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REVIEW ARTICLE

Ridinilazole: a novel, narrow-spectrum antimicrobial agent targeting *Clostridium* (*Clostridioides*) *difficile*

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Abstract

Clostridium (Clostridioides) difficile infection (CDI) remains an urgent threat to patients in health systems worldwide. Recurrent CDI occurs in up to 30% of cases due to sustained dysbiosis of the gut microbiota which normally protects against CDI. Associated costs of initial and recurrent episodes of CDI impose heavy financial burdens on health systems. Vancomycin and metronidazole have been the mainstay of therapy for CDI for many years; however, these agents continue to cause significant disruption to the gut microbiota and thus carry a high risk of recurrence for CDI patients. Treatment regimens are now turning towards novel narrow spectrum antimicrobial agents which target *C. difficile* while conserving the commensal gut microbiota, thus significantly reducing risk of recurrence. One such agent, fidaxomicin, has been in therapeutic use for several years and is now recommended as a first-line treatment for CDI, as it is superior to vancomycin in reducing risk of recurrence. Another narrow spectrum agent, ridnilazole, was recently developed and is undergoing evaluation of its potential clinical utility. This review aimed to summarize experimental reports of ridinilazole and assess its potential as a first-line agent for treatment of CDI. Reported results from *in vitro* assessments, and from hamster models of CDI, show potent activity against *C. difficile*, non-inferiority to vancomycin for clinical cure and non-susceptibility among most gut commensal bacteria. Phase I and II clinical trials have been completed with ridinilazole showing high tolerability and efficacy in treatment of CDI, and superiority over vancomycin in reducing recurrence of CDI within 30 days of treatment completion. Phase III trials are currently underway, the results of which may prove its potential to reduce recurrent CDI and lessen the heavy health and financial burden *C. difficile* imposes on patients and healthcare systems.

Background

Worldwide, antimicrobial-resistant bacteria currently cause 700 000 deaths per year, and it is predicted that without an intervention 10 million people will die of infections caused by antimicrobial-resistant bacteria every year by 2050 (O'Neill, 2014). *Clostridium* (also called *Clostridioides*) *difficile* is the most common antimicrobial-resistant bacterium causing healthcare-associated infections in high-income countries (Miller *et al.* 2011), listed by the US Centers for Disease Control as an 'urgent' antimicrobial resistance (AMR) threat (CDC, 2019). First described in 1978 as the causative agent of pseudomembranous colitis (Bartlett *et al.* 1978), *C. difficile* causes toxin-mediated diarrhoeal disease which can progress to irreversible damage to the colon, or even death. *C. difficile* infection (CDI) most frequently occurs following antimicrobial use which disturbs the gut microbiome which normally protects against

C. difficile (Martin *et al.* 2016). Risk factors for CDI include advanced age, antimicrobial use and prolonged hospital stay (Martin *et al.* 2016).

The reported incidence of CDI varies widely and is heavily influenced by testing practices, antimicrobial prescribing policies and differing infection control approaches around the world. In general, CDI rates have increased significantly over the past 20 years (Slimings *et al.* 2014; Guh *et al.* 2020). Major outbreaks occurred in the early 2000s, attributed to a particular strain of *C. difficile*, ribotype (RT) 027/PFGE type NAP1/ REA group BI, which had developed fluoroquinolone resistance (He *et al.* 2013). This enhanced resistance profile appears to have driven spread worldwide.

Currently, *C. difficile* causes an estimated >460 000 cases per year in the USA (Guh *et al.* 2020), and costs USD1 billion to the health system (CDC, 2019). Incidence rates are aggregated at 7 cases per 10,000 patient days (PD) in Europe (Davies *et al.* 2014) and 4·6 per 10,000 PD in Australia (ACSQHC, 2020). *C. difficile* RT 027 remains an important strain in North America; however, many other strains, some of which also exhibit enhanced antimicrobial resistance, predominate in certain regions of the world, including RTs 012, 014/020, 017, 018, 078, 106, 356 and 369 (Tickler *et al.* 2019; Freeman *et al.* 2020; Lew *et al.* 2020).

C. difficile is ubiquitous among animals, most frequently asymptomatically colonizing infant animals, but also causing disease in several species including pigs and horses (Weese, 2020). The widespread use of antimicrobials in animal husbandry appears to drive high rates of colonization among production animals including pigs and cows. Increasing evidence is showing likely zoonotic transmission of *C. difficile* via contact with animals and use of animal manure as fertilizer in agriculture and horticulture (Knight *et al.* 2016; Lim *et al.* 2021).

Antimicrobial exposure presents the highest risk for development of CDI. The greatest relative risk for CDI is associated with clindamycin; however, notably, the greatest attributable risk is presented by cephalosporins (O'Grady *et al.* 2021). A meta-analysis of studies identified that carbapenems and third generation cephalosporins are associated with the highest risk for CDI, followed by clindamycin, fluoroquinolones and ß-lactamase inhibitor–penicillin combinations (Slimings and Riley, 2021). This aim of this review is to compile reported evidence of the activity of ridinilazole and examine its potential for use as a first-line agent for treatment of CDI.

Pathogenesis of CDI

Several toxins are produced by *C. difficile* in various combinations. Toxin A (enterotoxin) and toxin B (cytotoxin) are frequently found together, their encoding genes (*tcdA* and *tcdB*) carried on the Pathogenicity Locus (PaLoc). Some strains have partial or complete deletions in the PaLoc resulting in variant strains, most commonly toxin A-negative and toxin B-positive strains, which still elicit disease. Strains carrying *tcdA* only are rare. Toxins A and B glucosylate and inactivate epithelial cell GTPases including Rac, Rho and Cdc42, altering cell signalling that induces disruption of actin cytoskeleton and inducing apoptosis. A third toxin, *C. difficile* binary toxin (CDT), has been linked with emergent 'hypervirulent' *C. difficile* strains including RT 027, 078 and 244 (Lim *et al.* 2014). Its mode of action is not well understood, but its presence is associated with more severe disease (Gerding *et al.* 2014), and some rare strains which produce CDT only may still be capable of causing CDI (Androga *et al.* 2015; Eckert *et al.* 2015).

Spore production is another key virulence factor for *C. difficile*, with 'hypervirulent' strains possibly associated with increased sporulation rates (Akerlund *et al.* 2008). These spores give *C. difficile* the ability to withstand extreme environmental conditions including UV light, high temperatures and dessication, and their resistance to many disinfectants, including alcohol-based cleaning agents. Dormant spores thus survive for long periods of time in both healthcare settings and the community. Spores germinate in the gut when they encounter certain bile acids, and an ecological niche is provided by a disrupted gut microbiota. Conjugated and unconjugated bile acids, taurocholate and cholate, respectively, promote germination of *C. difficile* spores, while secondary bile acids (including lithocholate and deoxycholate) usually inhibit *C. difficile* germination (Qian *et al.* 2020). Different *C. difficile* strains appear to show varying growth responses to bile acids.

The gut microbiota contributes to colonization resistance against *C. difficile* through direct and indirect mechanisms including nutrient metabolism, modulation of bile salts, production of antimicrobial peptides and modulation of the host immune system. Antimicrobial use disrupts the diversity and volume of the gut microbiota. Resulting dysbiosis of the microbiota leads to functional changes in the host gut environment that reduce colonization resistance, and render the host susceptible to CDI, with the greatest risk of CDI occurring during and immediately after antimicrobial use (Figure 1). Colonization resistance has been associated with certain taxonomic groups of bacteria, particularly firmicutes, *Bacteroides fragilis* and *Bifidobacterium longum* (Vickers *et al.* 2016). It is likely that various diverse microbiota compositions can exhibit colonization resistance.

AMR in *C. difficile*

Another key to the virulence and success of *C. difficile* is AMR. *C. difficile* possesses many mechanisms for AMR,

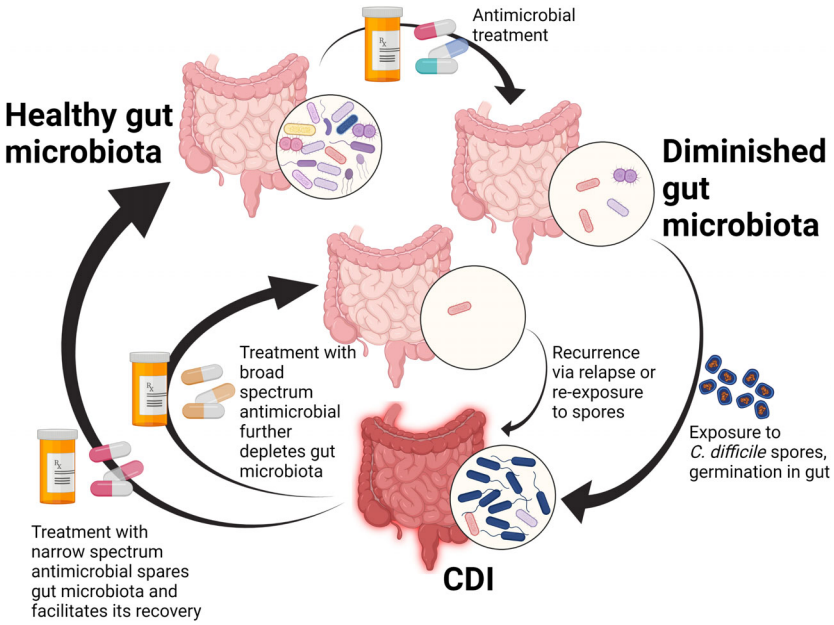

Figure 1 Interplay between antimicrobials, the gut microbiota and *C. difficile*. Antimicrobial treatment diminishes the normal gut microbiota, increasing the risk of CDI if exposed to *C. difficile* spores. Treatment with broad spectrum antimicrobials such as vancomycin or metronidazole further depletes the gut microbiota and increases risk of recurrent CDI. Treatment with narrow spectrum antimicrobials such as fidaxomicin or ridinilazole preserves and facilitates recovery of the gut microbiota, reducing risk of recurrence

some of which are intrinsic and some acquired. These include biofilm formation, mobile genetic elements (e.g. transposons including Tn*5398* carrying *ermB* conferring resistance to erythromycin), and alterations in metabolic pathways or antimicrobial targets (e.g. *rpoB* conferring resistance to rifamycins) (Spigaglia *et al.* 2018; O'Grady *et al.* 2021). Fluoroquinolone resistance (primarily to ciprofloxacin, and to a lesser extent against later generation fluoroquinolones) is conferred by mutations in the quinolone-resistance determining region of the DNA gyrase subunits GyrA and GyrB (O'Grady *et al.* 2021). The most common mutation is the substitution of Thr82Ile in GyrA. This mutation is likely responsible for the success of both the FQR1 and FQR2 lineages of RT 027, both of which acquired an altered *gyrA* gene independently (He *et al.* 2013). Cephalosporin resistance is intrinsic in *C. difficile* and is mediated via production of class D ß-lactamases (Toth *et al.* 2018). Further mechanisms of cephalosporin resistance may involve antibiotic-degrading enzymes and modification of target sites, and may be strain-specific (Spigaglia *et al.* 2018).

AMR rates for *C. difficile* vary widely, depending on strain and antimicrobial usage in different regions. Most *C. difficile* clinical strains show resistance to early generation fluoroquinolones and cephalosporins, while resistance to clindamycin and erythromycin is variable. Resistance to vancomycin, metronidazole, fidaxomicin, meropenem or piperacillin/tazobactam is rarely reported, but strains of RT 027 with reduced susceptibility to metronidazole and vancomycin have been found, particularly in Israel (Adler *et al.* 2015). Reduced susceptibility to metronidazole has also been reported in RT 078 and RT 126 strains (Spigaglia *et al.* 2018).

A recent meta-analysis of 111 studies of antimicrobial susceptibilities in *C. difficile* published between 1992 and 2019 has identified weighted pooled resistance (WPR) to metronidazole and vancomycin as 1% for breakpoint >2 μg mL^{-1}. High WPRs were found for fluoroquinolones: 95% for ciprofloxacin and 32% for moxifloxacin. Among other high-risk antimicrobials for development of CDI, clindamycin had a WPR of 59% and ceftriaxone had WPR of 47%. For fidaxomicin, the WPR was 0·08% (one isolate reported) (Sholeh *et al.* 2020).

Recurrent CDI

Recurrent CDI is a major issue facing CDI patients and treating physicians, defined as a recurrence of CDI symptoms within 8 weeks of resolution of a previous episode (McDonald *et al.* 2007). Recurrence results from relapse of initial CDI infection or reinfection with the same or a new strain of *C. difficile* and arises from slow recovery of the gut microbiota following use of antimicrobials, including vancomycin and metronidazole (Figure 1). Recurrent CDI is associated with increased morbidity and

mortality, is difficult to resolve and thus poses a considerable health and financial burden to patients and health systems. Each episode of recurrence is also associated with increased risk of further recurrent episodes. Approximately one third of recurrent CDI cases require re-admission to hospital (Sheitoyan-Pesant *et al.* 2016) and additional costs of USD10 000-11 000 per case are attributed to recurrent CDI in the USA (Dubberke *et al.* 2014; Zhang *et al.* 2018). Risk factors for recurrent CDI include gastric acid suppression due to proton pump inhibitor use, immunosuppression, older age, previous CDI episode and infection with certain strains of *C. difficile*.

Treatment of initial and recurrent episodes of CDI

Therapies for CDI ideally should target *C. difficile* while conserving the commensal gut microbiota. This would maintain a healthy microbiota and restore colonization resistance during treatment; however, narrow-spectrum therapies of this nature have not been developed until relatively recently. In mild cases of CDI, discontinuation of infection-eliciting antimicrobials can resolve the infection, but in many cases broad-spectrum agents have been administered for initial episodes of CDI. For many years, guidelines recommended oral metronidazole for non-severe disease, and oral vancomycin for severe CDI cases due to apparent superiority over metronidazole (Debast *et al.* 2014). Both vancomycin and metronidazole promote gut dysbiosis and thus are associated with high risk of recurrent CDI (up to 30% of patients) (Johnson *et al.* 2021).

Later iterations of guidelines recommended vancomycin over metronidazole (McDonald *et al.* 2018; Ooijevaar *et al.* 2018), due to findings from multi-centre randomized controlled trials (RCTs) that metronidazole was inferior to vancomycin in the treatment of all CDI (non-severe and severe combined) (Johnson *et al.* 2014). Metronidazole is no longer recommended as first-line treatment for either non-severe or severe CDI by the Infectious Diseases Society of America (IDSA) and the European Society for Clinical Microbiology and Infectious Diseases (ESCMID); both recommend fidaxomicin before vancomycin for initial episodes of CDI, subject to availability and feasibility (Johnson *et al.* 2021; van Prehn et al. 2021).

Fidaxomicin was approved for clinical use in 2011 and has shown potent activity against *C. difficile* while preserving the commensal gut microbiota. This narrow spectrum of activity results in superiority over vancomycin with regard to recurrence/sustained clinical response up to 25 days after treatment completion (Louie *et al.* 2011; Cornely *et al.* 2012); however, it has been reported as inferior to vancomycin for clinical response to RT 027 infections (Louie *et al.* 2011; Cornely *et al.* 2012). Its high cost (USD1 767 for 10 day treatment) initially raised concerns about its benefit compared with treatment with vancomycin (USD14) or metronidazole (USD8), but multiple cost effectiveness analyses demonstrated that it is cost effective due to high cure rates and prevention of recurrent episodes, reducing re-admissions to hospital (Rajasingham *et al.* 2020; Jiang *et al.* 2021).

For recurrent CDI, fidaxomicin is recommended (standard or extended-pulsed regimen) and vancomycin (tapered or pulsed regimen) is listed as an acceptable alternative. For multiply recurrent cases, vancomycin is recommended, followed by rifaximin and faecal microbiota transplant (FMT) (Johnson *et al.* 2021). FMT involves preparation of stool from healthy donors and introduction to the gut of recurrent CDI patients via various routes (capsule vs duodenal vs colonic). FMT has high cure rates, even in cases of fulminant CDI (>76%) (Tariq *et al.* 2019). Despite high cure rates, long-term health effects are still not well-understood and costs are high (USD 1 500–2 000) (Rajasingham *et al.* 2020).

Bezlotoxumab, a human monoclonal antibody which targets toxin B, also significantly decreases recurrence rates of CDI (Wilcox *et al.* 2017). Bezlotoxumab is now recommended as a co-intervention along with antimicrobials in cases of recurrent CDI in the USA, particularly for certain patients considered at high risk for recurrent CDI (age ≥65 years, immunocompromised or severe CDI) (Johnson *et al.* 2021). Some economic analyses again favour use of bezlotoxumab despite its high cost due to its high rates of clinical cure (Lam *et al.* 2018), although the recently released National Institute for Health and Care Excellence recommendations on treatment do not endorse the use of bezlotoxumab (NICE, 2021).

Ridinilazole

Ridinilazole [2,2'bis(4-pyridyl) 3H,3'-H5,5-bibenzimidazole (Figure 2), previously known as SMT19969] (Summit Therapeutics Inc, Oxfordshire, United Kingdom), is a novel non-absorbable narrow-spectrum antimicrobial agent which is currently under clinical evaluation for treatment of CDI. It has demonstrated potent activity against multiple strains of *C. difficile*, including RT027, both *in vitro* and *in vivo*, and in gut models (Weiss *et al.* 2014; Baines *et al.* 2015). It does not appear to have a typical mode of action compared with many antimicrobial classes, such as inhibition of RNA or DNA or cell wall synthesis.

Mode of action of ridinilazole

The mechanism of action of ridinilazole is not fully understood, but investigations using confocal microscopy

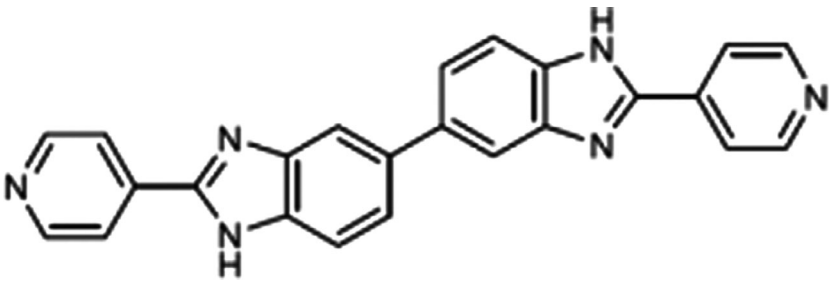

Figure 2 Chemical structure of ridinilazole [2,2′bis(4-pyridyl) 3H,3′-H5,5-bibenzimidazole] (Weiss *et al*. 2014)

and fluorescent labelling identified disruption of septum formation with filamentous phenotype with replicated nucleoids along the length of the cell, which suggests that ridinilazole impairs cell division of *C. difficile* (Basseres *et al*. 2016). Production of toxin A by impaired cells was reduced by 91% and of toxin B by 100%, which in turn elicited a reduction of 74% in the inflammatory response of human intestinal cells (Caco-2). Thus, its activity against *C. difficile* appears to be potent and effectively reduces toxin production, thereby attenuating the host inflammatory response (Basseres *et al*. 2016).

Activity of ridinilazole against *C. difficile* and gut commensals

Few *in vitro* studies measuring the activity of ridinilazole against *C. difficile* have been reported to date, but MIC appears to be low, ranging from 0·015 to 0·5 μg mL^{-1}. Reported MIC_{50} values range from 0·06 to 0·25 μg mL^{-1} and MIC_{90} values range from 0·125 to 0·25 μg mL^{-1} (Table 1). Ridinilazole is active against *C. difficile* at concentrations generally comparable to fidaxomicin (MIC range 0·004 to 1 μg mL^{-1}) (Table 1). Strain-specific MICs have been reported for several of the predominant *C. difficile* strains in the world, many of which show multi-resistance to ≥3 antimicrobials (Table 2). For *C. difficile* RT 027, MICs were 1 dilution lower for ridinilazole compared with fidaxomicin (MIC_{50} and MIC_{90} 0·25 μg mL^{-1} vs 0·5 μg mL^{-1}) in a study by Goldstein *et al*., however, Freeman *et al*. identified lower MIC_{50} (0·06 μg mL^{-1} vs 0·25 μg mL^{-1}) for fidaxomicin compared with ridinilazole for four RT 027 strains collected across Europe (Table 2). The lowest MICs for ridinilazole were recorded for RT 001 strains from Europe (MIC_{50} 0·06 μg mL^{-1}) which also had low MIC_{50} for fidaxomicin (0·008 μg mL^{-1}).

Goldstein *et al*. examined the activity of ridinilazole against 350 Gram-positive and Gram-negative aerobic and anaerobic commensal bacteria. Like fidaxomicin, ridinilazole was less active against Gram-negative anaerobes, particularly *B. fragilis*, than vancomycin and metronidazole. It also had low activity against other Gram-positive anaerobes including *Bifidobacteria* species, *Eggerthella lenta*, *Finegoldia magna* and *Peptostreptococcus anaerobius*. *Clostridium innocuum* was susceptible to ridinilazole (MIC_{90} 1 μg mL^{-1}) while *Clostridium ramosum* and *Clostridium perfringens* were non-susceptible. Gram-positive aerobes including *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium* and streptococci were also non-susceptible to ridinilazole, demonstrating its potential as a clinical therapeutic that preserves the host commensal microbiota (Goldstein *et al*. 2013).

Another study from Goldstein *et al*. evaluated the activity of ridinilazole against 162 strains of *Clostridium* representing clusters I to XIX, and 13 other *Clostridium* species typically found among the commensal gut microbiota. MICs ranged from 0·06 to >512 μg mL^{-1}, but predominantly showed a narrow spectrum of activity. Resistance was not related to cluster or species, occurring again in *C. ramosum* (10/10 strains) and *C. perfringens* (9/11 strains) as well as *Clostridium rectum* (3/3), *Clostridium sardiniense* (1/1), *Clostridium paraputrificum* (6/8), *Clostridium sporogenes* (3/5), *Clostridium colicanis* (1/2), *Clostridium glycolicum* (2/5), *Clostridium scindens* (1/5), *Clostridium sordellii* (1/6) and *Clostridium cadaveris* (2/6). Several species with MIC ≥ 32 μg mL^{-1} for fidaxomicin were also recorded, among different strains compared with those exhibiting resistance to ridinilazole (Goldstein *et al*. 2014).

In vivo studies

In a standard clindamycin-induced male golden Syrian hamster model of CDI, ridinilazole administered orally once daily for 5 days demonstrated full protection on dosing days up to day 12, and 70% survived to day 21 when infected with epidemic *C. difficile* (strain VA5/UNT106-1, BI/NAP1/RT 027), compared with 60% survival for vancomycin-treated hamsters. Survival rates of ridinilazole-treated hamsters ranged from 80 to 95% for non-epidemic *C. difficile* (strain VA11/UNT103-1, REA type J). Apparent relapse also occurred earlier among the

Table 1 Reported activity of ridinilazole, fidaxomicin, vancomycin and metronidazole against *C. difficile*

Isolates (*n*)	Activity (μg mL^{-1})	Ridinilazole	Fidaxomicin	Vancomycin	Metronidazole	Reference
50	MIC_{50}	0·25	0·25	1	0·5	Goldstein *et al*. (2013)
	MIC_{90}	0·25	0·5	4	2	
	Range	0·125–0·5	0·06–1	1–8	0·25–8	
82	MIC_{50}	0·125	0·03	1	2	Corbett *et al*. (2015)
	MIC_{90}	0·125	0·06	2	8	
	Range	0·06–0·125	0·008–0·125	0·5–4	0·125–8	
107	MIC_{50}	0·03	0·06	1	0·5	Freeman *et al*. (2016)
	MIC_{90}	0·125	0·125	2	2	
	Range	0·015–0·5	0·004–0·125	0·5–8	<0·125–2	
44*	MIC_{50}	0·12	0·12	1	0·5	Snydman *et al*. (2018)
	MIC_{90}	0·25	0·5	2	2	
	Range	0·06–0·5	0·06–1	1–4	0·12–4	
3†	Range	0·12–0·25	0·12–0·5	2–4	0·25–2	
45‡	MIC_{50}	0·12	0·25	1	0·25	
	MIC_{90}	0·5	0·5	2	1	
	Range	0·06–0·5	0·06–1	0·5–2	0·12–2	
5§	MIC_{50}	0·12	0·25	1	0·25	
	Range	0·12–0·5	0·12–0·5	1–2	0·12–0·5	
140	MIC_{50}	0·125	0·125	1	0·25	Collins *et al*. (2021)
	MIC_{90}	0·25	0·25	2	0·25	
	Range	0·03–0·25	0·015–0·25	0·06–4	0·06–0·5	

*Isolates collected in ridinilazole treatment group, day 1 of treatment.
†Isolates collected in recurrent cases, ridinilazole treatment group.
‡Isolates collected on day 1 of treatment, vancomycin treatment group.
§Isolates collected in recurrent cases, vancomycin treatment group.

vancomycin treatment group, occurring on day 11 with mortality. Toxin was not detected from surviving hamsters treated with ridinilazole. Plasma sample concentrations of ridinilazole were below the limit of quantification (25 ng mL^{-1}) at all time points demonstrating low bioavailability of the compound, while caecal concentrations were significantly above the MIC (96–172 μg mL^{-1}) (Weiss *et al*. 2014).

In another hamster study, animals infected with either *C. difficile* BI1 (RT 027) or *C. difficile* 630 (RT 012) were then treated with ridinilazole, fidaxomicin or vancomycin for 5 days. Treatment with all three agents resulted in 100% survival during the treatment period for hamsters infected with *C. difficile* BI1. Mortality was recorded on day 11 in animals treated with vancomycin, with 10% survival occurring by day 28. Similar results were found for ridinilazole and vancomycin when infected with *C. difficile* 630; respective day 28 survival rates were 80–100% and 0% (Sattar *et al*. 2015).

Clinical trials

In a phase I, double-blind, randomized placebo-controlled trial ridinilazole showed high tolerance and safety among 56 healthy human male volunteers, with 88% of adverse events limited to self-limiting gastrointestinal upsets. Pharmacokinetic analysis showed little systemic absorption with levels in plasma generally below the limit of quantification for fasted individuals and low concentrations when the drug was administered with food. Ridinilazole concentration in faeces increased with increasing dose, but no notable metabolites were detected. There was also minimal disruption to commensal gut microbiota other than total clostridia (among which a >3 $\log_{10}$ reduction was recorded) with single or multiple doses up to 2 000 mg (Vickers *et al*. 2015).

Based on the positive findings from phase I, phase II trials of ridinilazole were completed in 2015. In a randomized, double-blind active-controlled study of 100 patients, 10 days treatment with ridinilazole showed non-inferiority in comparison with 10 days treatment with vancomycin. A treatment difference of 21·1% (90% CI 3·1–39·1, $P = 0·0004$) was found, where 66·7% of patients treated with ridinilazole vs 42·4% of those treated with vancomycin had a sustained clinical response defined as clinical cure (≤3 unformed bowel movements within a 24 h period) at the end of treatment with no recurrence within 30 days. Ridinilazole was also well tolerated with adverse events reported in 82% of the ridinilazole group compared with 80% of the vancomycin group, none of which required discontinuation of the trial. Minimal systemic exposure was recorded for ridinilazole-treated

Table 2 Reported activities of ridinilazole, fidaxomicin and five comparator antibiotics against specific strains of *C. difficile* that are often multi-drug resistant

Ribotype	*n*	Activity (μgmL^{-1})	Ridinilazole	Fidaxomicin	Vancomycin	Metronidazole	Clindamycin	Moxifloxacin	Rifaximin/ Rifampin*	Reference
RT 001	7	MIC_{50}	0·06	0·008	0·5	1	128	16	0·002	Freeman
		Range	0·03–0·125	0·008–0·015	0·5–2	0·5–1	64–128	16–32	0·001–0·002	*et al.* (2016)
	16	MIC_{50}	0·125	0·03	1	0·25	>32	8	0·03	Collins *et al.*
		MIC_{90}	0·125	0·06	2	0·25	>32	16	>32	(2021)
		Range	0·06–0·125	0·015–0·25	0·06–4	0·125–0·25	0·25to >32	1to >32	0·008 to >32	
RT 002	8	MIC_{50}	0·25	0·25	1	0·5				Goldstein
		Range	0·125–0·25	0·06–0·25	1–2	0·25–0·5				*et al.*,
										(2013)
	17	MIC_{50}	0·125	0·06	1	0·25	>32	32	0·015	Collins *et al.*,
		MIC_{90}	0·25	0·125	2	0·25	>32	>32	0·03	(2021)
		Range	0·03–0·25	0·015–0·25	0·125–2	0·125–0·5	0·25 to >32	1 to >32	0·008–0·03	
RT 012	13	MIC_{50}	0·125	0·125	2	0·25	>32	2	0·015	Collins *et al.*,
		MIC_{90}	0·125	0·25	2	0·25	>32	2	0·03	(2021)
		Range	0·06–0·25	0·03–0·25	1–2	0·125–0·5	4 to >32	2	0·008–0·03	
RT 014/ 020	8	MIC_{50}	0·125	0·25	1	0·5				Goldstein
		Range	0·125–0·25	0·06–0·5	1–2	0·25–0·5				*et al.*,
										(2013)
	19	MIC_{50}	0·125	0·125	1	0·25	4	2	0·015	Collins *et al.*,
		MIC_{90}	0·125	0·125	2	0·25	16	16	0·03	(2021)
		Range	0·06–0·25	0·03–0·25	0·5–2	0·06–0·25	0·25 to >32	1–32	0·008–0·03	
RT 017	2	Range	0·125–0·25	0·06	0·5	0·125–0·25	128	32	0·001–32	Freeman
										et al.,
										(2016)
	23	MIC_{50}	0·125	0·06	1	0·125	>32	32	>32	Collins *et al.*,
		MIC_{90}	0·25	0·125	2	0·25	>32	32	>32	(2021)
		Range	0·03–0·125	0·015–0·125	0·5–2	0·06–0·25	8 to >32	1to >32	0·008 to >32	
RT 018	12	MIC_{50}	0·125	0·06	1	0·125	>32	32	0·015	Collins *et al.*,
		MIC_{90}	0·125	0·125	2	0·25	>32	32	0·03	(2021)
		Range	0·06–0·125	0·03–0·125	0·25–2	0·125–0·25	0·25 to >32	2–32	0·008–16	
RT 027	11	MIC_{50}	0·25	0·5	2	2				Goldstein
		MIC_{90}	0·25	0·5	4	8				*et al.*,
		Range	0·25–0·5	0·5–1	1–8	2–8				(2013)
	4	MIC_{50}	0·25	0·06	0·5	1	8	32	32	Freeman
		Range	0·06–0·25	0·06–0·125	0·5–1	1	8–128	16–32	0·002–32	*et al.*,
										(2016)
RT 369	18	MIC_{50}	0·125	0·125	1	0·25	>32	16	0·03	Collins *et al.*,
		MIC_{90}	0·25	0·25	1	0·25	>32	16	0·03	(2021)
		Range	0·06–0·25	0·03–0·25	0·5–2	0·125–0·25	0·25 to >32	8 to >32	0·015–0·03	

*Rifaximin was tested by Collins *et al.*, rifampin by Freeman *et al.*

patients, suggesting that inflamed GI tract does not precipitate increased ridinilazole levels (Vickers *et al.* 2017).

A nested cohort study in the phase II trial assessed changes in the microbiota at multiple time points: days 1, 5 and 10 (treatment period), day 24 and day 40 (end of the study), and at CDI recurrence. Vancomycin-treated individuals had significant reductions in *Bacteroides*, *Clostridium coccoides*, *Clostridium leptum* and *Prevotella* groups at day 10, and increases in Enterobacteriaceae, which persisted beyond day 10. Ridinilazole-treated individuals had modest decreases in *C. leptum* by day 10, which were recovering by day 25. Alpha diversity decreased with both antimicrobials by day 10, but was significantly less altered with ridinilazole ($P < 0·0001$). Microbiota composition also returned to pre-treatment levels sooner with ridinilazole than with vancomycin (Thorpe *et al.* 2018).

At day 10, intestinal bile acid profiles were measured and compared with gut bacteria. Subjects receiving vancomycin had nearly 100-fold increases in the ratio of conjugated to secondary bile acids in their stool compared with baseline, while ridinilazole-treated subjects had ratios similar to baseline (Qian *et al.* 2020). Significant positive associations were detected between secondary bile acids and members of the Bacteroidales and Clostridiales families, which were depleted in the vancomycin group but preserved in the ridinilazole group. Enterobacteriaceae correlated negatively with secondary bile acids and positively with conjugated bile acids. Bile acid ratios were significantly different among patients who experienced recurrent CDI and those who did not (Thorpe *et al.* 2018).

Phase III clinical trials assessing the utility of ridinilazole are currently underway, aiming to enroll 680 participants in a randomized, double-blind active-controlled study comparing 10-day treatment regimens with ridinilazole vs vancomycin in CDI patients. The safety and efficacy of ridinilazole will be evaluated with reference to the primary outcome of clinical cure and no recurrence of CDI within 30 days post end of treatment. The secondary outcome measures of the study are to determine clinical cure at 12 days and sustained clinical response (no recurrence) within 60 and 90 days following end of treatment. The study was completed in November 2021 (https://clinicaltrials.gov/ct2/show/NCT03595566, accessed 10 February 2022).

Conclusions

CDI incidence rates continue to rise in many regions of the world and the associated high costs and burden to health systems are thus increasing. Moreover, recurrent CDI is an important unmet need, therefore new treatments are required to ensure faster, complete recovery and reduce recurrent CDI events. Protecting the commensal gut microbiota is essential to reduce risk of both initial and recurrent episodes of CDI. Therefore, new therapies must focus on effectively eliminating *C. difficile* while conserving the gut microbiota. Ridinilazole shows significant promise as a narrow-spectrum agent that preserves the gut microbiome and efficaciously treats CDI. Results from *in vitro* and *in vivo* studies, and phase I and II clinical trials, show that ridinilazole has great potential as a first-line agent, effectively reducing the risk of recurrent CDI. Pending results of phase III clinical trials ridinilazole could dramatically change the prognosis for many CDI patients.

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Author Contributions

D.A. Collins planned the review, performed the literature review and drafted the manuscript. T.V. Riley planned the review and edited the manuscript.

Conflicts of Interest

The authors have previously received research funding from Summit Therapeutics Inc., and Merck & Co.

Data Availability Statement

No data were generated during the writing of this literature review.

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