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Narrative review

Update of treatment algorithms for *Clostridium difficile* infection

R.E. Ooijevaar [1, 2, *, †], Y.H. van Beurden [1, 2, †], E.M. Terveer [4], A. Goorhuis [3], M.P. Bauer [5], J.J. Keller [6, 7], C.J.J. Mulder [2], E.J. Kuijper [4, 8]

[1] *Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands*
[2] *Department of Gastroenterology and Hepatology, VU University Medical Center, Amsterdam, The Netherlands*
[3] *Department of Internal Medicine, Academic Medical Center, Amsterdam, The Netherlands*
[4] *Department of Medical Microbiology, Centre for Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands*
[5] *Department of Internal Medicine and Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands*
[6] *Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, The Netherlands*
[7] *Department of Gastroenterology and Hepatology, Haaglanden Medical Center, The Hague, The Netherlands*
[8] *European Study Group of C. difficile (ESGCD), European Society of Clinical Microbiology and Infectious Diseases, UK*

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ABSTRACT

Background: *Clostridium difficile* is the leading cause of antibiotic-associated diarrhoea, both in healthcare facilities and in the community. The recurrence rate of *C. difficile* infection (CDI) remains high, up to 20%. Since the publication of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidance document on CDI treatment in 2014, new therapeutic approaches have been developed and tested to achieve higher sustained clinical cure in CDI.
Aim: To review novel treatments and approaches for CDI, except probiotics and vaccines. We focused on new antibiotics, antibiotic inactivators, monoclonal antibodies and gut microbiota modulating therapies.
Sources: A literature review was performed for clinical trials published in PubMed, Embase or Cochrane Library between January 2013 and November 2017.
Content: We analysed 28 clinical trials and identified 14 novel agents. Completed phase 2 studies were found for cadazolid, LFF571, ridinilazole and nontoxigenic *C. difficile* strains. Four phase 3 active comparator studies comparing vancomycin with bezlotoxumab, surotomycin ($n = 2$) and rifaximin have been published. Seven clinical trials for treatment of multiple recurrent CDI with faecal microbiota transplantation were analysed, describing faecal microbiota transplantation by upper or lower gastrointestinal route ($n = 5$) or by capsules ($n = 2$).
Implications: Metronidazole is mentioned in the ESCMID guideline as first-line therapy, but we propose that oral vancomycin will become the first choice when antibiotic treatment for CDI is necessary. Fidaxomicin is a good alternative, especially in patients at risk of relapse. Vancomycin combined with faecal microbiota transplantation remains the primary therapy for multiple recurrent CDI. We anticipate that new medication that protects the gut microbiota will be further developed and tested to prevent CDI during antibiotic therapy. **R.E. Ooijevaar, Clin Microbiol Infect 2018;▪:1**

Introduction

Clostridium difficile, recently reclassified as *Clostridioides difficile*, is the most common cause of hospital-acquired infectious diarrhoea and is strongly associated with antibiotic use [1]. The clinical symptoms associated with *C. difficile* infection (CDI) range from mild, self-limiting diarrhoea to fulminant (pseudomembranous) colitis and toxic megacolon, leading to bowel perforation, sepsis and/or multiple organ failure [2]. Approximately 20% of CDI reoccurs [3], most likely associated with a persisting dysbiosis of the

* Corresponding author. R. E. Ooijevaar, Department of Medical Microbiology and Infection Control, VU University Medical Center, PO Box 7057, 1081 HZ Amsterdam, The Netherlands.
E-mail address: r.ooijevaar@vumc.nl (R.E. Ooijevaar).
† The first two authors contributed equally to this article, and both should be considered first author.

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microbiota in the intestinal tract and insufficient serum levels of antibodies against *C. difficile* toxins [2]. The main virulence factors of *C. difficile* are high-molecular-weight clostridial toxins: toxin A (TcdA) and toxin B (TcdB) [2]. TcdA and TcdB bind and enter the colonic epithelium, causing proinflammatory chemokine and cytokine production, influx of neutrophils, disruption of tight junctions, fluid secretion and epithelial cell death [2]. Some strains, including so-called hypervirulent strains (PCR ribotypes 027 and 078), additionally produce a binary toxin, the significance of which remains to be elucidated [4].

In 2014 Debast et al. [5] published a guideline on CDI treatment, approved by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Metronidazole, vancomycin and to a lesser extent fidaxomicin were considered to be the cornerstone of antibiotic treatment for CDI. The European guidance document agreed with guidelines of the Infectious Diseases Society of America and the American College for Gastroenterology that metronidazole could be considered as the first agent of choice for mild CDI [5–7], but novel agents and CDI treatments have been further developed and studied, including faecal microbiota transplantation (FMT) [8,9]. Here we review and discuss novel treatments and approaches to prevent CDI, except probiotics and vaccines. We focus on new antibiotics, antibiotic inactivators, monoclonal antibodies and gut microbiota modulating therapies and discuss their efficacy in clinical trials.

Definitions by ESCMID

CDI is defined as a clinical picture compatible with CDI such as diarrhoea, ileus and toxic megacolon in combination with either microbiologic evidence of free toxins in stool or the presence of toxigenic *C. difficile* in stool without reasonable evidence for an alternative cause of diarrhoea; or pseudomembranous colitis diagnosed during endoscopy, after colectomy or at autopsy [5].

Recurrent CDI (rCDI) is defined as a recurrence of CDI symptoms within 8 weeks after the onset of a previous episode, provided the symptoms from the previous episode resolved after completion of the initial treatment [5]. This definition does not include specific clinical and microbiologic criteria.

Severe CDI is defined as an episode of CDI with (one or more specific signs and symptoms of) severe colitis or a complicated course of disease, with significant systemic toxin effects and shock, resulting in need for intensive care unit admission, colectomy or death [5]. This ESCMID definition differs from definitions of two other guidelines (Table 1) by incorporating the definition of complicated CDI into the definition of a severe CDI syndrome [5–7].

Initial cure is defined as no diarrhoea for two consecutive days after completion of standard-of-care antibiotic therapy. Sustained (or global) cure is defined as initial clinical cure of the baseline episode of CDI and no recurrent infection through 12 weeks' follow-up.

Literature search

A literature search was performed on PubMed, the Cochrane Library and Embase on 17 July 2017 (Fig. 1). The following MeSH terms were used: *'Clostridium difficile,'* 'therapy,' 'therapeutics,' 'treatment.' In addition, the following filters were applied: publication date from 1 January 2013 and clinical trials. The publication date filter was chosen to identify novel treatment strategies not reviewed by the ESCMID guideline on CDI treatment [5]. EndNote X8 software (Clarivate Analytics, Philadelphia, PA, USA) was used to compile a database. The search led to a final inclusion of 28 clinical trials identifying 14 novel agents (Table 2).

In addition to these results, various other articles were identified and included after searching by agent-specific terms, as well as articles cited in other articles.

Comments on current CDI treatment guideline treatment by ESCMID

A summary of the treatment guideline from 2014 is shown in Table 3. Metronidazole was advised as first-line treatment for nonsevere CDI and vancomycin as the first choice for severe CDI [5].

Table 1
Definitions of severe and complicated CDI

Guideline	Severe CDI	Complicated CDI
European Society of Clinical Microbiology and Infectious Diseases [5]	Episode of CDI with one or more specific signs and symptoms of severe colitis or a complicated course of disease, with significant systemic toxin effects and shock, resulting in need for ICU admission, colectomy or death. One or more of following unfavourable prognostic factors can be present without evidence of another cause: • Marked leucocytosis (leucocyte count >15 000 cells/mm^3). • Decreased blood albumin (<30 g/L). • Rise in serum creatinine level (≥133 μM/L or ≥1.5 times premorbid level).	Incorporated in definition of severe CDI
American College of Gastroenterology [7]	Serum albumin <3 g/dL, plus either: • White blood count ≥15 000 cells/mm^3. • Abdominal tenderness.	Any of following events attributable to CDI: • ICU admission. • Hypotension. • Temperature ≥38.5°C. • Ileus. • Significant abdominal distension. • Alteration of mental status. • White blood count ≥35 000 cells/mm^3 or <2000 cells/mm^3. • Serum lactate level >2.2 mmol/L. • End organ failure.
Infectious Diseases Society of America [6]	Leukocytosis (white blood cell count of 15 000 cells/mL or higher) OR Serum creatinine level ≥1.5 times premorbid level	Hypotension or shock, ileus, megacolon

CDI, *Clostridium difficile* infection; ICU, intensive care unit.

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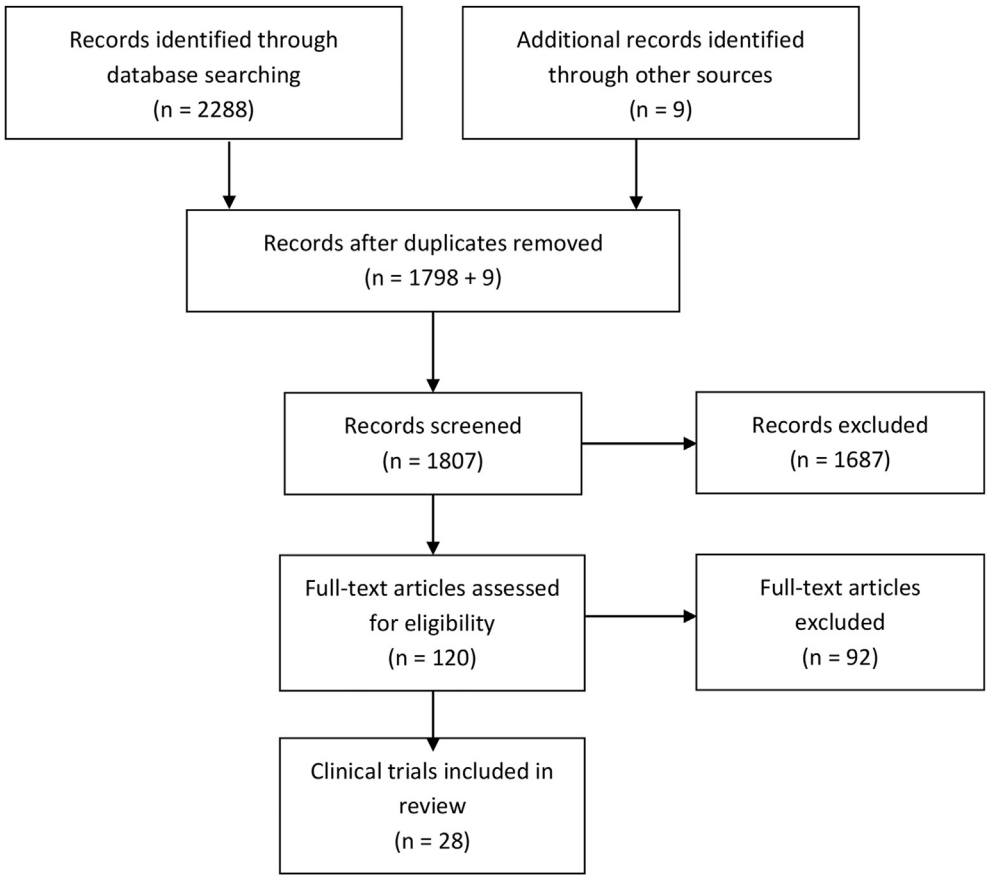

Fig. 1. PRISMA flowchart showing search of clinical trials.

Table 2
New treatments tested for CDI

Agent	Manufacturer	Type or class	Clinical trial	Registered	Indication
Actoxumab	Merck & Co.	Antitoxin A (MK-3415) human monoclonal antibody	Phase 3 terminated	No	rCDI
Bezlotoxumab	Merck & Co.	Antitoxin B (MK-6072) human monoclonal antibody	Phase 3 completed	Yes, FDA approved	rCDI
Cadazolid	Actelion Pharmaceuticals	Hybrid antibiotic, consisting of fluoroquinolone and oxazolidinone moieties	Phase 3 completed[a]	No	CDI/first rCDI
CRS3123/REP3123	Crestone Inc.	Antibiotic/methionyl-tRNA synthetase inhibitor	Phase 1 completed	No	CDI
LFF571	Novartis Pharmaceuticals	Semisynthetic thiopeptide antibiotic, related to elfamycins	Phase 2 completed	No	CDI/First rCDI
Ridinilazole (SMT19969)	Merck & Co	Antibiotic/pyridyl-bibenzimidazole	Phase 2 active	No	CDI
Rifaximin	Salix Pharmaceuticals	Antibiotic/rifamycins	Phase 3 completed	Yes[b]	rCDI
Surotomycin	Cubist Pharmaceutics	Antibiotic/lipopeptides	Phase 3 completed	No	CDI
Tigecycline	Pfizer	Antibiotic/glycylcycline	Phase 2 discontinued	Yes[c]	CDI/rCDI
Faecal Microbiota Transplantation	Self-provided/stool banks	Organic microbiota	Phase 3 completed	Yes	rCDI
SERES-109	Seres Therapeuticals	Organic microbiota	Phase 3 active	No	rCDI
SERES-232	Seres Therapeuticals	Synthetic microbiota	Phase 1 active	No	CDI
VP20621	Shire	Orally administered nontoxigenic *Clostridium difficile*	Phase 2 completed	no	rCDI
Ribaxamase/SYN004	Synthetic Biologics	Class A β-lactamase designed to protect gut microbiota from action of systemically administered β-lactam antibiotics	Phase 2 completed	No	CDI

CDI, *Clostridium difficile* infection; FDA, US Food and Drug Administration; rCDI, recurrent *Clostridium difficile* infection.
[a] Awaiting publication.
[b] Rifaximin is registered for use in hepatic encephalopathy.
[c] Tigecycline is registered for complicated skin infections.

However, since the publication of the ESCMID guideline, results of a large multicentre randomized controlled trials (RCT) show that metronidazole is inferior to vancomycin in the treatment of CDI (nonsevere and severe combined, with severe CDI defined as white blood cell count ≥20 001/mm^3, ten or more bowel movements per day and severe abdominal pain) [10]. Clinical success occurred in 202 (73%) of 278 patients who were treated with metronidazole compared to 210 (81%) of 259 patients treated with vancomycin (p 0.02) [10]. However, subgroup analysis per severity of CDI did not yield statistically significant results. A 2017 meta-analysis by

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Table 3
Current treatment guideline of CDI by ESCMID [5]

Episode	Treatment			Nonantibiotic treatment
	First choice	Second choice	Third choice	
First episode of nonsevere CDI	Metronidazole orally 500 mg three times a day for 10 days	Vancomycin orally 125 mg four times a day for 10 days[a]	Fidaxomicin orally 200 mg two times a day for 10 days[a]	For mild cases; stop inducing antibiotic and observe clinical response at 48 hours
Severe episode of CDI	Vancomycin orally 125 mg four times a day for 10 days	Fidaxomicin orally 200 mg two times a day for 10 days		In case of colon perforation or severe systemic inflammation, surgery is indicated
Severe episode when oral treatment is not possible	Metronidazole 500 mg three times a day 10 day and oral vancomycin 500 mg four times a day for 10 days			In case of colon perforation or severe systemic inflammation, abdominal surgery is indicated
First recurrence of CDI	Vancomycin orally 125 mg four times a day for 10 days[a]	Fidaxomicin orally 200 mg two times a day for 10 days[a]		
Multiple recurrences of CDI	Fidaxomicin orally 200 mg two times a day for 10 days[a]	Vancomycin orally 125 mg four times a day for 10 days, followed by vancomycin pulse strategy or taper strategy[a]		FMT added to antibiotic treatment

CDI, *Clostridium difficile* infection; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; FMT, faecal microbiota transplantation.
[a] Equally effective.

Nelson et al. [11] also concludes that metronidazole is inferior compared to vancomycin in the treatment of CDI. Since the publication of the ESCMID guidance document in which fidaxomicin was reserved for patients with relapsing CDI, a published meta-analysis and indirect treatment comparison suggested that fidaxomicin may be considered as first-line therapy for CDI [83]; these observations may reflect the slow and poor intestinal concentration of metronidazole in the lower gastrointestinal tract [12]. A recent study encompassing seven hospitals in the UK reported on the use of fidaxomicin as the first agent of choice in all forms of CDI, but it also mentioned that justification for severe CDI was not well studied [13]. Guery et al. [14] in 2017 showed that a tapered treatment schedule with fidaxomicin (days 1–5, treatment with 200 mg two times a day, followed by once daily on alternating days during days 7–25) is superior (p 0.03) in resulting in a sustained clinical cure (30 days after end of treatment) in CDI. Sustained clinical cure was experienced by 124 (70%) of 177 patients compared to 125 mg vancomycin four times a day for 10 days in 106 (59%) of 179 patients. These observations may have further impact on the use of fidaxomicin as the first agent of choice, especially for patients at high risk of relapse.

New agents for treatment of CDI

An overview of the reviewed agents is shown in Table 2.

Monoclonal antibodies bezlotoxumab and actoxumab

Bezlotoxumab (MK-6072) is a recombinant human IgG1/kappa isotype monoclonal antibody. In 2016 it was approved globally for use as an adjunctive treatment in patients at risk for rCDI (including old age and/or use of antibiotics other than anti-CDI treatment) [15]. Bezlotoxumab binds to regions of the combined repetitive oligopeptide domains of the toxin that partially overlap with putative receptor binding pockets. It blocks the action of *C. difficile* toxin B and potentially averts the damage and inflammation that can lead to the symptoms associated with CDI [16]. Actoxumab (previously known as MK-3415) binds specifically to toxin A and was developed in conjunction with bezlotoxumab (previously known as MK-3415A) [17]. The combined administration of these two fully human monoclonal antibodies is designated actoxumab and bezlotoxumab (previously known as MK-3415A). The half-life of bezlotoxumab is 19 days; the C_{max} measured following a 10 mg/kg dose iv was 185 μg/mL [18]. Studies of bezlotoxumab concentrations in stool are very limited and inconclusive, as are studies describing the bezlotoxumab concentration required for inactivation of toxin B in the gut lumen to prevent rCDI.

Clinical trials

After analysing the results of the phase 1 trials for bezlotoxumab and actoxumab, a combined single dose of 10 mg/kg was recommended for further studies [19,20]. In the phase 2 multicentre double-blind RCT, rCDI occurred in seven (7%) of 101 patients treated with combined therapy with bezlotoxumab and actoxumab added to a standard treatment regimen of vancomycin or metronidazole compared to 25 (25%) of 99 patients in the placebo group ($p < 0.001$). No difference in number of days to resolution of CDI or severity of infection was observed [21]. Two phase 3 studies were conducted, MODIFY 1 and MODIFY 2, the results of which were also published as pooled data (Table 4) [17]. In the interim analysis of MODIFY 1, the rate of rCDI was found to be significantly higher in the actoxumab group than in the combined group. Moreover, a higher rate of serious adverse events and deaths was found to have occurred in the actoxumab group compared to the placebo group. Enrollment in the actoxumab group was therefore stopped. Pooled results of MODIFY 1 and 2 showed a statistically significant decrease in the occurrence of rCDI. Bezlotoxumab monotherapy was found to be equally effective as combined actoxumab and bezlotoxumab therapy. Therefore, only bezlotoxumab is registered for treatment of CDI [17]. Searching ClinicalTrials.gov yielded one trial that is currently recruiting to investigate the efficacy of bezlotoxumab in children (NCT03182907).

Safety

In phase 3 studies, eight patients in the bezlotoxumab group experienced congestive heart failure, versus two in the placebo group [17]. This difference was not statistically significant. However, caution in patients with cardiovascular disease, and congestive heart failure in particular, should be warranted.

Antibiotics

Surotomycin

Surotomycin (CB-183,315; MK-4261) is an orally administered, minimally absorbed semisynthetic narrow-spectrum cyclic peptide. Surotomycin is formed by enzymatical cleavage of daptomycin [24]. It disrupts the bacterial membrane by acting as a calcium-

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Table 4
Phase 3 studies completed and published before 20 September 2017

Characteristic	Wilcox et al. [17], bezlotoxumab	Boix et al. [22], surotomycin	Daley et al. [23], surotomycin	Major et al., rifaximin[a]
Study design	RCT	RCT	RCT	RCT
No. centres enrolled	322	115	104	23
No. treatment arms	4	2	2	2
No. participants (controls)	2559 (773)	570 (280)	577 (292)	151 (77)
Dose	10 mg/kg iv	250 mg two times a day	250 mg two times a day	400 mg three times a day, 200 mg three times a day
Treatment regiment	Single dose	10 days	10 days	2 + 2 weeks
Comparator	Placebo	Vancomycin 125 mg four times a day	Vancomycin 125 mg four times a day	Placebo
Inclusion criteria[b]	Adults with primary or recurrent CDI who received oral standard-of-care antibiotics (metronidazole, vancomycin or fidaxomycin, chosen by treating physician) for 10 to 14 days	Adults with primary CDI	Adults with primary CDI	Adults with resolution of CDI after treatment with metronidazole or vancomycin
Exclusion criteria[b]	• Patient with planned surgery for CDI within 24 hours • Life expectancy <72 hours	• Toxic megacolon and/or small bowel ileus • More than two episodes of CDI within 90 days of trial therapy	• Toxic megacolon and/or small bowel ileus • More than 2 episodes of CDI within 90 days of trial therapy	Life expectancy <4 weeks
Diagnostics for CDI (stool samples)	Cytotoxicity assays, culture with toxin detection or strain typing and commercial assays that detect (at least) toxin B or its gene	Enzyme immunoassay, PCR or cell culture cytotoxin neutralization assay	Enzyme immunoassay, PCR or cell culture cytotoxin neutralization assay	Evidence of toxin production or pseudomembranes at endoscopy
Positive laboratory test needed for inclusion	Yes	Yes	Yes	No
Severe CDI included according to ESCMID definition	No	No	No	Unknown
Primary end point	rCDI within 12 weeks after resolution of initial CDI	End of treatment cure rate, noninferiority	End of treatment cure rate, noninferiority	rCDI within 12 weeks after start of treatment
Primary outcome				
Investigational product	17%	79%	83%	16%
Comparator	27%	84%	82%	30%
Outcome	p <0.001	Inferior to vancomycin	Noninferior to vancomycin	p 0.06
Initial cure rate				
Investigational product	80%	79%	83%	NA
Comparator	80%	84%	82%	NA
Sustained cure[c]				
Investigational product	64%	60%	63%	84%
Comparator	54%	61%	59%	70%

CDI, *Clostridium difficile* infection; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; NA, not applicable; rCDI, recurrent *Clostridium difficile* infection; RCT, randomized controlled trial.

[a] Major G et al., "PWE-050 follow-on rifaximin for the prevention of recurrence in Clostridium difficile associated diarrhoea: a randomised controlled trial," *Gut* 2017; 66: abstract 150.

[b] Summary of most important criteria.

[c] Sustained cure is defined as rate (%) of participants without rCDI upon initial cure in follow-up period.

dependent cell membrane depolarizing agent [25]. Surotomycin has a fourfold greater *in vitro* potency than vancomycin against *C. difficile* and other Gram-positive bacteria, with minimal impact on the Gram-negative organisms of the intestinal microbiota [24,26]. The half-life time of surotomycin ranges between 14.8 and 21.1 hours [27]. The effect of surotomycin on the composition and diversity of the gut microbiota is limited [28].

Clinical trials

A treatment dose of 125 or 250 mg surotomycin administered two times a day was recommended as result of the phase 1 trials [27]. The phase 2 trial that followed showed end-of-treatment cure rates of 92% in the 125 mg surotomycin group, 87% in the 250 mg surotomycin group and 89% in the vancomycin group [29]. The recurrence rate was significantly lower in patients treated with surotomycin. Two parallel phase 3 studies comparing surotomycin to oral vancomycin (Table 4) were conducted [22,23]. The results could not confirm the observations of the phase 2 study. Interestingly, in subjects infected with the *C. difficile* BI/NAP1/027 strain at baseline, the cure rate and sustained clinical response rate were numerically higher, with lower recurrence rates in patients treated with surotomycin versus vancomycin, although this was not statistically significant. These results stopped the development of surotomycin in CDI treatment.

Rifaximin

Rifaximin is a minimally absorbable antibiotic [30]. Rifaximin is related to the rifamycin class of antibiotics, but rifaximin possesses an extra pyridoimidazole ring [31,32]. Rifaximin shows *in vitro* activity against *C. difficile* [33], but high-level resistance to rifaximin in *C. difficile* (associated with *rpoB* mutations) has been reported [34]. The effects on the microbiota are not clear, though it does not induce dramatic shifts in the microbiota composition [35,36].

Clinical trials

After primary therapy of CDI with either metronidazole or vancomycin, in one phase 2 study, rifaximin was found to be equally effective as placebo in avoiding relapse (relative risk, 0.61; 95% confidence interval 0.36–1.02). The quality of evidence was

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low as a result of a high risk of bias and imprecision [37]. In a phase 3 trial (available only as an abstract; Table 4), 16% of patients treated with rifaximin experienced rCDI, versus 30% in the placebo group (p 0.06) (Major G et al., "PWE-050 follow-on rifaximin for the prevention of recurrence in Clostridium difficile associated diarrhoea: a randomised controlled trial," Gut 2017; 66: abstract 150). Recently a second randomized placebo controlled phase 3 trial has been completed (RAPID study: rifaximin for preventing relapse of *Clostridium* associated diarrhoea) which is awaiting data analysis (National Institute for Health Research RfPB, PB-PG-1010-23257).

Cadazolid

Cadazolid is a bacterial protein synthesis inhibitor and is classified as an oxazolidinone antibiotic. Cadazolid also contains parts of the chemical structure of the fluoroquinolone class of antibiotics in the form of a quinolone nucleus [38]. The quinolone nucleus in cadazolid causes only a weak DNA synthesis inhibition. A preclinical study shows that cadazolid has bactericidal activity against *C. difficile* [39]. Single and multiple (twice daily for 10 days) oral doses of cadazolid up to 3000 mg administered two times a day to healthy volunteers revealed that 81% to 86% of cadazolid was measured unchanged in faecal samples, suggesting minimal systemic absorption [39]. A study by Chilton et al. [40] shows that cadazolid has little effect on the commensal gut microbiota.

Clinical trials

In the phase 2 study, a better cure rate was observed for patients treated with cadazolid compared to vancomycin (Table 5). The recurrence rate was only provided in a modified intention-to-treat analysis: two (18%) of 11, three (25%) of 12 and two (22%) of nine in the cadazolid groups versus seven (50%) of 14 in the vancomycin group, respectively [41]. The sustained cure rate for all doses of cadazolid was significantly higher than vancomycin, but the observed cure rate in patients treated with vancomycin was lower than reported elsewhere. Currently two phase 3 trials have been completed: NCT01983683 and NCT01987895 (ClinicalTrials.gov), the results of which will be available soon. One clinical trial investigating the effect of cadazolid in children is recruiting patients (NCT03105479).

LFF571

LFF571 is a semisynthetic thiopeptide antimicrobial with potent *in vitro* antibacterial activity against Gram-positive bacteria, including *C. difficile* [42]. LFF571 targets the essential process of translation through impairment of elongation factor-Tu function. It is related to the family of elfamycins, a relatively understudied group of antibiotics [43]. Serum and faecal levels of LFF571 have been evaluated following a 200 mg four times a day dose for 10 days in patients with moderate CDI. High levels of LFF571 measured in faeces (median 3240 μg/mg) and low levels measured in serum (maximum 41.7 ng/mL) suggest minimal systemic absorption [44].

Clinical trials

A phase 1 trial evaluated the safety of a single dose of LFF571 up to a 1000 mg, as well as 200 mg four times a day for 10 days [45]. In a phase 2 trial, patients treated with LFF571 had better initial cure rates [46], but no significant difference was found in the occurrence of rCDI cases confirmed with toxin testing (Table 5). It was concluded that LFF571 was noninferior to vancomycin treatment [46]. Currently no phase 3 trials are underway.

Ridinilazole

Ridinilazole (formerly known as SMT19969) is a novel small-spectrum, nonabsorbable antibiotic specifically developed for CDI treatment. *In vitro* studies have shown its high inhibitory activity against *C. difficile* and minimal activity against both Gram-positive and Gram-negative aerobic and anaerobic intestinal microorganisms [47]. The working mechanism of ridinilazole has not yet been completely elucidated, but it is suggested that it may impair cell division [48]. Nearly all ridinilazole is passed unchanged through faeces. The effects on the gut microbiota were found to be minimal [49].

Clinical trials

In a phase 2 study (Table 5), the primary end point was resolution of CDI and no rCDI 30 days after the end of the trial [50]. The end point was met in 32 (64%) of 50 participants in the ridinilazole group versus 25 (50%) of 50 in the vancomycin group (p 0.002). RCDI occurred in four (11%) of 36 participants in the ridinilazole group versus 12 (32%) of 37 participants in the vancomycin group. Further analyses were done in a modified intention-to-treat analysis (Table 5). One phase 2 trial (NCT02784002) comparing the efficacy of ridinilazole versus fidaxomicin has just been completed (Mitra S et al., "Preservation of gut microbiome following ridinilazole versus fidaxomicin treatment of *Clostridium difficile* infection," poster abstract presented at IDWeek, San Diego, CA, 7 October 2017).

Tigecycline

Tigecycline has an expanded broad-spectrum antibiotic activity and acts as a protein synthesis inhibitor [51]. Similar to tetracycline antibiotics, tigecycline exerts bacteriostatic activity against *C. difficile* [33]. Currently tigecycline is not registered for use in CDI, but it is approved for complicated skin, soft tissue and complicated intra-abdominal infections [52]. In CDI, a retrospective cohort study analysing 45 patients with severe CDI (severity defined by clinical criteria) receiving tigecycline monotherapy and 45 patients receiving standard therapy alone revealed that patients treated with tigecycline had significantly better outcomes of clinical cure, less complicated disease course and less CDI-associated shock [53]. However, two retrospective cohort studies failed to demonstrate a difference in outcome of patients receiving adjunctive tigecycline and those who did not [54,55]. It is clear that RCTs are needed to elucidate the role of tigecycline in the management of severe CDI. One phase 2 trial was started but discontinued because of slow enrollment. The results were never published (NCT01401023).

CRS3123

CRS3123 has currently completed two phase 1 studies. CRS3123 inhibits bacterial methionyl-tRNA synthetase, thereby preventing growth and toxin production in *C. difficile.* It has shown potent activity against *C. difficile* (minimum inhibitory concentration 0.5–1 μg/mL), aerobic Gram-positive bacteria and Gram-negative bacteria, including anaerobes [56]. In a first phase 1 study, plasma concentrations of CRS3123 peaked after 2 to 3 hours and rapidly declined after 12 hours. Further systemic and faecal exposure will be investigated in future studies [57]. CRS3123 doses up to 1200 mg were found to be safe and well tolerated, with no serious adverse events reported. The most common adverse events in the CRS3123 group were decreased haemoglobin (23%) and headache (20%) [57]. Phase 2 studies are expected to start in the near future.

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Table 5
Phase 2 completed and no results of phase 3 available before 20 September 2017

Characteristic	Louie et al. [41], cadazolid	Mullane et al. [46], LFF571	Vickers et al. [50], ridinilazole	Gerding et al. [78], nontoxigenic *Clostridium difficile*
No. centres enrolled	9	25	33	44
No. treatment arms	4	2	2	4
No. participants (controls)	84 (22)	72 (26)	100 (50)	173 (44)
Dose	• 250 mg 2 times a day • 500 mg 2 times a day • 1000 mg 2 times a day	200 mg four times a day	200 mg 2 times a day	• 10^4 spores per day • 10^7 spores per day • 10^7 spores per day
Treatment regimen	10 days	10 days	10 days	7 or 14 days
Comparator	Vancomycin 125 mg four times a day	Vancomycin 125 mg four times a day	Vancomycin 125 mg four times a day	Placebo
Inclusion criteria[a]	Adults with primary CDI or first rCDI	Adults with mild to moderately severe primary CDI or first rCDI	Adults with primary CDI	Adults with primary or first rCDI who clinically recovered from a standard treatment
Exclusion criteria[a]	• Ileus • Severe abdominal tenderness • Toxic megacolon	Severe CDI	Life-threatening or fulminant CDI with evidence of hypotension (systolic blood pressure <90 mm Hg), septic shock, peritoneal signs or ileus or toxic megacolon	• rCDI • Other treatments than vancomycin or metronidazole • Presence of bowel disease or previous (6 weeks) bowel surgery • Toxic megacolon • Planned administration of antibiotics after randomization
Diagnostics for CDI (stool samples)	*C. difficile* toxin A/B assay and/or PCR	*C. difficile* toxin A/B or B assay	Toxigenic strain by nucleic acid amplification tests or free toxin by enzyme immunoassay	Free faeces toxin detection or PCR
Severe CDI included according to ESCMID definition	No	No	No	Yes
Primary end point	No additional CDI treatment necessary after 10 days of treatment, superiority	Clinical cure within 1–3 days after end of treatment, noninferiority	Resolution of CDI symptoms and no rCDI within 30 days after end of treatment, noninferiority	Safety and tolerability of NTCD-M3 within 7 days of treatment; clinical: rCDI from day 1 through week 6
Primary outcome				
Investigational product	• 250 mg: 77% • 500 mg: 80% • 1000 mg: 68%	91%	64%	rCDI: • 10^4 spores 7 days: 15%: • 10^7 spores 7 days: 5% • 10^7 spores 14 days: 15%
Comparator	68%	78%	50%	30%
Outcome	Not superior	Noninferior	Noninferior	p 0.006
Initial cure rate	• 250 mg: 77% • 500 mg: 80% • 1000 mg: 68%	91%	MITT: 78%	NA
Comparator	68%	78%	70%	NA
Sustained cure[b]			MITT:	
Investigational product	• 250 mg: 60% • 500 mg: 56% • 1000 mg: 47%	57%	67%	• 10^4 spores 7 days: 85%: • 10^7 spores 7 days: 95% • 10^7 spores 14 days: 85%
Comparator	33%	65%	42%	70%

CDI, *Clostridium difficile* infection; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; MITT, modified intention-to-treat analysis; NA, not applicable; rCDI, recurrent *Clostridium difficile* infection.
[a] Summary of most important criteria.
[b] Sustained cure is defined as rate (%) of participants without rCDI upon initial cure in follow-up period.

Gut microbiota modulating therapies

Faecal microbiota transplantation

Recurrences of CDI are associated with an impaired immune response to *C. difficile* toxins and/or alteration of the colonic microbiota [58,59]. FMT restores the composition and functionality of the gut microbiota, including restoration of colonization resistance, recovery of the secondary bile acid synthesis and inhibiting secondary bile acids direct suppression by antimicrobial peptides and/or reintroduction of bacteriophages [60,61].

Donor faeces can be administered by nasogastric tube, duodenal tube, colonoscopy, enema and capsules. Before FMT, patients are treated with antibiotic therapy directed at CDI for at least 4 days. Additionally, 1 day before FMT, bowel lavage is performed in most patients [62]. It has been suggested that at least 50 g of donor faeces should be used for a single treatment with FMT [63].

Clinical trials

After the first RCT by van Nood et al. [64], many clinical trials have been performed to study the efficacy and safety of FMT in the treatment of rCDI [65–70]. In the study by Orenstein et al. [69], the faecal microbiota product was provided by Rebiotix, a commercial biotechnology company, whereas in the other trials noncommercial products were used [64–70]. The reported cure rates vary between 44% and 94% (Table 6). A recent meta-analysis by Moayyedi et al. [71] underlines the efficacy of FMT in the treatment of rCDI (pooled risk ratio of 0.41 for the persistence of CDI). However, great

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Table 6
Clinical trials concerning treatment of rCDI with FMT

Study	Participants (FMT)	Route of administration	Bowel lavage	Follow-up	Antibiotic treatment before FMT	Volume of donor faeces per FMT	Resolution of rCDI
Van Nood et al. [64]	42 (16)	Duodenal tube	Yes	10 weeks	4–5 days vancomycin 500 mg four times a day	Faeces diluted to 500 mL	• 1st FMT: 81% • 2nd FMT: 94%
Cammarota et al. [65]	39 (20)	Colonoscopy	Yes	10 weeks	3 days vancomycin 125 mg four times a day	Faeces diluted to 500 mL	• 1st FMT: 65% • 2nd FMT: 80% • 3rd FMT:85% • 4th FMT: 90%
Kelly et al. [68]	46 (22)	Colonoscopy	Yes	8 weeks	>10 days vancomycin	100 g faeces diluted to 500 mL	• 1st FMT: 91%
Orenstein et al. [69]	40	Enema	No	8 weeks	7 days vancomycin 125 mg four times a day	50 g faeces diluted to 150 mL	• 1st FMT: 52% • 2nd FMT: 87%
Hota et al. [66]	30 (16)	Enema	No	120 days	14 days vancomycin 125 mg four times a day	50 g faeces diluted to 500 mL	• 1st FMT: 44%
Youngster et al. [70]	180 (180)	Capsules	No	8 weeks	>1 day of vancomycin, metronidazole or fidaxomicin	48 g faeces diluted into 30 capsules	• 1st FMT: 82% • 2nd FMT: 91% • 3rd FMT: 93%
Kao et al. [67]	116 (57)	Capsules	Yes	>8 weeks	>10 days vancomycin 125 mg four times a day	80–100 g faeces diluted into 40 capsules	• 1st FMT: 96.2%

FMT, faecal microbiota transplantation; NTCD-M3, nontoxigenic *Clostridium difficile* strain M3; rCDI, recurrent *Clostridium difficile* infection.

heterogeneity existed among the included trials with respect to the used donor faeces volume, route of administration, pretreatment and number of FMTs.

Currently FMT is mainly used to prevent further recurrences in rCDI and is performed after initial anti-CDI antibiotic therapy. A recently published review, however, concluded that FMT with or without additional antibiotic CDI treatment might also be a promising curative treatment alternative in patients with severe CDI or rCDI [90]. One currently active clinical trial (NCT02570477) is studying the efficacy of FMT in severe CDI.

Safety

FMT is generally safe and well tolerated. The most commonly noted adverse events are bloating, abdominal cramps, nausea, diarrhoea or constipation [64–66,68–70]. Most serious adverse events are procedure related according to the route of administration, or according to colonoscopy or duodenal tube placement. Aspiration during sedation for colonoscopy, septic shock with toxic megacolon and aspiration pneumonia due to regurgitation of faecal matter have been reported [72,73]. Less is known about the long-term effects of FMT. The development of long-term effects, including malignancies, autoimmune diseases and other gut microbiota–associated diseases in patients who received FMT, should be investigated in the future. A recent publication stimulates the development of national centres that also provide long-term follow-up data on patients treated with FMT [74]. These centres should only administer FMT after appropriate approval from the competent body. Unfortunately, the legal and regulatory framework relating to FMT is highly variable between countries.

Other microbiome therapeutics

Several commercial organizations are providing faecal microbiota transplants as microbiome therapeutics. For the purpose of this review, we only include products composed of cultured microorganisms from which data have been presented or published. SERES-109 is composed of bacterial spores from healthy human donors. It is designed to restore dysbiosis in the gut microbiota, thereby preventing rCDI [75]. The first preliminary results were presented of a placebo-controlled phase 2 trial of a single-dose SER-109 to reduce rCDI up to 8 weeks after treatment (Trucksis M, "An analysis of results from the first placebo-controlled trial of single-dose SER-109, an investigational oral microbiome therapeutic to reduce the recurrence of *Clostridium difficile* infection (CDI)," paper presented at the 27th European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 2017). However, the primary end point did not show a statistically significant difference between SER-109 and placebo arms regarding recurrence rates (44.1% vs. 53.3% recurrence, respectively). It was concluded that a dose increase may be necessary and that diagnostic accuracy needed improvement by direct toxin testing instead of PCR analysis, as recommended by 2016 ESCMID CDI guidelines [76]. SERES-262 is a new synthetically derived microbiome therapeutic and therefore does not require human donor material. At present, a phase 1 study is evaluating the safety and efficacy of SERES-262 for the prevention of rCDI in patients with primary CDI ([http://www.serestherapeutics.com/clinical-trials/overview] 2017).

Nontoxigenic Clostridium difficile strains

Nontoxigenic *C. difficile* (NTCD) strains lack the genes for toxin production. NTCD strains are capable of colonizing patients and preventing CDI by a toxigenic strain [77]. One of these NTCD strains, M3 (VP20621; NTCD-M3), has shown to safely colonize healthy volunteers [77]. In a completed phase 2 study (Table 5), treated participants experienced resolution of CDI after metronidazole or vancomycin treatment with differing doses of NTCD-M3 spores [78]. The treatment was well tolerated and appeared to be safe. NTCD-M3 colonized the gastrointestinal tract and significantly reduced CDI recurrence, though the highest dosage prescribed for 14 days was less effective than a similar dosage for 7 days, and not all treated individuals became colonized. This difference was associated with less colonization of NTCD-M3 in the 14-day treatment group. A phase 3 trial is currently not underway.

Antibiotic inactivator to prevent CDI development

Ribaxamase

Ribaxamase (SYN-004) is a β-lactam cleaving enzyme. It is engineered as a pH-dependent formulation and is released in the proximal small intestine [79]. Ribaxamase is designed for oral administration concomitantly with iv β-lactam antibiotics to prevent their disruption of gut microbiota [80]. Ribaxamase acts via enzymatic degradation of excess β-lactam antibiotics that are excreted in the small intestine, thus preventing alterations of the gut microbiota. A phase 1 study showed no adverse events in healthy volunteers [81]. A recent phase 2a study shows proof of concept in humans [82]. A phase 2b study is currently underway, but preliminary results have been presented (Kokai-Kun JF, "SYN-

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004 (ribaxamase) significantly reduced the incidence of *Clostridium difficile* infection in a phase 2b clinical study," paper presented at the 27th European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 2017). Released top-line data show a relative risk reduction of 71% for CDI and 41% for colonization with multidrug-resistant organisms. Ribaxamase also significantly reduced dysbiosis in the gut microbiota. Ribaxamase did not affect the efficacy of iv ceftriaxone treatment.

Conclusions

The cornerstones of CDI treatment, metronidazole and vancomycin, are associated with a 20% risk of rCDI after primary infection. Metronidazole is mentioned in the ESCMID guideline as first-line therapy, but it appears less effective than vancomycin in inducing initial cure, especially for severe clinical forms of CDI [10,11,91]. Compared to vancomycin, fidaxomicin results in a similar initial cure rate in patients with a first episode of CDI but a significant reduction of rCDI [13,14,83]. Nonetheless, treatments with an even higher sustained cure rate are needed. In recent years, multiple novel treatment modalities for (r)CDI have been investigated.

Although some show promising results, limitations of the studies with new agents should be addressed. Firstly, most of the studies were active comparator studies comparing vancomycin with other antibiotics. This makes it difficult to assess performance compared to fidaxomicin or FMT. Secondly, only a few studies follow the ESCMID recommendations to use a two-step algorithm with a toxin detection test as an important tool to determine the activity of CDI [76]. Inappropriate testing algorithm may result in treatment of *C. difficile* carriers instead of CDI [84,85]. The third limitation is the lack of a standardized definition for a rCDI. Preferably, both clinical criteria (e.g. more than 2 days of at least three loose stools per day) and microbiologic criteria (positive toxin test and exclusion of other enteropathogens) should be used. The large variation of reported recurrence rates indicate that standardization is urgently needed. Lastly, we did not include cost-effectiveness studies, as costs are highly variable between countries and institutions, and costs are often biased by industry and greatly depend on the chosen effectiveness [86].

No firm recommendations can be given for the efficacy of antibiotic treatment in severe CDI, as most studies excluded patients with severe disease, following the ESCMID definition [5]. Severity of CDI has been measured using many different methods, sometimes specifically defined for a treatment study [87]. The definition varies between different guidance documents, as summarized in Table 1. It should be emphasized that none of the definitions has been validated. In patients with mild CDI, the lack of 'no treatment' control studies does not allow for any conclusions to be drawn regarding the need for treatment beyond withdrawal of the initiating antibiotic. We still consider this a first approach for the treatment of the individual patient with mild CDI. We propose that oral vancomycin becomes the first choice when antibiotic treatment for nonsevere CDI is necessary due to a higher efficacy in inducing initial cure [10,11,87]. Fidaxomicin is a good alternative for vancomycin in patients at risk for development of rCDI, such as elderly patients, those with severe comorbidity and those with low serum antibodies to *C. difficile* toxins [13,14,83]. Bezlotoxumab is an interesting new therapeutic approach using a human monoclonal antibody against *C. difficile* toxin B. The studied patients seem to have mainly experienced mild to moderate CDI, thus causing us to question the applicability or generalizability of the study results to patients with more severe CDI [17]. Furthermore, the clinical relevance of the difference of 12% to 13% reduction in rCDI by fidaxomicin and 10% by bezlotoxumab must be questioned.

It is expected that new products that influence the serum antibody response or the human microbiota composition will be further developed. Though vaccination trials are currently being performed, a recent phase 3 trial (using inactivated *C. difficile* toxins A and B) has been preliminary ended by Sanofi SA after analysis by an independent data monitoring committee. To date, FMT remains the primary therapy for multiple rCDI. Faecal microbiota products do not differ greatly in terms of efficacy [88], but standardized mixtures of bacteria to replace FMT have not proven successful so far. The encapsulation of donor faeces may further simplify the treatment of patients with FMT in the future [67,70]. We also anticipate that new medication that protects the microbiota, such as ribaxamase or DAV132 (an adsorbent for antibiotic residue in the colon), will be further developed and tested to prevent CDI development during antibiotic therapy (Kokai-Kun JF, "SYN-004 (ribaxamase) significantly reduced the incidence of *Clostridium difficile* infection in a phase 2b clinical study," paper presented at the 27th European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 2017) [82,89].

In conclusion, recent progress in the treatment of CDI is modest, although promising agents are being tested. Interestingly, nonantibiotic treatment strategies—such as microbiota targeting approaches, microbiota preserving preventive strategies and toxin targeting antibodies—may change the battlefield in the fight against CDI.

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