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T.V. Riley
Edith Cowan University

D. Lyras

G. R. Douce

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Status of vaccine research and development for *Clostridium difficile*

T.V. Riley [a,b,c], D. Lyras [d], G.R. Douce [e,*]

[a] Edith Cowan University, Joondalup, Western Australia, Australia
[b] Murdoch University, Murdoch, Western Australia, Australia
[c] PathWest Laboratory Medicine, Nedlands, Western Australia, Australia
[d] Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton, Victoria, Australia
[e] Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, Sir Graham Davies Building, University Place, University of Glasgow, Glasgow G12 8TA, United Kingdom

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ABSTRACT

Clostridium difficile associated disease is fundamentally associated with dysbiosis of the gut microbiome as a consequence of antibiotic use. This is because this sporulating, obligate anaerobe germinates and proliferates rapidly in the dysbiotic gut, which is an indirect consequence of their use. During its growth, *C. difficile* produces two toxins, toxin A (TcdA) and toxin B (TcdB), which are responsible for the majority of clinical symptoms associated with the disease. Three parenterally delivered vaccines, based on detoxified or recombinant forms of these toxins, have undergone or are undergoing clinical trials. Each offers the opportunity to generate high titres of toxin neutralising antibodies. Whilst these data suggest these vaccines may reduce primary symptomatic disease, they do not in their current form reduce the capacity of the organism to persist and shed from the vaccinated host. The current progress of vaccine development is considered with advantages and limitations of each highlighted. In addition, several alternative approaches are described that seek to limit *C. difficile* germination, colonisation and persistence. It may yet prove that the most effective treatments to limit infection, disease and spread of the organism will require a combination of therapeutic approaches. The potential use and efficacy of these vaccines in low and middle income countries will be depend on the development of a cost effective vaccine and greater understanding of the distribution and extent of disease in these countries.

1. An overview

1.1. The disease and pathogen

Clostridium difficile is the leading cause of healthcare-associated diarrhoeal disease in the developed world [1], and a major cause of community-associated infection (CAI) [2]. This organism, which is found naturally in the gastrointestinal tracts of many domesticated animals, and consequently the environment [3,4], is strongly associated with disease in patients with increasing age and frailty [5], immunodeficiency and in particular modification of the normal microbiota through antibiotic use [6]. Extended stay in healthcare facilities is associated with an increased risk of disease, with infection linked to localised environmental contamination with metabolically inactive spores that are resistant to most cleaning protocols [7]. Elderly patients treated with antibiotics are particularly vulnerable as such treatments modify the composition and complexity of the intestinal bacterial population [8]. Loss of microbiome diversity as a consequence of antibiotic use has been linked to a reduced capacity to process and modify primary bile salts (including taurocholate and cholate) to their secondary state and these are reabsorbed. Loss of organisms that perform this function results in failure to process these compounds resulting in increased concentrations of primary bile salts, which act as germinants for *C. difficile* spores. Further, as secondary bile salts inhibit *C. difficile* vegetative growth, their loss is significant in disease progression [9]. Clinically, infection varies from asymptomatic carriage to mild, single episode infections, through to severe, recurrent and disabling disease. Rates of morbidity and mortality differ widely, reflecting differences in both host vulnerability and the genetic composition of the infecting bacterium.

Disease is largely associated with the production of two large glucosyltransferase exotoxins, Toxin A (TcdA) and Toxin B (TcdB) [10,11] that modify the cellular architecture of the epithelial surface of the colon through interactions with members of the superfamily of Rho GTPases [12] that are involved in maintenance of cell cytoskeleton. The resulting loss of integrity of epithelial cells of the

* Corresponding author.
E-mail address: Gillian.Douce@glasgow.ac.uk (G.R. Douce).

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mucosal barrier not only limits absorption of water but also induces a prolific inflammatory response including an influx of high numbers of polymorphonucleocytes (PMNs). Whilst symptoms can be alleviated through treatment with metronidazole, fidaxomicin and vancomycin, which destroy the toxin-producing bacteria, further complications including pseudomembranous colitis, toxic megacolon and sepsis also occur in a number of cases. TcdA and B are highly homologous, multi-domain proteins whose structure and function are well described [13,14]. The genes for TcdA and B (*tcd*A and *tcd*B) are encoded on a 19.6 kB pathogenicity island and amplification of sequences within this locus has identified multiple 'toxinotypes' [15]. In general, differences are associated with point mutations in the catalytic domain of TcdB and deletions in the C-terminus of TcdA. Naturally occurring PaLoc negative strains exist and have been used effectively to restrict colonisation with toxin producing strains [16]. However, the observation that the genes encoding these toxins are linked to mobile genetic elements [17] has raised concerns that acquisition of these genes by non-toxic strains can add to disease burden. The relative contribution of each toxin in disease has been the subject of some controversy, with early studies indicating TcdA alone was responsible for diarrhoeal symptoms [10]. However, recent re-evaluation suggests that TcdB is a key factor in *C. difficile* disease severity [11].

In addition, a small but clinically relevant number of strains encode a third toxin, commonly referred to as *C difficile* binary toxin (CDT) [18]. This toxin is composed of two domains, an enzymatically active A component (CDTa), which causes ADP-ribosylation of G-actin, and a cell-binding and translocation B component, CDTb. CDT alone does not induce severe disease in a hamster [19] or mouse model of CDI [20] but appears to enhance colonisation [21] and contribute to virulence [22] Although the biological role of CDT in infection appears to be adjunctive, enhanced protection in preclinical vaccine trials following inclusion of this antigen was seen [23].

1.2. Current diagnosis and treatment

New technologies that allow rapid diagnosis of infection have largely replaced microbiological culture of the organism, although culture currently remains a requirement for molecular epidemiological investigations. Detection depends on recognition of two targets; glutamate dehydrogenase (GDH), a common conserved antigen in *C. difficile* and the A and B toxins (or genes) (most frequently but not exclusively TcdB) in diarrheal stool samples [24]. These antigens or genes are recognised through the use of specific antibodies in commercial EIA based tests or amplification of specific sequences through nucleic acid amplification tests or NAAT. Ideally, identification of a positive sample initiates an algorithmic approach to confirm diagnosis. Metronidazole and vancomycin remain first line drugs for treatment of confirmed disease (if simple withdrawal of the inciting antimicrobial cannot be done), with metronidazole typically prescribed for mild disease and vancomycin for severe disease [25]. Recovery can be complicated by recurrence of symptomatic disease following completion and withdrawal of antibiotic treatment. Persistence of dysbiosis within the gut is thought to support germination of either resident spores or spores from the contaminated environment. Typically, patients that suffer one recurrence have a significant risk of enduring further and frequently multiple recurrences [26]. Fidaxomicin, recently introduced as an alternative treatment to the standard antibiotics, offers a reduced risk of recurrence as it has less impact on the normal microbiota [27]. However, its high cost is limiting widespread use. In patients who suffer multiple recurrences, a faecal transplant can be a highly effective treatment [28,29]. Stools provided from a healthy 'donor' and introduced via the upper or lower gastrointestinal tract allow the diversity of gastrointestinal microbiota to re-establish, limiting further growth of *C. difficile* and eliminating symptoms [30].

1.3. Economic burden of disease

The global increase in incidence and severity of *C. difficile* infection (CDI) over the past 20 years reflects a combination of factors including the greater use and misuse of antibiotics, a larger vulnerable population as mean average age increases and a greater awareness and diagnosis of disease. Studies performed in North America and Europe have reported two- to four-fold increased incidence of CDI in the past decade. In the United States, alone, an estimated 453,000 new cases of CDI arise per year, with the infection linked directly to approximately 29,000 deaths within 30 days of diagnosis of CDI [31]. In direct comparisons between patients with and without CDI, the resultant increased in hospital stay elevated hospital costs (3.5-fold) with patients six times more likely to die as a consequence of infection. Reports from United States National Vital Records reveal that from 1999 to 2008 death certificates listing C. difficile enterocolitis as the primary cause of death increased from 793 to 7483 with the majority of deaths from CDI occurring in persons >65 years of age [32]. The economic burden of this disease is thus significant [31] (multi-billion dollars in healthcare costs) and set to rise further as life expectancy in developing countries increases. This is not a problem limited to the US and Europe. In Korea, the prevalence of *C. difficile* increased from 1.43 cases per 10,000 in 2008 to 5.6/10,000 in 2011, with an increased economic burden over that time of $13.4 million [33]. In Australia, a large-scale study using data from 89 hospitals within Victoria reported 6736 cases of CDI between Oct 2010 and Dec 2014; rates were comparable to those in the US and Europe. Of these, 4876 cases (2.49/10,000 occupied bed days) were linked to hospital stay, with the remaining linked to community associated infections CAI [34]. In this study, severe disease was significantly higher in CAI with links to food contaminated with the epidemic outbreak strain [35]. This observation further supports the increased need for surveillance of disease within both the hospital and community settings.

1.4. Geographical distribution

Traditionally, CDI has been largely a disease of the hospitalised elderly, although the extent and impact of *C. difficile* disease has predominantly been limited to descriptions from high income countries (HICs). This is because there are very few reliable and quantitative countrywide evaluations of *C. difficile* disease in most low and middle-income countries (L + MIC). This failure was recently highlighted by a systematic review of the literature, which combined key terms '*Clostridium difficile*', '*C. difficile*', antibiotic associated colitis and pseudomembranous colitis, with countries recognised as low and middle human development index (LMHDI) countries by the United Nations Development Programme [36]. Of the 150 studies identified using this approach between Jan 2000 and Mar 2016, 125 were excluded on grounds of relevance and lack of demographic information. Of the remaining 25, 20 (80%) were observational and over half were conducted in India. Only 4/25 studies were multi-institutional. Individual studies within particular hospitals in Asian countries such as Indonesia [37], Thailand [38] and South Korea [39] suggested the organism is widespread and capable of causing clinical disease, although given the widespread availability of antibiotics, the number of cases was lower than expected. In sub-Saharan Africa, carriage of toxigenic strains linked to diarrhoeal disease, antibiotic usage and HIV status have been reported in the few studies that have been performed [40]. This paucity of epidemiological data from these countries makes extrapolation of the impact of vaccine introduction difficult to

predict. Further, this highlights a significant and important gap in our knowledge that needs to be addressed rapidly. This information will provide an accurate understanding of disease burden within these countries but will also be essential for deciding whether widespread vaccination is warranted for which patients and at what age. More significantly, understanding the baseline of infection is essential for determining the impact of vaccine introduction. However, this will require standardisation of terms and sampling techniques to ensure comparative analysis is valid. Further, greater understanding of epidemiology within these countries should be supported by the use of a sensitive and reproducible typing system. This will eliminate issues associated with the use of different typing methods (pulse field electrophoresis, restriction endonuclease analysis and PCR ribotyping) that have been previously employed in different countries [41].

Over the last 10 years, the epidemiology of CDI has changed dramatically with the emergence of several 'hyper-virulent' ribotypes including the BI/NAP1/027, which have been associated with significant outbreaks globally [42]. Enhanced pathogenicity and risk of infection has been linked to a variety of factors including the production of binary toxin [22] and resistance to fluoroquinolones [43]. Strains with similar features and associated with high incidence of severe disease have also emerged in other countries, including ribotype 244 (RT244) in Australia [44] and RT176 in Eastern Europe [45]. Both of these RTs belong to the same genetic clade (clade 2) as RT027 but are distinct from RT027 strains. Although modification of prescribing policies can significantly reduce the number of outbreaks [46] associated with ribotype 027 in particular, and infections more generally (55,000 infections in 2008 to less than 10,000 in 2013), less prevalent but clinically relevant ribotypes have emerged and are becoming dominant in several countries in Europe. These include RTs 056, 078 and 126 that are commonly associated with infections in farm animals [47]. Changes in clinically relevant types may additionally reflect the significant rise in CAI, which comprises more than 40% of all cases in some studies [48]. Interestingly, within individual countries, particular types appear to dominate, for example 017 isolates account for 20% of Shanghai isolates whilst in Stockholm, 005 strains are prevalent [49]; in Scotland 078 isolates account for over 10% of infections [50]. Recent studies performed in large tertiary hospitals in Malaysia [51], Indonesia [52] and Thailand [38] underline the importance of using a combined diagnostic approach. In these studies non-toxic strains and those expressing only TcdB were identified when a combination of immunoassays for detection of glutamate dehydrogenase (GDH) and TcdA/B were used. These data highlighted the prevalence of the 017 ribotype in Asia [53,54] and draw attention to the presence of non-toxigenic *C. difficile* in this population. In summary, these data suggest local or regional circulation of particular ribotypes implying that infection may be a consequence of an unidentified reservoir of infection. Furthermore, antibiotic resistance, both intrinsic and associated with acquisition of specific genes, appears to be increasing, with high levels of resistance to fluoroquinolones associated with the global spread of the BI/NAP1/027 [42] strain. However, more concerning is the reported observation of resistance to metronidazole [55] and vancomycin, which are the front line treatments for this infection [56]. These reports highlight and focus our vulnerability with respect to treatment options and are very much associated with the drive to develop an effective vaccine against this disease.

1.5. Target groups for vaccination

Increasing age, exposure to antibiotics and long-term stay within a health facility are all risk factors associated with CDI. There is also some evidence that pregnant women and some children are vulnerable to this infection [57] possibly reflecting greater recent exposure to the organism rather than changes in susceptibility. One problem associated with vaccination of the elderly is immune senescence that can cause impaired recognition of antigens. This can make vaccination of this vulnerable population particularly difficult.

2. Overview of current efforts: status of vaccine research and development activities

2.1. Toxin based vaccines

Protection against symptomatic (diarrhoeal) disease is dependent on the production of high levels of neutralising antibody to both TcdA and TcdB [58]. In general, high levels of systemic antibodies to TcdA correlate with protection from diarrhoea whilst neutralising antibodies to TcdB reduce disease severity and recurrence [59,60]. As such vaccines with the capacity to generate strong neutralising activity to both toxins offer an opportunity to reduce symptomatic disease. However, in animal models these vaccines do not appear to prevent colonisation of the organism or onward transmission through shedding of the organism in the stool [61].

Several vaccines that have completed, or are currently undergoing, clinical evaluation in humans are outlined in Table 1 with detailed descriptions given below.

2.1.1. Sanofi Pasteur toxoid vaccine

Until October 2017, the most advanced vaccine formulation in human trials was a toxoid-based vaccine produced by Sanofi Pasteur. This vaccine had been tested in several clinical phase I (NCT00214461, NCT00127803, NCT00772954) and II trials (NCT01230957, NCT00772343), and a large scale phase III trial was initiated in October 2013 (NCT01887912). This vaccine contained formalin-inactivated preparations of TcdA and TcdB, purified from the naturally high toxin producing *C. difficile* strain VP110463, admixed with $AlOH_3$ adjuvant. In reported phase II studies (June 2011–2013), approximately 90% of patients (aged 40–64) showed high levels of neutralising activity when immunised on 3 occasions (0, 7 and 30 days) with 100 μg total antigen. A subsequent open labelled study in the older patients (aged 65–75 years of age) revealed an approximate 60% conversion rate which could be enhanced to approximately 90% following a fourth boost on day 180 [62]. The manufacture of these vaccines at global scale was considered achievable, given that it was based on conventional and proven methodologies. However, following primary interim reporting of phase 3 data, it was concluded that the primary objective, the prevention of primary CDI, was unlikely to be achieved. This has resulted in the entire programme of vaccine development within Sanofi Pasteur being halted. This significantly changes the landscape in vaccine development as failure, at this relatively late stage in development, has implications for the remaining vaccines currently undergoing clinical evaluation that are also based on similar approaches. These approaches are described as are other approaches, the value and importance of which may gain increased attention in this changing climate

2.1.2. Pfizer genetically detoxified toxin vaccine

In contrast to the Sanofi Pasteur vaccine, this formulation contains modified versions of both TcdA and TcdB with reduced toxicity as a consequence of amino acid substitutions in the glucosyltransferase domains of both toxins (D285A/D287A for TcdA; D286A/D288A for TcdB) [63]. These toxins are generated from an episome expression vector used to transform a non-sporulating *C. difficile* that lacks the *tcdA* and *tcdB* genes [63]. Analysis of these recombinant proteins revealed a low level of residual toxic activity, requiring the proteins to be additionally chemical

Table 1
Development status if current vaccine candidates.

Candidate Name/identifier	Developer/manufacturer	Approach	Phase of development	References/ clinical trial ID
Cdiffense	Sanofi/Pasteur	Chemically detoxified TcdA and TcdB	Terminated at phase III	NCT 01,887,912 Ref. [62]
PF-06425090	Pfizer	Genetically and chemically detoxified TcdA and TcdB	Phase III	NCT03090191 Ref. [63,64]
VLA84	Valneva	Recombinant chimeric protein linking the binding domains of TcdA and TcdB	Phase II	NCT02316470 Ref. [65]
VP20621	Shire	Mucosally delivered, live non toxigenic strain of *C. difficile*	Phase II	NCT01259726 Ref. [16]

detoxified to increase safety. In phase 1 studies, these vaccines were safe and highly immunogenic, generating strong and sustainable neutralising activities in both 40–64 and 65–75 age groups with antigen alone vaccines more potent han those containing the $Al(OH)_3$ adjuvant (NCT 01706367) [64]. This vaccine was granted fast track designation by the FDA in 2014 and, based on interim reporting of data from a phase II study looking at the impact of an accelerated and non-accelerated immunisation schedule of responses in individuals aged 65–85 (NCT02561195), a phase III study to establish the impact of the vaccine on primary CDI was initiated in early 2017 (NCT03090191). Results from this study are expected in the third quarter of 2020, with data on the incidence of recurrence following vaccination being included as a secondary measurement of efficacy in this trial.

2.1.3. Valneva recombinant chimeric vaccine VLA84

In contrast to the toxoided vaccines, the Valneva vaccine is based on a recombinant chimeric protein, designed to capture neutralising epitopes of both toxins within a single protein. This approach avoids issues associated with residual toxicity by genetically fusing the binding (C-terminal) domains from both toxins (TcdA, 15 of 31 repeats; TcdB 23 of 24 repeats) via a short linking sequence of 12 amino acids. Consequently this vaccine can be generated in heterologous expression systems avoiding the need for *C. difficile* culture. This vaccine is anticipated to be less complex to manufacture than the equivalent toxoided vaccines. In phase I studies (NCT01296386), unadjuvanted antigen appeared as immunogenic as adjuvanted formulations. Antibodies raised to these antigens were able to neutralise the activity of both native toxins produced by VPI10463 [65]. This data has been confirmed in a successful phase II study (NCT02316470) in two cohorts of patients (aged 50–64/aged 65+).

One disadvantage of this chimera is that it lacks several neutralising epitopes that have been located elsewhere in the toxin [66,67]. This includes epitopes within the glucosyltransferase domain that are not included in the chimera. Also it does not target the other host receptor binding regions identified in TcdB, which facilitate uptake of the toxin independently of the C-terminal repeat region [68]. Further, some isolates of *C. difficile* express variants of TcdB with amino acid substitutions in the binding domain, which may limit the capacity of this vaccine to be effective against diverse clinical isolates [69,70]. The relevance of this variation with respect to neutralising activity is unclear at present but should be considered, especially for those vaccines based on the binding domains alone.

2.2. Pre-clinical consideration of additional vaccine antigens

2.2.1. Addition of CDT

Several strains of *C. difficile*, including many associated with outbreaks or greater severity of disease (ribotypes 027, 078, 244), encode a third toxin, CDT. Combining this antigen within toxoided vaccines in hamsters enhances protection against these strains when compared to vaccines based only on TcdA and TcdB [23]. Whilst inclusion of this antigen offers an opportunity to improve the response to a number of clinically important strains, including the epidemic 027 ribotype, its late identification as a vaccine candidate may limit its inclusion in those vaccines likely to be licensed in the next 5 years.

2.2.2. Addition of vaccine antigens to limit colonisation

Prevention of primary CDI is the stated objective of all of the current vaccines undergoing clinical evaluation. Success in this context is determined by production of toxin neutralising antibodies and the absence of diarrheal disease. However, data from animal models suggests that protected animals continue to shed the organism for up to 3 weeks post infection [61]. Therefore, use of a vaccine that prevents toxin-mediated symptoms but does not limit germination, outgrowth, sporulation and release of the spores into the environment could indirectly result in increased transmission. Further, if diagnosis of infection is partly reliant on detection of toxin in the faeces, its neutralisation may lead to underreporting of cases. An ideal vaccine should therefore be formulated to include bacterial factors that target germination, colonisation or sporulation. Several antigens have been proposed and tested preclinically, including several surface exposed cell wall proteins. These include SlpA, Cwp66, Cwp84 and flagellar antigens, which have been identified as being actively involved in early colonisation of the gut [71,72]. Whilst all appear to be immunogenic, in preclinical testing none has generated high levels of protection against *C. difficile* colonisation or disease [73,74].

2.2.3. Use of non-toxigenic strains of C. difficile to limit disease

The potential of non-toxigenic strains, delivered mucosally to limit primary and recurrent disease, has been evaluated preclinically, with impact on recurrent disease determined clinically (NCT01259726) [16]. This approach offers direct competition for the colonisation niche within the host and can easily be used in L + MICs. Administration of this vaccine was well tolerated and reduced recurrence from 30% in patients receiving the placebo compared to 11% receiving the vaccine (odds ratio [OR], 0.28; 95% CI, 0.11–0.69; P = .006). This vaccine is attractive as it offers the opportunity to stimulate mucosal responses that may limit colonisation. However, the observation that the toxin genes can be acquired via horizontal gene transfer raises the concern that this approach may result in evolution of new toxigenic strains [17].

2.2.4. Spore coat proteins as vaccine candidates

Several proteins located within the exosporium coat of *C. difficile* including CotA, CotE, CdeC and CdeH have been identified as possible vaccine targets [75]. It is predicted that generation of antibodies to these proteins may offer a combined impact, encouraging uptake and destruction of spores prior to germination, and limiting efficient sporulation and excretion from the host [76].

2.2.5. Mucosal delivery of recombinant spores

Whilst systemically generated anti-toxin responses reduce or eliminate toxin-associated symptoms, activation of mucosal responses at the immediate site of infection may reduce symptoms through the prevention of toxin action within the gut. One approach that has shown preclinical success is the oral delivery of recombinant *B. subtilis* spores expressing fragments of TcdA, which limited colonisation and disease in hamsters [77]. Further protection was associated with high levels of toxin specific sIgA in the faeces of these animals highlighting the importance of mucosal response in protection.

2.2.6. Carbohydrate-based vaccines

Three phosphorylated polysaccharides; PSI, PSII and PSIII, first revealed during structural analysis of the *C. difficile* cell wall have also been considered as potential vaccine candidates [78]. In particular PSII, a common antigen to many strains, appears immunogenic with antibodies to this antigen found in the stools of naturally infected horses and man [79]. Experimental synthetic vaccines generated through conjugation to diphtheria toxoid (crm197) or recombinant fragments of generate high level of PsII specific antibody in mice and farm animals [80,81]. Whilst these antibodies recognise *C. difficile* vegetative cells in culture, their capacity to protect against primary CDI remains unclear.

3. Therapeutic approaches for primary and recurrent disease

Whilst effective vaccination offers an opportunity to prevent symptomatic disease, several alternative prophylactic and therapeutic approaches are undergoing clinical and preclinical evaluation. Although adoption of some of these approaches may be limited by cost and lack of infrastructure, the opportunities they offer should be considered in the context of effective vaccine development.

3.1. Microbiome modification

Whilst prevention of disease should be the key objective of any vaccine, prevention of recurrent disease, which places a further financial burden on the healthcare system of any individual country, should be considered. In this context, our increasing knowledge of functional activity of particular bacteria within the microbiome would suggest that treatments based on replacement of key members of the gut microbiota are feasible. Whilst faecal microbiota transplantation (FMT) is reported to be clinically effective [28,30], the unpleasant nature of the procedure and the level of screening required to ensure the transplant is free from significant enteric pathogens continue to limit its use therapeutically. In the future, identification of key organisms with specific metabolic functions will allow specific combinations of bacteria to be prepared. Such an approach is effective at limiting colonisation in mice [82] but similar combinations for treatment of human disease have still to be evaluated. Currently, 41 clinical trials to evaluate the effectiveness of FMT for *C. difficile* are ongoing. Most are focussed on modes of delivery; fresh versus frozen samples, impact of encapsulation and use of defined bacterial formulations. Rebiotix, who have focussed on the commercialisation of this approach, have completed a phase 2 open label study using a standardised microbiota suspension (RBX2660) (NCT01923417). This trial reported 87% efficacy in 31 patients 6 months after treatment with bacterial suspension delivered by enema. This formulation prevented recurrence in 50% of patients following a single treatment and in 87% of patients after a second dose [83]. Whilst this approach has potentially wide application, some caution is warranted regarding its widespread application in patients whose general health status is likely to be poor. Further, the increasing number of links that are being made between microbiome composition and conditions such as diabetes, obesity and mental health issues [84–86].

3.2. Antibody therapy

An alternative approach to treatment of recurrent disease is the direct infusion of humanised monoclonal antibodies capable of neutralising toxin activity. Merck have pioneered this approach and clinical trials using their monoclonal antibody formulations Bezlotoxumab (raised to TcdB) and Actoxumab (raised to TcdA) have been completed (NCT00350298, NCT01241552). Results from phase II clinical studies (NCT00350298) showed that a single infusion of the Bezlotoxumab antibody given in conjunction with standard metronidazole or vancomycin treatment reduced the recurrence of *C. difficile* recurrence compared to antibiotic treatment alone [87]. In contrast, use of actoxumab alone did not appear to show any clinical benefit. These results have been supported by data produced in recent phase III trials (NCT01241552), in which treatment with Bezlotoxumab compared to the placebo-controlled group reduced recurrence by around 10% [88]. However, as administration of this intravenous treatment will require specialist healthcare facilities, generation of antibodies is likely to be costly and its efficacy limited, it is unlikely to become a routine treatment for most patients.

An alternative approach is the use of orally delivered colostrum-derived antibodies, which are generated by immunisation of pregnant cows with spores, vegetative cells and TcdB purified from *C. difficile* [89]. In preclinical evaluation of mouse infection and relapse models, administration of these antibodies prevented 80% of primary CDI and reduced disease recurrence by 67%.

3.3. Phage/phage tails

Several bacteriophages with lytic activity to *C. difficile* have been identified and studied in preclinical studies [90,91]. Phages are attractive as therapeutics as their specificity ensures minimal disruption and loss of diversity within the microbiome. Whilst currently identified natural phages encode integrases, which limit their long-term lytic effectiveness, the potential to genetically modify such phages to improve efficacy is promising. An alternative approach is the use of *C. difficile* R type bacteriocins, which resemble Myoviridae phage, structurally encoding a contractile sheath, a nanotube core and tail fibers. However, instead of delivering DNA across the bacterial membrane, injection of the nanotube core disrupts the cell membrane potential and kills the cell. These protein antibiotics are effective in mouse models at reducing the number of organisms persisting in the gut [92]. Further, these proteins can be manipulated to redirect their specificity using phage sequences from existing lysogenic phages within the genome. As a consequence, recombinant versions have been generated with killing activity against the majority of current clinical types [93].

4. Likelihood for vaccine implementation

Given the extent of testing of the vaccines currently in clinical trials, it is feasible that a licensed *C. difficile* vaccine may be available within the next 5 years. However, this will depend on the demonstrable level of efficacy generated by the two remaining vaccines. Given the apparent failure of the Sanofi vaccine in phase 3 trials, it is essential that, moving forward, improvements to the existing vaccine formulations should be considered. This may

include the addition of further antigens that limit colonisation or sporulation, or identification of alternative or combined treatment regimens. Most importantly, we need to identify approaches that limit both primary and recurrent disease burden, which will reduce suffering and lower the economic impact on worldwide rising health-care costs. Whilst the burden of disease is currently associated with the increasing elderly population within high income countries (HICs), greater understanding of the extent of disease within M + LICs will allow appropriate decisions regarding the best approach to limit disease. Implementation of vaccine programmes or other interventional strategies will depend on the availability and use of standardised systems of surveillance, many of which are currently not available. In many countries, significant organisational and financial effort will be required including the adoption of established methodologies that allow direct correlation with existing data. Building this resource now will allow more rapid use and dissemination of effective treatments, including vaccines, to limit CDI.

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