDI episode in the past 6 months, and cases with clinically severe CDI.

A FDA Antimicrobial Drugs Advisory Committee voted 10 to 5 (1 abstention) in June 2016 to recommend bezlotoxumab injection for the prevention of CDI recurrence (http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Anti-InfectiveDrugsAdvisoryCommittee/UCM505290.pdf). If approved for the prevention of recurrent CDI (decision expected in late 2016), bezlotoxumab would be the first therapeutic to attain this indication.

Microbiome based therapeutics

Live biotherapeutics drugs (as opposed to foodstuffs) are regulated by the Food and Drug Administration (FDA) in a similar manner to drugs. An investigational new drug (IND) application is required, and the drug must be safe, pure, potent, and consistently produced according to good manufacturing practices.

Faecal microbiota transplantation

The evidence base concerning the effectiveness of FMT continues to grow, notably following the publication of the first randomised controlled trial in 2013.[58] FMT is still essentially an experimental, non-standardised procedure, but attracts considerable interest and support, not least due to unmet patient needs for effective therapies for multiple recurrences of CDI. The potential benefits and risks of FMT are summarised in Table 2. Organisations such as Openbiome aim to overcome some of the practical barriers to FMT, by facilitating access to screened faecal transplant material (http://www.openbiome.org/impact/).

Potential safety issues surrounding the administration of live faeces-derived mixtures of micro-organisms, are a concern; such biotherapeutics may include species of unknown significance, which could be beneficial or harmful, including non-cultivable/unknown microbes. The longer term consequences of manipulation of the gut microbiota are unknown. Such issues underlie the restrictions that different regulatory authorities have imposed on the use of FMT, primarily to safeguard patient interests. These include in some settings the need for an IND, and requirements for consenting subjects, screening of donors and recipients. Furthermore, the need for faecal

material preparation and delivery via either rectal or nasogastro/duodenal routes mean that there are intensive endeavours to develop alternatives to FMT, which can reduce risk and/or simplify delivery, and yet still harness the beneficial properties of the gut microbiota

Recent studies have examined the use of freeze-thawed and encapsulated material for FMT.[59,60] Freeze-thawed versus fresh faeces delivered via an enema achieved rates of clinical resolution without recurrence up to 13 weeks compared with that were not significantly different in per-protocol (83.5% *vs.* 85.1%) and mITT (75.0% *vs.* 70.3%) populations.[59] However, about one third of the subjects in each treatment group, who were ultimately classified as resolved according to the study protocol, required two FMTs. It is a relatively common finding that repeat FMT administration is required to prevent recurrences.

A small non-blinded, non-randomised study of encapsulated (and freeze-thawed) faeces was carried out in 20 subjects with $\geq$3 episodes of mild-to-moderate CDI and failure of a 6-8-weeks of vancomycin therapy or $\geq$2 episodes of severe CDI requiring hospitalization.[32] Resolution of diarrhoea was achieved in 14 patients (70%; 95% CI, 47%-85%) after a single capsule-based FMT; 4/6 re-treated non-responders had resolution of diarrhea, resulting in an overall 90% (95% CI, 68%-98%) response rate. No serious adverse events were attributed to FMT.

Live biotherapeutic microbiota preparations

RBX2660 is a live biotherapeutic microbiota suspension that aims to harness the effectiveness of FMT, but within a standardised, regulated product, for the treatment of recurrent CDI. In the first of two phase 2 studies, 34 subjects (with $\geq$2 recurrent CDI episodes or $\geq$2 severe episodes resulting in hospitalization) received at $\geq$1 dose of RBX2660 and 31 completed 6 months follow up.[61] Following a 10-14 day course of CDI antibiotics and a 24-48 hour washout period, RBX2660 was administered as a single dose via enema. Further recurrent CDI occurred in 48% of subjects after one dose of RBX2660, with 15/31 patients receiving a second enema; of these, 78.6% were considered to be treatment successes, contributing to an overall success rate of 27/31 (87.1%). A second phase 2 study has recently been completed, but results are not yet available.

SER-109 (Seres) is also a live biotheraputic that comprises an encapsulated mixture of purified *Firmicutes* spores, derived from human faeces. As the spores are resilient to manipulation, ethanol treatment has been used to theoretically reduce the risk of transmissible, harmful infectious agents contaminating the therapeutic product. Seres has completed two phase 2 studies of SER-109. The

first of these was a non-comparative study in patients with ≥3 CDI episodes in the previous 12 months.[62] 26/30 patients (86.7%) across two dosing groups met the primary efficacy end point (absence of *C. difficile*-positive diarrhoea during 8 weeks of follow-up). 96.7% of subjects were considered to have achieved clinical resolution, as 3 patients with early, self-limiting *C. difficile*-positive diarrhoea did not require antibiotic treatment. Notably, the loss of diversity of gut microbiota that is typical in CDI patients rapidly reversed after receipt of SER-109. The interim results of the second, recently completed, phase 2 (ECOSPORE) study of SER-109 have just been released (http://ir.serestherapeutics.com/phoenix.zhtml?c=254006&p=irol-newsArticle&ID=2190006). SER-109 was not effective overall at reducing CDI recurrence, but was efficacious in subjects aged ≥65 years old, in whom CDI recurrence occurred in 14/31 (45%) of subjects who received SER-109 versus 12/15 (80%) of those who received placebo. Further analyses will be important to understand these results.

Non-toxigenic C. difficile

A randomized, double-blind, placebo-controlled, dose-ranging study examined the efficacy of a non-toxigenic *C difficile* (NTCD) strain to prevent recurrent *C difficile* infection (CDI), presumably by displacing (blocking) toxigenic bacteria remaining after CDI antibiotic treatment.[63] About two thirds (69%) of NTCD recipients were colonized, with only a 2% CDI recurrence rate in these patients. However, of those not colonized by NTCD, recurrence occurred in 31% (similar to placebo). Of note, none of the subjects who were colonized at week 6 remained colonized by week 26.

Summary

There are a number of promising approaches aimed at tackling the global challenge of CDI. However, further trials are required to determine which, if any, can take a role in the routine primary prevention, treatment and/or secondary prevention of CDI.

Key points (3-5)

1. *C. difficile* remains a significant cause of morbidity and mortality worldwide. Current approaches to reduce CDI using antimicrobial stewardship and infection prevention programmes have not led to a consistent marked decline in disease rates.
2. Historically, two antibiotics (metronidazole and vancomycin) and a recent third (fidaxomicin) have been used routinely for CDI treatment; convincing data are now available showing that metronidazole is the least efficacious agent.

3. Novel antimicrobial agents are being evaluated in Phase III trials; it is not yet clear what will be the roles of these agents in future CDI treatment.
4. Prophylaxis is an optimum approach to reduce the impact of CDI especially in high-risk populations; monoclonal antibodies, antibiotic blocking approaches and multiple vaccines are currently in advanced clinical trials.
5. The treatment of recurrent CDI is particularly troublesome, and several different live biotherapeutics are being developed, in addition to faecal microbiota transplantation (FMT).

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Conflicts of Interest

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