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Antibiotic Treatment Pipeline for *Clostridioides difficile* Infection (CDI): A Wide Array of Narrow-Spectrum Agents

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Abstract

Purpose of Review *Clostridioides difficile* infection (CDI) represents a major burden on the U.S. healthcare system. Despite the U.S. Food and Drug Administration (FDA) approval of fidaxomicin in 2011, observed rates of clinical cure and CDI recurrence suggest there is room to improve. As a result, there are many antibiotic treatments in the developmental pipeline for CDI. The purpose of this focused review is to summarize these antibiotic therapies in all stages of development.

Recent Findings Here, we discuss 10 antibiotics in development, including three that have completed phase II trials, five in phase II trials, and two still undergoing preclinical trials.

Summary The antibiotic treatment pipeline for CDI contains a plethora of narrow-spectrum agents with unique mechanisms of action and potent activity against *C. difficile*. Only ridinilazole, LFF571, and ramoplanin have completed phase II trials, and ridinilazole is the only antibiotic to begin recruitment for a phase III trial. While the future of CDI treatment appears bright, the healthcare community will have to await the results from phase III trials.

Keywords *Clostridium difficile* · Antibiotic · Microbiome · Drug pipeline · Narrow spectrum · Clinical trial

Introduction

Clostridioides difficile infection (CDI) is a gastrointestinal infection with symptoms ranging from mild diarrhea to pseudomembranous colitis and toxic megacolon [1]. CDI, which is caused by the toxin-producing, spore-forming, gram-positive, anaerobic bacillus *C. difficile*, is recognized as one of the most common healthcare-associated infections in the USA [2•]. Although the annual incidence of CDI is decreasing, *C. difficile* continues to cause an estimated 130 infections per 100,000 persons per year [3•]. The financial burden is accordingly significant with more than $1 billion of attributable healthcare costs annually, including attributable hospital costs of $10,275–$17,933 per episode [4]. Despite an influx of attention and resources over the past several decades, CDI persists as a major issue in the USA and remained one of five Centers for Disease Control and Prevention (CDC)–designated urgent threats in 2019 [4].

Antibiotic use has been identified as the most important modifiable risk factor for the development of CDI [5], yet the treatment of CDI has historically relied on two relatively broad-spectrum antibiotics, metronidazole and vancomycin, as mainstays [1, 6, 7]. In fact, all agents previously used for the treatment of CDI have been antibiotics [8•], except for tolevamer, a polystyrene binder of *C. difficile* toxins A and B, which failed to demonstrate efficacy in its phase III trials [9]. Fidaxomicin represents the first narrow-spectrum CDI antibiotic to successfully navigate the drug development pipeline [10]. The future of CDI treatment appears to be much of the same; however, efforts are being made to develop antibiotics that are increasingly narrow spectrum and in turn limit concomitant dysbiosis and the rate of recurrent CDI (rCDI)

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following treatment. This review aims to summarize these efforts by briefly documenting pre-clinical and clinical trials of antibiotics being developed to treat CDI.

Current Treatment Landscape

There are two U.S. Food and Drug Administration (FDA)–approved treatments for CDI, vancomycin and fidaxomicin [10, 11]. A third antibiotic, metronidazole, is not FDA approved for CDI but continues to be used off-label. Current U.S. clinical practice guidelines recommend using one of the two FDA-approved medications while avoiding metronidazole due to inferior clinical cure rates [12•].

The diminishing niche for metronidazole represents the most notable recent shift in the CDI treatment landscape. Metronidazole had served as the preferred first-line therapy since demonstrating similar clinical outcomes to vancomycin in a randomized controlled trial published in 1983 [13]. However, increasing rates of treatment failure and rCDI when compared to vancomycin observed in contemporary clinical trials led the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) to recommend reserving its use for when other therapies are unavailable [9, 12, 14]. While the clinical benefit of duel CDI therapy is a topic of recent debate [15, 16], metronidazole is the only guideline-recommended antibiotic available as an intravenous (IV) product, and it remains recommended as adjunctive therapy to oral vancomycin in cases of fulminant disease [12•].

Vancomycin, administered exclusively via the oral or rectal route, was the first FDA-approved treatment for CDI and has been continually used for decades due to its high cure rates, relatively low rates of recurrence, tolerability, and high colonic concentrations relative to *C. difficile* minimum inhibitory concentrations (MICs) [1, 9, 14, 17, 18]. Fidaxomicin (formerly OPT-80), an orally administered antibiotic approved by the FDA in 2011, has a narrower spectrum of activity and lower rates of recurrence when compared to vancomycin [17, 18]. Fidaxomicin is more expensive than vancomycin and metronidazole and contrasting cost-effectiveness analyses affected its utilization in clinical practice between 2011 and 2017 [19–21]. However, its emergence as a guideline-recommended first-line treatment agent in 2018 appears to have increased its use [22], and results from a recent cost-effectiveness analysis support the widespread use of fidaxomicin [23•]. The only other currently guideline-recommended antibiotic is rifaximin, which is given as a chaser regimen following a standard-of-care CDI treatment course [12, 24].

Three promising CDI treatment options, tolevamer, cadazolid, and surotomycin, have demonstrated exciting potential in their early clinical trials over the past 15 years [25–27]. However, each of them failed to meet key efficacy endpoints during phase III clinical trials [9, 28–30]. Tolevamer (formerly GT267-004 and GT160-246), the only non-antibiotic developed for the treatment of CDI, showed promise when compared with vancomycin in its phase II trial [25]. Unfortunately, tolevamer consistently showed inferiority to both metronidazole and vancomycin in both of its phase III trials, abruptly ending its development for CDI [9]. Surotomycin (formerly CB-183,315 and MK-4261), a cyclic lipopeptide that disrupts the bacterial membrane during both the logarithmic and stationary growth phases of *C. difficile*, is another notable failure [31]. Despite a narrow spectrum of activity and positive phase II results, its development was halted in 2017 after failing to demonstrate non-inferiority to vancomycin in one of its two phase III trials [27–29, 32]. Similarly, cadazolid (formerly ACT-179811), an oxazolidinone–quinolone hybrid antibiotic, failed to demonstrate non-inferiority to vancomycin in one of its two phase III trials, and further development is not anticipated [30•].

An ideal antibiotic for the treatment of CDI should have potent, bactericidal effects against vegetative *C. difficile* and spores while sparing the remainder of the gut microbiota, have little to no systemic exposure, and remain equally effective at treating CDI and preventing recurrence regardless of *C. difficile* ribotype. While fidaxomicin is an improvement over metronidazole and vancomycin, imperfect rates of sustained clinical response (i.e., clinical cure without recurrence of CDI within 30 days after end of treatment) leave room to improve [17, 18]. Many investigators have now dedicated years pursuing discovery of a more ideal agent, resulting in an exciting pipeline of antibiotics in development. Here, we briefly summarize this pipeline.

Antibiotic Pipeline

Ridinilazole (Formerly SMT19969)

Ridinilazole (Summit Therapeutics PLC) is an oral antibiotic with a novel but not fully understood mechanism of action [33]. Ridinilazole has completed two phase II trials (ClinicalTrials.gov identifiers NCT02092935 and NCT02784002) [34, 35], and two identical phase III trials, denoted Ri-CoDIFy 1 (NCT03595553) and Ri-CoDIFy 2 (NCT03595566), are currently recruiting patients [36, 37].

In vitro data suggest that ridinilazole is a narrow-spectrum antibiotic [38–41]. Although it is highly active against *C. difficile* (MIC_{90} of 0.25 μg/mL), it spares other aerobic and anaerobic bacteria commonly found in the gut. Ridinilazole has undergone study in an in vitro gut model confirming previous in vitro findings regarding potency and spectrum [42]. In addition, two in vivo hamster models were conducted, which demonstrated higher rates of survival in

ridinilazole-treated animals than those treated with either vancomycin or fidaxomicin [43, 44]. Significant human gut microbiota analyses were also completed using stool samples collected from participants in ridinilazole's phase I and phase II trials [45, 46]. Participants in the phase I trial that received 200 mg twice daily, which was the dose chosen for subsequent phase II and III trials, experienced a decrease in lactobacilli and an increase in *Bacteroides* spp., total aerobes, and lactose-fermenting Enterobacteriaceae [45]. Total anaerobe and bifidobacteria counts were unchanged. Quantitative polymerase chain reaction (qPCR) and high-throughput DNA sequencing from stool samples collected during the CoDIFy phase II trial showed that alpha diversity indices were significantly lower in vancomycin-treated participants when compared to ridinilazole-treated patients [46••]. Beta diversity analysis showed that while all participants had a significantly different baseline gut microbiota from healthy controls, only vancomycin-treated participants experienced further dysbiosis from baseline following treatment. Clinically, ridinilazole demonstrated a sustained clinical response that was superior to vancomycin (66.7% vs. 42.4%, $P = 0.0004$), and no study drug-related adverse effects led to discontinuation [34••]. Ridinilazole's phase III trials intend to demonstrate a superior sustained clinical response rate over vancomycin-treated patients [36, 37].

LFF571

LFF571 (Novartis Pharmaceuticals Co.) is an oral semisynthetic thiopeptide antibiotic that inhibits bacterial growth by binding to protein synthesis elongation factor Tu (EF-Tu) [47]. It has completed one phase I and one phase II trial (NCT01232595) [48, 49]. Although the results from the phase II trial were published in 2015, Novartis dropped its antibiotic development program in 2018 and no data are available to suggest an upcoming phase III trial [50].

LFF571 has been shown to be narrow spectrum with high in vitro potency against *C. difficile* (MIC_{90} of 0.25 μg/mL) [51]. It has little activity against other anaerobic bacteria commonly found in the gut, including gram-positive (e.g., *Bifidobacterium* spp.) and gram-negative (e.g., *Bacteroides* spp.) organisms. In a hamster model, LFF571 demonstrated a significantly lower hazard of death and recurrence when compared to vancomycin [52]. Like many of the other antibiotics discussed in this review, LFF571 obtains high fecal concentrations and low levels of systemic exposure, leading healthy volunteers and patients with CDI receiving 10 days of LFF571 to experience mild gastrointestinal adverse effects [48, 49, 53]. In a phase II trial comparing LFF571 to vancomycin, LFF571 was shown to be non-inferior to vancomycin in terms of clinical cure (85% vs. 80%, respectively), but rates of rCDI during the 30-day follow-up were similar between groups (31% vs. 30%) [49]. It is worth noting that there were numerically more patients being treated for rCDI in the LFF571 group, and all patients were diagnosed through use of a nucleic acid amplification test (NAAT). This diagnostic method is highly sensitive and detects the *C. difficile* toxin genes rather than the toxins themselves, which may have led to the inclusion of patients who were colonized with *C. difficile* rather than those with true CDI [54]. There were no unanticipated safety signals [49].

Ramoplanin (Formerly A-16686, MDL 62,198, and NT-851)

Ramoplanin (Ology Bioservices, Inc.) is a glycolipodepsipeptide antibiotic that has completed phase I and II trials [55]. No data are available to suggest an upcoming phase III trial.

The MIC_{90} of ramoplanin to *C. difficile* is 0.5 μg/mL, including in strains with reduced susceptibility to vancomycin and metronidazole [56], and it appears to affect organisms associated with the indigenous gut microbiota to a similar extent as vancomycin [57]. What differentiates ramoplanin from vancomycin is its ability to suppress *C. difficile* spore counts, which has been demonstrated through plating of ramoplanin-exposed spores on agar and in an in vitro gut model of CDI [58, 59]. The ability of ramoplanin to suppress spores is thought to be due to its ability to adhere to the organism's outer exosporium coat and kill vegetative *C. difficile* cells as they germinate [59]. In an in vivo hamster model, ramoplanin performed similarly to vancomycin regarding the total viable *C. difficile* counts and symptom reduction, but *C. difficile* spores were recovered significantly less often in the caecal contents of ramoplanin-treated hamsters ($P < 0.05$) [58]. Lastly, in a phase II trial comparing ramoplanin (200 or 400 mg twice daily) to vancomycin 125 mg four times daily for 10 days, sustained clinical response rates were similar between groups (83%, 85.2%, and 85.7% for ramoplanin 200 mg, 400 mg, and vancomycin, respectively) [55].

MGB-BP-3

MGB-BP-3 (MGB Biopharma Ltd.) is an oral synthetic polyamide antibiotic with a novel mechanism of action. It has completed one phase I trial (NCT02518607) and has completed recruitment for its phase IIa trial (NCT03824795); however, nothing has been published in the peer-reviewed literature [60, 61]. The phase IIa open-label trial is made up of three cohorts of patients with CDI who will receive ascending doses of MGB-BP-3 twice daily for 10 days' duration [61]. According to a press release published on MGB Biopharma's website, preliminary data from the first cohort suggest "high efficacy and good tolerability" [62]. In addition, they state that they will be assessing the impact of MGB-BP-3 on the gut microbiota.

DNV3837/DNV3681 (Formerly MCB3837/MCB3681)

Like cadazolid, DNV3837 (DEINOVE) is an oxazolidinone–quinolone hybrid prodrug that is converted to the active substance DNV3681 following IV administration [63••]. It has completed one phase I trial and has begun recruitment for its phase II trial (NCT03988855) [63–65].

The MIC_{90} of DNV3681 to *C. difficile* is 0.064 μg/mL [66]. In healthy volunteers, a reduction of ≥ 2 log_{10} in viable counts of *Clostridium* spp., *Bifidobacterium* spp., *Lactobacillus* spp., and *Enterococcus* spp. following 5 days of exposure was observed [63, 64]. Conversely, counts of *Escherichia coli* increased and *Bacteroides* spp. counts were not impacted. DNV3681 was well tolerated in healthy volunteers except for two instances of moderate phlebitis [64]. The first part of its open-label phase II trial will study the safety and efficacy of 10 days DNV3681, infused over 12 h, in patients with non-severe CDI followed by a second part in patients with severe CDI [65].

Ibezapolstat (Formerly ACX-362E and GLS-362E)

Ibezapolstat (Acurx Pharmaceuticals, LLC) is an oral antibiotic that inhibits the DNA polymerase IIIC (pol IIIC) of *C. difficile* [67]. Ibezapolstat has completed a phase I study and is recruiting for an open-label phase IIa study (NCT04247542) [68, 69].

While extensive in vitro studies demonstrating the effects of ibezapolstat on the broader gut microbiome are lacking, it has an MIC_{90} to *C. difficile* of 4 μg/mL [70]. In an in vivo hamster model, ibezapolstat had identical survival rates (33%) to vancomycin at 34 days following only 3 days of therapy [70]. However, when the treatment was extended to 14 days, survival was 100% in the ibezapolstat-treated hamsters. The phase I trial of ibezapolstat recruited 68 subjects to determine the safety, pharmacokinetics, food, and fecal microbiome effects of ibezapolstat [68]. This trial uniquely included an arm of healthy subjects receiving oral vancomycin in order to compare the gut microbiome effects of both agents. Overall, fecal concentrations were high (up to ~ 2500× MIC) while systemic exposure was low (< 1 μg/mL). Furthermore, qPCR demonstrated that ibezapolstat had minimal effects on the beneficial phylum Bacteroidetes and caused significantly less overall gut dysbiosis than vancomycin [68]. A 10-day course of ibezapolstat will be compared to vancomycin in its phase II trial, which will include an open-label 2a segment of patients receiving ibezapolstat followed by a double-blinded vancomycin-controlled 2b segment [69].

CRS3123 (Formerly REP3123)

CRS3123 (Crestone, Inc.) is an oral, fully synthetic diaryldiamine antibiotic that acts by inhibiting a factor involved in bacterial protein synthesis, type 1 methionyl-tRNA synthetase (MetRS). It has completed two phase I trials (NCT01551004 and NCT02106338) and there are plans for a phase II trial [71–73].

The MIC_{90} of CRS3123 to *C. difficile* is 1 μg/mL [74, 75]. It also has excellent in vitro activity against aerobic and anaerobic gram-positive organisms (e.g., *S. aureus*, *Enterococcus* spp., and *Clostridium* spp.) but lacks activity against gram-negative organisms. As a protein synthesis inhibitor, CRS3123 inhibits *C. difficile* toxin production and sporulation in vitro [76]. The first phase I trial was a single ascending dose study which demonstrated safety of CRS3123 given a similar frequency and severity of adverse effects when compared to those that received placebo [71]. However, the investigators noted a decrease in hemoglobin in 7/30 (23%) patients that received CRS3123. In addition, systemic absorption was observed, but accurate pharmacokinetic analysis was limited due to the presence of coeluting metabolites. The second phase I trial included a multiple ascending dose investigation for safety, pharmacokinetic, and microbiome analyses [72]. Of the 24 patients randomized to receive CRS3123, only 4 (16%) experienced a decrease in hemoglobin. As expected, fecal concentrations were high and dose dependent (median 2115–8280 μg/g) while systemic exposure was low (< 1 μg/mL for all doses at steady state). Beta diversity analysis via 16S rRNA gene sequencing showed significantly more dysbiosis in all three CRS3123 groups when compared to placebo-treated patients. Among CRS3123 treated patients, the investigators did not note any significant proportional changes at the genus level from baseline except for the target genus *Clostridium*. It is worth noting that the microbiome effects of CRS3123 were not compared with patients receiving a standard-of-care agent (e.g., vancomycin).

DS-2969b

DS-2969b (Daiichi-Sankyo Co., Ltd.) is an antibiotic that acts by inhibiting DNA gyrase B (GyrB) and can be administered orally or intravenously. The results of its phase I trial were published in 2018 and no data are available to suggest an upcoming phase II trial [77, 78].

The MIC_{90} of DS-2969b to *C. difficile* is 0.125 μg/mL [79, 80]. Propensity for the development of resistance was tested in vitro using five *C. difficile* isolates and none developed spontaneous mutations following exposure to DS-2969b at 4× MIC [80]. In addition, DS-2969b performed better than fidaxomicin and vancomycin in an in vivo hamster model [80]. Both phase I trials reported that DS-2969b was well tolerated when given as a single dose or multiple doses for 14 days [77, 78]. Unlike many of the other antibiotics discussed in this review, DS-2969b is rapidly absorbed following oral administration and is primarily excreted in the urine [77, 78]. Furthermore, a similar proportion of the

administered dose was excreted in the feces of rats following IV and oral administration suggesting it could serve as an IV alternative to metronidazole in patients who cannot tolerate oral medication [80]. Microbiome analyses were performed using 16S rRNA gene-targeted qPCR in the phase I trial and suggest that DS-2969b decreases *Bifidobacterium* spp. and *Clostridium coccoides* counts while sparing *Bacteroides fragilis*, *Clostridium leptum*, and *Prevotella* spp. [77, 78].

Clofazimine

Clofazimine (KamTek, Inc.) is an oral antibiotic that has been FDA approved for the treatment of leprosy since 1986 but has recently been appreciated to have in vitro activity against *C. difficile* (MIC_{90} of 0.25 μg/mL) and is undergoing development for the treatment of CDI [81, 82].

Although clofazimine's hydrophobicity limits its delivery to the lower gastrointestinal tract, several oral reformulations have demonstrated promise in overcoming solubility issues in both in vitro bioactivity assays and in hamster models [82–84]. Survival rates were compared in a hamster model, which showed survival at 27 days was numerically higher in the clofazimine group (6 mg/kg twice daily) than in placebo and vancomycin-treated animals (80% vs. 0% vs. 20%) [84]. Clofazimine has not yet undergone clinical trials for CDI in humans and no information has been released regarding developmental plans.

Ramizol

Ramizol (Boulos & Cooper Pharmaceuticals) is an oral styrylbenzene antibiotic that targets the mechanosensitive ion channel of large conductance (MscL) [85]. Similar to clofazimine, ramizol suffers from solubility issues and is still undergoing preclinical investigation [86].

The MIC_{90} of ramizol to *C. difficile* is 4 μg/mL [87]. In an in vivo hamster model, the 28-day survival rate in the ramizol group receiving 100 mg/kg twice daily was statistically higher than in placebo-treated animals (57% vs. 0%), but numerically lower than in vancomycin-treated animals, of which 86% survived [85]. Another group of hamsters were treated with ramizol 100 mg/kg four times daily, and 71% of them survived. Most recently, a dose finding study was done in rats in which doses up to 1500 mg/kg/day were well tolerated with only slight decreases in white blood cells and increases in total cholesterol and triglycerides [88].

Repurposing of Other Antibiotics for CDI

In addition to novel agents in development, several antibiotics already available on the market have been used off-label for CDI treatment. Nitazoxanide and fusidic acid, both of which have evidence from randomized controlled clinical trials supporting efficacy, are addressed in the current treatment guidelines as probably effective in CDI but lacking enough clinical evidence to recommend their use as first-line agents [12, 89–92]. Tigecycline and bacitracin are also addressed as having inadequate data to support any efficacy in CDI [12•]. A recent network meta-analysis detailing the data of 13 antibiotics concluded teicoplanin was the most likely to lead to a sustained clinical response, which was calculated as the number of patients with resolution of diarrhea at the end of therapy, minus the number of patients with recurrence or death during the follow-up period [8•]. However, this conclusion was based on small, randomized clinical trials conducted in the 1990s [93, 94].

Another class of antibiotics, the tetracyclines, have an increasing amount of evidence emerging to support their use for CDI. tigecycline (previously GAR-936), a semisynthetic glycylcycline antibiotic, has the most data of the tetracyclines supporting its use as an anti-*C. difficile* antibiotic [95–101]. While it demonstrates excellent in vitro activity against *C. difficile* (MIC_{90} = 0.06 μg/mL) [97, 98], it may be limited as a treatment agent by its broad spectrum of activity, including other bacteria known to be present among healthy gut microbiota, e.g., *Bacteroides* spp. and *Bifidobacterium* spp. [95–98]. Tigecycline has been tested in an in vitro gut model to assess its effect on the gut microbiota and propensity to induce CDI. While tetracycline significantly reduced the counts of healthy gut microbiota, e.g., *Bacteroides* spp. and *Bifidobacterium* spp., it appeared to have a low risk of inducing CDI as demonstrated by the reduction in both vegetative and spore forms of *C. difficile* cell counts as well as the absence of cytotoxin [99]. In addition, tigecycline has been used as adjunctive therapy to standard of care anti-*C. difficile* antibiotics in cases of severe and/or refractory disease [100, 101]. Eravacycline (formerly TP-434), a fully synthetic fluorocycline antibiotic, has been tested against *C. difficile* in two large in vitro studies. It also demonstrates potent activity against *C. difficile* (MIC_{90} = 0.12 μg/mL), but its otherwise broad spectrum of activity has the potential to cause collateral damage [102, 103]. Lastly, the MIC_{90} of omadacycline, an aminomethylcycline antibiotic, to *C. difficile* is 0.5 μg/mL [104]. Like tigecycline, omadacycline has been tested in an in vitro gut model with similar results [105]. While the low propensity for inducing CDI is positive, it is unlikely that any of these agents will undergo further investigation for the treatment of CDI given their broad spectrum of activity and high chance of collateral damage.

Conclusions

A general theme of narrowing the spectrum of activity and thus limiting gut dysbiosis is apparent when looking at the antibiotic treatment pipeline for CDI. Like their predecessors,

antibiotics in the pipeline have potent activity against *C. difficile* and attain high colonic concentrations. Refer to Table 1 for antibiotic susceptibility and selected pharmacokinetic data. While the effects of fidaxomicin on the human gut microbiota were investigated in phase II and III studies [108, 109], ridinilazole is the first to use robust metagenomic data, including alpha and beta diversity analyses, to compare its effects on the gut microbiota versus the standard of care in its phase II trial [46••]. Investigators studying CRS3123, DNV3681, DS-2969b, and ibezapolstat have included similar analyses in their phase I trials [63, 64, 68, 72, 77, 78]. Current data suggest that less gut dysbiosis will lead to lower rates of rCDI [46, 109], so it is increasingly important for investigators to explore the microbiome effects of these agents in the early stages of development.

Except for metronidazole, all antibiotics used to treat CDI have tolerable adverse effect profiles given their lack of systemic absorption. With few additional exceptions, antibiotics currently in development have limited systemic absorption and have demonstrated safety in humans [34, 53, 68, 72]. Exceptions include DS-2969b, which was orally administered in its phase I study and is rapidly absorbed with excretion primarily via the urine [78]. Animal studies suggest that IV administration of DS-2969b also achieves adequate levels in the gut [80]. DNV3681, administered intravenously as the prodrug DNV3837, has completed its phase I trial and is the closest to providing an IV alternative to metronidazole in cases where a patient with CDI cannot tolerate oral medications [63–65].

With so many promising agents in development, it may be tempting to directly compare the potency of these agents using susceptibility data, including MIC_{90} values (Table 1). However, they must be evaluated in the context of achievable antibiotic concentrations obtained in the gut of a patient with CDI. Thus far, only three agents have completed phase II trials and have published such data (Table 1). Additionally, the potential for sporicidal activity is offered by several protein synthesis inhibitors (CRS3123, DNV3681, LFF571, ramoplanin) in development. Published data demonstrating sporicidal effects for CRS3123 and ramoplanin are available [58, 59, 76]. These sporicidal effects could give these agents an edge by decreasing the number of spores in the gut and therefore limiting rates of rCDI. However, these preclinical observations must be tested in large, phase III trials before any final conclusions can be drawn.

As several of these antibiotics move into phase III trials, it will be important to learn from those antibiotics that previously failed. Cadazolid, surotomycin, and tolevamer all randomized few to no "severe" cases in their phase II trials, which may have impacted their ability to meet key efficacy endpoints in phase III trials [25–27]. In addition, the diagnosis of CDI must remain consistent throughout development and follow current clinical practice guideline recommendations [12•]. Specifically, the use of nucleic acid amplification tests

Table 1 Overview of in vitro and pharmacokinetic studies of antibiotics in development for the treatment of CDI

Antibiotic	Reference(s)	Number of isolates	*C. difficile* MIC_{90} (μg/mL)	Fecal concentrations in humans on day 10 (μg/g) (mean (SD))*
Ridinilazole	[41]	$n = 107$	0.125	–
	[40]	$n = 82$	0.125	–
	[38]	$n = 50$	0.25	–
	[34]	–	–	1298 (1302)**
		–	–	1373 (1390)
LFF571	[106]	$n = 398$	0.25	–
	[51]	$n = 50$	0.25	–
	[49]	–	–	3950 (2810)
Ramoplanin	[56]	$n = 105$	0.25	–
	[107]	$n = 18$	0.25	–
	[57]	$n = 72$	0.5	–
DNV3681	[66]	$n = 114$	0.064	–
Ibezapolstat	[70]	$n = 23$	4	–
CRS3123	[74]	$n = 108$	1	–
	[75]	$n = 50$	1	–
DS-2969b	[79]	$n = 101$	0.125	–
Clofazimine	[82]	$n = 36$	0.25	–
Ramizol	[87]	$n = 100$	4	–

*Data only included from phase II trials

**Concentration on day 5

Table 2 Antibiotics in development for the treatment of CDI

Antibiotic	Mechanism of action	Formulation under study	Dose(s) under study	Clinical trials
Ridinilazole	Unknown	Oral capsule	200 mg twice daily × 10 days	*Complete*: – Phase I – CoDIFy phase II *Ongoing*: – Ri-CoDIFy phase III
LFF571	Elongation factor Tu (EF-Tu) inhibitor	Oral capsule	200 mg four times daily × 10 days	*Complete*: – Phase I – Phase II
Ramoplanin	Peptidoglycan synthesis inhibitor	Oral	400 mg twice daily × 10 days	*Complete*: – Phase I – Phase II
MGB-BP-3	Unknown	Oral	Unknown dose × 10 days	*Complete*: – Phase I *Ongoing*: – Phase II
DNV3837/DNV3681	Protein synthesis inhibitor (oxazolidinone–quinolone hybrid)	Intravenous solution	6 mg/kg infused at a rate of 0.5 mg/kg/h over 12 h × 10 days	*Complete*: – Phase I *Ongoing*: – Phase II
Ibezapolstat	DNA polymerase IIIC (pol IIIC) inhibitor	Oral capsule	450 mg twice daily × 10 days	*Complete*: – Phase I *Ongoing*: – Phase II
CRS3123	Type 1 methionyl-tRNA synthetase (MetRS) inhibitor	Oral capsule	Multiple doses twice daily × 10 days	*Complete*: – Phase 1
DS-2969b	DNA gyrase B (GyrB) inhibitor	Oral suspension	Multiple doses once daily × 14 days	*Complete*: – Phase I

(NAAT) may identify patients without true CDI and thus lead to an overestimation in the efficacy of the antibiotic being developed [54]. Tolevamer was the only agent to use diagnostics that detect toxin directly in stool (e.g., enzyme immunoassay (EIA) or cell culture cytotoxicity neutralization assay) consistently throughout phase II and III trials, and therefore, testing was not likely a contributing factor to its failure [9, 25]. Both cadazolid and surotomycin used NAAT for diagnosing randomized patients, and cadazolid even switched diagnostic methods from NAAT in their phase II trial to EIA in their phase III trials, potentially leading them to include more "true" cases of CDI [26–30]. Ridinilazole defined their phase II modified intention-to-treat as those randomized with a confirmed EIA, and it is doing the same in its phase III trial, which is currently enrolling patients [34, 36, 37]. Hopefully other candidates will take such factors into account to ensure replicable and accurate phase III trial findings.

The antibiotic treatment pipeline for CDI contains a plethora of narrow-spectrum agents with unique mechanisms of action and potent activity against *C. difficile* (Table 2). Only ridinilazole, LFF571, and ramoplanin have completed phase II trials, and ridinilazole is the only antibiotic to begin phase III trials. While the future of CDI treatment appears bright, the healthcare community will have to await the results from phase III trials to see how the treatment landscape may change in coming years.

Compliance with Ethical Standards

Conflict of Interest T.J.C. and A.J.G.-L. declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

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