World Journal of Clinical Infectious Diseases

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DOI: 10.5495/wjcid.v6.i3.28

World J Clin Infect Dis 2016 August 25; 6(3): 28-36
ISSN 2220-3176 (online)

REVIEW

Challenges in management of recurrent and refractory *Clostridium difficile* infection

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Author contributions: Meehan AM, Tariq R and Khanna S planned and researched the topic; Meehan AM and Tariq R wrote the paper; Meehan AM and Khanna S revised the paper.

Conflict-of-interest statement: Sahil Khanna serves as a consultant to Rebiotix Inc and Summit Pharmaceuticals.

Manuscript source: Invited manuscript

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Received: March 24, 2016
Peer-review started: March 24, 2016
First decision: April 15, 2016
Revised: May 5, 2016
Accepted: May 31, 2016
Article in press: June 2, 2016
Published online: August 25, 2016

Abstract

Clostridium difficile infection (CDI) is the most common nosocomial infection in the United States and is associated with a high mortality. One quarter of patients treated for CDI have at least one recurrence. Spore persistence, impaired host immune response and alteration in the gastrointestinal microbiome due to antibiotic use are factors in recurrent disease. We review the etiology of recurrent CDI and best approaches to management including fecal microbiota transplantation.

Key words: *Clostridium difficile* infection; Epidemiology; Outcomes; Treatment; Fecal microbiota transplantation

Core tip: Recurrent *Clostridium difficile* infection (RCDI) is common and can be difficult to treat. Clostridia spores transmit disease. They are ubiquitous and hard to eradicate. The composition of the gut microbiome plays an essential yet poorly understood role in maintaining overall health, and in protecting against *Clostridium difficile* (*C. difficile*) infection. Antibiotic induced dysbiosis of the microbiome is a key contributor to RCDI. Here we review how *C. difficile* spores and alterations in the microbiome contribute to RCDI.

Meehan AM, Tariq R, Khanna S. Challenges in management of recurrent and refractory *Clostridium difficile* infection. *World J Clin Infect Dis* 2016; 6(3): 28-36 Available from: URL: http://www.wjgnet.com/2220-3176/full/v6/i3/28.htm DOI: http://dx.doi.org/10.5495/wjcid.v6.i3.28

INTRODUCTION

Clostridium difficile (*C. difficile*) is a gram positive, anaerobic, spore forming bacteria first associated with antibiotic-associated and pseudomembranous colitis in 1978[1,2]. Originally isolated from meconium and feces of newborn infants in 1935, it was dubbed "*Bacillus difficilis*" due to its poor culture growth characteristics[3]. Although

C. difficile culture is achievable now using Cycloserine Cefoxitin Fructose Agar media[4], the moniker remains apt, albeit for different reasons. A diagnosis of *Clostridium difficile* infection (CDI) adds considerably to healthcare cost, length of stay, complications and mortality[5,6].

CDI diagnosis is based on symptoms and toxin detection, and initial treatment involves oral metronidazole for mild-moderate cases or oral vancomycin if severe[7]. Both metronidazole and vancomycin lead to intestinal dysbiosis and impair "resistance to colonization" actually facilitating recurrence[8].

Recurrent CDI (RCDI) is defined as recurrence of clinical symptoms with a positive *C. difficile* stool test within 8 wk of symptom resolution[9]. Twenty to twenty five percent of CDI patients will have at least one recurrence[10] and subsequent risk can be as high as 40%-65%[11]. Reinfection *vs* relapse are indistinguishable clinically, however based on serotyping and PCR ribotyping up to 50% of patients recur with a strain that is different to the original one[12]. RCDI relates to spore production and persistence, the host immune response (or lack of it) to toxins, and alterations in the gut microbiome.

C. DIFFICILE SPORES: RESISTANCE AND PERSISTENCE

C. difficile spores are the agents of disease transmission[13]. They are ubiquitous and may survive on contaminated surfaces for months, possibly years[14-16]. *C. difficile* pathophysiology relates to spore exposure and ingestion, spore vegetation and toxin production in the setting of an altered host gut microbiome[17]. A healthy gut flora is protective against colonization and infection from *C. difficile*[18]. Asymptomatic colonization with toxin negative and positive strains has been described[8,19].

Anaerobic bacteria form spores when conditions are not conducive to growth (*i.e.*, starvation), specifically when deprived of carbon or nitrogen[16]. Clostridial spores are metabolically inactive (dormant) and impervious to most environmental assaults (except bleach)[7]. Anaerobic spore DNA is protected from damage by several mechanisms that have been established in related clostridial and bacteroides species and extrapolated to *C. difficile*. These include the fact that the spore core is anhydrous (water content 25%) and acidic (pH 6.5), which inhibits enzymatic activity and immobilizes most proteins[16]. There are high levels of ionic calcium-dipicolinic acid in the spore core, which forms a 1:1 complex with DNA. Deletion experiments suggest that saturation of DNA with α/β small acid soluble spore proteins (SASPs) is the dominant protective mechanism[20]. Mutants spores that lack α/β SASPs and calcium-dipicolinic acid lose viability rapidly during sporulation due to DNA damage[16].

Spores are the main vehicle of disease transmission, persistence and recurrence in CDI[14]. The environmental spore load necessary to infect 50% of mice after 1 h in one series of experiments was 5-10 spores/cm^2[21,22]. Spores shed through stool contaminate skin, bed clothes and even air, reaching 53-426 colony forming units/m^3 of air[15]. Mutants unable to produce Spo0A (a transcription regulatory protein essential for sporulation) do not persist or transmit disease in mice[23]. Thus elimination of spores can interrupt disease transmission. Presently this is most often pursued in the health care setting in the context of a known case (we don't as yet target spores in the community)[7]. Sodium hypochlorite (*i.e.*, bleach) is the most commonly used agent, with far UV light and vapor hydrogen peroxide also effective[14].

There are several additional issues of note. In murine gut *C. difficile* sporulates at a rapid rate - 56% relative to vegetative cells at 14 h post infection[24]. The murine colonic environment supports sporulation by phosphorylation of the master regulator Spo0A[14,23,25]. Presumably similar unknown triggers are present in the human gut.

Recent whole genome sequencing of CDI isolates in > 1200 patients with disease showed only 35% of cases were related to known cases, which suggests alternate routes of exposure (animals/food), outside of health care settings[26] (Presumably patients got the disease from spores in the community). The prevalence of asymptomatic carriage in hospital admission ranges from 7%-18%[27].

This has great clinical implications. Widespread community colonization with toxigenic *C. difficile* suggests that attempts to restrict spore spread only in the context of known exposure in healthcare settings may be insufficient. For meaningful interruption, universal modified contact precautions for all admissions may be necessary. Measures to prevent spore formation may alter the transmission cycle. Further study of the mechanism of spore formation may identify new targets. Thus far only fidaxomicin has been shown to decrease spore formation most likely by inhibiting transcription of sporulation genes[28]. Its high cost however precludes widespread use, as discussed below.

VEGETATIVE FORMS: TOXIN PRODUCTION AND CDI

Germination of spores to toxin producing vegetative forms can occur within minutes of exposure to specific triggers deemed germinants (*i.e.*, taurocholate)[14,16]. Taurocholate (a primary bile acid) is both necessary and sufficient to trigger *C. difficile* germination. L-glycine acts as a cogerminant[29]. In contrast, certain secondary bile acids, *i.e.*, deoxycholate can inhibit vegetative growth[30]. Secondary bile acids are derived by the action of endogenous flora on primary bile acids[31] and the relative ratio of each in the colon may determine spore/vegetative balance.

Toxins A (TcdA) and B (TcdB) and binary toxin (CDT) are the major virulence factors that contribute to pathogenesis[32]. Toxins A and B are multi-domain proteins that share a high degree of homology and comprise an N terminal catalytic domain with glucosyltransferase activity, a middle translocation domain and a C-terminal host cell binding region[33]. The toxin receptor remains unknown.

Both A and B are proinflammatory and cytotoxic and it is not clear if both are needed for pathogenesis[34]. Both alter the actin cytoskeleton, disrupt the epithelial barrier and cause apoptosis by glucosylation and inactivation of GTPases-Rac, Rho and Cdc42[35]. This induces mucosal damage and inflammation. Toxin expression derives from a 19.6 kb pathogenicity chromosomal locus (PaLoc) that encodes *TcdA* and *TcdB* in addition to *TcdR* (RNA polymerase sigma factor that positively regulates toxin expression), *TcdC* (putative negative regulator-deletion in 027 ribotype may increase toxin production), and *TcdE* (related to bacteriophage holins)[32,35]. The role of the toxins in the bacterial life cycle is unclear. Different *PaLoc* variants are called toxinotypes: 34 are described[36]. *PaLoc* has features of both stable integration and a mobile genetic element[37]. The CDT-binary toxin expressed in 027 ribotype ADP ribosylates G actin in target cells leading to protrusion bodies of microtubules that contact *C. difficile* and possibly increase colonization efficiency[38].

Toxigenic *C. difficile* causes disease: However colonization with toxigenic *C. difficile* can be asymptomatic[27]. After successful treatment many patients will continue to shed spores without manifesting disease. Colonization is a critical step in the pathogenic process and depends on adherence to gut epithelial cells by adhesion and flagellin proteins[39-41].

Colonization with non-toxin forming *C. difficile* may out-compete toxin forming *C. difficile*[27]. In one recent study, administration of nontoxigenic *C. difficile* spores (NCTD-M3) to patients after treatment of either first CDI episode or first recurrence, showed a 3-fold reduction (from 30% to 11%) in recurrent disease compared to placebo[42]. Patients given 10^7 spores/day for 7 d had the lowest recurrence rate (5%)[42]. The study does raise some concerns, primarily the possible acquisition of toxin containing PaLoc sequences by toxin negative strains, an event that has been shown to occur *in vitro*[43].

In theory, non-antibiotic toxin binders could ameliorate disease without disrupting intestinal flora. Cholestyramine, which binds toxin has been tried[44]. One difficulty is that it also binds vancomycin (as does colestipol and other anion exchange resins), complicating its use[45]. It can also bind bile salts and potentially stimulate *C. difficile* growth[46]. Given lack of efficacy data and possible harmful interactions use of cholestyramine or colestipol is not recommended.

Tolevamer, a polymer of styrene-sulfate that binds *C. difficile* toxin *in vitro*, was inferior to both metronidazole and vancomycin in 2 phase Ⅲ trials[47]. Only 44% of patients who took tolevamer had resolution of diarrhea or abdominal pain compared to 73% for metronidazole and 81% for vancomycin[47].

IMMUNE RESPONSE TO TOXINS AND CDI

Only half of hospitalized patients colonized with *C. difficile* develop CDI, and initial disease is associated with lack of anti-toxin A IgG[48]. The host immune response also plays a part in recurrent disease- patients with antibodies to toxin are less likely to relapse than those with undetectable toxin antibody[49,50]. Passive immunization by administration of intravenous immunoglobulin may have a role in patients with hypogammaglobulinemia[51,52], or in patients with severe disease[53].

Specific anti-toxin antibodies prevent mortality independent of antibiotic treatment. In one study a 3-fold reduction in relapse (25% to 7%) was seen when anti-toxin antibodies were used[54]. Data in animal models supports the efficacy of toxin-targeted vaccines[55]. Formalin inactivated toxin A/B (toxoid) protected hamsters from lethal *C. difficile* challenge[56]. Currently there are 2 vaccines in human trials. Sanofi Pasteur formalin inactivated toxins A/B vaccine was safe, well tolerated and immunogenic (generated antibodies to toxin)[57]. It is now in phase Ⅲ trial for primary prevention (https://clinicaltrials.gov/ct2/show/NCT01887912). An alternate approach involves a recombinant fusion protein of toxins A/B. A phase 1 trial of escalating doses of this recombinant is completed and results are pending (https://clinicaltrials.gov/ct2/show/NCT01296386).

There is some evidence of efficacy of vaccines in secondary prevention of RCDI[58], but more data is needed.

STANDARD ANTIMICROBIAL TREATMENT OF RCDI

Antimicrobial stewardship remains a key element of any RCDI management strategy. The reader is directed to other reviews for further discussion[59-61]. This review will focus on RCDI specific treatment.

Standard antimicrobial therapy targets the vegetative forms of *C. difficile*[7,52]. Spore vegetation and recurrent CDI are intricately linked. Favoring germination (by altering the germinant/sporulation ratio towards vegetation) would in theory allow eradication with antibiotics. Depending on antibiotic used however, this can also alter the microbiome and could increase the likelihood of relapse. Alternatively inhibiting germination, *i.e.*, by altering the gut flora towards secondary bile acids that inhibit vegetative forms[46] might also be a therapeutic option.

The use of vancomycin to treat CDI predates recognition of *C. difficile* as the causative agent of antibiotic associated colitis. First recurrence of CDI is treated with the same agent used for the initial episode. If clinically severe then vancomycin is used[7,52]. For second recurrence, pulsed and/or tapered vancomycin is recommended. Metronidazole is not used beyond the first recurrence due to possible cumulative neuropathy[62] (Table 1 is a summary of general clinical approach to RCDI).

Data supporting these recommendations is recognized as weak and poor quality with no corroborative randomized controlled trials.

Tedesco *et al*[63] reported on 22 patients treated for 21 d with a vancomycin taper and pulse and noted

Table 1 Management outline for recurrent *Clostridium difficile* infection[7]

General
Stop/minimize antibiotics (if possible, to allow gut flora to repopulate)
Rule out other causes of diarrhea, *i.e.*, post-infectious IBS
(check stool for C diff only in context of symptoms, not as test of cure)
Antibiotic treatment
Use the same antibiotic as initial regimen (depending on disease severity and response to initial treatment)[7,52]
Consider Vancomycin taper ± pulse[11]
Vancomycin followed by rifaximin chaser[67]
Fidaxomicin[80]
Probiotics
Probiotics with antibiotics may help[99]. Consider adding to last 2 wk of vancomycin pulse/taper and continue for 4 wk after
(caution in immunocompromised patients- may cause fungemia. Don't use in isolation. Not standardized, doses/active agents may vary)
Immunotherapy
Monoclonal antibody (neutralize toxin)[54]
IVIG[51]
Toxoid vaccine[58]
Non toxigenic strains[42]
Bacteriotherapy
Fecal microbiota transplant[111,114]

IBS: Irritable bowel syndrome; IVIG: Intravenous immunoglobulin.

no relapses (average follow-up 2-12 mo). In McFarland *et al*[11], 83 patients treated with 10-14 d course of vancomycin had an average relapse rate of 55% (range 42%-71%, depending on vancomycin dosing). Twenty-nine patients were treated with a vancomycin taper over an average of 21 d and 31% relapsed. If vancomycin taper was followed by vancomycin pulse (drug dosed every 48 or 72 h) then relapse decreased to 20% (10 patients). Lastly, 7 patients treated only with vancomycin pulse had 14% relapse[11]. The theory behind pulsed doses is to target vegetative forms of *C. difficile* but still allow restitution of the gut flora[11]. These numbers are small and the approach is not standardized. Oral vancomycin is also expensive: A 6 wk tapered course can cost hundreds of dollars[64].

Management of those who fail pulsed/tapered vancomycin is challenging.

ALTERNATIVE AGENTS FOR RCDI

Rifaximin is a synthetic rifamycin derivative that inhibits transcription[65]. It has little (< 0.4%) systemic absorption[65]. It is not used as monotherapy due to rapid emergence of resistance[66,67]. It has been used as an adjunct to vancomycin after 2 wk of standard treatment or taper[67]. Dosed at 400 mg BID for 2 wk after vancomycin taper, cure was described in 17/20 patients in 3 reports[67-69]. Recurrence rate was similar (15%) in a small (68 patients) RCT[70].

Fidaxomicin is the first macrolide antibiotic with an 18 membered macrocyclic lactone ring[71]. It is bactericidal and acts at an early step of RNA synthesis (it stops DNA strand separation)[72]. The *C. difficile* minimum inhibitory concentration is lower than that for vancomycin or metronidazolel[73]. A prolonged post antibiotic effect of at least 10 h allows twice daily dosing[74]. It is not absorbed systemically and has minimal effect on the gut microbiome. The effect on transcription inhibits both sporulation and toxin production[28,75]. The effect on sporulation may impact recurrences.

In vitro then and based on mechanism of action fidaxomicin should be an attractive option for RCDI. Indeed, in a phase 3 trial fidaxomicin was non inferior to vancomycin in terms of clinical cure[76]. Moreover, in the same study it strikingly decreased recurrence rates from 24%-25% to 13%-15%. Adverse event profiles were similar.

Subset analysis looking specifically at RCDI confirmed both the efficacy of fidaxomicin and decreased recurrence[77]. The stumbling block with fidaxomicin is the prohibitive cost ($140 per pill, 2800 for ten day course)[52].

Cadazolid, a novel hybrid antibiotic with a quinolone pharmacophore incorporated in an oxazolidinone ring has potent anti *C. difficile* activity and decreased propensity to induce antibiotic resistance[78,79]. It has a dual mechanism of action, both inhibiting translation and DNA synthesis[78,80]. Phase 1 studies with doses up to 3000 mg indicated the drug to be generally well tolerated with headache and diarrhea being most common SE.

A phase Ⅱ multi-center, double-blind, randomized study was conducted in 84 CDI patients. Cadazolid was dosed at 250, 500, or 1000 mg and deemed comparable or superior to vancomycin with respect to clinical and sustained cure rates[79,81]. Lower recurrence rates (18%-25% *vs* 50%) were noted for all doses[82]. Although there is no data as yet in RCDI, given decreased recurrence rate, and reported impact on spore production efficacy in RCDI is of significant interest.

GASTROINTESTINAL MICROBIOME: ROLE IN CDI

The adult gastrointestinal tract has 10^{14} bacterial cells from > 1000 different bacterial species[83,84], which comprise the microbiome, or gut flora. Composition varies depending on diet, age and health[85]. A "healthy" microbiome has a large number of different species of microorganisms with more of certain phyla, *i.e.*, *Firmicutes* and *Bacteroides* and less of others, *i.e.*, *Proteobacteria*[86]. Gut bacteria play critical roles in immunity, epithelial barrier function (resist pathogens) and nutrient absorption[87]. Any imbalance (in number, species, or composition) can distort this symbiosis leading to the converse, known as dysbiosis[88,89]. The microbiome varies between individuals but is generally stable over time[90].

C. difficile can be part of the normal microbiome[88], but is generally contained by other more dominant anaerobes. A healthy microbiome may protect against CDI in different ways. One may simply be due to numbers and competition for nutrients and mucosal niches[30].

Alternatively, the microbiome may elicit substances, *i.e.*, short chain fatty acids that actively inhibit *C. difficile*[91]. Normal intestinal flora primes a Myd88 TLR-5 dependent innate immune response which protects against CDI[92]. More recent data shows that certain bacteria (*i.e.*, *Clostridium schindens*) change the primary and secondary bile acids ratio[46].

The most common cause of alteration in the microbiome is antibiotic use, which can affect "mutualistic" interactions[93]. The gut microbiome in patients with *C. difficile* is indeed dysbiotic[94,95]. Probiotics have been used in an attempt to redress this.

Probiotics are preparations containing live microbial agents that may be beneficial to the host when ingested. They range from yoghurt to specific microbial extractions (*i.e.*, *lactobacillus*, *Saccharomyces boulardii*). Efficacy in RCDI may be multifactorial and comprise restitution of gut flora[96], specific anti *C. difficile* effect (*i.e.*, *S. boulardii* protease cleaves Toxin A)[96] and/or immune modulation[97].

At present preparations are not standardized or regulated, and may have no live organisms or organisms not listed on label[52]. There is risk of fungemia or bacteremia- even in immunocompetent hosts[98].

Staggered and tapered vancomycin with daily kefir (yoghurt) led to resolution of symptoms in 21/25 patients with RCDI[99]. This was a retrospective study and remains to be confirmed.

FECAL MICROBIOTA TRANSPLANTATION

Administration of exogenous fecal material *via* fecal microbiota transplant (FMT) to correct intestinal dysbiosis has been used successfully to treat CDI. FMT for pseudomembranous colitis was performed in 1950s by Eiseman *et al*[100] using fecal enemas. Successful use of FMT to treat CDI was reported in 1983[101]. A proof of principle study reported by Silverman *et al*[102] in 2010 described 7 patients with RCDI who self-administered fecal enemas at home. At an average of 14 mo follow-up there were no recurrences[102]. Brandt *et al*[103] reported long term follow-up of 77/94 patients administered colonoscopic FMT for RCDI with primary cure rate of 91% (resolution of symptoms without recurrence). Since then multiple case reports and small series have been published showing efficacy in CDI[102,104]. An open label randomized clinical trial comparing fecal transplant to vancomycin was stopped early when interim analysis showed that 94% patients in the transplant group had improvement of diarrhea compared to 31% in the vancomycin alone group[105]. FMT has been reported for more than 1000 cases worldwide with > 90% efficacy[106], including patients with severe CDI[107]. Current guidelines recommend FMT for 3rd recurrence (*i.e.*, after vancomycin taper)[7,52].

Also deemed "bacteriotherapy", FMT restores both the microbiome and favorable bile acid composition[31,108].

Barriers to mainstream use of fecal transplants have included general aversion to knowing ingestion of feces, technical issues with standardization of material (route of administration, donor, volume, preparation) and concern for transmission of disease/infection[109]. Donors are screened and stool tested for transmissible pathogens[110].

An attempt to standardize FMT involving frozen oral FMT capsules led to 90% clearance of diarrhea[111]. A recent trial from Canada directly compared efficacy of frozen- thawed *vs* fresh FMT administered *via* enema and showed equivalent outcomes (70%-75% overall cure)[112]. An alternative approach involved SER-109, a novel *Firmicutes* spore containing oral agent derived from healthy stool[113]. Thirty patients with RCDI received SER-109 after standard CDI antibiotic treatment. At 8 wk 29/30 patients showed clinical resolution and diversification of gut flora[113].

If borne out, these approaches would negate concerns for procedural risk, donor variability and disease transmission and allow standardization of transplanted material.

Many questions remain with respect to the microbiome and its role in RCDI. If indeed the main protective effect relates to bile acid composition then perhaps administration of favorable agents, *i.e.*, deoxycholate may suffice. Defined microbial systems (*i.e.*, a mixture of known specified microbes) have been used to treat CDI also[114]. The optimal composition remains to be defined. Current use of FMT is for those who have failed standard RCDI therapy. Use as first line therapy or indeed as prophylaxis in patients receiving antibiotics is possible. The role of microbiome modulation with FMT in other disease states ranging from obesity to multiple sclerosis[106] is being explored.

CONCLUSION

Recurrent/relapsing *C. difficile* remains a therapeutic challenge. *C. difficile* spores are the agents of persistence and disease and additional efforts to minimize spread are warranted. Further research on factors that affect sporulation and vegetation may yield additional therapeutic targets. The role of the gut microbiome remains mysterious; however it is clearly of great importance not only in RCDI, but in myriad disease states. FMT is an effective therapeutic modality, but long term follow-up is needed.

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