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REVIEW

Management of adult *Clostridium difficile* digestive contaminations: a literature review

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Abstract

Clostridium difficile infections (CDI) dramatically increased during the last decade and cause a major public health problem. Current treatments are limited by the high disease recurrence rate, severity of clinical forms, disruption of the gut microbiota, and colonization by vancomycin-resistant enterococci (VRE). In this review, we resumed current treatment options from official recommendation to promising alternatives available in the management of adult CDI, with regard to severity and recurring or non-recurring character of the infection. Vancomycin remains the first-line antibiotic in the management of mild to severe CDI. The use of metronidazole is discussed following the latest US recommendations that replaced it by fidaxomicin as first-line treatment of an initial episode of non-severe CDI. Fidaxomicin, the most recent antibiotic approved for CDI in adults, has several advantages compared to vancomycin and metronidazole, but its efficacy seems limited in cases of multiple recurrences. Innovative therapies such as fecal microbiota transplantation (FMT) and antitoxin antibodies were developed to limit the occurrence of recurrence of CDI. Research is therefore very active, and new antibiotics are being studied as surotomycin, cadazolid, and rinidazole.

Keywords *Clostridium difficile* · Fidaxomicin · Fecal microbiota transplantation · Antitoxin antibodies · Surotomycin · Cadazolid

Introduction

Clostridium difficile is a strict and sporulated anaerobic gram-positive *bacillus* described in 1935 by Hall and O'Toole [1]. Infection occurs by oro-fecal contamination with ingestion of spores and vegetative cells of *C. difficile* in the environment (Fig. 1). In the stomach, vegetative cells are killed by gastric acidity, but spores are resistant. The germination of spores is dependent on bile acids and will take place in the duodenum. Then, there is a colonization of the colon by vegetative forms, toxin production, sporulation, adhesion of a part of the spores to the colon cells, and elimination of spores in the feces. Thus, spores can remain dormant in the colon and resist to antibiotics. Germination in vegetative cells can occur after the end of the antibiotic treatment and cause recurrences [2]. The pathogenicity of *C. difficile* is related to the production of two particularly virulent toxins in the colon: an enterotoxin (toxin A) and a cytotoxic toxin (toxin B). This bacterium is responsible for a wide variety of clinical manifestations ranging from asymptomatic carrier state to post-antibiotic diarrhea which can be complicated by pseudomembranous colitis, major ileus, toxic megacolon, or sepsis.

In more than 70% of cases, nosocomial cause is responsible of hospital-acquired *C. difficile* infections (CDI) [3]. These CDI cause a major public health problem with 500,000 infections reported in the USA in 2011 and 7711 cases reported in 20 European Union countries in 2016, according to American and European surveillance protocols of CDI [4, 5]. These surveillance protocols found a mortality rate attributable to

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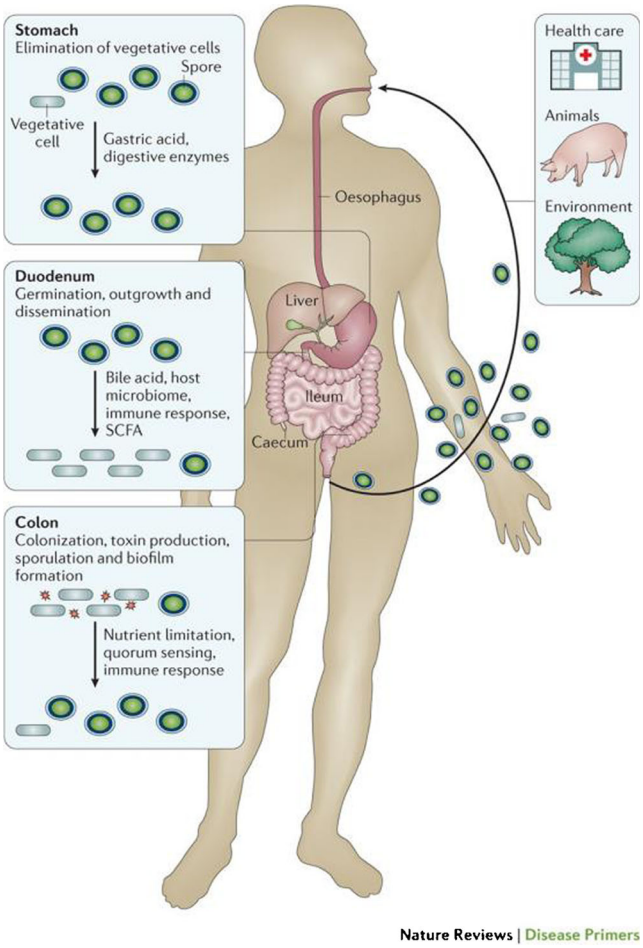


Fig. 1 *C. difficile* life cycle [2]

CDI infection of 7.9% in Europe and 5.8% in the USA. One of the major problem in the management of these infections is the occurrence of recurrences estimated to 33% after a first episode of CDI, that complicates the therapeutic strategies [6]. Since 2003, the emergence of a *C. difficile* virulent strain named NAP1/027 resulted in the increased incidence and severity of clinical forms [7, 8]. According to a Netherland study, the mortality risk among patient with *C. difficile* 027 was evaluated to 21.8%, higher than other most common strains (10.8% for 014, 14.5% for 078, and 15.8% for 001) [9]. In the last European epidemiologic data, 027 was the most common PCR ribotypes found in CDI (22.9%), followed by 014 (6.7%), 078 (5.1%) and 001 (4.5%) [4]. We report herein the management of CDI according to the severity, complications, and recurrences as well as the new therapeutic options and research pathways.

Management of asymptomatic carriers

Asymptomatic patient carriers represent a reservoir of *C. difficile* transmission for other patients, by the hand of health care workers or from the spread of *C. difficile* spores in the environment [10]. Some studies have therefore been conducted to demonstrate the possible benefit of treatment of these healthy carriers.

In 1987, a significant reduction in new CDI in a hematology department after initiation of oral vancomycin antibiotherapy (500 mg q.i.d for 7 days) in patients colonized with *C. difficile* and clinically asymptomatic was demonstrated [11]. Another open randomized study was conducted in 30 patients with toxigenic *C. difficile* colonization without clinical symptoms (neither diarrhea nor abdominal pain). Patients were randomized to receive either oral vancomycin (125 mg q.i.d for 10 days), oral metronidazole (500 mg b.i.d), or placebo (t.i.d) for 10 days. The results showed a negative culture of *C. difficile* during and immediately after the end of the treatment for 9 of 10 patients who received vancomycin compared to 3 of 10 patients in the metronidazole group and 2 of 10 in the placebo group. However, of the nine patients who had a negative culture after vancomycin antibiotherapy, eight had positive cultures again within 20 days after the end of treatment [12].

These studies showed that the culture conversion and disappearance of toxins in the stool were only transient. So, current recommendations do not recommend screening and management of healthy carriers because of insufficient data [13]. However, prevention rules to prevent CDI are strongly recommended such as isolation of affected patients, environmental cleaning and cleaning of medical equipment, wearing gloves and gowns for healthy personnel, and having a good hand hygiene after contact with a patient contaminated by *C. difficile* [14].

Treatment of a first non-severe episode of CDI

Stopping the current antibiotherapy

Discontinuation of the responsible antibiotherapy associated with rehydration and electrolytic reequilibration leads in 25% of cases to a complete recovery in 2 to 3 days [13, 15].

Antimotility medications are not recommended because they extend the duration of toxin elimination.

An observational study showed that in a small sample of patients treated with metronidazole for symptomatic CDI, symptomatic recovery was achieved in 100% of patients who stopped the prescribed antibiotics, against only 59% in patients who keep on antibiotic treatment [16]. Among antibiotics promoting the occurrence of CDI, cephalosporins and clindamycin are the most involved in hospitalized patients. For community-associated infection, the most antibiotics involved in CDI are cephalosporins, clindamycin, and quinolones [17].

Therapeutic strategies according to official recommendations

According to the recommendations of "the European Society of Clinical Microbiology and Infectious Diseases" of 2013

and "the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA)" of 2010, the first-line treatments for non-severe CDI are oral metronidazole (500 mg t.i.d for 7 to 14 days) and oral vancomycin (125 mg q.i.d for 14 days) [10, 15].

Four randomized clinical trials compared vancomycin and metronidazole in terms of efficacy and relapse rates after treatment discontinuation (Table 1). Two of them showed equivalent efficacy and relapse rates between metronidazole and vancomycin [18, 19]. However, two more recent studies distinguishing severe and non-severe CDI showed similar efficacy and relapse rates in non-severe CDI, but a greater efficacy of vancomycin compared to metronidazole in severe CDI [20, 21]. These studies led to the conclusion that metronidazole can be used as first-line therapy in the management of non-severe CDI, with equivalent efficacy to vancomycin. However, bias in metronidazole regimen was found in these comparative studies. Indeed, some of them used a metronidazole regimen different from that used in clinical practice of 500 mg t.i.d for 10 days.

The emergence of a particularly virulent *C. difficile* 027 strain is related to the severity of the clinical CDI resulted in a reduced susceptibility to metronidazole and vancomycin [22, 23]. Significant therapeutic failure rates have been reported, reaching 38% for metronidazole and 19% for vancomycin [24]. A metronidazole resistance rate of 6.3% was observed in a study conducted between 1993 and 2003 on 415 isolates of *C. difficile* strains [25]. Moreover, a US study conducted between 2011 and 2013 showed that out of 87 strains of *C. difficile* 027, 34 had a reduced susceptibility to vancomycin (39.1%) [26].

Another difficulty in the management of CDI is the occurrence of relapses, up to 50% after treatment with metronidazole and 37% after treatment with vancomycin [24, 27].

Before the publication of the latest American recommendations, metronidazole was preferred to vancomycin as soon as possible for economic reasons, oral availability and to limit the emergence of vancomycin-resistant enterococci (VRE) [28]. The latest US recommendations published in 2017 recommend the use of oral vancomycin (125 mg q.i.d for 10 days) or oral fidaxomicin (200 mg b.i.d for 10 days) for non-severe CDI instead of oral metronidazole (500 mg t.i.d for 10–14 days) [13]. The European recommendations retain metronidazole (500 mg t.i.d for 10 days) with the highest level of evidence, followed by vancomycin (125 mg q.i.d for 10 days) or fidaxomicin (200 mg b.i.d for 10 days) as first-line treatment [15]. The use of fidaxomicin is reserved for documented CDI with the presence of A and/or B toxin in the stools, either in first intention in case of high risk of recurrence, or in second intention in case of documented recurrence after a first-line regimen with metronidazole or vancomycin [29].

Fidaxomicin is the first antibiotic approved by the US Food and Drug Administration (FDA) in the management of documented CDI in the last 20 years. It is a macrocyclic antibacterial agent with narrow-spectrum and bactericidal activity against *C. difficile* [29]. The non-inferiority of fidaxomicin compared to vancomycin was demonstrated in two phase III clinical trials (Table 1) [30, 31]. These studies also showed that the recurrence rate was significantly lower with fidaxomicin than with vancomycin except in the case of NAP1/027 strain for which the recurrence rate was not significantly different (24.4% *vs* 23.6%; $p = 0.93$). Three additional randomized, multicentric studies confirmed the superiority and lowest recurrence rate for fidaxomicin compared to vancomycin (Table 1) [32–34]. Extended-pulsed fidaxomicin (200 mg b.i.d days 1–5 followed by 200 mg t.i.d days 7–25) was very interesting to obtain sustained recovery for CDI compared to vancomycin (Table 1) [33]. The lowest recurrence rate observed with fidaxomicin can be explained by two mechanisms: it spares *Bacteroides* species that normally colonize the fecal flora allowing the preservation of human gut microbiota [35, 36], and reduces the secretion of toxins A and B of *C. difficile* during and after discontinuation of treatment (Thabit *et al* (Table 1)) [32]. Indeed, fidaxomicin is the only antibiotic that inhibits the transcription of genes coding for the secretion of toxins A and B [32]. Moreover, fidaxomicin would have an effect on enterococci against many VRE [37]. A multicenter randomized clinical trial evaluated the effect of fidaxomicin on VRE and modification of concentration in case of pre-existing enterococcal colonization. Five hundred forty-eight patients were included in this study, with CDI confirmed by the presence of A and/or B toxin in the stools (265 received fidaxomicin and 283 vancomycin). In comparison with vancomycin-treated patients, fidaxomicin-treated patients acquired less VRE (31% *vs* 7%; $p < 0.001$). Among patients already colonized by VRE before treatment, the concentration of VRE decreased significantly after treatment with fidaxomicin (from 5.9 to 3.8 log10 CFU/g stool; $p = 0.001$). In contrast, the decrease in VRE concentrations in the vancomycin group was not significant (from 5.3 to 4.2 log10 CFU/g stool; $p = 0.20$) [38].

Fidaxomicin is therefore a first-line therapeutic alternative in the treatment of mild to moderate CDI with high-risk recurrences. However, in the most severe clinical forms, the lack of efficacy and tolerance data limits its use. Only vancomycin was compared to fidaxomicin, and no comparative study with metronidazole was conducted nowadays. A Cochrane analysis published in 2018 reviewed 19 studies on antibiotic treatment for CDI [39]. This meta-analysis concludes that fidaxomicin is the most effective treatment in the management of CDI with five supporting publications including four phase III clinical studies [30–34]. Its use in Europe remains regulated because of its higher acquisition costs, and its limited effectiveness in

Table 1 Randomized controlled trials of antibiotics used in the management of an initial episode of non-severe CDI

Study/design	Number of patients	Antibiotic regimen	Results		
			Efficacity (cure)	Recurrence	
Teasley 1983 [18]	42	Metronidazole 250 mg q.i.d 10 days	88%	5%	
Prospective, randomized, monocenter study	52	Vancomycin 500 mg q.i.d 10 days	86%	11% $p = 0.17$	
	Total 94				
Wenisch 1996 [19]	31	Metronidazole 500 mg t.i.d 10 days	94%; $p > 0.05$[a]	16%; $p = 0.27$ *vs* fusidic acid $p = 0.75$ *vs* teicoplanin $p = 0.8$ *vs* vancomycin	
Prospective, randomized, monocenter study	31	Vancomycin 500 mg t.i.d 10 days	94%	16%; $p = 0.27$ *vs* fusidic acid $p = 0.75$ *vs* teicoplanin	
	28	Teicoplanin 400 mg b.i.d 10 days	96%	7%; $p = 0.042$ *vs* fusidic acid	
	29	Fusidic acid 500 mg t.i.d 10 days	93%	28%	
	Total 119				
Zar 2007 [20]	**79**	Metronidazole 250 mg q.i.d 10 days	84%; $p = 0.06$[a]	14%; $p = 0.27$[a]	
Prospective, randomized, double-blind, monocenter study	41[NS] 38[S]		90%[NS]; $p = 0.36$[a] 76%[S]; $p = 0.02$[a]	8%[NS]; $p = 0.67$[a] 21%[S]; $p = 0.30$[a]	
	71	Vancomycin 125 mg q.i.d 10 days	97%	7%	
	40[NS] 31[S]		98%[NS] 97%[S]	5%[NS] 10%[S]	
	Total 150				
Johnson 2014 [21]	143	Metronidazole 375 mg q.i.d 10 days	72% 76.7%[NS] 64.9%[S]; $p = 0.042$[a]	27.1%	
Phase III, randomized, double-blind, multicenter clinical trial					
Protocol 301	133	Vancomycin 125 mg q.i.d 10 days	82% 81%[NS] 84.8%[S]	23.4%	
	266	Tolevamer 3 g t.i.d 14 days	46.6%; $p < 0.001$[a] 52.7%[NS] 36.8%[S]	3.4%; $p < 0.001$[a]	
	Total 542				
Johnson 2014 [21]	135	Metronidazole 375 mg q.i.d 10 days	73.3% 75.5%[NS] 68.6%[S]	18.9%	
Phase III, randomized, double-blind, multicenter clinical trial					
Protocol 302	125	Vancomycin 125 mg q.i.d 10 days	80.8% 84%[NS] 71.9%[S]	17.6%	
	268	Tolevamer 3 g t.i.d 14 days	41.8%; $p < 0.001$[a] 43.8%[NS] 37.7%[S]	5.7%; $p < 0.05$[a]	
	Total 528				
Louie 2011 [30]	309	Vancomycin 125 mg q.i.d 10 days	85.8%	25.3%	
Phase III, randomized, prospective, multicenter, double-blind clinical trial	287	Fidaxomicin 200 mg b.i.d 10 days	88.2%	14.5% $p = 0.005$	
	Total 596				
Cornely 2012 [31]				No prior episode:	1 prior episode:
Phase III randomized, multicenter, double-blind clinical trial	76	Vancomycin 125 mg q.i.d 10 days	91.6%	22.6%	35.5%
	74	Fidaxomicin 200 mg b.i.d 10 days	93.7%	11.7%; $p < 0.001$	19.7%; $p = 0.045$
	Total 150				
Thabit 2016 [32]	5	Vancomycin 125 mg q.i.d 10 days	Fecal concentration of *C. difficile* toxins		
Randomized, monocenter, open-label clinical trial	7			Baseline	End-off therapy	Follow-up
	Total 12	Fidaxomicin 200 mg b.i.d 10 days	Toxin A			
			Vanco	27.1 ± 31.5	19.1 ± 26.6	35.5 ± 35.2
			Fidaxo	34.5 ± 39.4	0.3 ± 0	0.3 ± 0
			Toxin B			
			Vanco	32.2 ± 39	26.9 ± 37.5	25.3 ± 25
			Fidaxo	45.5 ± 27.9	0.3 ± 0	5 ± 8.2
Guery 2017 [33]					Day 40	Day 55	Day 90
	181	Vancomycin 125 mg q.i.d 10 days	80%; $p = 0.721$		17%	18%	19%

Table 1 (continued)

Study/design	Number of patients	Antibiotic regimen	Results			
			Efficacity (cure)	Recurrence		
Phase IIIb/IV randomized, controlled, parallel, multicenter, superiority, open-label clinical trial	181	Fidaxomicin 200 mg b.i.d days 1–5 followed by 200 mg t.i.d days 7–25	82%	2% $p < 0.001$	4% $p < 0.001$	6% $p < 0.00073$
	Total 362					
Mikamo 2018 [34]	108	Vancomycin 125 mg q.i.d 10 days	65.7%	25.3%		
Phase III, randomized, multicenter in Japan, controlled, double-blind clinical trial	104	Fidaxomicin 200 mg b.i.d 10 days	67.3%	19.5%		
	Total 212					
De lalla 1992 [43]	20	Vancomycin 500 mg q.i.d 10 days	100%. $p = 0.56$	20%; $p = 0.21$		
Prospective, randomized, comparative study	26	Teicoplanin 100 mg b.i.d 10 days	96.2%	7.7%		
	Total 46					
Wullt 2004 [45]	55	Metronidazole 400 mg t.i.d 7 days	93%; $p = 0.116$	29%; $p = 0.691$		
Prospective, randomized, double-blind clinical trial	59	Fucidic acid 250 mg t.i.d 7 days	83%	27%		
	Total 114					
Lagrotteria 2006 [48]	20	Metronidazole 500 mg t.i.d 10 days	65%	20%; $p = 0.66$		
Prospective, randomized, single-blind study	19	Metronidazole 500 mg t.i.d + rifampicin 300 mg b.i.d 10 days	63%	11%		
	Total 39					
Young 1985 [50]			Symptom resolution	Negative toxins		
Randomized, double-blind study	21	Vancomycin 125 mg q.i.d 7 days	86%	83%; $p = 0.04$		
	21	Bacitracin 20,000 UI q.i.d 7 days	76%	53%		
	Total 42					
Dudley 1986 [51]			Negative toxins			
Randomized, double-blind study	15	Vancomycin 500 mg q.i.d 10 days	71%			
	12	Bacitracin 25,000 UI q.i.d 10 days	30%			
	Total 27					

NS non-severe CDI, *S* severe CDI

[a] Comparison with vancomycin

clinical practice for patients with multiple recurrences [40–42].

Other studied antibiotics

The effect of different antibiotics on *C. difficile* was evaluated, and some of them showed interesting results.

Oral teicoplanin, another glycopeptide of the same class as vancomycin, showed equivalent cure rates compared to vancomycin according to two randomized clinical trials (Table 1) [19, 43], and two meta-analysis [39, 44]. Compared to vancomycin, intravenous teicoplanin has an indication for oral administration in Europe but is not available in the USA. So, only oral vancomycin appears on the current guidelines [13, 15].

Oral fusidic acid, a polysaccharide antibiotic, is effective mainly against gram-positive bacteria and showed equivalent activity to metronidazole on *C. difficile* in two randomized clinical trials (Table 1) [19, 45]. However, a prospective randomized, double-blind clinical trial compared metronidazole to fusidic acid and reported 55% of *C. difficile* resistance strains after fusidic acid therapy for a first episode of CDI [46]. Even if fusidic acid spares *Bacteroides* species and have a cost significantly lower compared to vancomycin (13 vs 66), the lack of randomized clinical trials and evidence of resistance have limited its use [45].

Rifampicin, an antibiotic with a good intracellular penetration, showed excellent in vitro activity against *C. difficile* [47]. The use of rifampicin in addition to

metronidazole for the treatment of CDI was evaluated in a prospective, randomized study, but no clinical benefit in terms of recovery and relapse rates was demonstrated (Table 1) [48]. Moreover, after oral administration, the absorption is rapid and complete inducing systemic effects not localized at the colon [49].

Bacitracin, a polypeptide antibiotic, is effective mainly against gram-positive bacteria and showed good symptom resolution for CDI compared to vancomycin in two randomized clinical trials (Table 1) [50, 51]. However, bacitracin was significantly less effective than vancomycin in the negativation of *C. difficile* toxins in stool samples collected at completion of therapy.

Also, among these other antibiotics with activity on *C. difficile* without additional data, only teicoplanin may represent an alternative to oral vancomycin. Clinical studies showed the non-inferiority of teicoplanin compared to vancomycin but no better efficacy. Although there is a tendency to decrease the recurrence rate, the non-significance of the results and the higher acquisition cost of teicoplanin compared to vancomycin do not justify its use for CDI in clinical practice [52].

Non-antibiotic treatments

Clinical trials have evaluated several non-antibiotic treatments to provide a prolonged recovery. The hyperproduction of toxins A and B for several *C. difficile* strains (in particularly for ribotypes 027 and 078) is correlated with the severity of CDI, and may contribute to antibiotic resistance [53]. Luminal toxin-binding agents (LTBAs) such as cholestyramine, colestipol, and tolevamer were tested as an alternative to conventional antibiotic treatment to reduce the cytotoxic effects of toxins A and B [54].

Tolevamer (GT160-246) is an orally high molecular weight anionic toxin-binding polymer that neutralizes *C. difficile* toxins A and B. One phase II clinical trial showed a non-inferiority of tolevamer 2 g t.i.d compared to vancomycin (Table 2) [55], but three phase III clinical trials showed a lower recovery rate for tolevamer 3 g t.i.d. (Tables 1 and 2) [15, 21]. These clinicals trials suggested a recurrence rate lower for tolevamer compared to vancomycin, but data were limited, and on the three phase III clinical trials, two were only published in posters. Because of limited efficiency and its ability to bind cations inducing hypokalemia as a side effect, tolevamer is not recommended in current practice for CDI.

Cholestyramine and colestipol are synthetic ion exchange resins. One randomized clinical trial and several case reports described the ability of these resins to bind the toxins A and B of *C. difficile* leading to decrease toxin concentration in samples of stools (Table 2) [54, 56]. However, current guidelines do not recommend the use of colestipol and cholestyramine in CDI because of their *in vitro* binding to vancomycin, which may reduce the effectiveness of antibiotic therapy.

Treatment of severe episodes of CDI

Therapeutic strategies according to official recommendations

According to the last US recommendations, severe CDI is defined by leukocytosis with a white blood cell count of ≥ 15,000 cells/μL and a serum creatinine level < 1.5 mg/dL. The European and American guidelines recommend for an initial episode of severe CDI the use of oral vancomycin (125 mg q.i.d for 10 days) or oral fidaxomicin (200 mg b.i.d for 10 days) [13, 15]. The criteria for severity of a CDI are not homogeneous according to clinical studies.

The first phase III clinical trial comparing metronidazole to vancomycin according to the severity of CDI was conducted between October 1994 and June 2002 (Table 3) [20]. Patients with *C. difficile* documented diarrhea were stratified into non-severe or severe CDI and randomized to have either oral metronidazole (250 mg q.i.d) or oral vancomycin (125 mg q.i.d) for 10 days. A severity score was established counting one point in case of fever > 38.3 °C, [albumin] < 2.5 mg/L, leucocytes > 15,000 cells/μL, and two points in case of hospitalization in intensive care unit or pseudomembranous colitis. Sixty-nine patients were included in the severe CDI group because they had a score ≥ 2. These patients with severe CDI were randomized to receive metronidazole (38 patients) or vancomycin (31 patients). The results showed a clinical success significantly more important for vancomycin compared to metronidazole (97% *vs* 76%; $p = 0.02$). The recurrence rate was lower for vancomycin compared to metronidazole without significantly difference (10% *vs* 21%; $p = 0.30$). Seven years later, metronidazole was compared to vancomycin for severe CDI in another phase III clinical study (Table 3) [21]. This study defined severe CDI when the patient had 10 or more bowel movements in 24 h or a white blood cell count of ≥ 20,000 cells/μL or severe abdominal pain due to CDI. The results suggested a better clinical success with vancomycin compared to metronidazole (78.5% *vs* 66.3%), without significantly difference ($p > 0.05$).

The main pharmacokinetic advantage of oral vancomycin over oral metronidazole is to be not absorbed. Therefore, bactericidal plasmatic concentrations at the colonization site of *C. difficile* in the colon are reached, contrary to metronidazole. Indeed, 80% of metronidazole administered orally is absorbed in less than 1 h, and the colic concentrations are very low because it mainly distributed in the liver and bile [57–59]. Thus, the limited efficacy of metronidazole in severe infections may be related to too low concentration at the site of action, the colon. However, oral vancomycin can be absorbed

Table 2 Randomized controlled trials of luminal toxin-binding agents for the management of an initial episode of CDI

Study/design	Number of patients	Treatment regimen	Results	
			Efficacity (cure rate)	Recurrence
Louie 2006 [55]	72	Tolevamer 1 g t.i.d 14 days	67%; $p = 0.53$[a]	23%; $p = 0.61$[b]
Phase II, randomized, double-blind, controlled, multicenter clinical trial	70	Tolevamer 2 g t.i.d 14 days	83%; $p = 0.02$[a]	10%; $p = 0.19$[b]
	80	Vancomycin 125 mg q.i.d 14 days	91%	19%
	Total 222			
Louie 2007 [15]	266	Tolevamer 3 g t.i.d 14 days	47%	3%
Phase III, randomized, double-blind, multicenter clinical trial	134	Vancomycin 125 mg q.i.d 10 days	81%	23%
	143	Metronidazole 375 mg q.i.d 10 days	72%	27%
	Total 543			
Bouza 2008 [15]	268	Tolevamer 3 g t.i.d 14 days	42%	6%
Phase III, randomized, double-blind, multicenter clinical trial	125	Vancomycin 125 mg q.i.d 10 days	81%	18%
	135	Metronidazole 375 mg q.i.d 10 days	73%	19%
	Total 528			
Mogg 1982 [56]			Disappearance of the toxin in stools[c]	
Randomized controlled trial	17	Colestipol 10 g q.i.d 5 days	1/6	
	21	Placebo	2/5	
	Total 38			

[a] p value vs the vancomycin 500 mg group by chow test of non-inferiority

[b] p value vs the vancomycin 500 mg group by the log-rank test

[c] Toxin detection before treatment was present in only six patients in colestipol group and five patients in placebo group

from the colon, due to compromised intestinal epithelium in patients with *C. difficile* colitis, resulting in systemic-related side effects (renal failure and ototoxicity) [60, 61].

A limited number of clinical studies compared fidaxomicin to metronidazole for severe infections and one phase III clinical trial compared fidaxomicin to vancomycin (Table 3) [30]. In this trial, patients were randomized in non-severe or severe CDI. Severe disease was defined by the presence of 10 or more unformed bowel movements per day or a white cell count of $\geq 15{,}001/\mu L$, and according to the strain type of *C. difficile*. This study showed equivalent clinical recovery and recurrence rates significantly lower with fidaxomicin compared to vancomycin (11.7% *vs* 23.2%; $p = 0.05$) [30].

Other studied antibiotics

Tigecycline, a broad-spectrum antimicrobial against gram-positive, gram-negative, and anaerobic bacteria, showed in vitro activity of 100% against several *C. difficile* isolates including multidrug-resistant strains [62]. This antibiotic

Table 3 Randomized controlled trials of antibiotics used in the management of an initial episode of severe CDI

Study/design	Number of patients	Treatment regimen	Results	
			Efficacity (cure rate)	Recurrence
Zar 2007 [20]	38	Metronidazole 250 mg q.i.d 10 days	76%; $p = 0.02$	21%; $p = 0.30$
Prospective, randomized, double-blind, monocentric study	31	Vancomycin 125 mg q.i.d 10 days	97%	10%
	Total 69			
Johnson 2014 [21]	92	Metronidazole 375 mg q.i.d 10 days	66.3%; $p = 0.059$	24.6%; $p = 0.41$
Phase III, randomized, double-blind, multicentric clinical trials	65	Vancomycin 125 mg q.i.d 10 days	78.5%	32.7%
Protocol 301 and 302	Total 157			
Louie 2011 [30]	115	Vancomycin 125 mg q.i.d 10 days	93%	23.2%; $p = 0.05$
Phase III, randomized, prospective, multicenter, double-blind clinical trial	101	Fidaxomicin 200 mg b.i.d 10 days	88.1%	11.7%
	Total 216			

appeared as a third-line therapy in Australian guidelines for severe CDI (100 mg IV as a single dose followed by 50 mg t.i.d for 14 days) in addition to oral vancomycin ± intravenous metronidazole [63]. Several case series/cohort are reported to evaluate efficacy of tigecycline in patients with recurrent and severe CDI (Table 4) [64–68]. Two of them do not show improvement of a dual therapy vancomycin/tigecycline compared to vancomycin alone (Table 4) [65, 66]. Only one study showed an improvement of the recovery rate (Table 4) [64]. The latest study showed a low mortality rate and a high clinical recovery rate with vancomycin + tigecycline for severe CDI but did not compare these results with vancomycin alone [68]. These different studies are not comparable because the severity criteria are different. Tigecyclin oral bioavailability is limited, and it is used only by intravenous administration [69]. So, its bioavailability is to 100% unlike oral vancomycin that can concentrate at the colon. The interest of tigecycline for severe CDI is actually under clinical evaluation in a phase III clinical trial started in 2011 [70, 71].

Nitazoxanide is a broad spectrum nitrothiazole benzamide anti-infective agent used as antiparasitic compound that showed interesting activity against anaerobic bacteria as *C. difficile* [71, 72]. Equivalent efficacy to metronidazole was showed for non-severe CDI. A first prospective randomized, double-blind clinical trial randomized 110 patients to receive either oral metronidazole 250 mg q.i.d for 10 days, oral nitazoxanide 500 mg b.i.d for 7 days, or nitazoxanide 500 mg b.i.d for 10 days. The results showed sustained recovery rate, not significantly different 31 days after the beginning of treatment with, respectively, 57.6%, 65.8%, and 74.3% ($p = 0.34$). However, this study seems to lack robustness because the results are not significantly different ($p = 0.35$) despite a difference of 17% between clinical recovery rates of metronidazole and nitazoxanide 500 mg for 10 days [73]. Another study evaluated the effect of nitazoxanide for patients who failed to metronidazole treatment for CDI. This study showed interesting results with a recovery rate of 66% [74]. In 2009, nitazoxanide (500 mg b.i.d for 10 days) was compared to vancomycin (125 mg q.i.d for 10 days) for severe CDI in a prospective, randomized, double-blind, controlled trial. Among the 20 patients with severe CDI, the response rate at the end of the treatment and the sustained response rate at 31 days was lower for those who received vancomycin compared to nitazoxanide (70% *vs* 80% and 60% *vs* 70%) [75]. A case

Table 4 Tigecycline combinated with vancomycin for severe CDI

Study/design	Number of patients	Treatment	Severity criteria of CDI	Efficacity
Gergely 2016 [64] Retrospective, observational cohort study. Budapest, January 2014–December 2015	45 45 Total 90	Vancomycin Vancomycin + tigecycline	Fever (> 38.5 °C), chills, abdominal pain Respiratory failure or haemodynamic instability peritonitis Leucocytosis WBC > 15,000 cells/μL) Serum creatinine ≥ 1.5-fold rise compared with premorbid levels Serum lactate ≥ 5 mmol/L; serum albumin < 30 g/L Colonic distension or wall thickening, ascites, or pseudomembranous colitis.	Clinical cure 53.3% 75.6% $p = 0.02$	Recurrence 19% 13% $p = 0.2$
La Salvia 2017 [65] Retrospective cohort study. Boston, September 2009–June 2012	69 21 Total 90	Vancomycin Vancomycin + tigecycline	Intensive care unit (ICU) level of care, sepsis, ileus Serum lactate > 2.5 mmol/L Leucocytosis WBC > 50,000 cells/μL Hemodynamic instability, severe abdominal pain or rigidity Imaging with megacolon or pseudomembranous colitis on colonoscopy.	Mortality rate 28% 14%	
Manea 2017 [66] Retrospective cohort study. Romany, September 2014–August 2015	204 62 Total 264	Vancomycin Vancomycin + tigecycline	Toxic megacolon, sepsis Age > 65 Fever Acute renal insufficiency Leucocytosis WBC > 15,000 cells/μL Serum albumin < 30 g/L	Clinical cure 92% 80% $p = 0.06$	
Bishop 2018 [68] Retrospective observational study. Australia, February 2013 to October 2016	16	Vancomycin + tigecycline	Fever > 38.5 °C haemodynamic instability Peritonitis or evidence of bowel perforation, ileus or toxic megacolon Leucocytosis WBC > 15,000 cells/μL Elevated lactate, albumin < 25 g/L Creatinine level (> 50% above baseline) Imaging features of large intestine distension, ascites, or pseudomembranous colitis on colonoscopy	Clinical cure 77%	Mortality rate at 30 days 8%

report described the use of nitazoxanide for recurrent CDI after an initial episode of severe CDI treated with oral vancomycin and IV metronidazole. This patient was successfully treated with nitazoxanide followed by a tapering dose of oral vancomycin for a recurrent and severe CDI. However, designed clinical trial to explore the clinical utility of nitazoxanide for patients with CDI is needed [76].

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is currently used for recurrent CDI, but two recent reviews published several case series reporting FMT as a curative treatment of severe or complicated CDI. They conclude that FMT could be considered in patients with severe CDI unresponsive to conventional antibiotic treatment or to avoid surgery [77, 78]. A 3-year monocentric, retrospective cohort study including 111 patients hospitalized for colitis with *C. difficile* compared FMT after a short antibiotic regimen to antibiotic regimen alone. This study showed a lower mortality rate within 3 months with FMT compared to no-FMT (12.1% *vs* 42.2%; $p < 0.003$) [79]. It was shown that FMT allowed to improve prognosis of patients with severe CDI. However, randomized clinical trials were needed to confirm these results.

Treatment of complicated CDI

Therapeutic strategies according to official recommendations

In case of fulminant CDI with complications such as ileus or megacolon, standard oral therapies are ineffective because they cannot reach the right and transverse colon [10]. In these cases, French and American guidelines recommend the use of oral vancomycin (500 mg q.i.d) *via* a nasogastric tube, with or without IV metronidazole (500 mg t.i.d). The use of parenteral vancomycin administration to treat CDI is not recommended because it can promote rectal colonization with vancomycin-resistant enterococci [80]. On the other hand, IV metronidazole is preferred to oral metronidazole for complicated CDI because it allows to obtain higher colonic concentrations than with the oral route [57].

To overcome the lack of penetration of antibiotics in the colon during severe ileus, vancomycin could be administered by rectal instillation in combination with IV metronidazole [13, 81]. This route of administration was previously studied in 2002. Nine patients with complicated CDI (ileus, megacolon, or fulminant colitis) were randomized to receive a rectal instillation of a vancomycin solution (dissolution of 0.5 to 1 g of vancomycin for 1 to 2 L of saline solution) by enema in association with conventional therapeutics. The results showed a complete clinical resolution for eight patients (88.9%) [82]. Another case series on a larger sample of patients was reported in 2016. One hundred twenty-seven patients with moderate to severe CDI were included to receive intracolonic vancomycin by enema (from 0.25 g per day to 1 g q.i.d) [83]. Patients who received intracolonic vancomycin by enema were hospitalized over a longer period of time (39.1 ± 30.6 days *vs* 27.1 ± 33.3 days; $p = 0.09$). This longer duration of hospitalization with intracolonic vancomycin compared to conventional administration is correlated with the occurrence of complications such as toxic megacolon (34.6% *vs* 4%; $p < 0.0001$), colectomy (15.4% *vs* 1%; $p = 0.006$), and VRE infection (26.9% *vs* 5.9%; $p = 0.005$). The mortality rate is not significantly different (38.5% *vs* 38.6%; $p = 1$), but the associated morbidities are problematics, and the clinical benefit remains to be demonstrated by randomized clinical trials. The optimal dose and volume to be instilled are not clearly established and are different in the two studies cited.

Surgery

Total abdominal colectomy is the last resort for toxic megacolon, acute abdomen, and severe ileus or colonic perforation [10, 15]. To avoid total colectomy, another surgical technique is under evaluation and consists to make a diverting loop ileostomy followed by colonic lavage, combined with antibiotic treatment [84].

Treatment of recurrent episodes of CDI

A nosocomial origin is usually responsible of a first episode of CDI. Even though 80% of patients are successfully treated with metronidazole or vancomycin, approximately 20% have a recurrence of CDI after the end of antibiotherapy [85]. Recurrent CDI is defined by the complete resolution of symptoms after appropriate antibiotherapy, with subsequent relapse and return of symptoms within 8 weeks of the initial episode [86]. Several individual factors are involved in the occurrence of recurrence such as alteration of the composition of gut microbiota allowing colonization by pathogenic bacteria, alterations of the immune response by lower levels of immunoglobulin G antibodies against toxin A, advanced age, additional courses of antibiotics, use of medications such as proton pump inhibitors, prolonged hospitalization, and prior episodes of recurrent CDI. *C. difficile* life cycle plays also an important role in the occurrence of recurrences with the resistance of

spores to antibiotics and germination of these spores in vegetative cells after stopping antibiotic therapy [87].

These recurrences are most often due to a relapse with the same strain of *C. difficile* involved in the initial episode (persistence of spores insensitive to antibiotics), and more rarely to a reinfection with a different strain [88].

Therapeutic strategies according to official recommendations

The French guidelines (HAS) recommends to treat a first recurrence episode with the same antibiotics used in the initial episode of CDI if it was effective (oral metronidazole 500 mg t.i.d or oral vancomycin 250 to 500 mg q.i.d for 10–14 days) [81]. Indeed, a retrospective analysis of 463 patients with a first recurrence of CDI was conducted in Quebec between 1991 and 2005 [89]. It was found that the rate of a second recurrence within 60 days after therapeutic management of the first recurrent episode of CDI by metronidazole or vancomycin was not significantly different. If metronidazole was given to treat the initial episode, the administration of vancomycin for the first recurrence did not result in a lower risk of a second recurrence compared to metronidazole (HR = 1.16, 95% CI [0.74–1.82], $p = 0.52$). Conversely, if the initial episode of CDI was treated with vancomycin, the risk of a second recurrence after treatment of the first with metronidazole or vancomycin is not significantly different (HR = 0.68, 95% CI [0.32–1.46], $p = 0.32$). The treatment of a first recurrence of CDI with the same antibiotic used for the initial episode did not show a higher risk of second recurrence. Beyond the first recurrence, the use of metronidazole is not recommended due to the neurotoxicity of its metabolite [90].

On the other hand, the last American guidelines recommend to take account of the antibiotic used to manage the initial episode and to change molecule or posology to treat a first recurrence (Fig. 2) [13]. If metronidazole is used for the initial episode, vancomycin is preferred to treat a first recurrence. If vancomycin is used for the initial episode, tapered or pulse dosing regimens of vancomycin or another molecule as fidaxomicin are recommended.

The interest of tapered or pulsed dosing regimens of vancomycin for the management of recurrent episodes of CDI was demonstrated in a study conducted on 163 patients [91]. This study showed that the recurrence rate decreases according to the vancomycin doses used: it is higher with an average dose of vancomycin of 1 g per day (71.4% of recurrences), and lower with a very high dosage of 3 g per day (42.9%). Starting treatment at an initial dose and gradually reducing the dose over a given period (from 3 to 750 mg/day or 500 to 125 mg/day for 10–16 days) reduce the recurrence rate to 31%. Intermittent administration of vancomycin 125–500 mg per day every 2 to 3 days for 3 weeks brings the lowest recurrence rate (14.3%). A significant difference in recurrence rate was found in these different therapeutic strategies ($p = 0.05$). Intermittent administration of vancomycin and gradual dose decline appeared to be the two most promising methods for improving the management of CDI recurrence. A recent retrospective study evaluated vancomycin taper and pulse treatment on 100 patients. Patients received vancomycin regimen tapered from q.i.d to once a day followed by every other day (QOD) for a total duration of treatment of 60.3 ± 25.9 days or QOD and every third day (Q3D) for a total duration of treatment of 86.3 ± 27.8 days. The cure rate was higher when treatment was prolonged with QOD and Q3D compared to QOD alone (81.1% *vs* 61.1%; $p = 0.03$). The pulse regimen Q3D gives more time to the spores to germinate in vegetative cells and allows to kill the vegetative cells with the next dose. This scheme allows to limit the problem of spore germination after stopping antibiotic therapy [92]. One randomized phase 4 clinical trial is in progress to determine the benefit of a standard vancomycin treatment followed by taper and pulse vancomycin treatment compared to standard vancomycin treatment alone according to the following treatment regimen [93]:

The benefit of fidaxomicin compared to vancomycin in the management of a first recurrence of CDI was demonstrated in a phase III clinical trial. Of the 178 patients with a first CDI recurrence, 88 received fidaxomicin 200 mg b.i.d and 90 received vancomycin 125 mg q.i.d for 10 days [94]. The recovery rate was equivalent with fidaxomicin compared to vancomycin (respectively, 93.7% and 91.6%). The rate of a second recurrence within 4 weeks after the end of antibiotherapy was significantly lower in patients treated with fidaxomicin compared to those treated with vancomycin (respectively, 19.7% *vs* 35.5%; $p = 0.045$). After discontinuation of vancomycin treatment, 17 patients (28%) had recurrence within 14 days *vs* 5 patients (8%) treated with fidaxomicin ($p = 0.003$). On the other hand, between the 15th and the 28th day following cessation of treatment, the occurrence of recurrences is not significantly different between the two groups. Fidaxomicin for a first recurrent episode of CDI is associated to a lower risk and a longer delay in second recurrence compared to vancomycin.

The lower rate of recurrences of CDI with fidaxomicin could be explained by a lower alteration of the gut microbiota ecosystem. Its effects on intestinal microbiota were examined in a comparative study between fidaxomicin 200 mg b.i.d and vancomycin 125 mg q.i.d. [36]. At the beginning of the study, 89 stool specimens from patients with CDI were collected, analyzed, and compared to those from healthy donors. The number of *Bacteroides* species,

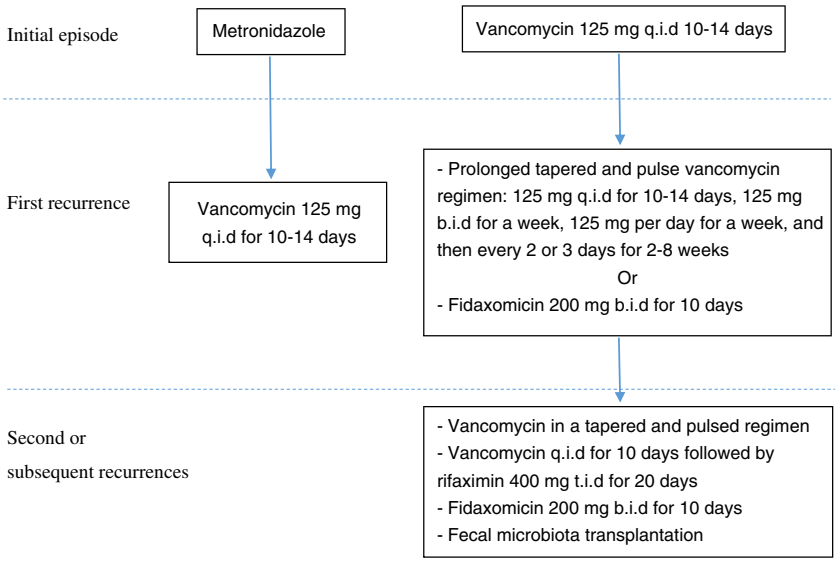


Fig. 2 Recommendations for the treatment of a first recurrence of CDI in adult according to the last American guidelines [13]

Prevotella species, *Clostridium coccoides*, and *Clostridium leptum* was lower in feces of patients with CDI compared to healthy donors. Fourteen days after the end of treatment, the rate of *Bacteroides* species and *Clostridium coccoides* was significantly lower with vancomycin regimen compared to fidaxomicin regimen ($p = 0.0001$ and $p < 0.03$). Another study confirmed these results, showing a significant decrease in the level of *Bacteroides* species after treatment with vancomycin from 4.1 to 2.6 log 10 CFU/g stool ($p < 0.001$) whereas after treatment with fidaxomicin, the level of this microorganism increases from 4.8 to 6.1 log 10 CFU/g stool ($p < 0.01$) [38].

Rifaximin is a non-absorbable antibiotic with positive result in reducing recurrence after an initial episode of CDI. Several case series described a decrease in recurrences when rifaximin was prescribed after the completing course of standard antibiotherapy regimen. In 2007, a case series study showed that four on six patients with multiple CDI recurrences did not have additional recurrences after administration of rifaximin therapy 6 months after the end of antibiotherapy [95]. Two years later, another case series study were published and showed no recurrence for seven on eight patients with history of four to eight episodes of CDI when rifaximin was used after completion therapy with vancomycin [96]. In 2011, a randomized, double-blind, placebo-controlled pilot study was conducted to confirm the positive results of the addition of rifaximin after standard antibiotic therapy for CDI [97]. Among 68 patients with confirmed CDI treated with oral vancomycin or metronidazole for a planned 10–14-day course, 35 received a placebo and 33 received rifaximin (400 mg t.i.d for 20 days) after the end of standard anti-CDI antibiotherapy. The results showed a significantly lower recurrent rate of diarrhea with rifaximin *vs* placebo (21% *vs* 49%; $p = 0.018$). CDI recurrence are also lower (15% *vs* 31%; $p = 0.15$). Rifaximin appeared to be a good alternative to reduce recurrent CDI after standard antibiotherapy.

Probiotics

It is well known that the modification of the gut microbiota caused by anti-CDI antibiotherapy is a factor of recurrences. The human gut is colonized by more of 500 different bacterial species living in symbiosis with their host. Antibiotic treatment can change this gut microbiota and promotes colonization by pathogenic species bacteria. Probiotics containing lactic acid bacteria (LAB; *lactobacilli* and *bifidobacteria*) produce antimicrobial agent substances such as bacteriocin. Probiotics decrease fecal pH and have many properties such as growth inhibition of pathogenic bacteria (*Clostridium*, *Salmonella*, *Campylobacter*, *Shigella*…) [98]. Several studies evaluated whether re-colonization and restoration of the intestinal microbiota concomitantly to anti-CDI antibiotics through the use of probiotics allowed to reduce the incidence of CDI recurrences [85, 88, 99]. Some of them showed positive results for prevention of recurrences of CDI, but others did not show significant results. In 2008, a Cochrane analysis concluded that there was insufficient evidence to recommend probiotics [100]. A recent meta-analysis assessed the effect of probiotic administration 1 to 7 days after the first anti-CDI antibiotic administration. Four main probiotic species were studied (*Lactobacillus*, *Saccharomyces*, *Bifidobacterium*, and *Streptococcus*) [101]. According to 19 published studies including 6261 patients, this meta-analysis showed a lower incidence of CDI when probiotics were administered within 2 days of the first anti-CDI antibiotic dose compared to placebo (1.6% *vs* 3.9%; $p < 0.001$) with a risk reduction of CDI recurrences > 50%. However, because of insufficient evidence, current Australian, European, and

American guidelines do not recommend actually the administration of probiotics to prevent recurrences of CDI [13, 15, 63].

Fecal microbiota transplantation

The instillation of stool from healthy donors to a patient with CDI by colonoscopy, enema, nasoenteric tube (NET), or capsules is effective to treat recurrent CDI by restoring the structure and function of the gut microbiota of the patient [102]. Two randomized clinical trials showed positive results for fecal microbiota transplantation [103, 104]. For the first study (Table 5), three regimens were compared: the infusion of donor's feces preceded by an abbreviated regimen of vancomycin (500 mg q.i.d for 4–5 days) and bowel lavage (4 L of macrogol solution) at the last day of antibiotic, standard vancomycin regimen (500 mg q.i.d for 14 days), and standard vancomycin with bowel lavage. The better recovery rate and the lower relapse rate after 5 weeks were observed when fecal microbiota transplantation was used after a short regimen of vancomycin and bowel lavage. In the second study, two modalities of fecal microbiota transplantation instillation were compared: colonoscopy and nasogastric tube. These two regimens showed a high recovery rate and no relapse after 8 weeks without significant difference. FMT is considered to be safe, and a recent study showed no long-term side effects in a cohort of 39 patients who received FMT for recurrent CDI [105].

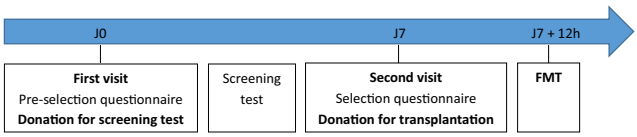
This procedure is subject to regulatory requirements due to its "experimental" character and requires guidance and standardization in terms of donor selection, preparation, and administration:

Collection of informed consent

Patients should be informed of the experimental nature of this procedure and the known and hypothetical risks.

Donor screening

The national french agency ANSM (Agence Nationale de Sécurité du Médicament et des Produits de Santé) recommends a two-stage selection process to minimize the risk of infections between the day of the pre-selection and the actual day of donation [106]:



The use of fresh stools is limited by the duration of donor selection. Freezing of stools of donors could simplify the procedure and allowed a rapid disposition of the transplant.

Screening test include blood and stool analysis. The purpose of the blood test is to detect bacterial (*treponema pallidum*), viral (HIV, HTLV, VHB, VHC, CMV, EBV), and parasitic (*Strongyloïdes stercoralis*, *Toxoplasma gondii*, *Trichinella* sp.) infections. The stool analysis allows to detect other bacteria (*Clostridium difficile*, *Listeria monocytogenes*, *Vibrio cholerae*/*Vibrio parahemolyticus*, *Salmonella*, *Shigella*, other multidrug-resistant bacteria), virus (adénovirus, astrovirus, calcivirus, picornavirus, rotavirus, VHA, VHE), and parasites (*Strongyloïdes stercoralis*, *Cryptosporidium* sp., *Cyclospora* sp., *Entamoeba histolytica*, *Giardia intestinalis*, *Isospora* sp., *Microsporidies*).

Preparation/administration

The FDA and the French authorities classified human feces as a drug [106, 107]. The drug qualification requires that its preparation and dispensation be managed by the hospital pharmacies.

New routes of administration have emerged, and the choice varies according to several studies. Instillation by

Table 5 Randomized clinical trials evaluating fecal microbiota transplantation (FMT)

Study	Regimen	Patients	Clinical outcomes	
Van Nood 2013 [103] Randomized, open-label, controlled trial			Cure rate	Relapse after 5 weeks
	- Infusion of donor's feces *via* nasoduodenal tube	16	94%	6%
	- Standard vancomycin (500 mg q.i.d 14 days)	13	31%	62%
	- Standard vancomycin + bowel lavage (4 L of macrogol solution)	13	23%	54%
		Total, 42	$p < 0.001$	
Youngster 2014 [104] Randomized, open-label, controlled pilot study	Frozen fecal suspension			
	- Colonoscopy	10	100%	0
	- NGTs	10	80%	0
			$p = 0.53$	

colonoscopy resulted in a risk of digestive perforation in ill and elderly patients, and instillation by enemas did not lead to a precise implantation of the donor's fecal microbiota in the colon [108]. So, administration by upper endoscopy, nasogastric, or nasojejunal tube were developed and have for advantage to eliminate the need for sedation, anesthetic risks, and colonic "cleanout" of colonoscopy instillation. Capsule formulations are the most recent method to facilitate administration and patient compliance. We compared in Table 6 the different FMT protocols described in recent large case report and randomized or no randomized clinical trials. Nasogastric tube instillation was used in most of cases, and showed equivalent results compared to colonoscopy administration [79, 103, 104, 109–111]. More recently, two studies reported capsule use to improve patient comfort compared to nasogastric tube administration [112, 113]. About the way of preparing feces, we observed different protocols in terms of volume and concentration, and a standardization is needed. The freezing of fecal material gives good results compared to fresh stool and have several advantages as identification and screening of donors ahead of time allowing establishment of a bank of donors readily available [109]. A preparation is performed before the FMT with a minimum of 5-day pre-treatment with vancomycin and a bowel lavage during colonoscopy administration. In the other hand, some studies described a proton pump inhibitor administration prior to NGT transplantation [104], and loperamide administration during transplantation [104, 111] without a real consensus.

The majority of current guidelines conclude to a strong recommendation of FMT for more than one relapse of CDI [13, 15, 63]. Only the American College of Gastroenterology recommends to consider FMT after three recurrences of CDI [102, 114]. From a regulatory point of view, protocol need to be standardized and regulated in terms of fecal material preparation and administration.

Immunotherapy

The level of antitoxin A IgG was shown to be significantly higher in asymptomatic carriers compared to those with *C. difficile* diarrhea [115]. Moreover, *C. difficile* toxins are immunogenic, and many healthy patients have detectable antibodies to *C. difficile* toxins A and B in their serum [116]. Passive immunization strategies were studied to improve outcome and decrease recurrent CDI [117].

Polyclonal antibodies

The efficacy of three commercial preparations of polyclonal antibody (IgG) was determined in the treatment of adult patients with protracted, recurrent, or severe CDI [116]. In this study, patients received standard antibiotic treatment for severe CDI (vancomycin 500 mg q.i.d + IV metronidazole 500 mg q.i.d) before IV Ig infusion (0.4 g/kg). The results showed only 41% of therapeutic response to IV Ig infusion in CDI patients.

Monoclonal antibodies

To prevent the occurrence of recurrences of CDI after a first cure episode, antitoxin antibodies of *C. difficile* were developed. The effect of *C. difficile* antitoxin antibodies in combination with conventional therapeutics in the prevention of recurrences was demonstrated in phase II clinical studies. One evaluated only the effect of antitoxin A (CDA1) antibodies and suggested a recurrence rate lower than placebo without significant results (17.2% *vs* 17.7%; $p = 1$) (Table 7) [118]. The other evaluated the effect of the association of antitoxin A (CDA1) and antitoxin B (CDB1) antibodies. The results showed a laboratory-documented recurrence rate significantly lower than placebo (Table 7) [119].

Two humanized monoclonal antibodies, bezlotoxumab and actoxumab, were included in a phase III clinical trial to evaluate their ability to protect against recurrent CDI [120]. Results showed a recurrent infection within 12 weeks after infusion of bezlotoxumab alone or associated with actoxumab significantly lower than placebo, including towards the hypervirulent strain NAP1/027 (Table 7). These results were only compared to placebo and not to another molecule as metronidazole or vancomycin. Bezlotoxumab was approved by the US Food and Drug Administration (FDA) in 2017 and the European Medicines Agency (EMA) 1 year later to reduce recurrence of CDI in adult patients when given with standard-of-care antibiotics [114]. However, monoclonal antibodies are expensive molecules for use in preventive treatment.

Vaccines

Different vaccine strategies were developed in recent years to prevent or improve outcome of CDI patients.

Vaccines undergoing clinical trials in humans are toxoid A and B vaccines to produce an immune response to neutralize the effects of *C. difficile* toxins A and B. A recent review summarized the results of efficacy and security of *C. difficile* toxoid vaccines under clinical trial for the prevention of CDI [121]. Safety and immunogenicity of *C. difficile* vaccine in healthy adults aged 50 to 85 years were demonstrated in four phase I and 5 phase II clinical trials [122–130]. One phase III clinical trial was initiated but discontinued because the high rates of seroconversion (over 90%) showed in phase II clinical trial was not

Table 6 Different administration strategies for fecal microbiota transplantation

References	Indication	Study/cohort	Infusion route/regimen	Preparation before transplantation	Outcome	Adverse event
Hamilton 2012 [109]	At least two relapses of CDI	Large single-case series on 43 patients	Colonoscopy 50 g of stool in 250 mL of NaCl were mixed, filtered, and passed through different sieves Fresh fecal material: fecal slurry was centrifugated and resuspended in 125 mL of NaCl Frozen fecal material: fecal slurry was mixed in sterile glycerol to a final concentration of 10% and stored at − 80 °C for 2 months	- Vancomycin 125 mg q.i.d until 2 days before FMT - Discontinuation of all antibiotics at least 48 h prior to the procedure - Bowel lavage the day before transplantation with PEG solution - Sedation during transplantation	Infection clearance Fresh fecal material 91.6% Frozen fecal material 90.4%	None
Van Nood 2013 [103]	At least one relapse of CDI	Open-label randomized controlled trial	Nasogastric tube At least 150 g of stool mixed with 500 mL of sterile NaCl 0.9%	- Vancomycin 500 mg q.i.d for 4–5 days followed by bowel lavage with 4 L of macrogol solution the last day of vancomycin regimen. - Discontinuation of vancomycin 24 h prior transplantation	Recovery rate without relapse: FMT 94%; $p < 0.001$ Vancomycin 31% Vancomycin + bowel lavage 23%	Minor AE: Diarrhea, cramping
Youngster 2014 [104]	At least two relapses of CDI	20 patients Randomized controlled feasibility study	Cf Hamilton protocol to fecal manipulation Colonoscopy Nasogastric tube	- Discontinuation of all antibiotics at least 48 h prior procedure Colonoscopy: - Bowel preparation with 4 L of PEG the day before transplantation - Single oral dose of loperamide 8 mg during the transplantation Nasogastric tube: - Esomeprazole 2 mg/kg/day 48 h prior the transplantation	Recovery rate 100% 80% $p = 0.53$	Minor AE Abdominal discomfort
Lagier 2015 [110]	- Early FMT combined with antibiotics for primary CDI - Tardive FMT after at least two relapses	16 patients 3 patients	Nasogastric tube 30 g of stool in 400 mL of NaCl 0.9% were mixed for 10 min and filtered	- Current antibiotic regimen before FMT: vancomycin 125 mg q.i.d or metronidazole 500 mg t.i.d for 14 days or fidaxomicin 200 mg b.i.d for 10 days - Bowel lavage the day before transplantation: 4 L of Klean prep or 2 glasses of Fast prep - Hydration: 200 mL of 1.4% bicarbonates for 15 min before transplantation	Globally mortality: Early FMT 19% Tardive FMT 64% $p < 0.001$	Minor AE: Diarrhea, nausea
Youngster 2016 [112]	At least two relapses of CDI	180 patients	Cf Hamilton 2012 protocol to fecal suspension preparation Fecal suspension was encapsulated in hydromellose capsule and stored at − 80 °C for 6 months Regimen: 30 capsules/patients	- Discontinuation of all antibiotics at least 48 h prior transplantation	Recovery rate 91%	Serious AE: 5% (fever, ulcerative colitis, relapse)
Khao 2017 [113]	Two relapses of CDI	Randomized, non-inferiority trial 57 patients 59 patients	80–100 g of fresh stool were mixed in 200 mL of sterile NaCl 0.9% and filtered to obtain fecal slurry **Capsules** Fecal slurry mixed with 40 mL of glycerol 100%, centrifugation, encapsulation, and storage at − 70 °C for 2 months. 40 capsules manufacturing from 1 donor Regimen: 40 capsules/patients **Colonoscopy** Fecal slurry mixed with 20 mL of glycerol 100% and stored at − 70 °C for 2 months Thawing one night at 4 °C and reconstitution with 160 mL of NaCl 0.9% Volume: 360 mL of fecal slurry in the cecum	- Vancomycin 125 mg q.i.d for 10 days followed by Vancomycin 125 mg b.i.d until 24 h before FMT - Discontinuation of all antibiotics at least 24 h prior transplantation - 4 L of PEG the night before FMT and remained fasting until the scheduled treatment	No recurrent rate 12 weeks after FMT: Capsules 96% Colonoscopy 96%	Minor AE: Nausea, fever, abdominal discomfort Capsules 5% Colonoscopy 12%

Table 6 (continued)

References	Indication	Study/cohort	Infusion route/regimen	Preparation before transplantation	Outcome	Adverse event
Goldenberg 2018 [111]	2 relapses of CDI	36 patients 27 patients	**Colonoscopy** 50–100 g of stool mixed with 150 mL of NaCl 0.9%, filtration and addition of NaCl 0.9% and glycerol 12.5% for cryoconservation to get a final volume of 250 mL Storage − 80 °C for 6 months **Nasojejunal tube** 80 g of stool with 400 mL of NaCl 0.9% mixed for 1 min, filtered decanted and stored in oral syringes for 4 h	- Before transplantation: Vancomycin 125 mg q.i.d or fidaxomicin 200 mg b.i.d for 5 days discontinued at least 24 h prior transplantation **Colonoscopy** Bowel lavage with macrogol and no solid food for 24 h before transplantation - During transplantation: sedation with midazolam and fentanyl - Pre-transplantation: Loperamide 8 mg to aid retention of transplant material **Nasojejunal tube (NJT)** Bowel preparation not given	Recovery rate Colonoscopy 94% NJT 96%	Minor AE: Diarrhea, abdominal discomfort
Hocquart 2018 [79]	Patients hospitalized for colitis with *C. difficile*	Retrospective cohort study 111 patients FMT 66 No FMT 45	NGT Cf Lagier 2015 protocol to fecal suspension preparation Fresh stool 45.5% Frozen stool 54.5%	Cf Lagier 2015 protocol prior transplantation	3-month mortality rate: FMT 12% No FMT 42% $p < 0.003$ No difference between fresh and frozen stool ($p = 0.28$)	

translate in this phase III study [131, 132]. Another phase III clinical trial is in progress [133].

Other strategies using different immunogenic surface components of *C. difficile* (spore proteins, surface membrane proteins, capsular polysaccharides and lipoteichoic acid compound, flagellar proteins, pili protein) were evaluated *in vivo* in animal model [134].

New molecules under clinical trials

Several novel antimicrobial molecules were discovered and are under evaluation for CDI treatment [71, 135]. Their antibacterial properties are linked to several mechanisms: the inhibition of protein synthesis (surotomycin, cadazolid, LFF-571, CRS3123) and DNA supercoiling (DS-2969b), the inhibition of peptidogycane synthesis (ramoplanin), an essential component of the bacterial wall, and of the regulation of the balance of bile acids (ridinilazole).

Surotomycin (CB-183.315) is a daptomycin analogue whose safety and efficacy were determined in two clinical trials [136, 137]. In the phase II study, the efficacy and safety of oral surotomycin 125 mg twice daily and surotomycin 250 mg b.i.d were compared to oral vancomycin 125 mg q.i.d in patients with CDI. The clinical response at the end of treatment was equivalent for these three regimens (92.4%, 86.6%, and 89.4%), but the sustained clinical response was better for surotomycin 250 mg b.i.d compared to vancomycin (70.1% *vs* 56.1%) with a significantly lower recurrence rate (17.2% *vs* 35.6%; $p = 0.035$). Two phase III clinical trials were initiated in parallel to confirm these results. The first phase III clinical trial included 570 patients randomized to receive surotomycin 250 mg b.i.d or vancomycin 125 mg q.i.d for 10 days. The clinical response at the end of treatment was lower for surotomycin compared to vancomycin (79% *vs* 83.6%), and the inferiority was not demonstrated. At 30–40 days of the follow-up period, clinical response was not superior for surotomycin (60.6% *vs* 61.4%; $p = 0.832$) [138]. The results were better in the second phase III clinical trial that demonstrated the non-inferiority of surotomycin compared to vancomycin with a clinical cure rate after the end of treatment of 83.4% *vs* 82.4%. Over time, clinical response seemed superior for surotomycin than vancomycin (63.3% *vs* 59%) [139]. According to the conflicting results between these two phase III clinical trials, the fate of surotomycin in the management of CDI is not clear.

Cadazolid (ACT-179811) is a novel oxazolidinone antibiotic substituted with a fluoroquinolone moiety that showed anti-CDI properties [140]. Tolerability and pharmacokinetic parameters of cadazolid up to 3 g b.i.d for 10 days were evaluated in a phase I clinical trial on 64 healthy volonters. Results of tolerability showed a good tolerance except some headaches. Pharmacokinetic studies showed a low absorption after

Table 7 Randomized clinical trials of human monoclonal antibodies to prevent recurrences of CDI

Study	Patients	Regimen	Results	
Leav 2010 [118]			Recurrence infection after discontinuation resolution of prior CDI episode	
Phase II, randomized, double-blind, placebo-controlled clinical trial	59	Human monoclonal antibody against toxin A	17.2%	
	17	Placebo	17.7%	
	Total 76		$p = 1$	
Lowy 2010 [119]			Laboratory documented recurrence	
Phase II, randomized, double-blind, placebo-controlled clinical trial	101	- Human monoclonal antibody	7%	
	99	- Placebo	23%	
	Total 200		$p < 0.001$	
Wilcox 2017 [120]			Clinical cure	Recurrent infection within 12 weeks after infusion
Phase III, randomized, placebo-controlled, double-blind clinical trial MODIFY 1	386	- Bezlotoxumab[a]	77%	17%; $p < 0.001$
	393	- Bezlotoxumab + actoxumab[a]	75%	16%; $p < 0.001$
	395	- Placebo	83%	28%
	Total 1174			
Wilcox 2017 [120]			Clinical cure	Recurrent infection within 12 weeks after infusion
Phase III, randomized, placebo-controlled, double-blind trial MODIFY 2	326	- Bezlotoxumab[a]	83%	16%; $p < 0.001$
	390	- Bezlotoxumab + actoxumab[a]	72%	15%; $p < 0.001$
	378	- Placebo	78%	26%
	Total 1094			

[a] 10 mg/kg/body weight

oral administration leading to high colonic concentration [141]. Its efficacy was demonstrated in a phase II clinical trial with the comparison of three regimens of cadazolid (250 mg b.i.d, 500 mg b.i.d, and 1 g b.i.d for 10 days) *vs* vancomycin (125 mg q.i.d for 10 days) on 82 patients with CDI [142]. Clinical recovery was defined as resolution of diarrhea and sustained clinical response when there was no recurrences within 26–30 days after the end of treatment. Clinical recovery and sustained response were higher for cadazolid 250 mg and 500 mg compared to vancomycin with, respectively, 76.5% and 80% *vs* 68.2%, and recurrence rates were lower (18.2% to 25% *vs* 50%). A phase III clinical trial is in progress to confirm these results of efficacy compared to vancomycin in a larger number of patients with *C. difficile*-associated diarrhea (CDAD) [143].

Ridinilazole (SMT 19969) is a heterocyclic antibacterial with a narrow-spectrum activity against gram-positive bacteria including *C. difficile* but not the other *clostridia* species [144]. The preservation of other *clostridium* species, in particular *C. scindens*, regulates bile acid homeostasis. Indeed, *C. scindens* is able to convert primary bile acids to secondary bile acid, and a decrease in *C. scindens* population increases the primary bile acid level that would promote germination of *C. difficile* spores [71]. Fifty-six healthy volonters were included in a phase I clinical trial to evaluate tolerability and pharmacokinetic parameters of ridinilazole single oral doses up to 2 g. Only non-severe gastrointestinal adverse events were reported, and this molecule was generally well tolerated [145]. A phase II clinical trial showed non-inferiority of rinidazole (200 mg b.i.d for 10 days) compared to vancomycin (125 mg q.i.d for 10 days) with a clinical response rate of 77.8% *vs* 69.7%, respectively, and a sustained clinical response with no recurrence in 30 days after the end of treatment of 66.7% *vs* 42.5%, respectively ($p < 0.004$) [146]. A phase III clinical trial is in progress to compare ridinilazole to vancomycin treatment for CDI [147].

LFF-571 is a thiopeptide antibiotic with an average MIC against 50 strains of *C. difficile* to 0.25 μg/mL, lower than fidaxomicin (0.5 μg/mL), vancomycin, and metronidazole (2 μg/mL) [148]. The first determination of *in vivo* activity on a hamster model of CDI showed interesting results compared to vancomycin [149]. Its development was stopped after phase II clinical trial because of a higher recurrence rate compared to vancomycin [150].

Ramoplanin is a lipoglycodepsipeptide antibiotic known since 1984 for its antibacterial properties against gram-positive bacteria and anaerobic organism [151]. The evaluation of its activity against *C. difficile* was initiated in 2004. Its efficacity was equivalent to vancomycin according to the results of a phase II clinical trial, but the rate of adverse events was higher. Two phase III clinical trials were initiated, but data have not been published: one for its use in prevention of bloodstream infections caused by VRE and one as a therapeutic agent for CDI [152, 153].

CRS3123 (REP 3123) is a novel diaryldiamine antibiotic with narrow spectrum activity and interesting

properties on *C. difficile* determined *in vitro* on 108 strains [154]. A phase I multicenter, placebo-controlled, double-blind clinical study evaluated security and tolerability of ascending dose of 100 to 1200 mg of CRS3123. No serious adverse effects were reported, and phase II clinical trial was needed to show efficacity on CDI compared to standard antibiotherapy [155].

MBX-500 is a new hybrid antibiotic with an anilinouracil scaffold (DNA polymerase inhibitor) linked to a fluoroquinolone moiety (DNA gyrase inhibitor). The antibacterial activity was evaluated *in vitro* on 16 different strains of *C. difficile* and showed average MIC between 0.5 and 2 μg/mL, equivalent to that of vancomycin. *In vivo* activity was determined on a hamster model of CDI. The results showed an equivalent efficacy and recurrence rate compared to vancomycin. Another *in vivo* study conducted on a murine model revealed a complete protection against recurrences of CDI within 12 days after the end of treatment with MBX-500 [156]. The bioavailability of this antibiotic is very low, and it acts locally with high intestinal concentrations. The other advantage of this molecule is its low activity on gram-negative anaerobic bacilli, *Bifidobacterium* and *Lactobacillus*, avoiding alteration of gut microbiota, a major risk factor of recurrences. No human randomized study was known to this date.

DS-2969b is a novel antibacterial GyrB inhibitor against gram-positive anaerobe bacteria including *C. difficile*. As *M. tuberculosis*, *C. difficile* does not have the gene coding for topoisomerase IV, and in these microorganisms, DNA supercoiling is dependent on DNA gyrase composed of two subunits: GyrA and GyrB. By blocking this enzyme, there is an inhibition of DNA supercoiling resulting in the death of the bacteria. *In vitro* activity of DS-2969b was evaluated against 55 isolates of *C. difficile* and showed lower MIC_{50} (0.03 μg/mL) compared to fidaxomicin (0.06 μg/mL), vancomycin (1 μg/mL), and metronidazole (0.25 μg/mL) [157]. *In vivo* efficacity was demonstrated on Syrian golden hamster CDI model infected with hypervirulent NAP1/027 strain and showed 100% survival without recurrences at 0.3 mg/kg once a day compared to 16.7% with fidaxomicin and 0% with vancomycin [157]. Because of its better activity than vancomycin and fidaxomicin and its lower alteration of the intestinal microbiota compared to vancomycin, two phase 1 clinical trials were conducted. Safety and tolerability were demonstrated for 71 healthy patients who received 6 to 600 mg of oral DS2969b. Mainly mild gastrointestinal adverse events were observed [158, 159]. These data encourage further development of DS-2969b.

Conclusion

During the last decade, the management of CDI was complicated by the emergence of hypervirulent strains (e.g., NAP1/027), the development of resistance to conventional antibiotic (metronidazole and vancomycin), and the multiplication of recurrences after the management of a first episode with appropriated antibiotic [160].

The discontinuation of the responsible antibiotherapy (cephalosporin and clindamycin most of the time) is the first step in the management of CDI. Then, specific antibiotherapy anti-CDI may be instituted if the symptoms are not resolved within 2 to 3 days after stopping the antibiotic incriminated. The choice of antibiotherapy depends on the clinical forms and the presence of an initial episode or a recurrence. The US recommendations have recently been updated, and the main change from the European recommendations concerns the management of an initial episode of non-severe CDI and recurrences:

	European guideline 2013 [15]	**American guideline 2018 [13]**
Initial episode of non-severe CDI	- Metronidazole 500 mg t.i.d 10 days- Vancomycin 125 mg q.i.d 10 days - Alternative: fidaxomicin 200 mg b.i.d 10 days	- Vancomycin 125 mg q.i.d 10 days - Fidaxomicin 200 mg b.i.d 10 days - Alternative: metronidazole 500 mg t.i.d 10 days
Initial episode of severe CDI	Vancomycin 125 mg q.i.d 10 days Alternative: fidaxomicin 200 mg b.i.d 10 days	Vancomycin 125 mg q.i.d 10 days or fidaxomicin 200 mg b.i.d 10 days
First recurrence	Vancomycin 125 mg q.i.d 10 days Fidaxomicin 200 mg b.i.d 10 days Alternative: metronidazole 500 mg t.i.d 10 days	- Vancomycin 125 mg q.i.d 10 days if metronidazole was used for the initial episode - Prolonged tapered and pulsed vancomycin regimen if a standard regimen was used for the initial episode (125 mg q.i.d 10–14 days, b.i.d for a week, once per day for a week, and then every 2 or 3 days for 2–8 weeks) - FDX 200 mg b.i.d 10 days if vancomycin was used for the initial episode
Multiple recurrences	Fidaxomicin 200 mg b.i.d 10 days Vancomycin 125 mg q.i.d for 10 days followed by pulse or taper strategy	- Vancomycin in a tapered and pulsed regimen - Vancomycin 125 mg q.i.d 10 days followed by rifaximin 400 mg t.i.d 20 days - Fidaxomicin 200 mg given b.i.d 10 days - Fecal microbiota transplantation

Due to the increasing rate of recurrences following discontinuation of these anti-CDI antibiotics, new therapeutic strategies have been developed such as fecal microbiota transplantation (FMT) which is recommended from the second recurrence of CDI.

It was shown that the pathogenicity of *C. difficile* was correlated with the effect of its toxins. So, different molecules able to neutralize toxins A and B of *C. difficile* were developed (ion exchange resins, polymers, or immunoglobulins directed against toxins A and B). Only antitoxin antibodies such as bezlotoxumab have shown a significant interest in the prevention of recurrences. However, their place in the therapeutic arsenal is to be discussed because of their high cost of acquisition for a preventive treatment.

Despite the discovery of innovative therapy (FMT and antitoxin antibodies) and the use of other broad-spectrum antibiotics (teicoplanin, tigecycline, nitazoxanide, rifaximin), vancomycin remains the first-line treatment in the majority of CDI. Research is therefore very active, and new antibiotics are being studied as surotomycin, cadazolid, and rinidazole. A vaccine is also under development to prevent contamination in at-risk patients.

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