Investigational New Treatments for *Clostridium difficile* Infection

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Teaser: With a remarkably diverse set of therapeutic approaches currently in clinical trials, how much potential is there for further innovation in developing new drugs to treat *Clostridium difficile* infection?

Significant progress has been made by the biotech and pharmaceutical industries in the last two years to address the unmet medical need posed by *Clostridium difficile* infection. These developments provide an excellent example of how patient need has driven a surge of cutting-edge innovation in drug discovery. Indeed, only two drugs have been approved for the infection in the last thirty years but there are thirteen treatment candidates undergoing clinical trials today. What makes the latter number even more remarkable is the diversity in the strategies that it includes: antibiotics, microbiota supplements, vaccines, antibiotics quenchers and passive immunization are all represented. In this Review, we provide a snapshot of the current stage of these breakthroughs and argue that there is still room for further innovation in the development of *Clostridium difficile* anti-infectives.

Clostridium difficile is now widely recognized as the leading cause of nosocomial diarrhea worldwide and is associated with substantial morbidity [1,2]. The reported incidence of *C. difficile* infection (CDI) has exploded in the last 15 years and is of the order of hundreds of thousands of cases per year in the United States and Europe (Fig. 1) [3,4]. The number of community-acquired cases is also on the rise [5]. An important factor causing these increases is the emergence of so-called hypervirulent strains such as NAP1/BI/ribotype 027 that are more resistant to antibiotics and produce more toxin. In addition, they have also been suggested to form spores more readily and to adhere to intestinal epithelium better than wild-type strains [6]. Reports of CDI cases outside of the United States and Europe have also been on the rise in recent years [7,8]. Accurate numbers for CDI prevalence are difficult to obtain for several reasons that complicate reporting of cases. These include a high rate of cases in patients with co-morbidities, lack of widespread testing of at-risk patients and variable diagnostic methods [9]. Furthermore, reporting of CDI cases is not mandatory in all countries [10].

CDI typically occurs when a patient is given broad-spectrum antibiotics, which deplete the gut flora, and is exposed to *C. difficile* spores that germinate in the colon after ingestion. *C. difficile*'s resistance to several commonly used antibiotics such as clindamycin and moxifloxacin is thus the root cause of infections [11]. Once established in the colon, *C. difficile* secretes two toxins, toxin A (TcdA) and toxin B (TcdB) that are the bacterium's main virulence factors. These toxins are large proteins containing four domains, three of which (the binding, translocation and cysteine protease domains) act sequentially to deliver the fourth (the glucosyltransferase domain) into the cytosol of target cells, namely cells of the intestinal epithelium [12]. The glucosyltransferase domain disrupts the actin cytoskeleton, ultimately causing cell death. At the macroscopic level, this results in diarrhea and pseudomembranous colitis, which can lead to severe and fatal complications such as toxic megacolon, bowel perforation, renal failure, systemic inflammatory response syndrome and sepsis [14]. The present Review offers a critical overview of chemical, biological and microbiological agents currently

in development for the treatment of CDI (Fig. 2), with a strong focus on drug candidates in clinical trials (Table 1).

[Figure 1]

FIGURE ERROR! NO TEXT OF SPECIFIED STYLE IN DOCUMENT. Number of diagnosed cases of *C. difficile* infection in the United States between 1997 and 2012 (data from hcupnet.ahrq.gov). This figure is an update of the data presented in reference [3]. The data were obtained by searching using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) code for "Intestinal infection due to *Clostridium difficile*": 008.45. All listed diagnoses for all patients in all hospitals in the US were searched.

Antibiotics for the Treatment of CDI

The antibiotics metronidazole and vancomycin have been the treatments of choice for CDI for over 30 years and are to this day still the recommended drugs in guidelines to physicians in Europe and the United States (metronidazole has not been approved for this indication but is nevertheless widely used) [15–17]. Metronidazole is a nitroimidazole prodrug that, once taken up and reduced by bacterial cells, binds DNA covalently to inhibit nucleic acid synthesis [18]. Vancomycin is a glycopeptide that inhibits the synthesis of peptidoglycans necessary for bacterial cell wall integrity and is a so-called antibiotic of last-resort [19]. Metronidazole and vancomycin are both broad-spectrum antibiotics, with the former being the reference therapy for infections with anaerobic bacteria and some parasites, while the latter displays activity against the majority of Gram-positive bacteria [20]. As such, they are active against *C. difficile* but also cause significant damage to the endogenous gut microbiota, which exposes patients to the risk of re-infection due to reduction of the protective barrier function of the microbiota. Indeed, a recent systematic review of treatment failures with the two antibiotics revealed that complete cure was achieved in 77.6 % and 85.8 % of cases, with 27.1 % and 24.0 % recurrence for metronidazole and vancomycin, respectively [21]. The fact that metronidazole is less efficacious for treatment of severe infections than vancomycin (but equivalent for mild disease) is the main reason for the small discrepancies in the figures obtained for each antibiotic. This, and the fact that metronidazole is roughly 10 times less costly than vancomycin, is also the reason why metronidazole is used to treat mild infections whereas vancomycin is recommended in cases of severe infection or of a lack of response to metronidazole [17]. Indeed, the recommendations for CDI management state that metronidazole 500 mg should be administered three times daily in cases of mild to moderate CDI, and vancomycin 125 mg should be administered four times daily for severe CDI, both orally for a duration of ten days. Since vancomycin is not absorbed, it tends to be associated with minimal side-effects. Metronidazole, on the other hand, can be absorbed and lead to mild-to-moderate systemic side effects such as nausea, vomiting and in rare cases, peripheral and optic neuropathy [16].

Emerging strains of *C. difficile* are becoming less susceptible to both metronidazole and vancomycin [11,22]. Exacerbating the harmful consequences of their use, it has also been shown that

these antibiotics enhance overgrowth of vancomycin-resistant enterococci, another healthcare-associated pathogen of serious concern [23]. Taken together with the incomplete efficacy and high rates of recurrence attained with metronidazole and vancomycin, it is clear that there is significant upside potential for novel therapies to treat CDI.

In 2011, fidaxomicin (Optimer Pharmaceuticals) became the second drug to gain approval from the U.S. Food and Drug Administration (FDA) for the treatment of CDI [24]. Fidaxomicin, whose discovery was first described in 1975, is a macrolide displaying a narrow-spectrum of activity against *C. difficile* and a few other Gram-positive anaerobes, which acts through inhibition of RNA polymerase. Importantly, fidaxomicin spares certain key species of the endogenous microbiota such as *Bacteroides* and *Bifidobacterium* and thus induces lower selection pressure for overgrowth of harmful bacteria [25,26]. Minimizing damage to the microbiota is also cardinal to protecting patients from disruptive, dangerous and costly recurrences, and thus constitutes the key value proposition of fidaxomicin and indeed all novel antibiotics in development for CDI treatment. Fidaxomicin resulted in 13.3 % recurrence of infection versus 24.0 % with vancomycin on a per-protocol basis for patients taking part in a phase 3 clinical trial [27]. A notable outcome of this study is that the difference in recurrence rates for patients infected with non-NAP1 (*i.e.* non-hypervirulent) strains was large (7.8 % with fidaxomicin versus 25.5 % with vancomycin), whereas the recurrence rates were indistinguishable in the 35.9 % of patients infected with NAP1 strains.

Despite the fact that fidaxomicin has clinically proven advantages over metronidazole and vancomycin, its use has remained limited because of high pricing: 10-day regimens of metronidazole, vancomycin and fidaxomicin pills respectively cost approximately $ 22, $ 680 and $ 2’800 [17]. The majority of recent cost-effectiveness studies conclude that fidaxomicin’s price is not justified despite the recurrences that it prevents in non-hypervirulent strains, and that it would need to be priced at less than half of its current price to be deemed cost-effective [28–30]. The hype and media storm surrounding fidaxomicin’s approval thus has yet to result in the patient benefit that society needs.

There are currently two antibiotics in phase 3 clinical trials for CDI: cadazolid (Actelion) and surotomycin (Cubist Pharmaceuticals), both of which have received Qualified Infectious Disease Product designations and Fast Track status by the FDA to accelerate their development (see: www.actelion.com and www.cubist.com). Cadazolid is a chimeric quinolonyl-oxazolidinone inhibitor of protein synthesis that also shows weak inhibition of DNA synthesis whereas surotomycin is a cyclic lipopolypeptide that acts by inducing cytoplasmic membrane depolarization [31,32]. Although structurally heterogeneous and possessing different mechanisms of action, the pharmacodynamics of both antibiotics appear to be similar to that of fidaxomicin in that they display strong bactericidal activity against *C. difficile*, are minimally absorbed and therefore readily reach and sustain therapeutic concentrations at their site of action in the colon, and largely spare key components of the gut flora such as *Bacteroides* species [33,34]. Both cadazolid and surotomycin appear to be more detrimental

than fidaxomicin to *Bifidobacterium* species and it will be interesting to see if this translates into a difference in recurrence rates once clinical trials are complete [26].

The only other antibiotics currently in clinical trials for treating CDI are SMT-19969 (Summit Corporation in collaboration with the Wellcome Trust) in clinical phase 2 and CRS-3123 (Crestone) in phase 1. Both are non-absorbable narrow-spectrum agents and SMT-19969 has been shown to give rise to lower rates of recurrence than fidaxomicin in hamsters infected with hypervirulent strains such as ribotype 012 [35]. After several clinical trials, a broad spectrum glycolipodepsipeptide antibiotic, ramoplanin (Nanotherapeutics), is being re-purposed for CDI prophylaxis (see: www.nanotherapeutics.com/ramoplanin). Phase 2 trials have also been completed with LFF-571 (Novartis) but its development for CDI treatment appears not to be actively pursued anymore (see: www.novartis.com/innovation/research-development/clinical-pipeline/index.shtml). Clinical testing of NVB-302 (Novacta Biosystems) also seems to have been recently discontinued after completion of phase 1 trials but the reasons for this are unclear.

Microbiological Approaches for CDI Treatments

A number of microbiological and biological approaches for treating CDI have emerged in recent years. Although highly different from a mechanistic point of view, they share the common property of allowing the endogenous gut flora to flourish [36]. Their development has been spurred by the high rates of recurrence, and particularly debilitating multiple recurrences, observed with standard antibiotic treatment: the cure rate for recurrent CDI using vancomycin or metronidazole drops to < 30 % [37]. By recently designating recurrent CDI as an orphan indication, the FDA has also provided a regulatory incentive for the development of new treatments.

Among microbiological approaches, fecal microbiota transplantation (FMT) has only emerged in the last few years as having remarkably high success rates (> 90 %) for treating recurrent CDI, despite that it was first tested clinically in the 1950s [38,39]. The first randomized clinical trial using FMT for recurrent CDI was carried out in 2013 and was interrupted early for ethical reasons, since the patient group receiving vancomycin had a 31 % response to treatment whereas the FMT group had an 81 % cure rate (with a 94 % response after the second administration of FMT) [40]. As its name suggests, FMT consists of transplanting processed stool from a healthy donor to a patient's colon *via* enema, colonoscopic or nasogastric tube administration. The procedure rapidly restores the diversity of the intestinal microbiota and thus prevents proliferation of *C. difficile*. To date, there are at least one company (Rebiotix) and one non-profit organization (OpenBiome) that provide clinicians with filtered and frozen pathogen-free stool for FMT. The sharp rise in number of reports of successful treatments using FMT and the concomitant lack of regulatory oversight for the procedure prompted the FDA to announce in May 2013 that it considered human feces as a drug, hence requiring the submission of an Investigational New Drug (IND) application [41]. Although this requirement would make FMT safer

by standardizing donor screening and treatment administration, the FDA withdrew the requirement 6 weeks after the announcement in order not to restrict access to a much-needed therapy. The regulatory status of FMT thus remains in limbo and the final decision will have a strong impact on whether or not FMT will become a widespread therapy [42]. The combination of high efficacy, regulatory uncertainty, limited access leading to numerous postings of do-it-yourself procedures on the Internet, and the unconventional esthetics of FMT have led to a media storm around the treatment that has had the beneficial effect of increasing public, patient and doctor awareness about CDI, a condition that until recently was referred to in the media as a "silent killer" because so few people had heard of it.

Although the advantages of FMT over antibiotics have been widely lauded, significant hurdles for its widespread use remain, not least of which is safe and standardized large scale production and formulation. Based on the fact that a key active ingredient in the stool samples used for FMT are the bacteria contained in it, efforts are now being made to define minimal consortia of a few tens of bacterial strains that can be formulated for oral administration as a replacement for FMT [39]. Identifying which of the *ca.* 5,000 species of bacteria found in the gut are key to providing colonization is by no means a trivial task and these efforts are being led by the venture capital-backed companies Seres Health (clinical stage) and Vedanta Biosciences (pre-clinical stage) [43,44]. Their approaches deviate from probiotics development programs of large food manufacturers such as Nestlé and Danone that have focused heavily on a limited number of strains of *Lactobacillus* and *Bifidobacterium* that are readily amenable to inclusion in foodstuffs because of their resistance to harsh conditions in production processes and storage but are not key members of the endogenous human microbiota. Isolated examples of randomized, placebo-controlled double-blind studies reporting positive results for probiotics do however exist, such as for Bio-K+ products [45]. Vedanta focuses on (non-toxigenic) bacteria from the *Clostridium* genus, which the founders have shown to play a key role in inducing regulatory T cells that maintain immune homeostasis [46]. By rebalancing activity of the immune system, Vedanta's therapy also has potential for treating conditions such as Crohn's disease, inflammatory bowel disease and allergies. Seres' core technology is an algorithm to analyze differences between the healthy and diseased state microbiome, based on which consortia of a few tens of bacteria species capable of equilibrating the microbiota can be defined. The company's lead candidate resulted in clinical cure of CDI in 29 out of 30 patients in a recently completed single-arm, open-label phase 1/2 trial (see: www.sereshealth.com/news).

It should be noted that there is an increasing amount of evidence showing the important role that non-bacterial components of fecal extracts playin conferring the protective effects observed through FMT. Experiments using the hamster model have demonstrated that sterile-filtered fecal extracts confer protection from CDI, which is abrogated upon ultrafiltration or heating [47]. Taken together with results demonstrating the role of taurocholate and its derivatives on *C. difficile* sporulation and toxin activity, it would appear that bile salts and enzymes that metabolize them into secondary bile

salts are key in mediating the therapeutic effects of FMT [48,49]. Taking these observations into consideration may be key to ensuring the efficacy of the microbiota supplementation therapies.

ViroPharma (acquired by Shire in January 2014) has pursued the development of another microbiological approach that consists of administering a non-toxigenic strain of *C. difficile* (VP20621) to recurrent CDI patients [50]. The therapeutic concept is based on the observation that hospital patients asymptomatically colonized with *C. difficile* have a significantly reduced risk of contracting CDI, possibly because the niche in the intestinal ecosystem that harmful *C. difficile* would occupy is already taken [51]. A phase 2 clinical trial to investigate safety, colonization and efficacy in reducing recurrence when administering VP20621 after a standard antibiotic treatment was completed in 2013 (see: clinicaltrials.gov/ct2/show/NCT01259726). Although the results were positive, the therapeutic use of non-toxigenic strains of *C. difficile* is questionable because it has been shown that the genetic region coding for *C. difficile* toxins, the pathogenicity locus, can be acquired by non-toxigenic strains by horizontal gene transfer from toxigenic strains, turning them into toxin producers [52]. Furthermore, the microbiota repopulation technologies described above offer a safer and more balanced means of re-establishing microbiota homeostasis than a single non-toxigenic strain of *C. difficile* and are therefore likely to be preferred modalities from a regulatory and efficacy perspective.

Bacteriophages constitute an entirely different class of microbiological treatment with potential as a therapy for CDI [53]. This approach has appealing properties such as high specificity but on the reverse side it is also susceptible to the development of resistance since *C. difficile*, similarly to other bacteria, have very effective natural defense mechanisms against phages, like the CRISPR/Cas system, that need to be thwarted [54]. The pre-clinical development of therapeutic *C. difficile*-specific phages has recently been financed by UK-based AmpliPhi Biosciences Corporation and it will be interesting to see whether or not this treatment modality will enter clinical testing (see: http://www.ampliphibio.com/news.html).

Given the rapid mutation rates and sensitivity to growth parameters of bacteria, a common challenge that all the microbiological approaches described above will ultimately face will be to develop scalable, GMP-compliant production processes with low batch-to-batch variability. Companies currently venturing into the microbiome-modulation market can benefit from the regulatory challenges and pitfalls that pioneering companies such as Osel and Actogenix have already faced in the last decade in other disease areas [39].

[Figure 2]

FIGURE 2 Schematic representation of the mode of action in the colon of clinical-stage drug candidates for CDI treatment and prevention.

Non-Microbial Biological Approaches for CDI Treatment and Prevention

The non-microbial biological approaches closest to market entry are passive immunization and vaccination. They are fundamentally different from antibiotics and microbiological therapies in that they target the symptom-causing toxins secreted by *C. difficile*. In addition, they do not possess antibiotic properties and therefore also avoid causing damage to the intestinal microbiota.

Passive immunization consists of the systemic administration of antibodies to a non-immune individual and was used as an anti-toxin treatment as early as 1890 by Emil Behring and Shibasaburo Kitasato to treat diphtheria, earning them a Nobel prize [55]. The rather crude so-called serum therapy, using blood serum from immunized animals for treatment, has evolved dramatically since then and the state-of-the-art are now highly purified antibodies engineered for high affinity and low non-specific activity. In the context of CDI, phase 3 clinical trials of a mixture of fully human monoclonal anti-TcdA and anti-TcdB antibodies administered intravenously to patients receiving either metronidazole or vancomycin for CDI are currently underway with recurrence of infection within 12 weeks as the primary endpoint. This drug candidate, developed by Merck (MSD) and Medarex, showed positive results in a phase 2 trial with the same endpoint where patients receiving the treatment had a recurrence rate of 7 %, whereas recurrence occurred in 25 % of patients in the placebo group. The exact mechanism of protection is unclear in terms of where in the body the neutralization effect is taking place: TcdA and TcdB are secreted in the colon lumen and exert their toxic effects locally, except in severe cases where the damage to the intestines is such that endothelial barriers are destroyed and the toxins can circulate systemically [56]. Antibodies, however, are commonly accepted to be unable to passively cross epithelia, although a few reports of increased fecal IgG after systemic administration of IgG at high doses do exist [57,58]. Nevertheless, the clinical data are very encouraging and other companies such as MicroPharm or UCB Pharma are also actively developing passive immunization drug candidates for CDI [59,60].

With mean half-lives of circulation in humans of 26 and 22 days for anti-TcdA and anti-TcdB respectively, passive immunization offers transient boosts of circulating anti-toxin IgG [61]. Several groups are now developing vaccines that can trigger sustained increases of serum anti-toxin. Sanofi Pasteur is carrying out a phase 3 trial with a bi-valent toxoid vaccine generated from formalin-inactivated TcdA and TcdB adsorbed on alum that increases serum IgG concentrations against the toxins [62]. The endpoint of the trial is efficacy in preventing onset of primary symptomatic CDI and is seeking to enroll 15,000 people (see: clinicaltrials.gov/ct2/show/study/NCT01887912). Development of the vaccine has also been granted Fast Track designation by the FDA. The rationale for the development of a vaccine against CDI is similar to that for passive immunization and stems from studies showing that there is a strong correlation between asymptomatic carriage of *C. difficile*

and high serum anti-TcdA IgG titers, and that patients that produce an anti-TcdA IgG response during a first episode of CDI are less likely to suffer recurrence [63,64]. Although data on the studies carried out to date are sparse, Sanofi Pasteur's vaccine has been reported to increase serum anti-TcdA IgG levels for at least 60 days [62]. Immune response to TcdB was consistently lower than for TcdB. The development of vaccines tailored for the elderly has been encouraged as an approach to reduce morbidity in the aging population but improvements in formulation are needed to obtain the strong and sustained immune responses seen in young and healthy individuals [65]. The Sanofi Pasteur study will provide valuable information about the quality of the immune response in a large, elderly (> 50 years old) population, and its correlation with protection from CDI. Multiple other companies such as Pfizer, Merck and Valneva are also developing vaccine candidates using recombinant fragments of the toxins or different adjuvants to obtain stronger immunes responses. One should note that all of these vaccines elicit a systemic immune response and the anti-toxin antibodies produced do not inhibit colonization by *C. difficile.* A radically different approach developed by Prof. Simon Cutting of Royal Holloway University of London and sponsored by the European Union 7th Framework Programme that should be entering clinical trials shortly consists of orally-administered inactivated bacterial spores that raise a mucosal response [66]. If successful, such a vaccine would be likely to offer a more thorough protection from CDI than injected toxoid vaccines due to the co-localization of the immune response and the infection site.

Antibiotic Inactivation for CDI Prevention

Addressing the root cause of *C. difficile*, namely antibiotic treatment leading to destruction of the endogenous microbiota, is also an attractive option for reducing incidence of CDI. Improved antibiotic stewardship programs are an obvious and necessary way of reducing exposure to antibiotics but very often, antibiotics cannot be avoided. In these cases, an indirect approach to spare the microbiota is to co-administer agents that inactivate or sequester antibiotics present in the large intestine, thus allowing systemic circulation of the antibiotic but preventing damage to the intestinal microbiota. Such approaches for prevention of CDI are being led by two companies, Synthetic Biologics and Da Volterra. Synthetic Biologics is entering phase 1 in the fourth quarter of 2014 with SYN-004, an oral formulation of β-lactamase that digests intravenously administered β-lactam antibiotics to ampicilloic acid (see: www.syntheticbiologics.com). Da Volterra has completed phase 1 trials with an activated charcoal sorbent coated with a pH-dependent enteric polymer that acts as a sequestrant in the colon [67]. This approach has the advantage of in principle being more universal than SYN-004. Pre-clinical testing was carried out with a clinical but non-epidemic strain of *C. difficile* (UNT-103-1) [68]. One possible negative consequence of reducing the concentration of antibiotic in the gut without complete elimination is that it could increase exposure of bacteria to sub-minimum inhibitory concentrations of antibiotic, which encourages emergence of resistance.

TABLE 1 Products currently in development for treating and preventing CDI. Details about the clinical trials can be found using the NCT number on http://clinicaltrials.gov. The data were retrieved on the 15th of September 2014.

Treatment/prophylactic type[a]	Product	Sponsor	Clinical phase
Antibiotic	Cadazolid	Actelion	3 (NCT01987895)
	Surotomycin	Cubist	3 (NCT01597505)
	SMT19969	Summit Corp.	2 (NCT02092935)
	CRS3123	Crestone	1 (NCT02106338)
Microbiota supplement	FMT[b]	Rebiotix	2 (NCT01925417)
	SER109	Seres Health	2 (not registered)
	VP20621	ViroPharma	2 (NCT01259726)
Passive immunization	MK3415A	Merck (MSD)	3 (NCT01513239)
Vaccine	CDiffense	Sanofi Pasteur	3 (NCT01887912)
	Adjuvanted vaccine	Pfizer	2 (not registered)
	IC84	Valneva	1 (NCT01296386)
Antibiotic inactivation	SYN004	Synthetic Biologics	1 (not registered)
	DAV132	Da Volterra	1 (NCT02176005)

Conclusion

The striking variety and number of targeting strategies against CDI in clinical tests at the time of writing makes predicting the standard of care for CDI ten years from now very difficult. The best case scenario for patients and society is that preventative measures, which include the prophylactics presented herein as well as improvements to hospital hygiene and antibiotic stewardship programs, will curb and ideally reverse the sharp rise in incidence that we have witnessed in the last fifteen years. However, as CDI continues to spread outside of the US and Europe, it is clear that a need for therapeutics on a global level will remain and the door thus remains open for new therapies to make a run towards market approval. Novel and differentiated drug candidates to enter clinical testing could include inhibitors of sporulation [69], aforementioned phage therapies or even new synthetic toxin inhibitors that could make up for the unfulfilled phase 3 potential of tolevamer and synsorb 90 [70]. Innovation will be further spurred on if the European Medicines Agency and other medicines authorization agencies worldwide follow the exemplary lead of the FDA by providing regulatory incentives for the development of new treatments for CDI and recurrent CDI through the provision of equivalents to Orphan Drug status and Fast Track status to relevant applicants. Ultimately, as for all

[a] Antibiotics and microbiota supplements are treatments (in the latter case only for recurrences); passive immunization prevents recurrence, vaccines and antibiotic inactivation can prevent both first cases and recurrences.

[b] A total of 11 non-commercial hospital-sponsored phase 1 and 2 clinical trials are also underway as revealed by a search for "FMT *Clostridium difficile*" on clinicaltrials.gov.

drugs, not only the empirical parameters of drug safety and efficacy, but also acceptance, pricing and reimbursement will end up being key drivers in dictating the benefit of new therapies for CDI patients.

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