**Name of Journal: *World Journal of Clinical Infectious Diseases***

**ESPS Manuscript NO: 25846**

**Manuscript Type: Review**

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

Meehan AM *et al.* Challenges in management of recurrent and refractory *C. difficile* infection

**Anne M Meehan, Raseen Tariq, Sahil Khanna**

**Anne M Meehan,** Division of Hospital Internal Medicine, Mayo Clinic, Rochester, MN 55905, United States

**Raseen Tariq, Sahil Khanna,** Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN 55905, United States

**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.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to: Sahil Khanna, MBBS, MS,** Division of Gastroenterology and Hepatology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, United States. khanna.sahil@mayo.edu

**Telephone:** +1-507-2664347

**Fax:** +1-507-2840538

**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:**

**Published online:**

**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

**© The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**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; In press

**INTRODUCTION**

*Clostridium difficile* (*C. difficile)* is a gram positive, anaerobic, spore forming bacteria first associated with antibiotic-associated and pseudomembranous colitis in 1978[[1](#_ENREF_1),[2](#_ENREF_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](#_ENREF_3)]. Although *C. difficile* culture is achievable now using Cycloserine Cefoxitin Fructose Agar media[[4](#_ENREF_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](#_ENREF_5),[6](#_ENREF_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](#_ENREF_7)]. Both metronidazole and vancomycin lead to intestinal dysbiosis and impair “resistance to colonization” actually facilitating recurrence[[8](#_ENREF_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](#_ENREF_9)]. Twenty to twenty five percent of CDI patients will have at least one recurrence[[10](#_ENREF_10)] and subsequent risk can be as high as 40%-65%[[11](#_ENREF_11)]. Reinfection versus 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](#_ENREF_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](#_ENREF_13)]. They are ubiquitous and may survive on contaminated surfaces for months, possibly years[[14-16](#_ENREF_14)]. *C. difficile* pathophysiology relates to spore exposure and ingestion, spore vegetation and toxin production in the setting of an altered host gut microbiome[[17](#_ENREF_17)]. A healthy gut flora is protective against colonization and infection from *C. difficile*[[18](#_ENREF_18)]. Asymptomatic colonization with toxin negative and positive strains has been described[[8](#_ENREF_8),[19](#_ENREF_19)].

Anaerobic bacteria form spores when conditions are not conducive to growth (*i.e.*, starvation), specifically when deprived of carbon or nitrogen[[16](#_ENREF_16)]. Clostridial spores are metabolically inactive (dormant) and impervious to most environmental assaults (except bleach)[[7](#_ENREF_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](#_ENREF_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](#_ENREF_20)]. Mutants spores that lack α/β SASPs and calcium- dipicolinic acid lose viability rapidly during sporulation due to DNA damage[[16](#_ENREF_16)].

Spores are the main vehicle of disease transmission, persistence and recurrence in CDI[[14](#_ENREF_14)]. The environmental spore load necessary to infect 50% of mice after 1 h in one series of experiments was 5-10 spores/cm2[[21](#_ENREF_21),[22](#_ENREF_22)]. Spores shed through stool contaminate skin, bed clothes and even air, reaching 53-426 colony forming units/m3 of air[[15](#_ENREF_15)]. Mutants unable to produce Spo0A (a transcription regulatory protein essential for sporulation) do not persist or transmit disease in mice[[23](#_ENREF_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](#_ENREF_7)]. Sodium hypochlorite (*i.e.*, bleach) is the most commonly used agent, with far UV light and vapor hydrogen peroxide also effective[[14](#_ENREF_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](#_ENREF_24)]. The murine colonic environment supports sporulation by phosphorylation of the master regulator Spo0A[[14](#_ENREF_14),[23](#_ENREF_23),[25](#_ENREF_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 knowncases, which suggests alternate routes of exposure (animals/food), outside of health care settings[[26](#_ENREF_26)] (Presumably patients got the disease from spores in the community*)*. The prevalence of asymptomatic carriage in hospital admission ranges from 7%-18%[[27](#_ENREF_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](#_ENREF_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](#_ENREF_14),[16](#_ENREF_16)]. Taurocholate (a primary bile acid) is both necessary and sufficient to trigger *C. difficile* germination*.* L-glycine acts as a cogerminant[[29](#_ENREF_29)]. In contrast, certain secondary bile acids, *i.e.*, deoxycholate can inhibit vegetative growth[[30](#_ENREF_30)]. Secondary bile acids are derived by the action of endogenous flora on primary bile acids[[31](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_34)]. Both alter the actin cytoskeleton, disrupt the epithelial barrier and cause apoptosis by glucosylation and inactivation of GTPases­-Rac, Rho and Cdc42[[35](#_ENREF_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](#_ENREF_32),[35](#_ENREF_35)]. The role of the toxins in the bacterial life cycle is unclear. Different *PaLoc* variants are called toxinotypes: 34 are described[[36](#_ENREF_36)]. *PaLoc* has features of both stable integration and a mobile genetic element[[37](#_ENREF_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](#_ENREF_38)].

Toxigenic *C. difficile* causes disease: However colonization with toxigenic *C. difficile* can be asymptomatic[[27](#_ENREF_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](#_ENREF_39)-[41](#_ENREF_40)].

Colonization with non-toxin forming *C. difficile* may out-compete toxin forming *C. difficile*[[27](#_ENREF_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](#_ENREF_42)]. Patients given 107 spores/day for 7 d had the lowest recurrence rate (5%)[[42](#_ENREF_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](#_ENREF_43)].

In theory, non-antibiotic toxin binders could ameliorate disease without disrupting intestinal flora. Cholestyramine, which binds toxin has been tried[[44](#_ENREF_44)]. One difficulty is that it also binds vancomycin (as does colestipol and other anion exchange resins), complicating its use[[45](#_ENREF_45)]. It can also bind bile salts and potentially stimulate *C. difficile* growth[[46](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_49),50]. Passive immunization by administration of intravenous immunoglobulin (IVIG) may have a role in patients with hypogammaglobulinemia[[51](#_ENREF_51),52], or in patients with severe disease[[53](#_ENREF_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](#_ENREF_54)]. Data in animal models supports the efficacy of toxin-targeted vaccines[[55](#_ENREF_55)]. Formalin inactivated toxin A/B (toxoid) protected hamsters from lethal *C. difficile* challenge[[56](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_59)]. This review will focus on RCDI specific treatment.

Standard antimicrobial therapy targets the vegetative forms of *C. difficile*[[7](#_ENREF_7),[52](#_ENREF_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](#_ENREF_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](#_ENREF_7),[52](#_ENREF_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](#_ENREF_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 (RCTs).

Tedesco *et al*[[63](#_ENREF_63)] reported on 22 patients treated for 21 d with a vancomycin taper and pulse and noted no relapses (average follow up 2-12 mo). In McFarland *et al*[[11](#_ENREF_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](#_ENREF_11)]. The theory behind pulsed doses is to target vegetative forms of *C. difficile* but still allow restitution of the gut flora[[11](#_ENREF_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](#_ENREF_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](#_ENREF_65)]. It has little (< 0.4%) systemic absorption[[65](#_ENREF_65)]. It is not used as monotherapy due to rapid emergence of resistance[[66](#_ENREF_66),[67](#_ENREF_67)]. It has been used as an adjunct to vancomycin after 2 wk of standard treatment or taper[[67](#_ENREF_67)]. Dosed at 400 mg BID for 2 wk after vancomycin taper, cure was described in 17/20 patients in 3 reports[[67-69](#_ENREF_67)]. Recurrence rate was similar (15%) in a small (68 patients) RCT[[70](#_ENREF_70)].

Fidaxomicin is the first macrolide antibiotic with an 18 membered macrocyclic lactone ring[[71](#_ENREF_71)]. It is bactericidal and acts at an early step of RNA synthesis (it stops DNA strand separation)[[72](#_ENREF_72)]. The *C. difficile* minimum inhibitory concentration (MIC) is lower than that for vancomycin or metronidazolel[[73](#_ENREF_73)]. A prolonged post antibiotic effect of at least 10 h allows twice daily dosing[[74](#_ENREF_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](#_ENREF_28),[75](#_ENREF_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](#_ENREF_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](#_ENREF_77)]. The stumbling block with fidaxomicin is the prohibitive cost ($140 per pill, 2800 for ten day course)[[52](#_ENREF_52)].

Cadazolid, a novel hybrid antibiotic with a quinolone pharmacophore incorporated in an oxazolidinone ring has potent anti *C. difficile* activityand decreased propensity to induce antibiotic resistance[[78](#_ENREF_78),[79](#_ENREF_79)]. It has a dual mechanism of action, both inhibiting translation and DNA synthesis[78,[80](#_ENREF_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 (https://trials.actelion.com/asp/Trial\_Registry/ListStudy.asp?PN=ACT-179811).

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](#_ENREF_79),[81](#_ENREF_81)]. Lower recurrence rates (18%-25% *vs* 50%) were noted for all doses[[82](#_ENREF_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 1014 bacterial cells from > 1000 different bacterial species[[83](#_ENREF_83),84], which comprise the microbiome, or gut flora. Composition varies depending on diet, age and health[[85](#_ENREF_85)]. A “healthy” microbiome has a large number of different species of micro-organisms with more of certain phyla, *i.e*., *Firmicutes* and *Bacteroides* and less of others, *i.e*., *Proteobacteria*[[86](#_ENREF_86)]. Gut bacteria play critical roles in immunity, epithelial barrier function (resist pathogens) and nutrient absorption [[87](#_ENREF_87)]. Any imbalance (in number, species, or composition) can distort this symbiosis leading to the converse, known as dysbiosis[[88](#_ENREF_88),[89](#_ENREF_89)]. The microbiome varies between individuals but is generally stable over time[[90](#_ENREF_90)].

*C. difficile* can be part of the normal microbiome[[88](#_ENREF_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](#_ENREF_30)]. Alternatively, the microbiome may elicit substances, *i.e.*, short chain fatty acids that actively inhibit *C. difficile*[[91](#_ENREF_91)]. Normal intestinal flora primes a Myd88 TLR-5 dependent innate immune response which protects against CDI[[92](#_ENREF_92)]. More recent data shows that certain bacteria (*i.e*., *Clostridium* *schindens*) change the primary and secondary bile acids ratio[[46](#_ENREF_46)].

The most common cause of alteration in the microbiome is antibiotic use, which can affect “mutualistic” interactions[[93](#_ENREF_93)]. The gut microbiome in patients with *C. difficile* is indeed dysbiotic[[94](#_ENREF_94),[95](#_ENREF_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](#_ENREF_96)], specific anti *C. difficile* effect (*i.e*., *S. boulardii* protease cleaves Toxin A)[[96](#_ENREF_96)] and/or immune modulation[[97](#_ENREF_97)].

At present preparations are not standardized or regulated, and may have no live organisms or organisms not listed on label[[52](#_ENREF_52)]. There is risk of fungemia or bacteremia- even in immunocompetent hosts[[98](#_ENREF_98)].

Staggered and tapered vancomycin (STAW) with daily kefir (yoghurt) led to resolution of symptoms in 21/25 patients with RCDI[[99](#_ENREF_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](#_ENREF_100)] using fecal enemas. Successful use of FMT to treat CDI was reported in 1983[[101](#_ENREF_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](#_ENREF_102)]. Brandt *et al*[[103](#_ENREF_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](#_ENREF_102),[104](#_ENREF_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](#_ENREF_105)]. FMT has been reported for more than 1000 cases worldwide with > 90% efficacy[[106](#_ENREF_106)], including patients with severe CDI[[107](#_ENREF_107)]. Current guidelines recommend FMT for 3rd recurrence (*i.e*., after vancomycin taper)[[7](#_ENREF_7),[52](#_ENREF_52)].

Also deemed “bacteriotherapy”, FMT restores both the microbiome and favorable bile acid composition[[31](#_ENREF_31),[108](#_ENREF_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](#_ENREF_109)]. Donors are screened and stool tested for transmissible pathogens[[110](#_ENREF_110)].

An attempt to standardize FMT involving frozen oral FMT capsules led to 90% clearance of diarrhea[[111](#_ENREF_111)]. A recent trial from Canada directly compared efficacy of frozen- thawed versus fresh FMT administered via enema and showed equivalent outcomes (70%-75% overall cure)[[112](#_ENREF_112)]. An alternative approach involved SER-109, a novel *Firmicutes* spore containing oral agent derived from healthy stool[[113](#_ENREF_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](#_ENREF_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](#_ENREF_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](#_ENREF_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.

**REFERENCES**

1 **Bartlett JG**, Moon N, Chang TW, Taylor N, Onderdonk AB. Role of Clostridium difficile in antibiotic-associated pseudomembranous colitis. *Gastroenterology* 1978; **75**: 778-782 [PMID: 700321]

2 **George WL**, Sutter VL, Goldstein EJ, Ludwig SL, Finegold SM. Aetiology of antimicrobial-agent-associated colitis. *Lancet* 1978; **1**: 802-803 [PMID: 85818 DOI: 10.1016/S0140-6736(78)93001-5]

3 [**Hall**](http://xueshu.baidu.com/s?wd=author%3A%28HALL%2C%20I.%20C.%29%20&tn=SE_baiduxueshu_c1gjeupa&ie=utf-8&sc_f_para=sc_hilight%3Dperson) **IC**, [O'Toole](http://xueshu.baidu.com/s?wd=author%3A%28O%27TOOLE%2C%20Elizabeth.%29%20&tn=SE_baiduxueshu_c1gjeupa&ie=utf-8&sc_f_para=sc_hilight%3Dperson) E. Intestinal flora in newborn infants with a description of a new pathogenic anaerobe Bacillus dificilis. *J Dis Child* 1935; **49**: 390-402 [DOI: 10.1001/archpedi.1935.01970020105010]

4 **George WL**, Sutter VL, Citron D, Finegold SM. Selective and differential medium for isolation of Clostridium difficile. *J Clin Microbiol* 1979; **9**: 214-219 [PMID: 429542]

5 **Bouza E**. Consequences of Clostridium difficile infection: understanding the healthcare burden. *Clin Microbiol Infect* 2012; **18** Suppl 6: 5-12 [PMID: 23121549 DOI: 10.1111/1469-0691.12064]

6 **Bagdasarian N**, Rao K, Malani PN. Diagnosis and treatment of Clostridium difficile in adults: a systematic review. *JAMA* 2015; **313**: 398-408 [PMID: 25626036 DOI: 10.1001/jama.2014.17103]

7 **Cohen SH**, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). *Infect Control Hosp Epidemiol* 2010; **31**: 431-455 [PMID: 20307191 DOI: 10.1086/651706]

8 **Kelly CP**, LaMont JT. Clostridium difficile--more difficult than ever. *N Engl J Med* 2008; **359**: 1932-1940 [PMID: 18971494 DOI: 10.1056/NEJMra0707500]

9 **Fekety R**, McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Mulligan ME. Recurrent Clostridium difficile diarrhea: characteristics of and risk factors for patients enrolled in a prospective, randomized, double-blinded trial. *Clin Infect Dis* 1997; **24**: 324-333 [PMID: 9114180 DOI: 10.1093/clinids/24.3.324]

10 **McFarland LV**, Surawicz CM, Greenberg RN, Fekety R, Elmer GW, Moyer KA, Melcher SA, Bowen KE, Cox JL, Noorani Z. A randomized placebo-controlled trial of Saccharomyces boulardii in combination with standard antibiotics for Clostridium difficile disease. *JAMA* 1994; **271**: 1913-1918 [PMID: 8201735]

11 **McFarland LV**, Elmer GW, Surawicz CM. Breaking the cycle: treatment strategies for 163 cases of recurrent Clostridium difficile disease. *Am J Gastroenterol* 2002; **97**: 1769-1775 [PMID: 12135033 DOI: 10.1111/j.1572-0241.2002.05839.x]

12 **Barbut F**, Richard A, Hamadi K, Chomette V, Burghoffer B, Petit JC. Epidemiology of recurrences or reinfections of Clostridium difficile-associated diarrhea. *J Clin Microbiol* 2000; **38**: 2386-2388 [PMID: 10835010]

13 **Barbut F**, Petit JC. Epidemiology of Clostridium difficile-associated infections. *Clin Microbiol Infect* 2001; **7**: 405-410 [PMID: 11591202 DOI: 10.1046/j.1198-743x.2001.00289.x]

14 **Barra-Carrasco J**, Paredes-Sabja D. Clostridium difficile spores: a major threat to the hospital environment. *Future Microbiol* 2014; **9**: 475-486 [PMID: 24810347 DOI: 10.2217/fmb.14.2]

15 **Roberts K**, Smith CF, Snelling AM, Kerr KG, Banfield KR, Sleigh PA, Beggs CB. Aerial dissemination of Clostridium difficile spores. *BMC Infect Dis* 2008; **8**: 7 [PMID: 18218089 DOI: 10.1186/1471-2334-8-7]

16 **Setlow P**. I will survive: DNA protection in bacterial spores. *Trends Microbiol* 2007; **15**: 172-180 [PMID: 17336071 DOI: 10.1016/j.tim.2007.02.004]

17 **Peniche AG**, Savidge TC, Dann SM. Recent insights into Clostridium difficile pathogenesis. *Curr Opin Infect Dis* 2013; **26**: 447-453 [PMID: 23982235 DOI: 10.1097/01.qco.0000433318.82618.c6]

18 **Johnson S**. Recurrent Clostridium difficile infection: causality and therapeutic approaches. *Int J Antimicrob Agents* 2009; **33** Suppl 1: S33-S36 [PMID: 19303567 DOI: 10.1016/S0924-8579(09)70014-7]

19 **Eyre DW**, Griffiths D, Vaughan A, Golubchik T, Acharya M, O'Connor L, Crook DW, Walker AS, Peto TE. Asymptomatic Clostridium difficile colonisation and onward transmission. *PLoS One* 2013; **8**: e78445 [PMID: 24265690 DOI: 10.1371/journal.pone.0078445]

20 **Leggett MJ**, McDonnell G, Denyer SP, Setlow P, Maillard JY. Bacterial spore structures and their protective role in biocide resistance. *J Appl Microbiol* 2012; **113**: 485-498 [PMID: 22574673 DOI: 10.1111/j.1365-2672.2012.05336.x]

21 **Lawley TD**, Clare S, Walker AW, Goulding D, Stabler RA, Croucher N, Mastroeni P, Scott P, Raisen C, Mottram L, Fairweather NF, Wren BW, Parkhill J, Dougan G. Antibiotic treatment of clostridium difficile carrier mice triggers a supershedder state, spore-mediated transmission, and severe disease in immunocompromised hosts. *Infect Immun* 2009; **77**: 3661-3669 [PMID: 19564382 DOI: 10.1128/IAI.00558-09]

22 **Lawley TD**, Clare S, Deakin LJ, Goulding D, Yen JL, Raisen C, Brandt C, Lovell J, Cooke F, Clark TG, Dougan G. Use of purified Clostridium difficile spores to facilitate evaluation of health care disinfection regimens. *Appl Environ Microbiol* 2010; **76**: 6895-6900 [PMID: 20802075 DOI: 10.1128/AEM.00718-10]

23 **Deakin LJ**, Clare S, Fagan RP, Dawson LF, Pickard DJ, West MR, Wren BW, Fairweather NF, Dougan G, Lawley TD. The Clostridium difficile spo0A gene is a persistence and transmission factor. *Infect Immun* 2012; **80**: 2704-2711 [PMID: 22615253 DOI: 10.1128/IAI.00147-12]

24 **Janoir C**, Denève C, Bouttier S, Barbut F, Hoys S, Caleechum L, Chapetón-Montes D, Pereira FC, Henriques AO, Collignon A, Monot M, Dupuy B. Adaptive strategies and pathogenesis of Clostridium difficile from in vivo transcriptomics. *Infect Immun* 2013; **81**: 3757-3769 [PMID: 23897605 DOI: 10.1128/IAI.00515-13]

25 **Underwood S**, Guan S, Vijayasubhash V, Baines SD, Graham L, Lewis RJ, Wilcox MH, Stephenson K. Characterization of the sporulation initiation pathway of Clostridium difficile and its role in toxin production. *J Bacteriol* 2009; **191**: 7296-7305 [PMID: 19783633 DOI: 10.1128/JB.00882-09]

26 **Eyre DW**, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, Ip CL, Golubchik T, Batty EM, Finney JM, Wyllie DH, Didelot X, Piazza P, Bowden R, Dingle KE, Harding RM, Crook DW, Wilcox MH, Peto TE, Walker AS. Diverse sources of C. difficile infection identified on whole-genome sequencing. *N Engl J Med* 2013; **369**: 1195-1205 [PMID: 24066741 DOI: 10.1056/NEJMoa1216064]

27 **Donskey CJ**, Kundrapu S, Deshpande A. Colonization versus carriage of Clostridium difficile. *Infect Dis Clin North Am* 2015; **29**: 13-28 [PMID: 25595843 DOI: 10.1016/j.idc.2014.11.001]

28 **Babakhani F**, Bouillaut L, Gomez A, Sears P, Nguyen L, Sonenshein AL. Fidaxomicin inhibits spore production in Clostridium difficile. *Clin Infect Dis* 2012; **55** Suppl 2: S162-S169 [PMID: 22752866 DOI: 10.1093/cid/cis453]

29 **Sorg JA**, Sonenshein AL. Bile salts and glycine as cogerminants for Clostridium difficile spores. *J Bacteriol* 2008; **190**: 2505-2512 [PMID: 18245298 DOI: 10.1128/JB.01765-07]

30 **Britton RA**, Young VB. Role of the intestinal microbiota in resistance to colonization by Clostridium difficile. *Gastroenterology* 2014; **146**: 1547-1553 [PMID: 24503131 DOI: 10.1053/j.gastro.2014.01.059]

31 **Weingarden AR**, Chen C, Bobr A, Yao D, Lu Y, Nelson VM, Sadowsky MJ, Khoruts A. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. *Am J Physiol Gastrointest Liver Physiol* 2014; **306**: G310-G319 [PMID: 24284963 DOI: 10.1152/ajpgi.00282.2013]

32 **Monaghan TM**. New perspectives in Clostridium difficile disease pathogenesis. *Infect Dis Clin North Am* 2015; **29**: 1-11 [PMID: 25573674 DOI: 10.1016/j.idc.2014.11.007]

33 **Rineh A**, Kelso MJ, Vatansever F, Tegos GP, Hamblin MR. Clostridium difficile infection: molecular pathogenesis and novel therapeutics. *Expert Rev Anti Infect Ther* 2014; **12**: 131-150 [PMID: 24410618 DOI: 10.1586/14787210.2014.866515]

34 **Kuehne SA**, Collery MM, Kelly ML, Cartman ST, Cockayne A, Minton NP. Importance of toxin A, toxin B, and CDT in virulence of an epidemic Clostridium difficile strain. *J Infect Dis* 2014; **209**: 83-86 [PMID: 23935202 DOI: 10.1093/infdis/jit426]

35 **Carter GP**, Rood JI, Lyras D. The role of toxin A and toxin B in the virulence of Clostridium difficile. *Trends Microbiol* 2012; **20**: 21-29 [PMID: 22154163 DOI: 10.1016/j.tim.2011.11.003]

36 **Rupnik M**, Janezic S. An Update on Clostridium difficile Toxinotyping. *J Clin Microbiol* 2016; **54**: 13-18 [PMID: 26511734 DOI: 10.1128/JCM.02083-15]

37 **Dingle KE**, Elliott B, Robinson E, Griffiths D, Eyre DW, Stoesser N, Vaughan A, Golubchik T, Fawley WN, Wilcox MH, Peto TE, Walker AS, Riley TV, Crook DW, Didelot X. Evolutionary history of the Clostridium difficile pathogenicity locus. *Genome Biol Evol* 2014; **6**: 36-52 [PMID: 24336451 DOI: 10.1093/gbe/evt204]

38 **Sun X**, Hirota SA. The roles of host and pathogen factors and the innate immune response in the pathogenesis of Clostridium difficile infection. *Mol Immunol* 2015; **63**: 193-202 [PMID: 25242213 DOI: 10.1016/j.molimm.2014.09.005]

39 **Hennequin C**, Janoir C, Barc MC, Collignon A, Karjalainen T. Identification and characterization of a fibronectin-binding protein from Clostridium difficile. *Microbiology* 2003; **149**: 2779-2787 [PMID: 14523111 DOI: 10.1099/mic.0.26145-0]

40 **Hennequin C**, Collignon A, Karjalainen T. Analysis of expression of GroEL (Hsp60) of Clostridium difficile in response to stress. *Microb Pathog* 2001; **31**: 255-260 [PMID: 11710845 DOI: 10.1006/mpat.2001.0468]

41 **Hennequin C**, Porcheray F, Waligora-Dupriet A, Collignon A, Barc M, Bourlioux P, Karjalainen T. GroEL (Hsp60) of Clostridium difficile is involved in cell adherence. *Microbiology* 2001; **147**: 87-96 [PMID: 11160803 DOI: 10.1099/00221287-147-1-87]

42 **Gerding DN**, Meyer T, Lee C, Cohen SH, Murthy UK, Poirier A, Van Schooneveld TC, Pardi DS, Ramos A, Barron MA, Chen H, Villano S. Administration of spores of nontoxigenic Clostridium difficile strain M3 for prevention of recurrent C. difficile infection: a randomized clinical trial. *JAMA* 2015; **313**: 1719-1727 [PMID: 25942722 DOI: 10.1001/jama.2015.3725]

43 **Brouwer MS**, Roberts AP, Hussain H, Williams RJ, Allan E, Mullany P. Horizontal gene transfer converts non-toxigenic Clostridium difficile strains into toxin producers. *Nat Commun* 2013; **4**: 2601 [PMID: 24131955 DOI: 10.1038/ncomms3601]

44 **Gerding DN**, Muto CA, Owens RC. Treatment of Clostridium difficile infection. *Clin Infect Dis* 2008; **46** Suppl 1: S32-S42 [PMID: 18177219 DOI: 10.1086/521861]

45 **Khanna S**, Pardi DS. Clostridium difficile infection: new insights into management. *Mayo Clin Proc* 2012; **87**: 1106-1117 [PMID: 23127735 DOI: 10.1016/j.mayocp.2012.07.016]

46 **Buffie CG**, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, No D, Liu H, Kinnebrew M, Viale A, Littmann E, van den Brink MR, Jenq RR, Taur Y, Sander C, Cross JR, Toussaint NC, Xavier JB, Pamer EG. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature* 2015; **517**: 205-208 [PMID: 25337874 DOI: 10.1038/nature13828]

47 **Johnson S**, Louie TJ, Gerding DN, Cornely OA, Chasan-Taber S, Fitts D, Gelone SP, Broom C, Davidson DM. Vancomycin, metronidazole, or tolevamer for Clostridium difficile infection: results from two multinational, randomized, controlled trials. *Clin Infect Dis* 2014; **59**: 345-354 [PMID: 24799326 DOI: 10.1093/cid/ciu313]

48 **Kyne L**, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. *N Engl J Med* 2000; **342**: 390-397 [PMID: 10666429 DOI: 10.1056/NEJM200002103420604]

49 **Kyne L**, Warny M, Qamar A, Kelly CP. Association between antibody response to toxin A and protection against recurrent Clostridium difficile diarrhoea. *Lancet* 2001; **357**: 189-193 [PMID: 11213096 DOI: 10.1016/S0140-6736(00)03592-3]

50 **Hunt JJ**, Ballard JD. Variations in virulence and molecular biology among emerging strains of Clostridium difficile. *Microbiol Mol Biol Rev* 2013; **77**: 567-581 [PMID: 24296572 DOI: 10.1128/MMBR.00017-13]

51 **O'Horo J**, Safdar N. The role of immunoglobulin for the treatment of Clostridium difficile infection: a systematic review. *Int J Infect Dis* 2009; **13**: 663-667 [PMID: 19186089 DOI: 10.1016/j.ijid.2008.11.012]

52 **Surawicz CM**, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, McFarland LV, Mellow M, Zuckerbraun BS. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. *Am J Gastroenterol* 2013; **108**: 478-98; quiz 499 [PMID: 23439232 DOI: 10.1038/ajg.2013.4]

53 **Shah N**, Shaaban H, Spira R, Slim J, Boghossian J. Intravenous immunoglobulin in the treatment of severe clostridium difficile colitis. *J Glob Infect Dis* 2014; **6**: 82-85 [PMID: 24926170 DOI: 10.4103/0974-777X.132053]

54 **Lowy I**, Molrine DC, Leav BA, Blair BM, Baxter R, Gerding DN, Nichol G, Thomas WD, Leney M, Sloan S, Hay CA, Ambrosino DM. Treatment with monoclonal antibodies against Clostridium difficile toxins. *N Engl J Med* 2010; **362**: 197-205 [PMID: 20089970 DOI: 10.1056/NEJMoa0907635]

55 **Baliban SM**, Michael A, Shammassian B, Mudakha S, Khan AS, Cocklin S, Zentner I, Latimer BP, Bouillaut L, Hunter M, Marx P, Sardesai NY, Welles SL, Jacobson JM, Weiner DB, Kutzler MA. An optimized, synthetic DNA vaccine encoding the toxin A and toxin B receptor binding domains of Clostridium difficile induces protective antibody responses in vivo. *Infect Immun* 2014; **82**: 4080-4091 [PMID: 25024365 DOI: 10.1128/IAI.01950-14]

56 **Giannasca PJ**, Zhang ZX, Lei WD, Boden JA, Giel MA, Monath TP, Thomas WD. Serum antitoxin antibodies mediate systemic and mucosal protection from Clostridium difficile disease in hamsters. *Infect Immun* 1999; **67**: 527-538 [PMID: 9916055]

57 **Ghose C**, Kelly CP. The prospect for vaccines to prevent Clostridium difficile infection. *Infect Dis Clin North Am* 2015; **29**: 145-162 [PMID: 25677708 DOI: 10.1016/j.idc.2014.11.013]

58 **Sougioultzis S**, Kyne L, Drudy D, Keates S, Maroo S, Pothoulakis C, Giannasca PJ, Lee CK, Warny M, Monath TP, Kelly CP. Clostridium difficile toxoid vaccine in recurrent C. difficile-associated diarrhea. *Gastroenterology* 2005; **128**: 764-770 [PMID: 15765411 DOI: 10.1053/j.gastro.2004.11.004]

59 **Bartlett JG**. A call to arms: the imperative for antimicrobial stewardship. *Clin Infect Dis* 2011; **53** Suppl 1: S4-S7 [PMID: 21795727 DOI: 10.1093/cid/cir362]

60 **Badger VO**, Ledeboer NA, Graham MB, Edmiston CE. Clostridium difficile: epidemiology, pathogenesis, management, and prevention of a recalcitrant healthcare-associated pathogen. *JPEN J Parenter Enteral Nutr* 2012; **36**: 645-662 [PMID: 22577120 DOI: 10.1177/0148607112446703]

61 **Chopra T**, Goldstein EJ. Clostridium difficile Infection in Long-term Care Facilities: A Call to Action for Antimicrobial Stewardship. *Clin Infect Dis* 2015; **60** Suppl 2: S72-S76 [PMID: 25922404 DOI: 10.1093/cid/civ053]

62 **Kapoor K**, Chandra M, Nag D, Paliwal JK, Gupta RC, Saxena RC. Evaluation of metronidazole toxicity: a prospective study. *Int J Clin Pharmacol Res* 1999; **19**: 83-88 [PMID: 10761537]

63 **Tedesco FJ**, Gordon D, Fortson WC. Approach to patients with multiple relapses of antibiotic-associated pseudomembranous colitis. *Am J Gastroenterol* 1985; **80**: 867-868 [PMID: 4050760]

64 **Patel NC**, Griesbach CL, DiBaise JK, Orenstein R. Fecal microbiota transplant for recurrent Clostridium difficile infection: Mayo Clinic in Arizona experience. *Mayo Clin Proc* 2013; **88**: 799-805 [PMID: 23910407 DOI: 10.1016/j.mayocp.2013.04.022]

65 **Rivkin A**, Gim S. Rifaximin: new therapeutic indication and future directions. *Clin Ther* 2011; **33**: 812-827 [PMID: 21741091 DOI: 10.1016/j.clinthera.2011.06.007]

66 **Carman RJ**, Boone JH, Grover H, Wickham KN, Chen L. In vivo selection of rifamycin-resistant Clostridium difficile during rifaximin therapy. *Antimicrob Agents Chemother* 2012; **56**: 6019-6020 [PMID: 22908175 DOI: 10.1128/AAC.00974-12]

67 **Johnson S**, Schriever C, Galang M, Kelly CP, Gerding DN. Interruption of recurrent Clostridium difficile-associated diarrhea episodes by serial therapy with vancomycin and rifaximin. *Clin Infect Dis* 2007; **44**: 846-848 [PMID: 17304459 DOI: 10.1086/511870]

68 **Garey KW**, Jiang ZD, Bellard A, Dupont HL. Rifaximin in treatment of recurrent Clostridium difficile-associated diarrhea: an uncontrolled pilot study. *J Clin Gastroenterol* 2009; **43**: 91-93 [PMID: 18385603 DOI: 10.1097/MCG.0b013e31814a4e97]

69 **Johnson S**, Schriever C, Patel U, Patel T, Hecht DW, Gerding DN. Rifaximin Redux: treatment of recurrent Clostridium difficile infections with rifaximin immediately post-vancomycin treatment. *Anaerobe* 2009; **15**: 290-291 [PMID: 19698797 DOI: 10.1016/j.anaerobe.2009.08.004]

70 **Garey KW**, Ghantoji SS, Shah DN, Habib M, Arora V, Jiang ZD, DuPont HL. A randomized, double-blind, placebo-controlled pilot study to assess the ability of rifaximin to prevent recurrent diarrhoea in patients with Clostridium difficile infection. *J Antimicrob Chemother* 2011; **66**: 2850-2855 [PMID: 21948965 DOI: 10.1093/jac/dkr377]

71 **Mullane KM**, Gorbach S. Fidaxomicin: first-in-class macrocyclic antibiotic. *Expert Rev Anti Infect Ther* 2011; **9**: 767-777 [PMID: 21810048 DOI: 10.1586/eri.11.53]

72 **Artsimovitch I**, Seddon J, Sears P. Fidaxomicin is an inhibitor of the initiation of bacterial RNA synthesis. *Clin Infect Dis* 2012; **55** Suppl 2: S127-S131 [PMID: 22752861 DOI: 10.1093/cid/cis358]

73 **Babakhani F**, Gomez A, Robert N, Sears P. Killing kinetics of fidaxomicin and its major metabolite, OP-1118, against Clostridium difficile. *J Med Microbiol* 2011; **60**: 1213-1217 [PMID: 21349983 DOI: 10.1099/jmm.0.029470-0]

74 **Babakhani F**, Gomez A, Robert N, Sears P. Postantibiotic effect of fidaxomicin and its major metabolite, OP-1118, against Clostridium difficile. *Antimicrob Agents Chemother* 2011; **55**: 4427-4429 [PMID: 21709084 DOI: 10.1128/AAC.00104-11]

75 **Babakhani F**, Bouillaut L, Sears P, Sims C, Gomez A, Sonenshein AL. Fidaxomicin inhibits toxin production in Clostridium difficile. *J Antimicrob Chemother* 2013; **68**: 515-522 [PMID: 23208832 DOI: 10.1093/jac/dks450]

76 **Louie TJ**, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, Gorbach S, Sears P, Shue YK. Fidaxomicin versus vancomycin for Clostridium difficile infection. *N Engl J Med* 2011; **364**: 422-431 [PMID: 21288078 DOI: 10.1056/NEJMoa0910812]

77 **Cornely OA**, Miller MA, Louie TJ, Crook DW, Gorbach SL. Treatment of first recurrence of Clostridium difficile infection: fidaxomicin versus vancomycin. *Clin Infect Dis* 2012; **55** Suppl 2: S154-S161 [PMID: 22752865 DOI: 10.1093/cid/cis462]

78 **Locher HH**, Seiler P, Chen X, Schroeder S, Pfaff P, Enderlin M, Klenk A, Fournier E, Hubschwerlen C, Ritz D, Kelly CP, Keck W. In vitro and in vivo antibacterial evaluation of cadazolid, a new antibiotic for treatment of Clostridium difficile infections. *Antimicrob Agents Chemother* 2014; **58**: 892-900 [PMID: 24277020 DOI: 10.1128/AAC.01830-13]

79 **Baldoni D**, Gutierrez M, Timmer W, Dingemanse J. Cadazolid, a novel antibiotic with potent activity against Clostridium difficile: safety, tolerability and pharmacokinetics in healthy subjects following single and multiple oral doses. *J Antimicrob Chemother* 2014; **69**: 706-714 [PMID: 24106141 DOI: 10.1093/jac/dkt401]

80 **Miller M**. Fidaxomicin (OPT-80) for the treatment of Clostridium difficile infection. *Expert Opin Pharmacother* 2010; **11**: 1569-1578 [PMID: 20446864 DOI: 10.1517/14656566.2010.485614]

81 **Kali A**, Charles MV, Srirangaraj S. Cadazolid: A new hope in the treatment of Clostridium difficile infection. *Australas Med J* 2015; **8**: 253-262 [PMID: 26392822 DOI: 10.4066/AMJ.2015.2441]

82 **Louie T**, Nord CE, Talbot GH, Wilcox M, Gerding DN, Buitrago M, Kracker H, Charef P, Cornely OA. Multicenter, Double-Blind, Randomized, Phase 2 Study Evaluating the Novel Antibiotic Cadazolid in Patients with Clostridium difficile Infection. *Antimicrob Agents Chemother* 2015; **59**: 6266-6273 [PMID: 26248357 DOI: 10.1128/AAC.00504-15]

83 **Eckburg PB**, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635-1638 [PMID: 15831718 DOI: 10.1126/science.1110591]

84 **Savage DC**. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol* 1977; **31**: 107-133 [PMID: 334036 DOI: 10.1146/annurev.mi.31.100177.000543]

85 **Hooper LV**, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu Rev Nutr* 2002; **22**: 283-307 [PMID: 12055347 DOI: 10.1146/annurev.nutr.22.011602.092259]

86 **Rupnik M**. Toward a true bacteriotherapy for Clostridium difficile infection. *N Engl J Med* 2015; **372**: 1566-1568 [PMID: 25875262 DOI: 10.1056/NEJMcibr1500270]

87 **Kau AL**, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. *Nature* 2011; **474**: 327-336 [PMID: 21677749 DOI: 10.1038/nature10213]

88 **Bien J**, Palagani V, Bozko P. The intestinal microbiota dysbiosis and Clostridium difficile infection: is there a relationship with inflammatory bowel disease? *Therap Adv Gastroenterol* 2013; **6**: 53-68 [PMID: 23320050]

89 **Petersen C**, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol* 2014; **16**: 1024-1033 [PMID: 24798552 DOI: 10.1111/cmi.12308]

90 Structure, function and diversity of the healthy human microbiome. *Nature* 2012; **486**: 207-214 [PMID: 22699609 DOI: 10.1038/nature11234]

91 **Bibbò S**, Lopetuso LR, Ianiro G, Di Rienzo T, Gasbarrini A, Cammarota G. Role of microbiota and innate immunity in recurrent Clostridium difficile infection. *J Immunol Res* 2014; **2014**: 462740 [PMID: 24995345]

92 **Jarchum I**, Liu M, Shi C, Equinda M, Pamer EG. Critical role for MyD88-mediated neutrophil recruitment during Clostridium difficile colitis. *Infect Immun* 2012; **80**: 2989-2996 [PMID: 22689818 DOI: 10.1128/IAI.00448-12]

93 **Jernberg C**, Löfmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME J* 2007; **1**: 56-66 [PMID: 18043614]

94 **Chang JY**, Antonopoulos DA, Kalra A, Tonelli A, Khalife WT, Schmidt TM, Young VB. Decreased diversity of the fecal Microbiome in recurrent Clostridium difficile-associated diarrhea. *J Infect Dis* 2008; **197**: 435-438 [PMID: 18199029 DOI: 10.1086/525047]

95 **Khanna S**, Tosh PK. A clinician's primer on the role of the microbiome in human health and disease. *Mayo Clin Proc* 2014; **89**: 107-114 [PMID: 24388028 DOI: 10.1016/j.mayocp.2013.10.011]

96 **Castagliuolo I**, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. Saccharomyces boulardii protease inhibits the effects of Clostridium difficile toxins A and B in human colonic mucosa. *Infect Immun* 1999; **67**: 302-307 [PMID: 9864230]

97 **Allen SJ**. The potential of probiotics to prevent Clostridium difficile infection. *Infect Dis Clin North Am* 2015; **29**: 135-144 [PMID: 25677707 DOI: 10.1016/j.idc.2014.11.002]

98 **Muñoz P**, Bouza E, Cuenca-Estrella M, Eiros JM, Pérez MJ, Sánchez-Somolinos M, Rincón C, Hortal J, Peláez T. Saccharomyces cerevisiae fungemia: an emerging infectious disease. *Clin Infect Dis* 2005; **40**: 1625-1634 [PMID: 15889360 DOI: 10.1086/429916]

99 **Bakken JS**. Staggered and tapered antibiotic withdrawal with administration of kefir for recurrent Clostridium difficile infection. *Clin Infect Dis* 2014; **59**: 858-861 [PMID: 24917658 DOI: 10.1093/cid/ciu429]

100 **Eiseman B**, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* 1958; **44**: 854-859 [PMID: 13592638]

101 **Schwan A**, Sjölin S, Trottestam U, Aronsson B. Relapsing clostridium difficile enterocolitis cured by rectal infusion of homologous faeces. *Lancet* 1983; **2**: 845 [PMID: 6137662 DOI: 10.1016/S0140-6736(83)90753-5]

102 **Silverman MS**, Davis I, Pillai DR. Success of self-administered home fecal transplantation for chronic Clostridium difficile infection. *Clin Gastroenterol Hepatol* 2010; **8**: 471-473 [PMID: 20117243 DOI: 10.1016/j.cgh.2010.01.007]

103 **Brandt LJ**, Aroniadis OC, Mellow M, Kanatzar A, Kelly C, Park T, Stollman N, Rohlke F, Surawicz C. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection. *Am J Gastroenterol* 2012; **107**: 1079-1087 [PMID: 22450732 DOI: 10.1038/ajg.2012.60]

104 **Yoon SS**, Brandt LJ. Treatment of refractory/recurrent C. difficile-associated disease by donated stool transplanted via colonoscopy: a case series of 12 patients. *J Clin Gastroenterol* 2010; **44**: 562-566 [PMID: 20463588 DOI: 10.1097/MCG.0b013e3181dac035]

105 **van Nood E**, Dijkgraaf MG, Keller JJ. Duodenal infusion of feces for recurrent Clostridium difficile. *N Engl J Med* 2013; **368**: 2145 [PMID: 23718168]

106 **Petrof EO**, Khoruts A. From stool transplants to next-generation microbiota therapeutics. *Gastroenterology* 2014; **146**: 1573-1582 [PMID: 24412527 DOI: 10.1053/j.gastro.2014.01.004]

107 **Zainah H**, Hassan M, Shiekh-Sroujieh L, Hassan S, Alangaden G, Ramesh M. Intestinal microbiota transplantation, a simple and effective treatment for severe and refractory Clostridium difficile infection. *Dig Dis Sci* 2015; **60**: 181-185 [PMID: 25052150 DOI: 10.1007/s10620-014-3296-y]

108 **Seekatz AM**, Aas J, Gessert CE, Rubin TA, Saman DM, Bakken JS, Young VB. Recovery of the gut microbiome following fecal microbiota transplantation. *MBio* 2014; **5**: e00893-e00814 [PMID: 24939885 DOI: 10.1128/mBio.00893-14]

109 **Lo Vecchio A**, Cohen MB. Fecal microbiota transplantation for Clostridium difficile infection: benefits and barriers. *Curr Opin Gastroenterol* 2014; **30**: 47-53 [PMID: 24275671 DOI: 10.1097/MOG.0000000000000023]

110 **Bakken JS**, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, Kelly C, Khoruts A, Louie T, Martinelli LP, Moore TA, Russell G, Surawicz C. Treating Clostridium difficile infection with fecal microbiota transplantation. *Clin Gastroenterol Hepatol* 2011; **9**: 1044-1049 [PMID: 21871249 DOI: 10.1016/j.cgh.2011.08.014]

111 **Youngster I**, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. *JAMA* 2014; **312**: 1772-1778 [PMID: 25322359 DOI: 10.1001/jama.2014.13875]

112 **Lee CH**, Steiner T, Petrof EO, Smieja M, Roscoe D, Nematallah A, Weese JS, Collins S, Moayyedi P, Crowther M, Ropeleski MJ, Jayaratne P, Higgins D, Li Y, Rau NV, Kim PT. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. *JAMA* 2016; **315**: 142-149 [PMID: 26757463 DOI: 10.1001/jama.2015.18098]

113 [**Khanna S**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Khanna%20S%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Pardi DS](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pardi%20DS%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Kelly CR](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kelly%20CR%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Kraft CS](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kraft%20CS%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Dhere T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Dhere%20T%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Henn MR](http://www.ncbi.nlm.nih.gov/pubmed/?term=Henn%20MR%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Lombardo MJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lombardo%20MJ%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Vulic M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Vulic%20M%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Ohsumi T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ohsumi%20T%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Winkler J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Winkler%20J%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Pindar C](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pindar%20C%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [McGovern BH](http://www.ncbi.nlm.nih.gov/pubmed/?term=McGovern%20BH%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Pomerantz RJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pomerantz%20RJ%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Aunins JG](http://www.ncbi.nlm.nih.gov/pubmed/?term=Aunins%20JG%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Cook DN](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cook%20DN%5BAuthor%5D&cauthor=true&cauthor_uid=26908752), [Hohmann EL](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hohmann%20EL%5BAuthor%5D&cauthor=true&cauthor_uid=26908752). A Novel Microbiome Therapeutic Increases Gut Microbial Diversity and Prevents Recurrent Clostridium difficile Infection. *J Infect Dis* 2016: Epub ahead of print [PMID: 26908752 DOI: 10.1093/infdis/jiv766]

114 **Tvede M**, Rask-Madsen J. Bacteriotherapy for chronic relapsing Clostridium difficile diarrhoea in six patients. *Lancet* 1989; **1**: 1156-1160 [PMID: 2566734]

**P-Reviewer:** García-Elorriaga G, Krishnan T, Moschovi MA **S-Editor:** Ji FF **L-Editor: E-Editor:**

**Table 1 Management outline for recurrent *Clostridium difficile* infection[**[**7**](#_ENREF_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](#_ENREF_52)]  Consider Vancomycin taper ± pulse[[11](#_ENREF_11)]  Vancomycin followed by rifaximin chaser[[67](#_ENREF_67)]  Fidaxomicin[[80](#_ENREF_80)] |
| Probiotics  Probiotics with antibiotics may help[[99](#_ENREF_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](#_ENREF_54)]  IVIG[[51](#_ENREF_51)]  Toxoid vaccine [[58](#_ENREF_58)]  Non toxigenic strains [[42](#_ENREF_42)] |
| Bacteriotherapy  Fecal microbiota transplant[[111](#_ENREF_111),[115](#_ENREF_115)] |

IBS: Irritable Bowel syndrome; IVIG: Intravenous immunoglobulin.