Send Orders for Reprints to reprints@benthamscience.net

Progress in the Discovery of Treatments for *C. difficile* Infection: A Clinical and Medicinal Chemistry Review

Lissa S. Tsutsumi[1], Yaw B. Owusu[2], Julian G. Hurdle[3] and Dianqing Sun[1,*]

[1]*Department of Pharmaceutical Sciences, The Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, 34 Rainbow Drive, Hilo, HI 96720, USA;* [2]*Department of Pharmacy Practice, The Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, 34 Rainbow Drive, Hilo, HI 96720, USA;* [3]*Department of Biology, University of Texas at Arlington, Arlington, TX 76019, USA*

Abstract: *Clostridium difficile* is an anaerobic, Gram-positive pathogen that causes *C. difficile* infection, which results in significant morbidity and mortality. The incidence of *C. difficile* infection in developed countries has become increasingly high due to the emergence of newer epidemic strains, a growing elderly population, extensive use of broad spectrum antibiotics, and limited therapies for this diarrheal disease. Because treatment options currently available for *C. difficile* infection have some drawbacks, including cost, promotion of resistance, and selectivity problems, new agents are urgently needed to address these challenges. This review article focuses on two parts: the first part summarizes current clinical treatment strategies and agents under clinical development for *C. difficile* infection; the second part reviews newly reported anti-difficile agents that have been evaluated or reevaluated in the last five years and are in the early stages of drug discovery and development. Antibiotics are divided into natural product inspired and synthetic small molecule compounds that may have the potential to be more efficacious than currently approved treatments. This includes potency, selectivity, reduced cytotoxicity, and novel modes of action to prevent resistance.

Keywords: Antibiotics, clinical, *Clostridium difficile*, *Clostridium difficile* infection, natural products, small molecules, treatment.

1. INTRODUCTION

Clostridium difficile (*C. difficile*) is a Gram-positive, spore-forming, anaerobic bacterium that was coined "difficile" based on the initial difficulty to culture it in the laboratory [1, 2]. Although originally described in 1935 as a commensal organism, by the late 1970s it was recognized as the main cause of pseudomembranous colitis (PMC), a severe gastrointestinal (GI) disease [3]. Acquisition of *C. difficile* occurs by ingestion of the acid-resistant spores [2] and after passing through the stomach, the spores germinate in the small bowel and inhabit the colon. In order for *C. difficile* to over-populate the colon, there must be a disruption of the normal bacterial flora, which provides colonization resistance to opportunistic pathogens [4, 5]. If *C. difficile* is able to colonize, it then reproduces in the intestinal crypts, and releases enterotoxin (toxin A) and cytotoxin (toxin B) [6-8]. Toxins A and B cause inflammation, attract neutrophils and monocytes, and degrade the colonic epithelial cells, leading to the clinical symptoms associated with *C. difficile* infection (CDI) [7, 9-12]. In contrast, there are asymptomatic carriers of *C. difficile* who do not exhibit any symptoms even though the organism is present in their stools. A review by Kachrimanidou and Malisiovas reported that about 5% of healthy adults and 50% of neonates carry the *C. difficile* bacteria with no symptoms of diarrhea [13]. Also, it is estimated that up to 57% of long term care facility (LTCF) residents carry *C. difficile* with no symptoms [14].

In the healthcare environment, *C. difficile* can easily be transferred from patient to patient *via* the hands of healthcare workers or from exposure to spores in the patient's milieu [15]. Furthermore, exposure to clindamycin and broad spectrum antibiotics (e.g., 2nd and 3rd generation cephalosporins, broad spectrum penicillins and fluoroquinolones), which disrupt the natural gut microbiota promote the overgrowth of toxigenic *C. difficile* from spores that survive drug exposure or colonize patients following drug treatment [15]. Other risk factors associated with the development of CDI include age >65 years, being a resident of a nursing home or LTCF, immunosuppression, previous episodes of CDI, leukocytosis, hypoalbuminemia, exposure to chemotherapeutic agents both inpatient and in the community, GI surgery, inflammatory bowel disease and probably the use of proton pump inhibitors (PPIs) [16-19]. Also, since 2000, there has been an increase in the number of outbreaks of CDI in hospitals following the emergence of seemingly more virulent strains that are more refractory to treatments [20]. However, CDI is no longer strictly a hospital-associated disease as its incidence has now increased in community settings and in many cases, patients are not displaying traditional risk factors such as recent antibiotic use [21] and age. These changes appear to be correlated with the changes in the epidemiology of *C. difficile* strains. Indeed, the emergence of the fluoroquinolone resistant hypervirulent *C. difficile* strain, designated as NAP1/BI/027 [15] in North America (U.S. and Canada)

*Address correspondence to this author at the Department of Pharmaceutical Sciences, The Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, 34 Rainbow Drive, Hilo, HI 96720, USA;
Tel: +1-(808)-933-2960; Fax: +1-(808)-933-2974;
E-mail: dianqing@hawaii.edu

1873-5294/14 $58.00+.00

and Europe, has increased the frequency and severity of CDI. Hypervirulence in NAP1/BI/027 is thought to result from the enhanced production of *C. difficile* toxins A and B and the carriage of a binary toxin whose function is currently unknown [13, 22]. The enhanced production of spores that are inherently resistant to antibiotics and most chemicals is also thought to contribute to the success of ribotype 027 [23], but this property does not appear to be exhibited by all isolates of this ribotype [24]. However, ribotype 027 is not the only *C. difficile* lineage responsible for outbreaks and severe diseases in North America and Europe, emphasizing the need for efforts to understand differences in the pathogenicity of *C. difficile* strains [25-27]. Other clinically relevant ribotypes of *C. difficile* have been identified in North America and Europe over the last decade, including the PCR ribotype 078, which has been found in food animals [28], affects younger patients [29], and has caused outbreaks in hospitals and nursing homes [30], ribotypes 001 and 106 [31], and the fluoroquinolone resistant PCR ribotype 018 [32]. A 2013 case study reported the isolation of a NAP12/ribotype 087 *C. difficile* strain in a fatal case of community-acquired (CA)-CDI (presumptive PMC) in a young 22-year-old female who contracted CDI after receiving clindamycin post tooth extraction due to an abscess [33].

The severity of CDI ranges from mild diarrhea to PMC and even death [34]. Diagnosis of CDI requires the detection of toxins A and B, with enzyme immunoassays (EIA) being a widely used technique [35]. Although the EIA testing yields rapid results, its use is limited by having lower sensitivity compared to cell cytotoxin assay, which also uses the stool to detect toxin activity [36]. However, alternative approaches have been developed and were recently reviewed by Burnham and Carroll [37]. In a study by Mushner and Stager [38], it was found that out of 122 specimens tested for *C. difficile*, only 13% were positive using an EIA while all samples were positive using PCR technology. The presence of toxin-producing *C. difficile* was then confirmed by culture in the samples that were shown to be positive using PCR. This indicated that *C. difficile* may be undetected by EIA and PCR may be a better diagnostic tool [38]. Additional methods of detecting *C. difficile* include culturing the unformed stool [36], nucleic acid amplification test [36], and detection of glutamate dehydrogenase enzyme (screening) produced by both toxic and nontoxic *C. difficile* [36, 39]. *C. difficile* stool culture is slow to yield results and is of limited diagnostic value since only toxigenic *C. difficile* causes the diarrhea associated with CDI [14].

According to the Centers for Disease Control and Prevention (CDC), the incidence, recurrence, and mortality from CDI have increased substantially in the U.S. over the last decade and the major risk factor was healthcare exposure [40]. Annually, *C. difficile* is linked to at least 400,000 infections and 14,000 deaths in the U.S. alone, with the risk of mortality being the highest in the elderly [41]. Previous research in the early 2000s had also shown that *C. difficile* resulted in $1.1 billion in health care costs annually in the U.S. [42]. Over the last decade in which the hypervirulent strains have become increasingly associated with more CDIs, the financial burden of managing the disease has almost tripled, with the average total cost for a single inpatient stay with CDI totaling more than $35,000, resulting in $3 billion in healthcare costs annually [43]. Similarly, in the European Union, substantial amount of money is spent on treating patients with CDI and the estimated annual cost (per 2006 data) was 3 billion euros [44].

2. CLINICAL TREATMENT STRATEGIES

There are several pharmacologic options for the management of CDI [36]. Supportive care [14, 45] is crucial in replacing the fluid, electrolytes, and nutrition lost through diarrhea. To avoid unnecessary treatment of CDI and premature interruption of a suspected offending antimicrobial agent, appropriate diagnosis of CDI should precede treatment [36]. In addition, appropriate environmental control measures of CDI need to be implemented (e.g., hand washing with soap and water, gloving, gowning, and using detergents that can kill *C. difficile* spores) to minimize the spread of CDI [15]. Although metronidazole (MTZ) is the conventionally accepted agent for treating the first episode (primary infection) of CDI, it is not one of the two medications approved by the U.S. Food and Drug Administration (FDA) for the treatment of CDI. The approved agents for the treatment of CDI are vancomycin (VAN) (oral or rectal retention enema) and oral fidaxomicin (FDX) [36, 45] (Fig. **1**).

After each episode, the risk of recurrence of CDI increases [46]. About 15%-30% [46-48] of patients get another infection after their first CDI and about 40%-60% after a second episode of the infection [13, 49, 50]. In cases of severe CDI (defined as white cell counts ≥15,000 cells/μL^3, or serum creatinine ≥50% above baseline) [49] or recurrent infection, VAN is the agent of choice [36, 45]. An alternative agent for cases refractory to VAN is FDX [45]. For multiple recurrent CDIs, adjunctive treatment options that may be used in addition to VAN, include cholestyramine (an anion exchange resin), nitazoxanide (NTZ), rifaximin, intravenous immunoglobulin (IVIG), and probiotics. When all agents fail, fecal microbiota transplant may be considered as the treatment of last resort [36, 45]. Finally, in patients who develop ileus or toxic megacolon, surgical management *via* partial or total colectomy is the most effective treatment strategy to reduce the risk of mortality [36]. Surgery is also considered in cases of fulminant or refractory CDI when patients satisfy one of the following: peritonitis, megacolon and/or worsening abdominal distension or pain, new onset ventilatory failure, new or increasing vasopressor requirement, mental status changes, inexplicable clinical deterioration, elevated serum (> 5 mmol/L) and leukocytosis (WBC >20,000/μL) or leucopenia (WBC < 3,000/μL) [51]. The Society for Healthcare Epidemiology of America/Infectious Diseases Society of America (SHEA/IDSA) recommended treatment for CDI is described in Table **1**. We will briefly review the evidence behind the use of each of the treatment options mentioned, and why certain interventions may be preferred over others in managing CDI in specific points of the disease progression.

2.1. Metronidazole

Metronidazole is a nitroaromatic prodrug that is active against protozoans as well as bacteria [53]. In order for MTZ to become cytotoxic to bacterial cells, reduction of the 5-nitro group of the imidazole ring needs to occur [54]. Due to

its low molecular weight, MTZ diffuses across the cellular membrane of bacteria. In the absence of MTZ, the pyruvate:ferredoxin oxidoreductase (PFOR) generates ATP when it oxidatively decarboxylates pyruvate. When MTZ is present within the cytoplasm, the nitro group captures electrons that are usually transferred to hydrogen ions. The reduced MTZ then creates a concentration gradient, driving the uptake of more MTZ [53, 55]. The nitroso free radical then interacts with DNA, resulting in DNA breakage and destabilization of the DNA helix [56-58].

Table 1. The Recommended Treatments for CDI [36, 52].

C. difficile severity and definition	Recommended treatment
Initial episode, mild or moderate (WBC count ≤15000 cells/μL and SCr<1.5 times premorbid level)	MTZ 500 mg by mouth tid for 10-14 d
Initial episode, severe, complicated (shock or hypotension, ileus, megacolon)	VAN 500 mg by mouth or nasogastric tube qid plus MTZ 500 mg intravenously tid. If complete ileus consider adding rectal instillation of VAN 500 mg qid.
First recurrence (i.e., second CDI episode)	Same as initial episode or VAN 125 mg by mouth qid × 10-14 d
Second recurrence (i.e., third CDI episode)	VAN in a tapered and/pulsed regimen [52] 125 mg po qid × 10–14 d, then 125 mg po bid per day × 1 week, then 125 mg po once daily × 1 week, then 125 mg po every 2 or 3 day for 2–8 week

Abbreviations: qid = four times a day; SCr = serum creatinine; WBC = white blood cells.

For mild to moderate cases of CDI, oral MTZ is preferred and is as effective as oral VAN [14, 36, 45]. The conventional adoption of MTZ as an agent of choice in the first case of CDI was bolstered by the U.S. CDC's caution in 1994 that the use of oral VAN could potentially induce enterococci resistance [52]. Potential properties of oral MTZ that may have contributed to its reduced effectiveness against the NAP1strains of *C. difficile* include the significantly lower concentration of MTZ reached in the colon due to extensive systemic absorption (>80%) compared to orally administered VAN (mean ± standard deviation, 9.3 ± 7.5 μg/g and 520 ± 197 μg/g, respectively) [59, 60]. In patients with no diarrhea, MTZ is not detected in their feces, suggesting higher absorption in this setting [61]. Compared to VAN, MTZ is associated with poorer clinical outcomes in severe infections of *C. difficile* [62]. Another concern with the use of MTZ is the possibility of inducing vancomycin resistant enterococcus (VRE), and this association has already been reported in case-control studies [59]. Al-Nassir *et al*. reported that MTZ promoted a transient overgrowth of VRE during CDI treatment in patients who had VRE stool colonization at baseline. However the VRE overgrowth dropped significantly within two weeks post CDI treatment [59]. Finally, MTZ may be ineffective against other clinically relevant *C. difficile* ribotypes as a recent study reported high MIC values for PCR ribotypes 001 and 010 [63]. Nevertheless, a high MIC of MTZ against toxigenic *C. difficile* may be not just due to the presence of specific genes, but rather due to multifactorial genetic mechanisms not fully understood [64]. One advantage of MTZ over oral VAN and the recently approved FDX is its very low cost [49].

2.2. Nitazoxanide

Similar to MTZ, NTZ (Fig. **1**) is a synthetic nitroaromatic antiparasitic drug bearing a nitrothiazolyl-salicylamide motif. However, its mode of action appears to be distinct from MTZ and is thought to be inhibiting PFOR, which is essential to anaerobic energy metabolism [65]. This is reflected by NTZ retaining activity against *C. difficile* that exhibits reduced susceptibility to MTZ [66]. Nitazoxanide is used for the treatment of parasitic intestinal infections, but it shows promise in the treatment of CDI (MIC = 0.06-0.125 mg/L) [66, 67]. A recent small prospective randomized study (N = 50) that compared NTZ 500 mg bid with oral VAN 125 mg qid could not determine non-inferiority of NTZ to VAN after 10 days of treatment. However, the results suggested that NTZ may be as effective as VAN for the treatment of CDI [68]. Prior to this study which compared NTZ to VAN, another small single center study (N = 35) evaluated the use of NTZ 500 mg twice daily for 10 days in patients with CDI who had failed MTZ and exhibited persistent symptoms of colitis. NTZ therapy cured 66% (composite cure) of the patients who had previously all failed MTZ and some cases also failed VAN [69].

2.3. Vancomycin

Vancomycin is a hydrophilic and rigid glycopeptide antibiotic, which consists of a glycosylated hexapeptide chain and cross linked aromatic rings by aryl ether bonds [70]. Notably, VAN has poor absorption in the GI tract and is not orally bioavailable; therefore, it can be used as an oral antibiotic for the treatment of CDI [71, 72]. Its mechanism of action involves tight binding to the D-Ala-D-Ala subunit of the precursor UDP-*N*-acetylmuramylpentapeptide of peptidoglycan, forming a complex *via* hydrogen bonds. This results in inhibition of the biosynthesis of peptidoglycan, an essential component of the bacterial cell wall envelope. Due to its large and rigid structure, VAN prevents the transglycosylase and transpeptidase enzymes from aligning correctly, therefore preventing peptidoglycan biosynthesis [73, 74]. Although resistance to VAN has emerged in other Enterococci and Staphylococci, there are currently no clinical reports of vancomycin-resistant *C. difficile*. Nonetheless, Leeds *et al.* recently showed that mutations in MurG, which converts lipid I to lipid II during peptidoglycan biosynthesis, confers *in vitro* resistance to VAN in *C. difficile* [75].

Oral VAN is effective for the treatment of CDI even though there is existing concern of selecting for VRE during treatment [52], which was reported in 8% of patients in one study [59]. Favorable properties of oral VAN include limited gut absorption, high fecal concentrations, and no evidence of *C. difficile* resistance. The main limitation is the high cost of oral VAN. A single center randomized controlled trial that

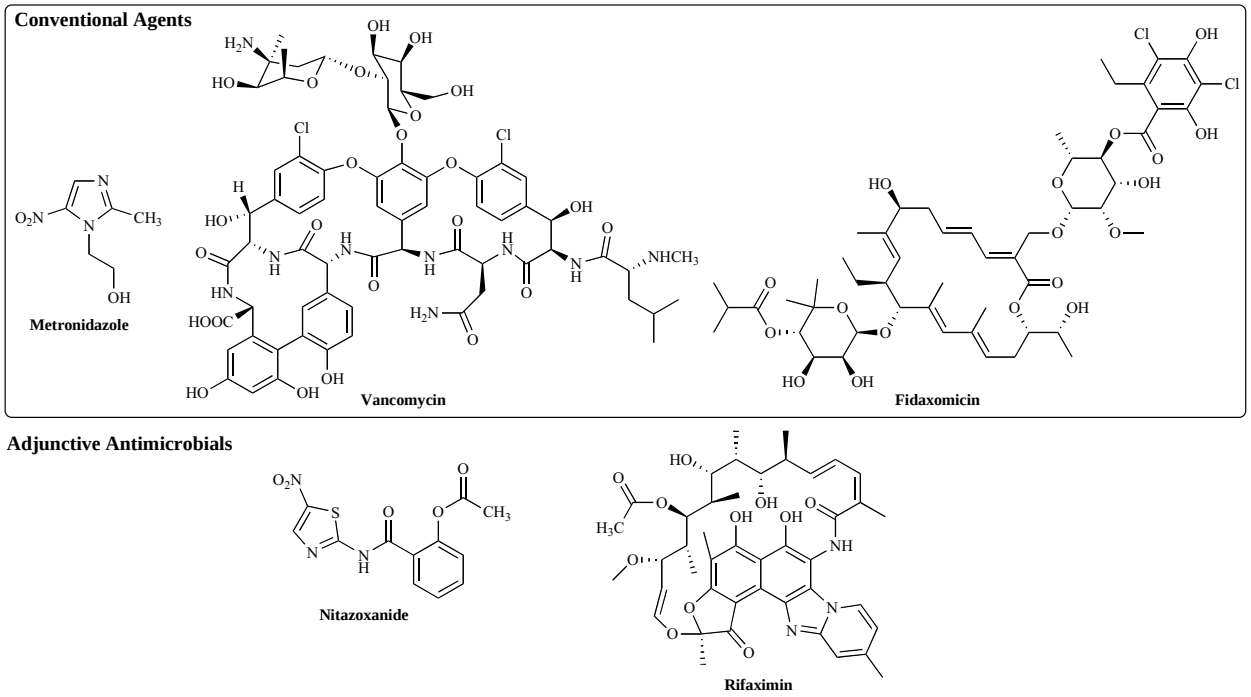

Fig. (1). Chemical structures of clinically used agents and adjunctive antibiotics for CDI.

compared oral VAN (125 mg qid) and oral MTZ (250 mg qid) for 10 days reported no difference in clinical cure (98% and 90%, respectively) for mild cases, but VAN was superior in curing severe cases (97% and 76%, respectively) [76]. Zar *et al.* supports the recommendations to use oral MTZ or oral VAN for mild CDI and to use oral VAN for severe CDI [36, 45]. In very severe CDI manifesting in ileus or toxic megacolon, high dose VAN (500 mg qid) is administered rectally in conjunction with IV MTZ [36, 45, 77].

2.4. Fidaxomicin

Fidaxomicin is a naturally occurring 18-membered macrocyclic antibiotic and it represents the first-in-class of new macrocyclic narrow spectrum antibiotics [78-80]. It was approved in 2011 by the U.S. FDA for the treatment of *C. difficile*-associated diarrhea (CDAD) [72]. Mechanistically, FDX inhibits bacterial RNA polymerase and subsequent RNA synthesis [81] by adopting a mechanism that appears different to rifamycins. There are multiple processes in the transcription of DNA to produce RNA, representing different points for compounds to inhibit transcription. In a study by Artsimovitch *et al.* [82], it was found that FDX binding to RNA polymerase occurred before the initial separation of the DNA strands, which happens before the synthesis of RNA begins. Notably, FDX's mechanism of action is different from other RNA synthesis inhibitors including rifamycins and streptolydigin, which are inhibitors of the initiation and elongation synthetic steps [82]. Interestingly, FDX retains activity against rifamycin-resistant strains and mutations causing resistance to FDX arises in RpoB gene at distinct loci to that causing resistance to rifamycins [83].

FDX has been used in clinical practice for treating CDI in the general population since 2011 and available data from several studies show it is more effective than oral VAN in reducing the second recurrence (i.e., a third episode) of CDI caused by non-NAP1 strains within four weeks of post treatment [84, 85], while both agents exhibit similar safety profiles [86]. In a well conducted prospective, randomized double-blind phase 3 trial evaluated by the U.S. FDA for approval of FDX, non-inferiority of FDX to VAN was demonstrated in the clinical cure of CDI following 10 days of treatment with FDX 200 mg twice a day or VAN 125 mg qid [48]. The cure rate of FDX was superior to VAN (90% and 74.9%, respectively) in a pooled analysis of two prospective, randomized double-blind non-inferiority studies, each evaluating the management of CDI in patients who simultaneously received antibiotics for an underlying infection [87]. A potential advantage of FDX over VAN is its minimal impact on the composition of indigenous fecal microbiota, in particular *Bacteroides* species [88] relative to VAN [89], while attaining a high local concentration in the gut [90] and feces [91]. Louie *et al.* reported mean fecal concentration of FDX of 1225.1 μg/g after 10 days of therapy and this concentration was about 4900 times the MIC_{90} [48]. The major limitation to the use of FDX as an alternative to VAN is cost, with a 10-day *C. difficile* treatment regimen of over $2,000 [14, 92], which is about twice the cost of using the oral VAN formulation. Furthermore, since FDX has a single target (RNA polymerase), resistance has already arisen during clinical use [93].

2.5. Rifaximin

Rifaximin (Fig. **1**) is a semisynthetic rifamycin derivative bearing a 25-membered macrolactam ring, which is linked by an aliphatic chain and a naphthalenic aromatic moiety [70]. It shows anti-difficile activity with a MIC value of 0.015 μg/mL [94] and is primarily used for the treatment of traveler's diarrhea caused by noninvasive strains of *Escherichia coli* [72, 95, 96]. Rifaximin exerts its antibacterial

activity by binding to the β-subunit of DNA-dependent RNA polymerase and thus inhibiting subsequent RNA synthesis [97, 98]. Because rifaximin is non-absorbed and shows potent activity against *C. difficile*, it is used as an adjunctive antibiotic for the treatment of CDI, in particular for patients with a history of multiple CDI recurrence [99]. It may have a potential role in managing CDIs in cases of recurrent CDI. In a small study, eight patients with recurrent CDI (4-8 episodes) were treated with a regular course of VAN, and immediately followed with rifaximin 400-800 mg daily for two weeks. There were no recurrent infections in seven of the eight patients [100]. However, the efficacy of rifaximin in preventing recurrent CDI may be limited by the BI/NAPI/027 hypervirulent strain of *C. difficile*, as a retrospective study by Mattila *et al.* reported higher MIC values (using rifampicin's susceptibility) for the BI/NAPI/027 strains (mean MIC 0.46 μg/mL) compared to the non-BI/NAPI/027 strains (MIC >0.002 μg/mL) [99]. However, like other rifamycins, resistance to rifaximin readily arises and is already found in the clinic [52, 101]. Resistance arises from point mutations in the drug target−the β-subunit of RNA polymerase [102]. Interestingly, commonly occurring rifamycin resistance alleles (e.g., $Arg_{505}Lys$) do not impose a biological fitness cost in *C. difficile* [102], which may enable these strains to further spread and cause CDI [103].

2.6. Intravenous Immunoglobulin (IVIG)

In response to *C. difficile* toxins A and B, the immune system makes antibodies against these toxins [104]. Therefore, the premise behind the use of IVIG in refractory CDI is to passively provide antibodies to neutralize the *C. difficile* toxins, primarily toxin A [104]. This may help reduce the severity of CDI's clinical manifestations and duration since it has been documented that patients with CDI mount a weak immune response by producing low levels of antibodies to *C. difficile* toxins A and B [104, 105]. A 2009 systematic review by O'Horo and Safdar [106] on the use of IVIG in the treatment of CDI examined literature from 1970-2008. The review could not make recommendations on the role of IVIG (dose range used in studies 150-400 mg/kg) in CDI management due to major limitations in available studies, such as small sample size, lack of control groups, or absence of randomization [106]. Respective clinical practice guidelines by Cohen *et al.* [36] and Wilcox *et al.* [45] suggest that IVIG (150-400 mg/kg) could potentially be used for severe or recurrent CDI.

2.7. Vaccine

Kyne *et al.* demonstrated that asymptomatic carriers of *C. difficile* mounted a strong immune response to the *C. difficile* toxin, as depicted by high serum concentrations of immunoglobulin G (IgG) antibodies against the toxin [10]. Also, there is scientific evidence of protection from CDI by high serum concentrations of IgG antibodies against *C. difficile* toxin A [107]. Furthermore, other studies have reported that the parenteral administration of *C. difficile* toxoid vaccine in humans or mice can induce high concentrations of serum anti-toxin A antibodies; based on results of some studies these antibodies should protect against CDI [10, 108, 109]. Sanofi Pasteur's novel *C. difficile* vaccine containing inactivated toxoids A and B demonstrated acceptable immunogenicity and safety in phase 1 study. It was also efficacious in the primary prevention of CDI and secondary prevention of recurrent CDI in two phase 2 studies [110]. In August 2013, Sanofi Pasteur announced it was commencing a phase 3 randomized controlled multi-national study of its experimental *C. difficile* vaccine. The study dubbed *Cdiffense* seeks to enroll subjects at least 50 years old with one of the following: an impending hospitalization or hospitalization at least two times in the preceeding one year where systemic antibiotics were administered. The purpose of *Cdiffense* is to evaluate the safety, immunogenicity and efficacy of the novel *C. difficile* vaccine in preventing the first episode of CDI [111].

2.8. Monoclonal Antibodies

The use of human monoclonal antibodies against *C. difficile* toxin is in the early clinical stages [39]. A phase 2 randomized, double-blind, placebo-controlled study evaluated the effect of a single infusion of human monoclononal antibodies against *C. difficile* toxins A and B in symptomatic CDI patients receiving treatment with MTZ or VAN. The addition of the monoclonal antibodies significantly reduced the recurrence of CDI caused by non-BI/NAP1/027 *C. difficile* strains within 84 days post administration of the antibodies [112]. Merck is currently recruiting patients for a phase 3 study to investigate the efficacy of a single infusion of human monoclonal antibodies against *C. difficile* toxin A, toxin B, and toxins A and B, respectively for preventing CDI recurrence in patients receiving antibiotics for CDI [113].

2.9. Probiotics

Probiotics are live microorganisms (bacteria or yeast) which may be consumed to aid in restoring the balance of indigenous GI microbiota that may be altered using antibiotics that are known to cause diarrhea [114]. Proposed mechanisms of probiotics to restore the normal flora or help prevent colonization by *C. difficile* include competing for nutrients, stimulating host's immune function, and inhibiting and maintaining integrity of GI mucosa by preventing adhesion and invasion by other pathogenic microbial flora [115, 116]. Data on the use of probiotics for prevention or treatment of CDI are variable. Both Cohen *et al.* [36] and Wilcox *et al.* [45] could not recommend the use of probiotics clinically for prevention of CDI due to lack of robust data. Large prospective randomized controlled trials of probiotics may be needed to determine the effectiveness of probiotics for prevention or treatment of CDI since meta-analyses of small and moderate sized randomized trials reported that probiotics of *Lactobacillus, Saccharomyces*, and *Bifidobacterium* species may be effective in preventing CDI [114, 117-119]. The use of the probiotic *Saccharomyces boulardii* has been associated with isolated cases of systemic fungal infections in patients with central venous catheters [120] and critically ill patients [121]. Therefore, it may be prudent to avoid *Saccharomyces boulardii* in these patients.

2.10. Fecal Microbiota Transplant

This is a treatment of last resort following multiple recurrent CDI (refractory), but turns out to be highly effective,

cheap, and safe. Fecal transplant involves replenishing of the gut flora with donated feces from a screened healthy donor such as close family members or spouse [122]. After preparation of the donated fecal material, it is delivered into the GI tract *via* nasogastric tube or rectal retention enema or colonoscopy [122]. According to a pooled review, both the nasogastric and rectal administrations of fecal microbiota appear to be safe and effective [123]. Landy *et al*. reviewed 22 studies of fecal transplantation and reported a response rate of 87% [124]. Similarly, Gough *et al.* [125] reviewed 27 case series and reports of intestinal microbiota transplantation and reported recurrent CDI cure rate of 92% after the transplantation. Of the 317 patients with recurrent CDI, only 11% required more than one intestinal microbiota transplantation to achieve resolution of symptoms. Finally, a small randomized study in the Netherlands comparing intestinal fecal transplantation with oral VAN had to be terminated prematurely due to significantly better outcomes in the fecal transplantation group [126]. The main drawbacks to the use of fecal transplantation are concerns of potential transmission of an unscreened transmittable infectious disease from the donor to the recipient [127] and the cost a patient incurs if the procedure is not paid for by insurance.

2.11. The Need for Newer Agents to Combat Resistance, Hypervirulence, and Recurrence of *C. difficile*

Only two pharmacologic agents (VAN and FDX) are approved by the U.S. FDA for the treatment of CDI and their use is limited by high cost. In addition, due to the increasing incidence, emergence of resistant and hypervirulent *C. difficile* strains, and the high rate of recurrence of CDI after treatment with the conventional agent for initial episode (i.e., MTZ), there is an urgent need to develop new therapies. Gastroenterologists have resorted to the use of fecal microbiota transplant as last resort to treat refractory CDI (≥ 3 recurrences) in order to avoid surgical intervention that typically removes part or the whole colon. Antibiotics to be used against *C. difficile* should meet several characteristics, which make drug discovery and development for these agents challenging. First, from medicinal chemistry point of view, the anti-difficile agents must often possess unique physiochemical chemical properties (e.g., high molecular weight, low solubility, high polarity, low permeability and absorption) and they should not function as substrates for efflux pumps [128]. Second, newer agents need to have a narrow spectrum of activity in order to avoid disruption of the normal gut flora. Third, they should have novel targets/mechanisms of action to prevent the emergence of resistance and recurrence. Fourth, they should be superior or at least comparable to the currently available treatments, including efficacy, tolerability, and achieve a high concentration at the site of action (decreased permeability).

There are currently several new antibiotics in clinical development for the treatment of CDI (Table **2** and Fig. **2**). Some of these clinical candidates have been previously reviewed by Johnson [129], Zucca *et al.* [115], Koo *et al.* [96], Sun *et al.* [130], Shah *et al.* [131], van Nispen tot Pannerden *et al.* [132], and Joseph *et al.* [133]. Here we review and highlight the chemistry, microbiology, and clinical relevance of these advanced agents for CDI.

2.12. Ramoplanin

Ramoplanin (Fig. **2**) is an oral lipoglycodepsipeptide antibiotic that has showed therapeutic potential for CDI [151, 152]. Ramoplanin exhibited good *in vitro* bactericidal activity against a broad panel of *C. difficile* clinical isolates (MIC_{90} = 0.5 μg/mL) [135] and showed comparable or superior *in vivo* efficacy compared to VAN in the hamster model of clindamycin-induced CDI [153]. Following oral administration, this agent is not absorbed from the GI tract and thus exerts its antibacterial activity locally in the gut with a favorable safety profile and minimal systemic side effects [134]. Ramoplanin works by inhibiting peptidoglycan biosynthesis [136].

2.13. Surotomycin (CB-183,315)

Surotomycin is a novel oral cyclic lipopeptide, which is currently under phase 3 clinical development by Cubist Pharmaceuticals [138]. Surotomycin is a structural analog of daptomycin. Mechanistically, surotomycin works by disrupting membrane potential [138]. The favorable microbiological and pharmacokinetic profiles of surotomycin include potent *in vitro* activity against *C. difficile* (MIC_{90} = 0.5 μg/mL) with good selectivity [154], a bactericidal property, low resistance development, *in vivo* efficacy in the hamster infection model, and low oral bioavailability (<1%) [137, 138]. In addition, surotomycin exhibited good antibacterial activity against a panel of *C. difficile* isolates (MICs ≤ 1 μg/mL) and showed greater activity than VAN and MTZ [155].

2.14. LFF571

LFF571 is a macrocyclic semisynthetic thiopeptide antibiotic, which possessed desirable physiochemical properties and exhibited potent *in vitro* activity (MICs ≤ 0.5 μg/mL) against *C. difficile* [156]. It had potent, relatively narrow spectrum anti-difficile activity with good selectivity [157]. In addition, LFF571 demonstrated more potent *in vivo* efficacy in the hamster model of CDI at a lower dose and with fewer recurrences compared to VAN [158]. From the phase 1 clinical trial, following an oral dose, LFF571 was generally safe with limited systemic exposure and it possessed favorable PK profiles including minimal serum and high fecal concentrations, which warrant further development toward the treatment of CDI [159]. LFF571 exerts its anti-difficile activity by specifically binding *C. difficile* translation elongation factor Tu and subsequently blocking protein synthesis [140].

2.15. Oritavancin

Oritavancin is a novel structural analog of VAN, which belongs to the lipoglycopeptide antibiotic family [160]. Although the major indication of oritavancin in completed phase 3 trials was not for the treatment of CDI, recent studies demonstrated high therapeutic potential of oritavancin as a novel anti-difficile agent. Oritavancin showed good *in vitro* and *in vivo* antibacterial activity against *C. difficile* with <0.1% bioavailability following oral dosing in hamsters [161]. Compared with VAN, oritavancin showed higher therapeutic potential in an *in vitro* human gut model to reduce sporulation and prevent associated symptomatic

Table 2. An Overview of Clinical Pipeline Agents for CDI.

Drug candidate	Chemical class	Company	Status	MIC_{90}	Mechanism
Ramoplanin	Lipoglycodepsipeptide	Nanotherapeutics, Inc.	Phase 3 [134]	0.5 μg/mL [135]	Bacterial cell wall biosynthesis inhibitor [136]
Surotomycin (CB-183,315)	Lipopeptide	Cubist Pharmaceuticals	Phase 3 [137]	0.5 μg/mL [138]	Disruption of membrane potential [138]
LFF571	Semisynthetic thiopeptide	Novartis	Phase 2 [139]	≤ 0.5 μg/mL [140]	Protein synthesis inhibitor [140]
Oritavancin	Semisynthetic lipoglycopeptide	The Medicines Co.	Phase 3 (investigational hospital-based broad spectrum antibiotic)	1 μg/mL [141]	Disruption of membrane potential; peptidoglycan biosynthesis inhibitor [141]
Cadazolid	Quinolonyl-oxazolidinone chimeric antibiotic	Actelion Pharmaceuticals Ltd.	Phase 2 completed	0.064-0.5 mg/L [142]	Protein synthesis inhibitor (primary); DNA synthesis inhibitor [143]
CRS3123 (REP3123)	Thienopyrimidone-tetrahydrochroman *via* a propanediamine linker	Crestone, Inc.	Phase 1 [144]	1 mg/L [145]	Protein synthesis inhibitor [146]
SMT19969	bis(4-pyridyl)bibenzimidazole	Summit PLC	Phase 2 (expected in Q1 2014)	0.125 μg/mL [147]	DNA synthesis inhibitor by binding to DNA [148, 149]
NVB302	Type B lanthionine-containing lantibiotic	Novacta Biosystems Ltd.	Phase 1 completed	1 mg/L [150]	Bacterial cell wall biosynthesis inhibitor by binding lipid II [150]

recurrence [162]. In 2012, Chilton *et al.* showed oritavancin was effective to treat simulated CDI following a 4 day course treatment with less observed recurrence than VAN in the human gut model [163]. Very recently, Chilton *et al.* evaluated oritavancin against *C. difficile* spore germination, outgrowth, and recovery; compared with VAN, oritavancin demonstrated higher potential for leading to early inhibition of germinated cells by adhering to spores, inhibiting subsequent vegetative outgrowth and spore recovery, and preventing recurrences of CDI [164]. Furthermore, in a recent comparative study with VAN, Freeman *et al.* showed that oritavancin exposure did not cause CDI in hamsters or a human gut model, which further supports the development of oritavancin as a potential treatment for CDI [165]. Mechanistically, oritavancin has dual modes of action, inhibiting cell wall peptidoglycan biosynthesis and disrupting membrane potential of Gram-positive bacteria, the latter of which is caused by the lipophilic *N*-alkyl-*p*-chlorophenylbenzyl side tail [141, 160].

2.16. Cadazolid (CDZ, ACT-179811)

Cadazolid (Fig. **2**) is a novel hybrid antibiotic of the oxazolidinone and fluoroquinolone antibacterial classes, which is covalently linked by a 4-methoxypiperidin-1-yl moiety [143]. Cadazolid has demonstrated excellent anti-difficile bactericidal activity (MICs = 0.03-0.25 mg/L) with a limited impact on the gut flora [166, 167]. In addition, cadazolid exhibited potent *in vitro* activity (MICs = 0.064-0.5 mg/L) against 133 *C. difficile* clinical strains isolated from primary and recurrent CDIs in Stockholm, Sweden [142]. Oral doses of cadazolid were well tolerated in phase 1 clinical trial with low absorption and minimal systemic exposure, and the majority of the dose was excreted from feces, leading to high concentrations in the colon [168]. Cadazolid primarily inhibits bacterial protein synthesis with DNA synthesis as a secondary effect [143].

2.17. CRS3123 (REP3123)

CRS3123 (formerly known as REP3123) is a diaryldiamine derivative with a narrow spectrum antibacterial activity (MIC_{90} = 1 mg/L) against *C. difficile* with high selectivity and specificity [145, 146]. Compared with VAN, CRS3123 demonstrated superior *in vivo* efficacy in the CDI hamster model, possibly resulting from its ability to inhibit both toxin production and spore formation [169]. CRS3123 targets methionyl-tRNA synthetase (MetRS) in *C. difficile* protein biosynthesis [146]. A homology model was recently constructed and used in computational docking studies to identify the key binding interactions and guide future design and synthesis of advanced MetRS inhibitors [170]. Resistance to CRS3123 arises from mutations in the MetRS target, but reduced the catalytic efficiency of the enzyme and the microbiological fitness of *C. difficile* [146]. CRS3123 is currently under phase 1 clinical trial as an oral nonabsorbable agent for the treatment of CDI. This study is being conducted by the Division of Microbiology and Infectious Disease (DMID) at NIAID/NIH [144].

2.18. SMT19969

SMT19969 is a novel oral, nonabsorbable small molecule antibiotic with a bis(4-pyridyl)bibenzimidazole scaffold,

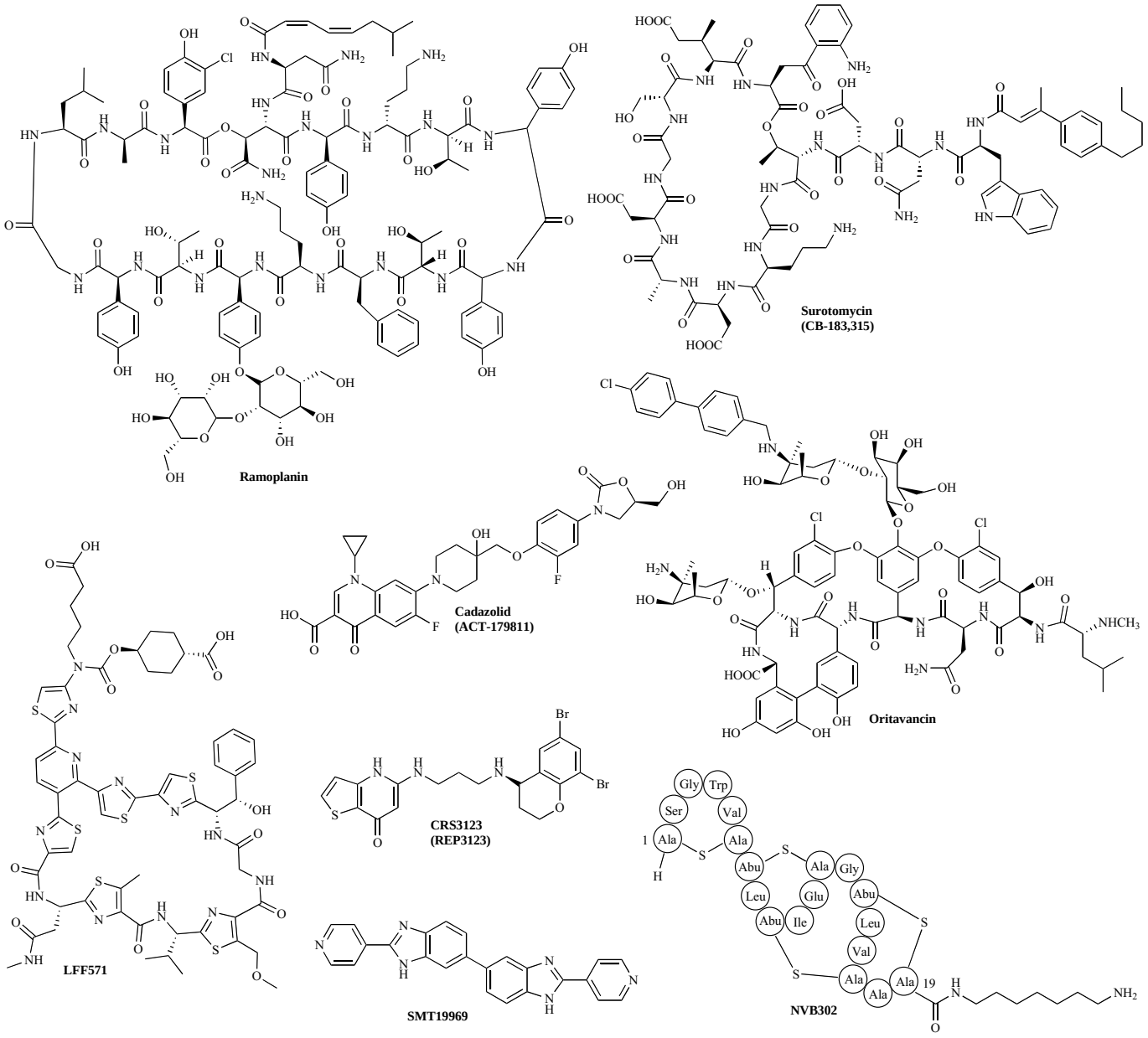

Fig. (2). Chemical structures of antibiotics under clinical development for the treatment of CDI.

which exhibited potent *in vitro* anti-difficile activity (MIC_{90} = 0.125 μg/mL; ranges 0.06-0.25 μg/mL) with favorable bactericidal, GI restricted, and narrow spectrum profiles [147, 171]. In addition, SMT19969 demonstrated greater *in vivo* efficacy than VAN in the hamster model of *C. difficile* with high concentrations in the GI tract and no exposure in plasma [172, 173]. In a 2013 comparative study, Goldstein *et al.* showed that SMT19969 was one-fold more active *in vitro* against a variety of *C. difficile* ribotype isolates than FDX [174]. Specifically, SMT19969 had a MIC_{90} of 0.25 μg/mL (ranges 0.125-0.5 μg/mL), while FDX had a MIC_{90} of 0.5 μg/mL (ranges 0.06-1 μg/mL). Moreover, SMT19969 outperformed MTZ and VAN against *C. difficile*, which displayed MIC_{90} values of 2 and 4 μg/mL, respectively. This agent further demonstrated high selectivity and specificity for *C. difficile* with limited impact on other Gram-positive and -negative anaerobes [174]. The completed phase 1 clinical trial showed that SMT19969 was well tolerated at therapeutically relevant doses in healthy volunteers [175].

2.19. NVB302

NVB302 (Fig. **2**), a semisynthetic peptide derivative (19AA) of deoxyactagardine B [176], is a novel, nonabsorbable type B lantibiotic with therapeutic potential for CDI. This agent showed good anti-difficile activity including the problematic 027 hypervirulent strain (MIC = 1 mg/L) with high selectivity over the normal gut flora, reaching high concentrations in the GI tract [150, 177]. From an *in vitro* gut model study, NVB302 showed non-inferiority to VAN in treating simulated CDI, leading to inhibition of cytotoxin production without affecting spore formation [150]. The completed phase 1 clinical trials revealed that NVB302 was safe and well tolerated in healthy volunteers at therapeuti-

cally relevant doses with minimal systemic absorption and high concentrations of the drug excreted in the feces [177].

3. NEWER AGENTS FOR CDI

3.1. Natural Product or Natural Product Inspired

These antibiotics are of natural origin, e.g., from bacterial sources or plant extracts. Although bacteria and fungi are currently the leading sources of antimicrobials, plants and extracts from plant materials have been used in traditional medicine worldwide and represent an underexploited resource for novel antibacterial therapeutics [178]. These naturally occurring compounds are often part of the plant defense towards attacks by pathogens. Commonly, these compounds are classified as phytoalexins, while other products termed phytoanticipins are inactive [179]. Some common classes of phytochemicals include flavonoids, alkaloids, lactones, and polyphenols which have been reported to show antibacterial activity [178, 180-183]. Due to the rising number of studies that showed activity of plants and their extracts, and the history of efficacy of traditional medicine, these sources may be able to provide a new generation of anti-difficile agents [181]. The antibiotics in Fig. **3** include natural products and natural product analogs with improved anti-difficile activity and/or more desirable physicochemical properties.

3.1.1. Solithromycin (CEM-101)

CEM-101 (Fig. **3**) is a novel macrolide fluoroketolide in the late stage of clinical development for the treatment of community-acquired pneumonia (CAP) [184]. In 2010, Putnam *et al.* [184] reported their results after testing CEM-101 against an expanded panel of bacterial pathogens, including anaerobes. CEM-101 displayed a MIC_{50} of 0.12 μg/mL against *C. difficile* and 0.06 μg/mL against other Clostridia species [184]. Although CEM-101 exhibited good potency against *C. difficile*, this agent lacks specificity, also being potent against *Staphylococcus* and *Enterococcus* spp. [185].

Although it is not a narrow spectrum agent, CEM-101 is promising because it is effective against various bacteria that are resistant to other antibiotics in its class [184]. It has been reported that CEM-101 has a higher activity compared to other macrolide-lincosamide-streptogramin B (MLS_B) compounds due to a higher binding affinity to bacterial ribosomes. It is suggested that the 11,12-cyclic carbamate-*n*-butyl-[1',2',3']-triazolyl-aminophenyl side chain plays an important role in enhancing its binding affinity with its ribosome target [186]. Similar to other ketolides, it was confirmed in a study by Rodgers *et al.* that CEM-101 exerts its antibacterial activity by impairing bacterial ribosome subunit formation [187]. This mode of action was examined in strains of *Streptococcus pneumoniae*, *S. aureus*, and *Haemophilus influenza*; it was found that the large 50S ribosomal subunit formation was reduced in all three test organisms, with IC_{50} values similar to those for solithromycin to inhibit cell viability, protein synthesis, and growth rate (7.5, 40, and 125 ng/mL for *Streptococcus pneumoniae*, *S. aureus*, and *Haemophilus influenzae*, respectively) [187].

3.1.2. RBx 14255

RBx 14255 is a novel ketolide antibiotic and it was reported by Kumar *et al.* to have potent anti-difficile activity in 2012 [188]. It displayed activity against 28 *C. difficile* strains including erythromycin- and clarithromycin-resistant and hypervirulent strains with MICs of 4 μg/mL (ranges 0.125-8 μg/mL). This observed MIC was 2-fold higher than MTZ and 2-fold lower than VAN. RBx 14255 also displayed a more narrow spectrum activity, inhibiting Gram-negative anaerobes *B. fragilis* and *F. nucleatum* at higher MIC ranges of 2-8 μg/mL and 8-16 μg/mL, respectively [188].

The *in vitro* time-kill kinetic study demonstrated that RBx 14255 was bactericidal against *C. difficile* at 4 × MIC and above and the two test *C. difficile* strains did not show any regrowth at the MIC level. In a hamster model of CDI, RBx 14255 treatment was able to extend the animal's survival more than MTZ or VAN treatment [188]. The mode of action of RBx 14255 was consistent with that of other ketolides, inhibiting protein synthesis by 70% at 6 h of incubation [188]. The activity of RBx 14255 against resistant *C. difficile* strains and its favorable *in vitro* activity and bactericidal property make this ketolide promising as an anti-difficile agent [188].

3.1.3. Rifalazil

Rifalazil is a poorly absorbed member of the notable rifamycin antibiotics family with a benzoxazinopiperazine side chain. This agent showed potent *in vitro* anti-difficile activity with the MIC_{90} values ranging from 0.004 to 0.03 μg/mL against a broad panel of *C. difficile* isolates [94, 189]. Rifalazil also demonstrated superior *in vivo* efficacy in a hamster animal model compared with VAN for the prevention of recurrent CDI [189] and has the therapeutic potential to treat CDI [189, 190], however it currently has fewer clinical data available [131]. Similar to the other rifamycin derivatives, rifalazil functions as bacterial RNA polymerase inhibitor [191] and may be rendered inactive by existing rifamycin-resistant strains found in the clinic [101].

3.1.4. Nosiheptide (Multhiomycin)

Nosiheptide, known as multhiomycin, was originally isolated in 1970 [192] and identified from an anti-MRSA screen of marine-derived actinomycete extract libraries by Haste *et al.* in 2012 [193]. Structurally, this compound is similar to the oral cyclic thiopeptide LFF571, a phase 2 clinical candidate for the treatment of moderate CDI [139]. Nosiheptide demonstrated extremely potent *in vitro* activity against a panel of contemporary and clinically significant bacterial strains, including the hypervirulent *C. difficile* BI/NAP1/027 strain with a MIC value of 0.008 mg/L [193]. Furthermore, nosiheptide was strongly active against MRSA and MSSA strains tested, remaining potent against strains that developed resistance to front-line antibiotics.

In addition to impressive MICs, nosiheptide exhibited favorable time-kill kinetics, being rapidly bactericidal against MRSA in a time- and concentration-dependent manner. Nosiheptide also displayed prolonged post-antibiotic effects (PAEs), a lack of cytotoxicity against the human cervical carcinoma HeLa cell line at up to 128 mg/L (about 1000-fold MIC against MRSA), and *in vivo* efficacy in a murine model of MRSA infection following intraperitoneal (IP) administration of nosiheptide [193]. The mode of action of nosiheptide was studied extensively by Cundliffe and Thompson [194] and it was found to inhibit bacterial protein synthesis.

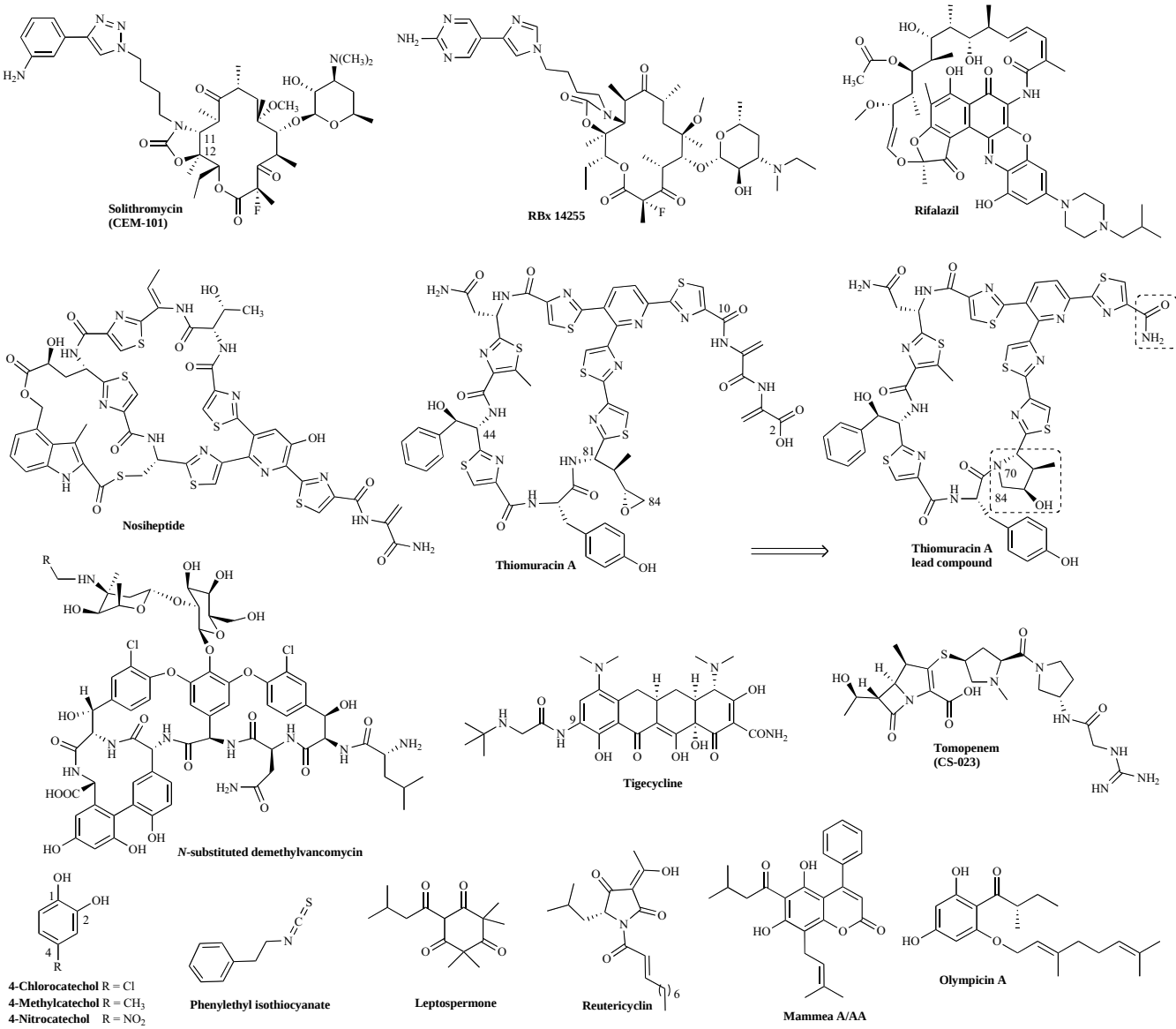

Fig. (3). Chemical structures of natural product inspired antibiotics with anti-difficile activity.

Specifically, nosiheptide binds to the large 50S ribosomal subunit, blocking the domain of the A site into which elongation factors Tu and G bind during protein synthesis [194]. Because of its potent *in vitro* activity against *C. difficile*, the lack of cytotoxicity against mammalian cells, and inactivity against most Gram-negative bacteria tested, nosiheptide may serve as a promising lead compound to be further developed for the treatment of CDI.

3.1.5. Thiomuracin A Analogs

Thiomuracin A (Fig. **3**), produced from a rare actinomycete bacterium *Nonomuraea* species, is a member of a novel family of bacterial secondary metabolites and macrocyclic thiazolyl peptide antibiotics [195]. In 2012, LaMarche *et al.* [196, 197] reported the synthesis and optimization of a series of thiomuracin A derivatives. This work led to the identification of a structurally simplified lead compound of thiomuracin A with retained antibacterial potency, improved chemical stability, and enhanced physicochemical properties. Notably, the structural simplicity and improved organic solubility of the thiomuracin A derivatives facilitated the isolation process and subsequent material supply and thus expedited drug discovery process. The semisynthetic strategies of thiomuracin A included protection of hydroxyl and acid functional groups and acid/base-mediated reactions, e.g., the removal of the C2-C7 side chain, derivatization of the C84 epoxide region, and the N70-84 cyclization of the pyrrolidine ring. The lead compound had a MIC of <0.008 μg/mL against *C. difficile* and a MIC range of 0.25-1 μg/mL against *E. faecalis*, *E. faecium*, *S. aureus*, and *S. pyogenes* [196]. This emerging lead also demonstrated *in vivo* efficacy in a mouse systemic model of *E. faecalis* and *S. aureus*.

The C2 esterified and hydroxyl protected thiomuracin analogs lost significant antibacterial activity with MICs ranging from 1 to >32 μg/mL, suggesting the importance of the two hydroxyl groups and the carboxylic acid functionality toward the antibacterial activity. In contrast, the chlorohydrins and epoxy analogs with the C2-C10 side chain re-

moved retained potent antibacterial activity against all test organisms (MICs = 0.03-0.5 μg/mL) except *S. pyogenes* (MIC = 2 μg/mL) [196]. Compared to the chlorohydrin derivative, the amino and hydroxyl derivatives of the epoxide had increased MICs. Notably, the thiomuracin lead compound with the cyclic pyrrolidine ring retained potent antibacterial activity against all five organisms tested (MICs = <0.008-1 μg/mL). Mechanistically, this class of thiopeptide antibiotics targets bacterial elongation factor Tu (EF-Tu) and blocks subsequent protein synthesis [198].

3.1.6. N-Substituted Demethylvancomycin

In 2012, a series of 17 novel *N*-substituted demethylvancomycin derivatives were synthesized and evaluated *in vitro* against *C. difficile* by Zhang *et al.* [199]. Structurally, demethylvancomycin differs from VAN by the replacement of a methyl group by a hydrogen atom of the *N*-terminal amino group and it demonstrated similar activity as VAN against Gram-positive pathogens [200]. Another structural modification of this glycopeptide is the introduction of a lipophilic side chain on the NH_2 group of the amino sugar moiety such as in telavancin, oritavancin, and dalbavancin [199]. These aforementioned agents have been reported to show greater activity against *C. difficile* than VAN [201, 202]. Based on these observations, a focused chemical library of semisynthetic demethylvancomycin derivatives bearing *N*-substituted arylmethylene and aliphatic motifs was synthesized and tested. Biological evaluation revealed that three *N*-substituted arylmethylene derivatives with structural similarity to oritavancin demonstrated more potent activity against four *C. difficile* strains than VAN or demethylvancomycin with MICs of 0.25, 0.125-0.25, and 0.25-0.5 μg/mL, respectively [199]. This class of glycopeptide antibiotics interacts with the bacterial membrane, dissipates membrane potential, and inhibits peptidoglycan biosynthesis [203].

Semisynthetic derivatives were produced using demethylvancomycin as a starting material following three steps (Fmoc protection, reductive amination, and Fmoc deprotection), with overall yields ranging from 3.2%-23.9%. In the preliminary SAR, semisynthetic derivatives with arylmethylene side chains showed more potent antibacterial activity against *C. difficile* than those with aliphatic side chains. The activity of compounds with benzyl groups improved when reducing the inductive effect of the halogen at the para position (Br>Cl>F). Derivatives with heterocyclic ring motifs had higher MICs against the ATCC 43255 strain (0.5-1 μg/mL) while fused aromatic analogs exhibited lower MICs (0.25 μg/mL). In general, the aliphatic derivatives of demethylvancomycin did not exhibit significant activity against *C. difficile* and the antibacterial activity was inversely related to side chain length [199].

3.1.7. Tigecycline

Tigecycline (Fig. **3**), an intravenous analog of minocycline, is the first glycylcycline antibiotic with glycylamido functionality at the 9 position [131]. It has been used successfully in conjunction with standard therapy in case reports as a salvage for severe refractory CDI [204-207]. Although tigecycline is a broad spectrum antibiotic with both Gram-positive and -negative aerobic and anaerobic bacteria coverage [52, 208], Wilcox *et al.* demonstrated that tigecycline use at regular doses (100 mg IV loading dose followed by 50 mg IV every 12 h) does not increase the growth of epidemic *C. difficile* bacteria or *C. difficile* toxin production [209]. In addition, tigecycline suppresses numerous *C. difficile* strains including the PCR ribotype 027 strain with low MICs of 0.06 mg/L, thereby making it an effective potential alternative agent for refractory CDI [209]. Tigecycline also suppresses other species of *Clostridium* such as *Clostridium tertium*, *Clostridium clostridioforme, Clostridium perfringens*, *Clostridium bifermentans*, *Clostridium butyricum, Clostridium hastiforme*, *Clostridium innocuum*, and *Clostridium sphenoides* with potent MICs of 0.25 μg/mL [210]. Another property of tigecycline which may contribute to its effectiveness against *C. difficile* is the high fecal concentration (mean of 6 mg/kg) at steady state in healthy individuals [211]. It should be noted, however, that an unsuccessful use of tigecycline in treating severe CDI has been reported by Kopterides *et al.* [212]. The fatal case involved an immunocompromised elderly patient with severe CDI who received tigecycline in combination with MTZ, VAN, and IVIG for about three weeks, ended up with two drug resistant Gram-negative bacteria, and eventually died of sepsis [212]. In July 2013, a small prospective, non-comparative, interventional, observational pilot study evaluating the safety and efficacy of IV tigecycline in conjunction with standard therapy was completed [213]. This trial enrolled 10 patients with known mild to severe confirmed CDAD [213]. However, the outcome of this study is not available yet.

Taken together, since all the clinical evidence of tigecycline use to treat CDI are from case reports, a large randomized controlled study is needed to determine the therapeutic potential of tigecycline for the treatment of CDI, as it is a broad spectrum antibiotic capable of disrupting the normal ecology of the human gut flora. Mechanistically, tigecycline inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit and subsequently blocking entry of amino-acyl tRNA into the A site of the ribosome [214].

3.1.8. Tomopenem

Tomopenem (CS-023/R04908463) is a carbapenem that has been previously shown to have broad spectrum activity against Gram-positive and -negative anaerobic organisms [215]. Tanaka *et al.* [216] investigated this agent against anaerobic bacteria, reporting their findings in 2009. Tomopenem had a MIC_{50} of 1 μg/mL and a MIC_{90} of 2 μg/mL against *C. difficile* [216].

Several studies have recently demonstrated that carbapenems with a longer side chain than commercially available β-lactams have a higher affinity to penicillin-binding protein 2a (PBP 2a). It is suggested that the longer side chain increases the interactions with the active site groove of PBP 2a. Although not studied in *C. difficile* extensively, the anti-MRSA activity of tomopenem is indicated to be related to its structural features, such as a 2-guanidoacetylamino pyrrolidine moiety. The presence of this moiety yields a molecule that could have better positioning within the groove, thereby allowing more rapidly acylation as compared to carbapenems currently available. This makes tomopenem more attractive as an antibacterial agent as the major resistance mechanism of β-lactams in MRSA is the low binding affinity to PBP 2a [215, 217].

3.1.9. Catechols

In 2009, Jeong *et al.* [218] isolated several catechol compounds from the roots of *Diospyros kaki*, which has been used in traditional Korean medicine to treat several ailments and for antioxidant purposes. The compounds were evaluated against harmful intestinal bacteria, including *C. difficile* using the paper disc agar diffusion method. At a concentration of 5.0 mg/disc, 4-chlorocatechol, 4-methylcatechol, 4-*t*-butyl-catechol, and tetrabromocatechol strongly inhibited the growth of *C. difficile* with a zone of inhibition of 21-30 mm [218].

Although the mechanism of action of the catechol compounds was not investigated in this study, because of its structural similarity to phenol, the compounds may have a similar mechanism of action. It has been agreed upon in the majority of the literature that phenols act on the membrane by destroying its permeability characteristics or by acting as uncouplers and increasing permeability of protons [219]. However, further studies are needed to confirm the mechanism of action of the catechol compounds as well as to evaluate and optimize the activity of these promising agents.

The preliminary SAR showed that the 3-substituted catechol compounds were generally less effective toward inhibiting the growth of intestinal bacteria than the 4-substituted catechols. The substitutions that led to the most selective growth-inhibiting activities at low concentrations were 4-nitro and *tert*-butyl functionalities [218]. Studies of the SARs of these catechols are still warranted as the 4-substituted compounds contained different functional groups compared with the 3- substituted catechols. Therefore it needs to be clarified as to whether the increased activity was due to position or functional group. Additionally, the mode of action of catechols need to be defined, determining whether these compounds cause membrane pore formation which would be an undesirable effect resulting in the leakage of intracellular toxins A and B.

3.1.10. Phenethyl Isothiocyanate

Phenethyl isothiocyanate was isolated from the seeds of *Sinapis alba* L. (white mustard), which has been used as a spice and in traditional medicine [220]. In 2009, Kim and Lee reported the activity of this compound and its derivatives against *C. difficile* [220]. The paper disc agar diffusion method was used to determine the growth inhibiting properties of the compounds; biological evaluation showed that phenethyl and benzyl isothiocyanates produced strong inhibition against *C. difficile* at 2 mg/disc with an inhibitory zone diameter of >30 mm [220]. In general, aromatic isothiocyanates produced greater inhibition of clostridia than aliphatic derivatives [220]. In a 2004 study by Hideki *et al.* [221], it was suggested that isothiocyanate compounds exhibit their antimicrobial effects by inhibiting the cell cycle and inducing apoptosis. However, the mode of action of phenethyl isothiocyanate was recently reported to arise from disturbance of membrane function, resulting in a loss of membrane integrity and cell death [222].

3.1.11. Leptospermone

In 2009, Jeong *et al.* [223] reported on the anti-difficile properties of leptospermone, a cyclic triketone that was isolated from the essential oil of *Leptospermum scoparium* seeds. *L. scoparium* is a medicinal plant that has been traditionally used by the Maori tribes of New Zealand to treat pain and fevers [223]. When tested against *C. difficile* using the paper disc agar diffusion method, leptospermone produced strong inhibition at 1.0 mg/disc and potent inhibitory effects at 5.0 mg/disc, corresponding to a zone of inhibition of 21-30 mm and >30 mm, respectively [223]. Four related compounds not isolated from *L. scoparium* were also tested against intestinal bacteria and 1,2,3-cyclohexanetrione-1,3-dioxime was found to strongly inhibit the growth of *C. difficile* but at higher concentrations of 5.0 and 2.0 mg/disc [223].

This work reported that leptospermone and 1,2,3-cyclohexanetrione-1,3-dioxime, containing cyclohexane conjugated trihydroxyl ketones, showed more inhibitory activity against *C. difficile* and *C. perfringens*. In contrast, 1,3-cyclohexanedione, 2-acetyl-1,3-cyclohexanedione, and 5,5-dimethyl-1,3-cyclohexanedione did not inhibit the growth of *C. difficile* to any significant extent. From these findings it was then concluded that the 1,3-dihydroxyl ketone motif played an important role in the antibacterial properties of these triketone compounds [223], which may function as ionophores. Based on the preliminary data, leptospermone may have the potential to serve as an anti-difficile natural product lead but further studies and optimization are currently warranted, including the improvement of antimicrobial stability as well as potency.

The mode of action of leptospermone and 1,2,3,-cyclohexanetrione-1,3-dioxime was not reported in this study but it has been suggested that triketones act on the cytoplasmic membrane. This would provide explanation as to why these compounds are generally more selective for Gram-positive bacteria, as Gram-negative bacteria have an outer membrane [224]. However, the mode of action of these particular triketones still warrants investigation.

3.1.12. Reutericyclin

Reutericyclin (Fig. **3**) is a naturally occurring tetramic acid that is produced by strains of *Lactobacillus reuteri* [225]. It is a small nonpeptide antibiotic that has a narrow spectrum activity against Gram-positive bacteria. Reutericyclin and several related analogs were explored by Hurdle *et al.* [225] for anti-difficile properties and the results were reported in 2011. Interestingly, the reutericyclins demonstrated concentration-dependent killing and were bactericidal against both logarithmic and stationary phase cells at concentrations of 0.09-2 mg/L [225]. While MTZ had some activity at elevated concentrations (>8 mg/L), reutericyclins were superior to VAN, which lacked activity against stationary phase *C. difficile* [225].

For synthetic reutericyclin analogs, the predicted intestinal absorption varied significantly from absorbed to non-absorbed when examined in the Caco-2 permeability model. In this model, reutericyclin's predicted permeability was comparable to that of VAN. However, the replacement of the α, β-unsaturated group in reutericyclin with a bicyclic myrtenyl motif showed an increased absorption, while a long alkyl chain substituent made the analog relatively non-absorbed. It is suggested that the non-absorption of the latter

analog resulted from efflux pumps, which may be desirable to retain the drug at the site of action in the colon [225]. Nonetheless, the high lipophilicity and poor solubility of these compounds hindered their *in vivo* efficacy in the hamster model of CDI [226].

Although the mechanism of action of the reutericyclin analogs was not reconfirmed in this study, reutericyclin's mechanism of action has been previously shown to result from dissipation of the bacterial transmembrane potential [227]. This has been identified as an advantageous mechanism of action for treating CDI, since toxins A and B are produced by slow and non-growing cells that are refractory to antibiotic killing [226].

3.1.13. Mammea A/AA

Mammea A/AA (4-phenyl-5,7-dihydroxy-6-(3-methylbutanoyl)-8-(3-methyl-2-butenyl)-2*H*-1-benzopyran-2-one) is a naturally occurring coumarin that was isolated from the stem bark of *Mammea Africana*, a plant used traditionally in Africa for its medicinal properties [228]. Canning *et al.* isolated this 4-phenylcoumarin and reported its antimicrobial activity against clinically significant bacteria in 2013 [228]. It was found that the pure mammea A/AA had an inhibitory effect on *C. difficile* that was comparable to MTZ, with a MIC of 0.25 μg/mL (crude extract: MIC = 1 μg/mL) [228]. Under the specified experimental conditions, the MIC was superior to that of VAN (MIC = 0.5 μg/mL). These results were promising as the *C. difficile* strain used in this study was resistant to ciprofloxacin (fluoroquinolone) but was still strongly inhibited by the isolated coumarin compound [228].

The mechanism of action of mammea A/AA and related coumarins was not elucidated in this study, but it has been agreed upon in numerous reports that the coumarins are a family of inhibitors of DNA gyrase B, inhibiting the ATPase activity of the β subunit [229-233]. Specifically, the coumarins and ATP bind competitively to gyrase. The amino acid side chains Asp73 and Thr165 are suggested to play key interactions between the target protein and the ligands. Another class for this binding site is the cyclothialidines, and although the binding sites for these two families of inhibitors overlap to some extent, the binding pockets/ interactions are not identical. Therefore, novel coumarin derivatives such as mammea A/AA may take advantage of these different interactions, which may aid in preventing drug resistance [231]. Although this compound displayed good potency and its mechanism may be able to overcome resistance, cell line treatment revealed that mammea A/AA was also cytotoxic against normal mammalian cells tested [228]. Future research should therefore be focused on the modification of these coumarins to maintain potency against bacteria while reducing toxicity.

3.1.14. Olympicin A Analogs

Flavonoids are a large family of polyphenol phytochemicals and they have been shown to mediate diverse activities [234]. In 2013, the anti-difficile activity of naturally occurring flavonoid compounds was reported by Wu *et al.* [235]. Synthetic olympicin A (Fig. **3**) displayed MICs of 1-2 mg/L against two genetically distinct strains of *C. difficile*. Four chemically related flavonoid analogs (three with a chalcone motif and one 4-chromanone analog) retained activity against *C. difficile* with MICs of 0.5-4 mg/L [235].

Out of the 22 naturally occurring flavonoids that were examined in this study, the majority displayed poor or no activity against *C. difficile*. Partially active compounds, with MICs in the range of 16-64 mg/L, included flavanone, 2'-hydroxyflavanone, 6-hydroxyflavanone, naringenin, hesperetin, taxifolin, and 6-aminoflavone. The most potent activity of this series of compounds tested was observed in those that were structurally related to olympicin A, suggesting that it may act as a pharmacophore [235].

Notably, these flavonoid lead compounds demonstrated concentration dependent killing of both logarithmic and stationary phase cultures [235]. Furthermore, the compounds may have the potential to minimize symptoms of CDI due to their reduction of toxin synthesis and sporulation, especially in hypervirulent strains that have up-regulated toxin and spore production. Dissipation of the membrane potential was observed for cells that were treated with olympicin A, two of the chalcone analogs and the 4-chromanone analog [235]. However, the analog with a chalcone motif and piperidyl moiety caused neither depolarization nor hyperpolarization. Therefore this compound may have a distinct mechanism of action from the other compounds tested as flavonoid molecules have been recognized to inhibit various targets such as DNA gyrase and energy metabolism [180].

3.2. Synthetic Small Molecule Antibiotics

Small molecules that are totally synthetic are the second component of the current antibiotic repository. One such example, the synthetic fluoroquinolones are a class of highly effective and broad spectrum antibiotics [236]. However, recent efforts based on high-throughput screens of novel targets identified by bacterial genomics to discover and develop new synthetic scaffolds have not yet been as successful as natural products [237, 238]. Despite this conclusion, synthetic small molecule antibiotics are still alluring to develop due to their structural simplicity and ease of production if found to be an effective therapeutic. Furthermore, advances in novel target identification and screening methodology may prove to find more active synthetic scaffolds in the future [238, 239]. The recently reported synthetic molecules found to be active against *C. difficile* are summarized in Fig. **4**.

3.2.1. Thiosemicarbazones

Costello *et al.* investigated the activity of a series of thiosemicarbazone derivatives, reporting a thiosemicarbazone lead compound (Fig. **4**) with promising activity in 2008 [240]. This compound was tested against a panel of clinically important organisms and showed a MIC of 4-8 μg/mL against the reference strain NCTC 11204 of *C. difficile* and MICs of 0.125-32 μg/mL against over 30 clinical strains tested. Moreover, it was largely inactive against the Gram-negative microorganism *Acinetobacter* spp. or *C. albicans* yeast strains [240].

The thiosemicarbazones were synthesized in two steps with yields of 59-93% for the nitrofuranyl compounds and 27-57% for the furanyl compounds [240]. Three other thiosemicarbazone compounds with noticeable antimicrobial

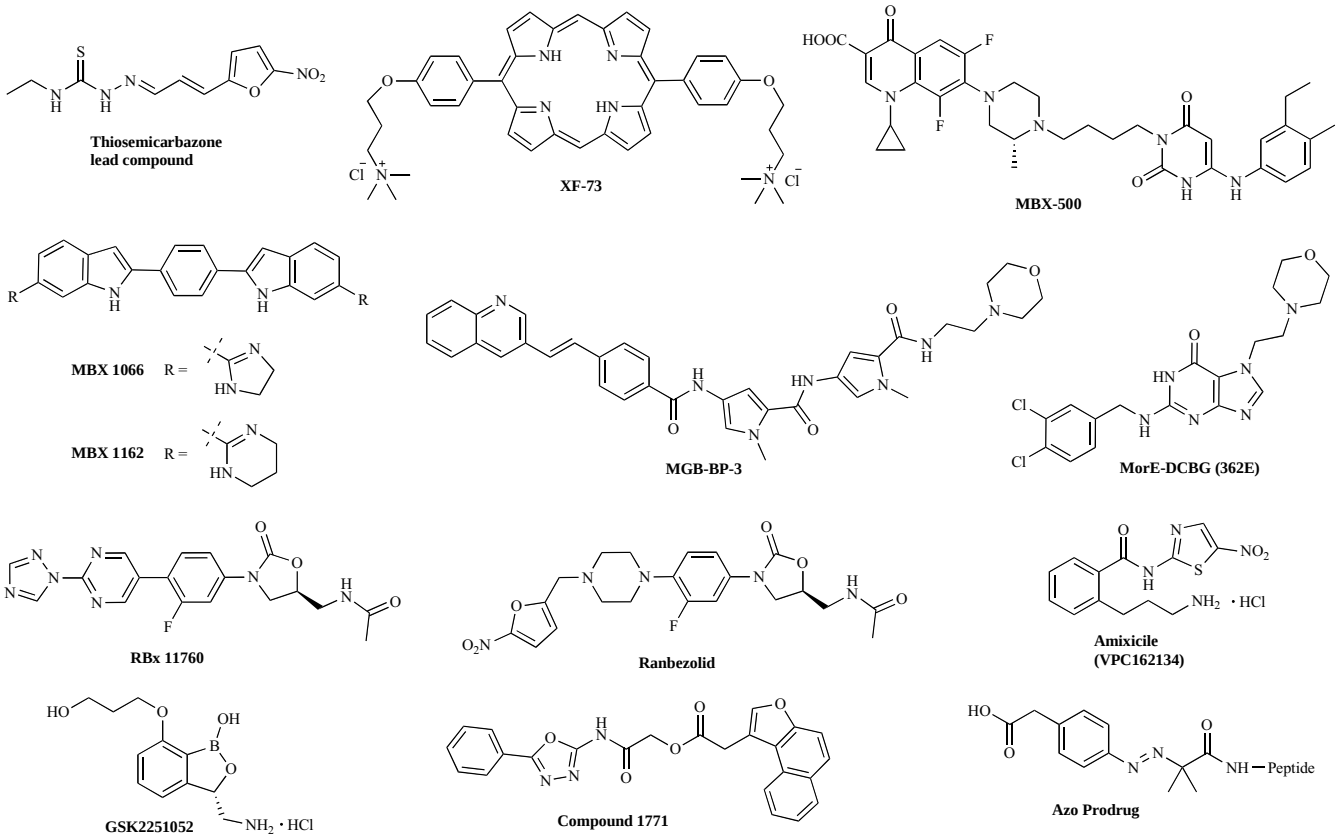

Fig. (4). Chemical structures of synthetic small molecule anti-difficile agents.

activity all share the 5-nitrofuran heterocyclic moiety. It was suspected that the observed SAR may be due to the differences in specific target interactions instead of the differences in cellular penetrations as the LogP values for the inactive thiosemicarbazones were very similar to that of the identified lead compound. The mechanism of action of this compound was not investigated in the study and currently warrants further investigation [240]. However, it can be speculated that the antibacterial property of this thiosemicarbazone lead compound is at least in part attributed to its nitroheterocyclic component. Compounds containing 5- and 2-nitroimidazoles and 5-nitrofurans have been clinically used in a variety of therapeutic agents to treat bacterial and parasitic infections [241]. A compound with a similar component, nifurtimox, leads to cellular damage by the formation of free radicals which then interact with macromolecules to cause DNA damage [241].

3.2.2. XF-73

Reported in 2010 by Farrell *et al.* [242], XF-73 is a dicationic porphyrin derivative that has been investigated against a broad panel of antibiotic resistant bacterial pathogens. Irrespective of the antibiotic resistance profile of the test isolates, XF-73 was active against all Gram-positive bacteria tested. The MIC against *C. difficile* specifically was 1.0 mg/L [242]. Furthermore, XF-73 demonstrated excellent bactericidal activity against all Gram-positive strains tested (MICs = 0.25-4 mg/L). Antibacterial activity against Gram-negative species was lower than for Gram-positive, with MICs ranging from 1 mg/L to >64 mg/L [242].

Although the mode of action of XF-73 is not fully understood, the mechanistic studies of XF-73 against *S. aureus* showed that the compound exhibits a rapid cell membrane-perturbing activity [242]. The drug may exhibit its action by rapidly disturbing the cell membrane, thereby inhibiting macromolecular synthesis [243]. Gram-negative bacteria are more difficult to penetrate due to their outer cell membrane [244], therefore, the less permeable outer cell wall of Gram-negative bacteria may prevent accumulation of XF-73 at the cytoplasmic membrane, resulting in a lower killing efficacy compared to Gram-positive pathogens [242].

3.2.3. MBX-500

MBX-500 is a fluoroquinolone/anilinouracil hybrid antibiotic connected by a 4-*n*-butyl-3-methylpiperazin-1-yl linker. It was reported to show *in vitro* and *in vivo* efficacy against toxigenic *C. difficile* in 2012 by Butler *et al.* [245]. From this study, this compound was evaluated against a panel of *C. difficile* strains with varying susceptibilities to antibiotics. The MIC_{90} against antibiotic-sensitive isolates was 2 µg/mL and increased to 4 µg/mL against antibiotic-resistant isolates. MBX-500 also had the same potency against the NAP1/027 strains that were resistant to the fluoroquinolone antibiotic, moxifloxacin [245]. These results were interesting as good potency was maintained against fluoroquinolone-resistant *C. difficile* isolates despite the fact that MBX-500's activity is partially attributed to the fluoroquinolone scaffold [245]. Importantly, MBX-500 exhibited narrow spectrum activity with good selectivity against other gut anaerobic bacteria. Furthermore, it demonstrated *in vivo*

efficacy in hamster and murine models of CDI without systemic absorption, when administered orally [245]. In addition, MBX-500 also showed *in vivo* efficacy in the gnotobiotic pig model for the treatment of CDI [246].

Mechanistically, due to its hybrid structural feature from an anilinouracil DNA polymerase inhibitor and a fluoroquinolone DNA gyrase/topoisomerase inhibitor, MBX-500 has multiple bacterial targets and functions as dual DNA polymerase inhibitor and DNA gyrase/topoisomerase inhibitor [245]. Taken together, this hybrid MBX-500 antibiotic represents a promising agent for CDI.

3.2.4. MBX 1066 and MBX 1162

Reported in 2010, MBX 1066 and MBX 1162 (Fig. **4**) are two novel bis-indole derivatives that were synthesized and evaluated by Butler *et al.* [247] for activity against Gram-positive and -negative pathogens. MBX 1066 was identified through a screen campaign of the NCI repository for activity against *Bacillus anthracis*. The MIC_{90} for MBX 1066 was 0.12 μg/mL with a range of 0.03-0.25 μg/mL against 18 isolates of *C. difficile* [247].

Although the exact molecular target(s) of the compound is currently unknown, this compound has a relatively rigid and planar structure, suggesting it may function as a DNA binding agent [248]. Indeed, Panchal *et al.* previously demonstrated that MBX 1066 was a potent inhibitor of DNA synthesis [249]. MBX 1162, an analog of MBX 1066, was evaluated and it showed enhanced anti-Gram-negative activity and similar anti-Gram-positive activity to MBX 1066. The MIC_{90} for MBX 1162 was 0.12 μg/mL with a range of 0.03-0.12 μg/mL against *C. difficile* strains tested [247].

3.2.5. MGB-BP-3

MGB-BP-3 belongs to a new class of antibacterial agents reported by Ravic *et al.* and it was shown to have efficacy in a hamster CDAD model [250]. *C. difficile* burdens were measured before and after a single oral dose of MGB, and the highest reductions were seen after 2 h in the small intestine and at 2-10 h in the cecum and colon. Furthermore, after oral delivery of MGB, C_{max} for the small intestine was within 15 min, 2-6 h for the cecum and 4-6 h for the colon, showing that MGB has a favorable PK profile to treat CDI with minimal systemic exposure [250]. Mechanistically, the MGB compound functions as a DNA binding agent, binding selectively to the minor groove of DNA and inhibiting DNA synthesis [250].

3.2.6. MorE-DCBG (362E)

Dvoskin *et al.* developed a series of 7-substituted-N^2-(3,4-dichlorobenzyl)guanines, reporting their results in 2012 [251]. The compound MorE-DCBG (362E) displayed the most promising anti-difficile activity as well as other desirable attributes including marginal GI absorption and no observable toxicity following oral administration. The MICs for this compound was 2-4 μg/mL, which was comparable to that of MTZ and VAN [251]. Although the SAR of this series of guanine derivatives was not studied extensively, the linker length between the guanine and morpholinyl group affected the antibacterial activity. MorE-DCBG (362E) had the shortest linker with n = 2. When increasing the length by one methylene group, the MIC value increased to 8 μg/mL. At n = 4, MIC was 4-16 μg/mL and at n = 5, MIC was 8 μg/mL [251].

In mode of action studies, it was found that MorE-DCBG (362E) selectively inhibits *C. difficile* DNA replication. Specifically, its mechanism of action is to inhibit the DNA polymerase IIIC [252], which gives this agent high potential to bypass resistance that *C. difficile* is likely to develop with currently used treatments. These findings establish the lead compound as a potential candidate for continued development for the treatment of CDI [251].

3.2.7. RBx 11760

Novel biaryl oxazolidinones have been previously demonstrated to have potent activity against Gram-positive bacteria [253]. RBx 11760 is a novel biaryl oxazolidinone compound that was reported by Mathur *et al.* in 2011 as a potential anti-difficile agent [254]. The *in vitro* activity of RBx 11760 was compared to VAN and MTZ, two currently used treatments, and linezolid, an oxazolidinone that is active against Gram-positive, anaerobic pathogens [254-257]. RBx 11760 was active against the *C. difficile* isolates with a MIC_{50} of 0.5 mg/L and a MIC_{90} of 1 mg/L. This activity was comparable to that of MTZ and VAN, and was more potent than linezolid under the experimental conditions. Furthermore, it was demonstrated that RBx 11760 was active against hypervirulent *C. difficile* strains, displaying high MIC_{90} (16 mg/L) against Gram-negative anaerobes [254]. Notably, RBx 11760 also demonstrated *in vivo* efficacy in the hamster infection model [254].

Kinetic studies of RBx 11760 demonstrated concentration-dependent killing of *C. difficile* (ATCC 43255) and *C. difficile* (6387) up to 2-4 × MIC (1-2 mg/L). Conversely to VAN, MTZ, and linezolid, RBx 11760 resulted in attenuation of *de novo* toxin production and sporulation in *C. difficile* isolates at low concentrations 0.25-0.5 mg/L. Furthermore, treatments of the *C. difficile* isolates with VAN and MTZ may have appeared to promote the formation of spores. This observation for MTZ and VAN promoting spore formation is supported by Ochsner *et al.* [169].

RBx 11760 was evaluated for protein synthesis inhibition and displayed a more potent inhibition of protein synthesis even at sub-MIC concentrations relative to linezolid. This mode of action may offer a possible explanation as to why RBx 11760 inhibits toxin production and protein coat synthesis, thereby reducing sporulation, which is not seen in MTZ or VAN. This may offer an advantage as a treatment option as spores contribute to the spread of infections, and as they are highly resistant and difficult to eradicate [254].

3.2.8. Ranbezolid

Ranbezolid belongs to an oxazolidinone chemical class of synthetic antimicrobial agents that have potent activity against Gram-positive and -negative bacterial pathogens [258]. Notably, this compound exhibited improved activity against Gram-negative anaerobes over linezolid, the first-in-class oxazolidinone antibiotic. Mathur *et al.* reported the MIC of ranbezolid against *C. difficile* (ATCC 700057) to be 0.03 μg/mL in 2013 [258]. The MICs of ranbezolid in this study were 32-128-fold lower than those of linezolid and 8-32-fold lower than those of MTZ against the anaerobic strains tested [258].

Although the mechanism of action and time-kill kinetics were not determined for *C. difficile*, it was studied in *B. fragilis*. Protein synthesis was inhibited at 2 h, demonstrating that ranbezolid is able to quickly impede protein synthesis even in slow growing bacteria. Furthermore, non-specific inhibition of cell wall biosynthesis was seen at 6 h after treatment with ranbezolid [258]. Structurally similar to linezolid, ranbezolid's piperazine ring makes van der Waals contacts with the 50S ribosome sugar residues. Docking studies showed that the nitrofuran moiety forms additional interactions with the residues of the 50S ribosome and may be one of the reasons why it functions as a potent protein synthesis inhibitor of both Gram-negative and -positive pathogens [259].

3.2.9. Amixicile

Amixicile is a derivative of NTZ, a FDA-approved drug for the treatment of diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia* [72, 260]. From a library of ~250 analogs of NTZ, Warren *et al.* identified lead compounds that displayed increased antibacterial potency, reporting their results in 2012 [261]. The selected derivative, amixicile, had MICs of 0.25-1.0 μg/mL against *C. difficile* strains [261].

Previous studies have identified the 2-aminonitrothiazole head group as being responsible for the antimicrobial activity of NTZ [262, 263]. Therefore, benzene ring substitutions as well as couplings of heterocyclic moieties to this head group were employed as the design strategy to produce derivatives with favorable physiochemical properties. For example, most analogs including NTZ had notable solubility issue (<10 μg/mL) while the new derivatives containing ether-linked aliphatic amines showed improved solubility (~0.4 mg/mL) with amixicile having the greatest improvement in solubility (10 mg/mL) [261].

Mechanistically, NTZ targets bacteria expressing PFOR. Specifically, NTZ inhibits the thiamine pyrophosphate (TPP) cofactor for PFOR by outcompeting its substrate, pyrvuvate, by nearly 2 orders of magnitude [261]. In docking simulations with the crystal structure of PFOR, it is shown that 2-aminonitrothiazole is positioned proximal to TPP, with the benzene tail group pointing outwards. From the docking study, it is suggested that amixicile's 1.5-fold increase in PFOR inhibition is correlated with a slight enhancement in binding as compared to NTZ, which may be the result of the propylamine tail. This tail group aids binding by forming additional interactions with the PFOR pocket amino acids [261].

3.2.10. GSK2251052

Goldstein *et al*. reported that GSK2251052 (Fig. **4**), a novel boron-containing antibacterial agent, showed *in vitro* activity against *C. difficile* [264]. Although this compound is currently in clinical development for the treatment of Gram-negative infections, it also shows good activity against anaerobic Gram-negative and -positive organisms found in intra-abdominal infections [264, 265]. Very recently, Goldstein *et al.* determined the *in vitro* activities of GSK2251052 against 916 strains of clinically important anaerobic organisms and the $MIC_{50/90}$ against *C. difficile* was found to be 4 μg/mL under the experimental conditions [264]. This compound and its related benzoxaborole compounds trap tRNA in the editing domain of leucyl tRNA synthetase, thereby inhibiting the enzyme [266].

3.2.11. Compound 1771

Compound 1771 [2-oxo-2-(5-phenyl-1,3,4-oxadiazol-2-ylamino) ethyl 2-naphtho[2,1-*b*]furan-1-ylacetate] was identified by Richter *et al.* [267] through the screening of small molecule libraries for compounds that selectively inhibited the growth of *S. aureus*. Reporting their findings in 2013, the MICs of compound 1771 against *S. aureus* and *B. anthracis* were 12.5-50 μM [267]. Its mechanism of action is to inhibit the biosynthesis of lipoteichoic acid (LTA), a cell wall polymer of Gram-positive bacteria. Because *C. difficile* synthesizes LTA with distinct phosphate-polymer structures, studies of the effects of compound 1771 against *C. difficile* are still warranted. In addition, compound 1771 demonstrated inhibition of growth of antibiotic-resistant Gram-positive bacteria and demonstrated *in vivo* efficacy in the mice model of *S. aureus* sepsis. Therefore it has the potential to be developed into a therapeutic agent and/or validates LTA synthase as an advanced target for antibiotic development [267]. A potential benefit to targeting LTA biosynthesis is that teichoic acids are lacking in Gram-negative bacteria making inhibitors such as 1771 narrow spectrum and suited for controlling Gram-positive *C. difficile* while sparing the major Gram-negative normal flora.

3.2.12. Azo Prodrug

In 2011, Kennedy *et al.* [268] reported the synthesis of azo mutual prodrugs containing 5-aminophenylacetic acid or 5-aminosalicylic acid (5-ASA) with antimicrobial peptides. The prodrugs were designed to deliver nonsteroidal anti-inflammatory agents (NSAIDs) with an antimicrobial agent to treat both the infection and symptoms caused by *C. difficile*. The aim was to protect the ammonium terminal of the antimicrobial peptide by an azo bond with an anilinic anti-inflammatory agent so that both components are maintained in an inactive state before reaching its site of action in the colon. This would potentially increase therapeutic outcomes, as it would avoid the ulceration side effects of the NSAID and reduce the disruption of the native microbiota by the antimicrobial peptide while it passes through the upper GI tract [268]. An analog of temporin A, L512TA was used as the antimicrobial peptide portion of the prodrug. It was previously reported by Wade *et al.* to have MICs of 2.8-5.2, 10.5, and 5.6 μM against strains of *S. aureus*, *E. faecalis*, and *E. faecium*, respectively [269]. However, the activity of this antimicrobial peptide and the produced azo mutual prodrug still needs to be extensively evaluated against *C. difficile* strains.

3.3. Other Miscellaneous Agents

In addition to natural product and small molecule-based anti-difficile agents, the other miscellaneous agents as potential treatments for CDI include antimicrobial peptides [115] (e.g., the peptide thuricin CD antibiotic [270, 271] bearing a two-component peptide motif with nanomolar anti-difficile activity and little impact on the commensal flora) and nonantimicrobial polymer-based toxin-binding and/or -neutralizing agents (e.g., cholestyramine, synsorb 90, and tolevamer**)**.

However, it should be noted that, consistent with poor efficacy in phase 3 clinical trials [272], tolevamer did not show efficacy in the neutralization of cytotoxin in a human gut model of CDI [273].

CONCLUSION

CDI is currently on the rise, with the bacterium posing many challenges to practitioners in the healthcare field and researchers in drug discovery and development. In the clinical setting, the bacterium is increasingly problematic in overcrowded hospitals, as the spores are difficult to eradicate and easily spread through healthcare personnel. Although the options currently available are able to manage mild-to-moderate cases of *C. difficile*, the handling of more severe cases may require prolonged treatment with conventional agents or combination of conventional and adjunctive agents and if not successful, more unorthodox and/or invasive options, such as fecal transplants and colectomy may be warranted. Therefore, drug discovery and the approval of new therapeutic agents may alleviate the increase in *C. difficile*-associated cases. First, developing more narrow spectrum antibiotics to be used for common infections can prevent the onset of CDI. These may serve as a replacement for the broad spectrum drugs that disrupt the normal gut flora and leave the host susceptible to *C. difficile*. Secondly, specific anti-difficile agents with novel mechanisms of action, increased potency, and enhanced selectivity and specificity may become superior to FDX, VAN, and MTZ in both curing of CDI and prevention of subsequent relapses. These goals achieved singularly or together will greatly improve the outcomes of those afflicted by *C. difficile* and its associated diseases.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

D.S. thanks financial support from the National Institutes of Health Grants P20RR016467, P20GM103466, and R15AI092315. J.G.H. acknowledges funding from Grant 5R01AT006732 from the National Center for Complementary and Alternative Medicine at the National Institutes of Health.

ABBREVIATIONS

5-ASA	=	5-aminosalicylic acid
bid	=	Two times a day
CDC	=	Centers for Disease Control and Prevention
CDAD	=	*Clostridium difficile*-associated diarrhea
C. difficile	=	*Clostridium difficile*
CDI	=	*C. difficile* infection
CA-CDI	=	Community-associated CDI
CDZ	=	Cadazolid
DMID	=	Division of Microbiology and Infectious Disease
DNA	=	Deoxyribonucleic acid
EF-Tu	=	Elongation factor Tu
EIA	=	Enzyme immunoassay
FDA	=	Food and Drug Administration
FDX	=	Fidaxomicin
GI	=	Gastrointestinal
SHEA/IDSA	=	Society for Healthcare Epidemiology of America/Infectious Diseases Society of America
IgG	=	Immunoglobulin G
IVIG	=	Intravenous immunoglobulin
LTA	=	Lipoteichoic acid
LTCF	=	Long term care facility
MIC	=	Minimum inhibitory concentration
MTZ	=	Metronidazole
NTZ	=	Nitazoxanide
NSAID	=	Nonsteroidal anti-inflammatory drug
PBP	=	Penicillin-binding protein
PCR	=	Polymerase chain reaction
PFOR	=	Pyruvate:ferredoxin oxidoreductase
PMC	=	Pseudomembranous colitis
qid	=	Four times a day
RNA	=	Ribonucleic acid
SAR	=	Structure activity relationship
SCr	=	Serum creatinine
tid	=	Three times a day
TPP	=	Thiamine pyrophosphate
VAN	=	Vancomycin
VRE	=	Vancomycin resistant enterococcus
WBC	=	White blood cells

REFERENCES

[1] Kelly, C. P.; Pothoulakis, C.; LaMont, J. T., *Clostridium difficile* colitis. *N. Engl. J. Med.,* **1994,** *330* (4), 257-262.

[2] McFarland, L. V.; Mulligan, M. E.; Kwok, R. Y.; Stamm, W. E., Nosocomial acquisition of *Clostridium difficile* infection. *N. Engl. J. Med.,* **1989,** *320* (4), 204-210.

[3] Bartlett, J. G.; Chang, T. W.; Gurwith, M.; Gorbach, S. L.; Onderdonk, A. B., Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. *N. Engl. J. Med.,* **1978,** *298* (10), 531-534.

[4] Bartlett, J. G., *Clostridium difficile*: old and new observations. *J. Clin. Gastroenterol.,* **2007,** *41* (Suppl. 1), S24-S29.

[5] Borriello, S. P., The influence of the normal flora on *Clostridium difficile* colonisation of the gut. *Ann. Med.,* **1990,** *22* (1), 61-67.

[6] Lyerly, D. M.; Krivan, H. C.; Wilkins, T. D., *Clostridium difficile*: its disease and toxins. *Clin. Microbiol. Rev.,* **1988,** *1* (1), 1-18.

[7] Sunenshine, R. H.; McDonald, L. C., *Clostridium difficile*-associated disease: new challenges from an established pathogen. *Cleve. Clin. J. Med.,* **2006,** *73* (2), 187-197.

[8] Lyerly, D. M.; Lockwood, D. E.; Richardson, S. H.; Wilkins, T. D., Biological activities of toxins A and B of *Clostridium difficile*. *Infect. Immun.,* **1982,** *35* (3), 1147-1150.

[9] Kelly, C.; Becker, S.; Linevsky, J.; Joshi, M.; O'Keane, J.; Dickey, B.; LaMont, J.; Pothoulakis, C., Neutrophil recruitment in *Clostridium difficile* toxin A enteritis in the rabbit. *J. Clin. Invest.,* **1994,** *93* (3), 1257-1265.

[10] Kyne, L.; Warny, M.; Qamar, A.; Kelly, C. P., Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. *N. Engl. J. Med.,* **2000,** *342* (6), 390-397.

[11] Riegler, M.; Sedivy, R.; Pothoulakis, C.; Hamilton, G.; Zacherl, J.; Bischof, G.; Cosentini, E.; Feil, W.; Schiessel, R.; LaMont, J., *Clostridium difficile* toxin B is more potent than toxin A in damaging human colonic epithelium *in vitro*. *J. Clin. Invest.,* **1995,** *95* (5), 2004-2011.

[12] Savidge, T. C.; Pan, W.-h.; Newman, P.; O'Brien, M.; Anton, P. M.; Pothoulakis, C., *Clostridium difficile* toxin B is an inflammatory enterotoxin in human intestine. *Gastroenterology,* **2003,** *125* (2), 413-420.

[13] Kachrimanidou, M.; Malisiovas, N., *Clostridium difficile* infection: A comprehensive review. *Crit. Rev. Microbiol.,* **2011,** *37* (3), 178-187.

[14] Surawicz, C. M.; Brandt, L. J.; Binion, D. G.; Ananthakrishnan, A. N.; Curry, S. R.; Gilligan, P. H.; McFarland, L. V.; Mellow, M.; Zuckerbraun, B. S., Guidelines for diagnosis, treatment, and prevention of *Clostridium difficile* infections. *Am. J. Gastroenterol.,* **2013,** *108* (4), 478-498.

[15] Kee, V. R., *Clostridium difficile* infection in older adults: a review and update on its management. *Am. J. Geriatr. Pharmacother.,* **2012,** *10* (1), 14-24.

[16] Khanna, S.; Pardi, D. S., *Clostridium difficile* infection: new insights into management. *Mayo Clinic proc.,* **2012,** *87* (11), 1106-1117.

[17] Cookson, B., Hypervirulent strains of *Clostridium difficile*. *Postgrad. Med. J.,* **2007,** *83* (979), 291-295.

[18] Dial, S.; Delaney, J. A. C.; Schneider, V.; Suissa, S., Proton pump inhibitor use and risk of community-acquired *Clostridium difficile*-associated disease defined by prescription for oral vancomycin therapy. *CMAJ,* **2006,** *175* (7), 745-748.

[19] Loo, V. G.; Bourgault, A.-M.; Poirier, L.; Lamothe, F.; Michaud, S.; Turgeon, N.; Toye, B.; Beaudoin, A.; Frost, E. H.; Gilca, R.; Brassard, P.; Dendukuri, N.; Béliveau, C.; Oughton, M.; Brukner, I.; Dascal, A., Host and pathogen factors for *Clostridium difficile* infection and colonization. *N. Engl. J. Med.,* **2011,** *365* (18), 1693-1703.

[20] Rupnik, M.; Wilcox, M. H.; Gerding, D. N., *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nat. Rev. Microbiol.,* **2009,** *7* (7), 526-536.

[21] Khanna, S.; Pardi, D. S.; Aronson, S. L.; Kammer, P. P.; Orenstein, R.; St Sauver, J. L.; Harmsen, W. S.; Zinsmeister, A. R., The epidemiology of community-acquired *Clostridium difficile* infection: a population-based study. *Am. J. Gastroenterol.,* **2012,** *107* (1), 89-95.

[22] Tonna, I.; Welsby, P., Pathogenesis and treatment of *Clostridium difficile* infection. *Postgrad. Med. J.,* **2005,** *81* (956), 367-369.

[23] Merrigan, M.; Venugopal, A.; Mallozzi, M.; Roxas, B.; Viswanathan, V. K.; Johnson, S.; Gerding, D. N.; Vedantam, G., Human hypervirulent *Clostridium difficile* strains exhibit increased sporulation as well as robust toxin production. *J. Bacteriol.,* **2010,** *192* (19), 4904-4911.

[24] Burns, D. A.; Heeg, D.; Cartman, S. T.; Minton, N. P., Reconsidering the sporulation characteristics of hypervirulent *Clostridium difficile* BI/NAP1/027. *PLoS ONE,* **2011,** *6* (9), e24894.

[25] Bacci, S.; Mølbak, K.; Kjeldsen, M. K.; Olsen, K. E. P., Binary toxin and death after *Clostridium difficile* infection. *Emerg. Infect. Dis.,* **2011,** *17* (6), 976-982.

[26] Carlson, P. E., Jr.; Walk, S. T.; Bourgis, A. E.; Liu, M. W.; Kopliku, F.; Lo, E.; Young, V. B.; Aronoff, D. M.; Hanna, P. C., The relationship between phenotype, ribotype, and clinical disease in human *Clostridium difficile* isolates. *Anaerobe,* **2013**, in press (doi: 10.1016/j.anaerobe.2013.04.003).

[27] Walk, S. T.; Micic, D.; Jain, R.; Lo, E. S.; Trivedi, I.; Liu, E. W.; Almassalha, L. M.; Ewing, S. A.; Ring, C.; Galecki, A. T.; Rogers, M. A. M.; Washer, L.; Newton, D. W.; Malani, P. N.; Young, V. B.; Aronoff, D. M., *Clostridium difficile* ribotype does not predict severe infection. *Clin. Infect. Dis.,* **2012,** *55* (12), 1661-1668.

[28] Keel, K.; Brazier, J. S.; Post, K. W.; Weese, S.; Songer, J. G., Prevalence of PCR ribotypes among *Clostridium difficile* isolates from pigs, calves, and other species. *J. Clin. Microbiol.,* **2007,** *45* (6), 1963-1964.

[29] Goorhuis, A.; Bakker, D.; Corver, J.; Debast, S. B.; Harmanus, C.; Notermans, D. W.; Bergwerff, A. A.; Dekker, F. W.; Kuijper, E. J., Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. *Clin. Infect. Dis.,* **2008,** *47* (9), 1162-1170.

[30] Burns, K.; Morris-Downes, M.; Fawley, W. N.; Smyth, E.; Wilcox, M. H.; Fitzpatrick, F., Infection due to *C. difficile* ribotype 078: first report of cases in the Republic of Ireland. *J. Hosp. Infect.,* **2010,** *75* (4), 287-291.

[31] Freeman, J.; Bauer, M. P.; Baines, S. D.; Corver, J.; Fawley, W. N.; Goorhuis, B.; Kuijper, E. J.; Wilcox, M. H., The changing epidemiology of *Clostridium difficile* infections. *Clin. Microbiol. Rev.,* **2010,** *23* (3), 529-549.

[32] Spigaglia, P.; Barbanti, F.; Dionisi, A. M.; Mastrantonio, P., *Clostridium difficile* isolates resistant to fluoroquinolones in Italy: emergence of PCR ribotype 018. *J. Clin. Microbiol.,* **2010,** *48* (8), 2892-2896.

[33] Heslop, O.; Roye-Green, K.; Coard, K.; Mulvey, M., A unique strain of community-acquired *Clostridium difficile* in severe complicated infection and death of a young adult. *BMC Infect. Dis.,* **2013,** *13* (1), 299.

[34] Gerding, D. N.; Johnson, S.; Peterson, L. R.; Mulligan, M. E.; Silva, J., Jr., *Clostridium difficile*-associated diarrhea and colitis. *Infect. Control Hosp. Epidemiol.,* **1995,** *16* (8), 459-477.

[35] Lessa, F. C.; Gould, C. V.; McDonald, L. C., Current status of *Clostridium difficile* infection epidemiology. *Clin. Infect. Dis.,* **2012,** *55* (Suppl. 2), S65-S70.

[36] Cohen, S., M.D. ; Gerding, D., M.D. ; Stuart Johnson, M. D.; Kelly, C. M. D.; Loo, V. M. D.; McDonald, C. M. D.; Pepin, J. M. D.; Wilcox, M. M. D., Clinical practice guidelines for *Clostridium difficile* infection in adults:, 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). *Infect. Control Hosp. Epidemiol.,* **2010,** *31* (5), 431-455.

[37] Burnham, C.-A. D.; Carroll, K. C., Diagnosis of *Clostridium difficile* infection: an ongoing conundrum for clinicians and for clinical laboratories. *Clin. Microbiol. Rev.,* **2013,** *26* (3), 604-630.

[38] Musher, D. M.; Stager, C., Diagnosis of *Clostridium difficile* infection. *Clin. Infect. Dis.,* **2012,** *54* (11), 1675-1676.

[39] Hedge, D. D.; Strain, J. D.; Heins, J. R.; Farver, D. K., New advances in the treatment of Clostridium difficile infection (CDI). *Ther. Clin. Risk Manag.,* **2008,** *4* (5), 949-964.

[40] Centers for Disease Control and Prevention (CDC). Vital signs: preventing *Clostridium difficile* infections. *MMWR Morb. Mortal. Wkly Rep.,* **2012,** *61* (09), 157-162.

[41] Centers for Disease Control and Prevention (CDC), *Clostridium difficile* infection. http://www.cdc.gov/hai/organisms/cdiff/cdiff_infect.html (accessed July 30, 2013).

[42] Kyne, L.; Hamel, M. B.; Polavaram, R.; Kelly, C. P., Health care costs and mortality associated with nosocomial diarrhea due to *Clostridium difficile*. *Clin. Infect. Dis.,* **2002,** *34* (3), 346-353.

[43] Walsh, N. *C. difficile* inpatient stays long, costly. http://www.medpagetoday.com/MeetingCoverage/ASHP/36339 (accessed July 30, **2013**).

[44] Kuijper, E. J.; Coignard, B.; Tüll, P., On behalf of the ESCMID study group for *Clostridium difficile* (ESGCD), emergence of *Clostridium difficile*-associated disease in North America and Europe. *Clin. Microbiol. Infect.,* **2006,** *12* (Suppl. 6), 2-18.

[45] Wilcox, M. H.; Hawkey, P. M.; Patel, B.; Planche, T.; Stone, S. Updated guidance on the management and treatment of *Clostridium difficile* infection. http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317138914904 (accessed August 4, **2013**).

[46] Gerding, D. N.; Muto, C. A.; Owens, R. C., Treatment of *Clostridium difficile* infection. *Clin. Infect. Dis.,* **2008,** *46* (Suppl. 1), S32-S42.

[47] Musher, D. M.; Aslam, S.; Logan, N.; Nallacheru, S.; Bhaila, I.; Borchert, F.; Hamill, R. J., Relatively poor outcome after treatment of *Clostridium difficile* colitis with metronidazole. *Clin. Infect. Dis.,* **2005,** *40* (11), 1586-1590.

[48] Louie, T. J.; Miller, M. A.; Mullane, K. M.; Weiss, K.; Lentnek, A.; Golan, Y.; Gorbach, S.; Sears, P.; Shue, Y.-K., Fidaxomicin

versus vancomycin for *Clostridium difficile* infection. *N. Engl. J. Med.,* **2011,** *364* (5), 422-431.

[49] Pepin, J., Vancomycin for the treatment of *Clostridium difficile* infection: for whom is this expensive bullet really magic? *Clin. Infect. Dis.,* **2008,** *46* (10), 1493-1498.

[50] Johnson, S., Recurrent *Clostridium difficile* infection: a review of risk factors, treatments, and outcomes. *J. Infect.,* **2009,** *58* (6), 403-410.

[51] Higa, J. T.; Kelly, C. P., New drugs and strategies for management of *Clostridium difficile* colitis. *J. Intensive Care Med.,* **2013**.

[52] Venugopal, A. A.; Johnson, S., Current state of *Clostridium difficile* treatment options. *Clin. Infect. Dis.,* **2012,** *55* (Suppl. 2), S71-S76.

[53] Edwards, D. I., Nitroimidazole drugs--action and resistance mechanisms. I. mechanisms of action. *J. Antimicrob. Chemother.,* **1993,** *31* (1), 9-20.

[54] Goldman, P., The development of 5-nitroimidazoles for the treatment and prophylaxis of anaerobic bacterial infections. *J. Antimicrob. Chemother.,* **1982,** *10* (Suppl. A), 23-33.

[55] Edwards, D. I., Reduction of nitroimidazoles *in vitro* and DNA damage. *Biochem. Pharmacol.,* **1986,** *35* (1), 53-58.

[56] Tocher, J. H.; Edwards, D. I., Evidence for the direct interaction of reduced metronidazole derivatives with DNA bases. *Biochem. Pharmacol.,* **1994,** *48* (6), 1089-1094.

[57] Löfmark, S.; Edlund, C.; Nord, C. E., Metronidazole is still the drug of choice for treatment of anaerobic infections. *Clin. Infect. Dis.,* **2010,** *50* (Suppl. 1), S16-S23.

[58] Knight, R. C.; Skolimowski, I. M.; Edwards, D. I., The interaction of reduced metronidazole with DNA. *Biochem. Pharmacol.,* **1978,** *27* (17), 2089-2093.

[59] Al-Nassir, W. N.; Sethi, A. K.; Li, Y.; Pultz, M. J.; Riggs, M. M.; Donskey, C. J., Both oral metronidazole and oral vancomycin promote persistent overgrowth of vancomycin-resistant Enterococci during treatment of *Clostridium difficile*-associated disease. *Antimicrob. Agents Chemother.,* **2008,** *52* (7), 2403-2406.

[60] Bergan, T., Antibacterial activity and pharmacokinetics of nitroimidazoles. A review. *Scand. J. Infect. Dis. Suppl.,* **1985,** *46*, 64-71.

[61] Kuijper, E. J.; Wilcox, M. H., Decreased effectiveness of metronidazole for the treatment of *Clostridium difficile* infection? *Clin. Infect. Dis.,* **2008,** *47* (1), 63-65.

[62] Wenisch, J. M.; Schmid, D.; Kuo, H.-W.; Allerberger, F.; Michl, V.; Tesik, P.; Tucek, G.; Laferl, H.; Wenisch, C., Prospective observational study comparing three different treatment regimes in patients with *Clostridium difficile* infection. *Antimicrob. Agents Chemother.,* **2012,** *56* (4), 1974-1978.

[63] Moura, I.; Spigaglia, P.; Barbanti, F.; Mastrantonio, P., Analysis of metronidazole susceptibility in different *Clostridium difficile* PCR ribotypes. *J. Antimicrob. Chemother.,* **2013,** *68* (2), 362-365.

[64] Peláez, T.; Cercenado, E.; Alcalá, L.; Marín, M.; Martín-López, A.; Martínez-Alarcón, J.; Catalán, P.; Sánchez-Somolinos, M.; Bouza, E., Metronidazole resistance in *Clostridium difficile* is heterogeneous. *J. Clin. Microbiol.,* **2008,** *46* (9), 3028-3032.

[65] Anderson, V. R.; Curran, M. P., Nitazoxanide: a review of its use in the treatment of gastrointestinal infections. *Drugs,* **2007,** *67* (13), 1947-1967.

[66] Freeman, J.; Baines, S. D.; Todhunter, S. L.; Huscroft, G. S.; Wilcox, M. H., Nitazoxanide is active against *Clostridium difficile* strains with reduced susceptibility to metronidazole. *J. Antimicrob. Chemother.,* **2011,** *66* (6), 1407-1408.

[67] McVay, C. S.; Rolfe, R. D., *In vitro* and *in vivo* activities of nitazoxanide against *Clostridium difficile*. *Antimicrob. Agents Chemother.,* **2000,** *44* (9), 2254-2258.

[68] Musher, D. M.; Logan, N.; Bressler, A. M.; Johnson, D. P.; Rossignol, J.-F., Nitazoxanide versus vancomycin in *Clostridium difficile* infection: a randomized, double-blind study. *Clin. Infect. Dis.,* **2009,** *48* (4), e41-e46.

[69] Musher, D. M.; Logan, N.; Mehendiratta, V.; Melgarejo, N. A.; Garud, S.; Hamill, R. J., *Clostridium difficile* colitis that fails conventional metronidazole therapy: response to nitazoxanide. *J. Antimicrob. Chemother.,* **2007,** *59* (4), 705-710.

[70] Yu, X.; Sun, D., Macrocyclic drugs and synthetic methodologies toward macrocycles. *Molecules,* **2013,** *18* (6), 6230-6268.

[71] Rybak, M.; Lomaestro, B.; Rotschafer, J. C.; Moellering, R.; Craig, W.; Billeter, M.; Dalovisio, J. R.; Levine, D. P., Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. *Am. J. Health Syst. Pharm.,* **2009,** *66* (1), 82-98.

[72] Lexi-Comp Online™, Hudson, Ohio: Lexi-Comp, Inc., **2013**.

[73] Reynolds, P. E., Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *Eur. J. Clin. Microbiol. Infect. Dis.,* **1989,** *8* (11), 943-950.

[74] Nagarajan, R., Antibacterial activities and modes of action of vancomycin and related glycopeptides. *Antimicrob. Agents Chemother.,* **1991,** *35* (4), 605-609.

[75] Leeds, J. A.; Sachdeva, M.; Mullin, S.; Barnes, S. W.; Ruzin, A., *In vitro* selection, via serial passage, of *Clostridium difficile* mutants with reduced susceptibility to fidaxomicin or vancomycin. *J. Antimicrob. Chemother.,* **2013**, in press (doi:10.1093/jac/dkt302).

[76] Zar, F. A.; Bakkanagari, S. R.; Moorthi, K. M. L. S. T.; Davis, M. B., A comparison of vancomycin and metronidazole for the treatment of *Clostridium difficile*-associated diarrhea, stratified by disease severity. *Clin. Infect. Dis.,* **2007,** *45* (3), 302-307.

[77] Kelly, C. P.; LaMont, J. T., *Clostridium difficile* - more difficult than ever. *N. Engl. J. Med.,* **2008,** *359* (18), 1932-1940.

[78] Whitman, C. B.; Czosnowski, Q. A., Fidaxomicin for the treatment of *Clostridium difficile* infections. *Ann. Pharmacother.,* **2012,** *46* (2), 219-228.

[79] Hardesty, J. S.; Juang, P., Fidaxomicin: a macrocyclic antibiotic for the treatment of *Clostridium difficile* infection. *Pharmacotherapy,* **2011,** *31* (9), 877-886.

[80] Gerber, M.; Ackermann, G., OPT-80, a macrocyclic antimicrobial agent for the treatment of *Clostridium difficile* infections: a review. *Expert Opin. Investig. Drugs,* **2008,** *17* (4), 547-553.

[81] Mullane, K. M.; Gorbach, S., Fidaxomicin: first-in-class macrocyclic antibiotic. *Expert Rev. Anti Infect. Ther.,* **2011,** *9* (7), 767-777.

[82] Artsimovitch, I.; Seddon, J.; Sears, P., Fidaxomicin is an inhibitor of the initiation of bacterial rna synthesis. *Clin. Infect. Dis.,* **2012,** *55* (Suppl. 2), S127-S131.

[83] Anti-Infective Drugs Advisory Committee Briefing Document, Optimer Pharmaceuticals, Inc. Dificid™ (fidaxomicin tablets) for the treatment of *Clostridium difficile* infection (CDI), also known as *Clostridium difficile*-associated diarrhea (CDAD), and for reducing the risk of recurrence when used for treatment of initial CDI. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Anti-InfectiveDrugsAdvisoryCommittee/UCM249354.pdf (accessed August 8, **2013**).

[84] Drekonja, D. M.; Butler, M.; MacDonald, R.; Bliss, D.; Filice, G. A.; Rector, T. S.; Wilt, T. J., Comparative effectiveness of *Clostridium difficile* treatments: a systematic review. *Ann. Intern. Med.,* **2011,** *155* (12), 839-847.

[85] Cornely, O. A.; Miller, M. A.; Louie, T. J.; Crook, D. W.; Gorbach, S. L., Treatment of first recurrence of *Clostridium difficile* infection: fidaxomicin versus vancomycin. *Clin. Infect. Dis.,* **2012,** *55* (Suppl. 2), S154-S161.

[86] Weiss, K.; Allgren, R. L.; Sellers, S., Safety analysis of fidaxomicin in comparison with oral vancomycin for *Clostridium difficile* infections. *Clin. Infect. Dis.,* **2012,** *55* (Suppl. 2), S110-115.

[87] Mullane, K. M.; Miller, M. A.; Weiss, K.; Lentnek, A.; Golan, Y.; Sears, P. S.; Shue, Y.-K.; Louie, T. J.; Gorbach, S. L., Efficacy of fidaxomicin versus vancomycin as therapy for *Clostridium difficile* infection in individuals taking concomitant antibiotics for other concurrent infections. *Clin. Infect. Dis.,* **2011,** *53* (5), 440-447.

[88] Tannock, G. W.; Munro, K.; Taylor, C.; Lawley, B.; Young, W.; Byrne, B.; Emery, J.; Louie, T., A new macrocyclic antibiotic, fidaxomicin (OPT-80), causes less alteration to the bowel microbiota of *Clostridium difficile*-infected patients than does vancomycin. *Microbiology,* **2010,** *156* (11), 3354-3359.

[89] Louie, T. J.; Cannon, K.; Byrne, B.; Emery, J.; Ward, L.; Eyben, M.; Krulicki, W., Fidaxomicin preserves the intestinal microbiome during and after treatment of *Clostridium difficile* infection (CDI) and reduces both toxin reexpression and recurrence of CDI. *Clin. Infect. Dis.,* **2012,** *55* (Suppl. 2), S132-S142.

[90] Goldstein, E. J.; Babakhani, F.; Citron, D. M., Antimicrobial activities of fidaxomicin. *Clin. Infect. Dis.,* **2012,** *55* (Suppl. 2), S143-S148.

[91] Sears, P.; Crook, D. W.; Louie, T. J.; Miller, M. A.; Weiss, K., Fidaxomicin attains high fecal concentrations with minimal plasma concentrations following oral administration in patients with *Clostridium difficile* infection. *Clin. Infect. Dis.,* **2012,** *55* (Suppl. 2), S116-S120.

[92] Venugopal, A. A.; Johnson, S., Fidaxomicin: a novel macrocyclic antibiotic approved for treatment of *Clostridium difficile* infection. *Clin. Infect. Dis.,* **2012,** *54* (4), 568-574.

[93] Goldstein, E. J.; Citron, D. M.; Sears, P.; Babakhani, F.; Sambol, S. P.; Gerding, D. N., Comparative susceptibilities to fidaxomicin (OPT-80) of isolates collected at baseline, recurrence, and failure from patients in two phase III trials of fidaxomicin against *Clostridium difficile* infection. *Antimicrob. Agents Chemother.,* **2011,** *55* (11), 5194-5199.

[94] Hecht, D. W.; Galang, M. A.; Sambol, S. P.; Osmolski, J. R.; Johnson, S.; Gerding, D. N., *In vitro* activities of 15 antimicrobial agents against 110 toxigenic *Clostridium difficile* clinical isolates collected from, 1983 to, 2004. *Antimicrob. Agents Chemother.,* **2007,** *51* (8), 2716-2719.

[95] Koo, H. L.; DuPont, H. L., Rifaximin: a unique gastrointestinal-selective antibiotic for enteric diseases. *Curr. Opin. Gastroenterol.,* **2010,** *26* (1), 17-25.

[96] Koo, H. L.; Garey, K. W.; DuPont, H. L., Future novel therapeutic agents for *Clostridium difficile* infection. *Expert Opin. Investig. Drugs,* **2010,** *19* (7), 825-836.

[97] Chopra, I., Bacterial RNA polymerase: a promising target for the discovery of new antimicrobial agents. *Curr. Opin. Investig. Drugs,* **2007,** *8* (8), 600-607.

[98] Jiang, Z.; DuPont, H., Rifaximin: *in vitro* and *in vivo* antibacterial activity-a review. *Chemotherapy,* **2005,** *51* (Suppl. 1), 67-72.

[99] Mattila, E.; Arkkila, P.; Mattila, P. S.; Tarkka, E.; Tissari, P.; Anttila, V. J., Rifaximin in the treatment of recurrent *Clostridium difficile* infection. *Aliment. Pharmacol. Ther.,* **2013,** *37* (1), 122-128.

[100] Johnson, S.; Schriever, C.; Galang, M.; Kelly, C. P.; Gerding, D. N., Interruption of recurrent *Clostridium difficile*-associated diarrhea episodes by serial therapy with vancomycin and rifaximin. *Clin. Infect. Dis.,* **2007,** *44* (6), 846-848.

[101] Curry, S. R.; Marsh, J. W.; Shutt, K. A.; Muto, C. A.; O'Leary, M. M.; Saul, M. I.; William, P. A.; Harrison, L. H., High frequency of rifampin resistance identified in an epidemic *Clostridium difficile* clone from a large teaching hospital. *Clin. Infect. Dis.,* **2009,** *48* (4), 425-429.

[102] Dang, T.-U.; Zamora, I.; Wu, X.; Hurdle, J. G., Fitness cost of rifamycin resistance in *Clostridium difficile*. In *113th General Meeting, American Society for Microbiology*, Denver, CO, USA, May 18-21, **2013**, Abstract No. 437.

[103] Huang, J. S.; Jiang, Z. D.; Garey, K. W.; Lasco, T.; Dupont, H. L., Use of rifamycin drugs and development of infection by rifamycin-resistant strains of *Clostridium difficile*. *Antimicrob. Agents Chemother.,* **2013,** *57* (6), 2690-2693.

[104] Abougergi, M.; Kwon, J., Intravenous immunoglobulin for the treatment of *Clostridium difficile* infection: a review. *Dig. Dis. Sci.,* **2011,** *56* (1), 19-26.

[105] Wullt, M.; Norén, T.; Ljungh, Å.; Åkerlund, T., IgG antibody response to toxins A and B in patients with *Clostridium difficile* infection. *Clin. Vaccine Immunol.,* **2012,** *19* (9), 1552-1554.

[106] O'Horo, J.; Safdar, N., The role of immunoglobulin for the treatment of *Clostridium difficile* infection: a systematic review. *Int. J. Infect. Dis.,* **2009,** *13* (6), 663-667.

[107] Aboudola, S.; Kotloff, K. L.; Kyne, L.; Warny, M.; Kelly, E. C.; Sougioultzis, S.; Giannasca, P. J.; Monath, T. P.; Kelly, C. P., *Clostridium difficile* vaccine and serum immunoglobulin G antibody response to toxin A. *Infect. Immun.,* **2003,** *71* (3), 1608-1610.

[108] Ghose, C.; Kalsy, A.; Sheikh, A.; Rollenhagen, J.; John, M.; Young, J.; Rollins, S. M.; Qadri, F.; Calderwood, S. B.; Kelly, C. P.; Ryan, E. T., Transcutaneous immunization with *Clostridium difficile* toxoid A induces systemic and mucosal immune responses and toxin A-neutralizing antibodies in mice. *Infect. Immun.,* **2007,** *75* (6), 2826-2832.

[109] Kotloff, K. L.; Wasserman, S. S.; Losonsky, G. A.; Thomas, W.; Nichols, R.; Edelman, R.; Bridwell, M.; Monath, T. P., Safety and immunogenicity of increasing doses of a *Clostridium difficile* toxoid vaccine administered to healthy adults. *Infect. Immun.,* **2001,** *69* (2), 988-995.

[110] Foglia, G.; Shah, S.; Luxemburger, C.; Pietrobon, P. J. F., *Clostridium difficile*: development of a novel candidate vaccine. *Vaccine,* **2012,** *30* (29), 4307-4309.

[111] *Clostridium difficile*: the hope to prevent a major nosocomial infection. http://en.sanofi.com/our_company/news/2013-08-05_vaccine_clostridium.aspx (accessed September 6, **2013**).

[112] Lowy, I.; Molrine, D. C.; Leav, B. A.; Blair, B. M.; Baxter, R.; Gerding, D. N.; Nichol, G.; Thomas, W. D.; Leney, M.; Sloan, S.; Hay, C. A.; Ambrosino, D. M., Treatment with monoclonal antibodies against *Clostridium difficile* toxins. *N. Engl. J. Med.,* **2010,** *362* (3), 197-205.

[113] A study of MK-3415, MK-6072, and MK-3415A in participants receiving antibiotic therapy for *Clostridium difficile* infection (MK-3415A-001) (MODIFY I). http://clinicaltrials.gov/show/NCT01241552 (accessed August 9, **2013**).

[114] Johnston, B. C.; Ma, S. S. Y.; Goldenberg, J. Z.; Thorlund, K.; Vandvik, P. O.; Loeb, M.; Guyatt, G. H., Probiotics for the prevention of *Clostridium difficile*-associated diarrhea: a systematic review and meta-analysis. *Ann. Intern. Med.,* **2012,** *157* (12), 878-888.

[115] Zucca, M.; Scutera, S.; Savoia, D., Novel avenues for *Clostridium difficile* infection drug discovery. *Expert Opin. Drug Discov.,* **2013,** *8* (4), 459-477.

[116] Alvarez-Olmos, M. I.; Oberhelman, R. A., Probiotic agents and infectious diseases: a modern perspective on a traditional therapy. *Clin. Infect. Dis.,* **2001,** *32* (11), 1567-1576.

[117] Goldenberg, J. Z.; Ma, S. S.; Saxton, J. D.; Martzen, M. R.; Vandvik, P. O.; Thorlund, K.; Guyatt, G. H.; Johnston, B. C., Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. *Cochrane Database Syst. Rev.,* **2013,** (5), CD006095.

[118] Johnson, S.; Maziade, P. J.; McFarland, L. V.; Trick, W.; Donskey, C.; Currie, B.; Low, D. E.; Goldstein, E. J., Is primary prevention of *Clostridium difficile* infection possible with specific probiotics? *Int. J. Infect. Dis.,* **2012,** *16* (11), e786-e792.

[119] Videlock, E. J.; Cremonini, F., Meta-analysis: probiotics in antibiotic-associated diarrhoea. *Aliment. Pharmacol. Ther.,* **2012,** *35* (12), 1355-1369.

[120] Hennequin, C.; Kauffmann-Lacroix, C.; Jobert, A.; Viard, J. P.; Ricour, C.; Jacquemin, J. L.; Berche, P., Possible role of catheters in *Saccharomyces boulardii* fungemia. *Eur. J. Clin. Microbiol. Infect. Dis.,* **2000,** *19* (1), 16-20.

[121] Lherm, T.; Monet, C.; Nougière, B.; Soulier, M.; Larbi, D.; Le Gall, C.; Caen, D.; Malbrunot, C., Seven cases of fungemia with *Saccharomyces boulardii* in critically ill patients. *Intensive Care Med.,* **2002,** *28* (6), 797-801.

[122] Allen-Vercoe, E.; Reid, G.; Viner, N.; Gloor, G. B.; Hota, S.; Kim, P.; Lee, C.; O'Doherty, K.; Vanner, S. J.; Weese, J. S., A Canadian working group report on fecal microbial therapy: microbial ecosystems therapeutics. *Can. J. Gastroenterol.,* **2012,** *26* (7), 457-462.

[123] Postigo, R.; Kim, J., Colonoscopic versus nasogastric fecal transplantation for the treatment of *Clostridium difficile* infection: a review and pooled analysis. *Infection,* **2012,** *40* (6), 643-648.

[124] Landy, J.; Al-Hassi, H.; McLaughlin, S.; Walker, A.; Ciclitira, P.; Nicholls, R.; Clark, S.; Hart, A., Review article: faecal transplantation therapy for gastrointestinal disease. *Aliment. Pharmacol. Ther.,* **2011,** *34* (4), 409-415.

[125] Gough, E.; Shaikh, H.; Manges, A. R., Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent *Clostridium difficile* infection. *Clin. Infect. Dis.,* **2011,** *53* (10), 994-1002.

[126] van Nood, E.; Vrieze, A.; Nieuwdorp, M.; Fuentes, S.; Zoetendal, E. G.; de Vos, W. M.; Visser, C. E.; Kuijper, E. J.; Bartelsman, J. F.; Tijssen, J. G., Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N. Engl. J. Med.,* **2013,** *368* (5), 407-415.

[127] Guo, B.; Harstall, C.; Louie, T.; Veldhuyzen van Zanten, S.; Dieleman, L. A., Systematic review: faecal transplantation for the treatment of *Clostridium difficile*-associated disease. *Aliment. Pharmacol. Ther.,* **2012,** *35* (8), 865-875.

[128] Filipski, K. J.; Varma, M. V.; El-Kattan, A. F.; Ambler, C. M.; Ruggeri, R. B.; Goosen, T. C.; Cameron, K. O., Intestinal targeting of drugs: rational design approaches and challenges. *Curr. Top. Med. Chem.,* **2013,** *13* (7), 776-802.

[129] Johnson, A. P., New antibiotics for selective treatment of gastrointestinal infection caused by *Clostridium difficile*. *Expert Opin. Ther. Pat.,* **2010,** *20* (10), 1389-1399.

[130] Sun, X.; Guiles, J.; Janjic, N., Recent development in the treatment of *Clostridium difficile* associated disease (CDAD). In *Annual Reports in Medicinal Chemistry*, Macor, J. E., Ed. Elsevier Science:, 2008; Vol. 43, pp 269-279.

[131] Shah, D.; Dang, M.-D.; Hasbun, R.; Koo, H. L.; Jiang, Z.-D.; DuPont, H. L.; Garey, K. W., *Clostridium difficile* infection: update

on emerging antibiotic treatment options and antibiotic resistance. *Expert Rev. Anti Infect. Ther.,* **2010,** *8* (5), 555-564.

[132] van Nispen tot Pannerden, C. M. F.; Verbon, A.; Kuipers, E. J., Recurrent *Clostridium difficile* infection. *Drugs,* **2011,** *71* (7), 853-868.

[133] Joseph, J.; Singhal, S.; Patel, G. M.; Anand, S., *Clostridium Difficile* colitis: review of the therapeutic approach. *Am. J. Ther.,* **2013**, in press (doi: 10.1097/MJT.0b013e318245992d).

[134] Nanotherapeutics, Inc. Products. http://www.nanotherapeutics.com/?q=products_ramoplanin (accessed July 29, **2013**).

[135] Peláez, T.; Alcalá, L.; Alonso, R.; Martín-López, A.; García-Arias, V.; Marín, M.; Bouza, E., *In vitro* activity of ramoplanin against *Clostridium difficile*, including strains with reduced susceptibility to vancomycin or with resistance to metronidazole. *Antimicrob. Agents Chemother.,* **2005,** *49* (3), 1157-1159.

[136] Fang, X.; Tiyanont, K.; Zhang, Y.; Wanner, J.; Boger, D.; Walker, S., The mechanism of action of ramoplanin and enduracidin. *Mol. Biosyst.,* **2006,** *2* (1), 69-76.

[137] Cubist Products and Pipeline. http://www.cubist.com/products/ (accessed July 29, **2013**).

[138] Mascio, C. T.; Mortin, L. I.; Howland, K. T.; Van Praagh, A. D.; Zhang, S.; Arya, A.; Chuong, C. L.; Kang, C.; Li, T.; Silverman, J. A., *In vitro* and *in vivo* characterization of CB-183,315, a novel lipopeptide antibiotic for treatment of *Clostridium difficile*. *Antimicrob. Agents Chemother.,* **2012,** *56* (10), 5023-5030.

[139] ClinicalTrials.gov Identifier: NCT01232595, Safety and efficacy of multiple daily dosing of oral LFF571 in patients with moderate *Clostridium Difficile* infections. http://clinicaltrials.gov/ct2/show/NCT01232595?term=lff571&rank=1 (accessed August 7, **2013**).

[140] Leeds, J.; Sachdeva, M.; Mullin, S.; Dzink-Fox, J.; LaMarche, M., Mechanism of action of and mechanism of reduced susceptibility to the novel anti-*Clostridium difficile* compound LFF571. *Antimicrob. Agents Chemother.,* **2012,** *56* (8), 4463-4465.

[141] Allen, N. E.; Nicas, T. I., Mechanism of action of oritavancin and related glycopeptide antibiotics. *FEMS Microbiol. Rev.,* **2003,** *26* (5), 511-532.

[142] Rashid, M. U.; Lozano, H. M.; Weintraub, A.; Nord, C. E., *In vitro* activity of cadazolid against *Clostridium difficile* strains isolated from primary and recurrent infections in Stockholm, Sweden. *Anaerobe,* **2013,** *20*, 32-35.

[143] Locher, H. H.; Ritz, D.; Schroeder, S.; Pfaff, P.; Knezevic, A.; Hubschwerlen, C.; Keck, W. Cadazolid, a novel quinolonyl-oxazolidinone antibiotic: mode of action and effect on *Clostridium difficile* toxin and spore formation. In *52nd Interscience Conference on Antimicrobial Agents and Chemotherapy,* San Francisco, CA, USA, September 9-12, 2012, Abstract No: C1-1347.

[144] Crestone, Inc. http://www.crestonepharma.com/ (accessed July 31, **2013**).

[145] Citron, D. M.; Warren, Y. A.; Tyrrell, K. L.; Merriam, V.; Goldstein, E. J. C., Comparative *in vitro* activity of REP3123 against *Clostridium difficile* and other anaerobic intestinal bacteria. *J. Antimicrob. Chemother.,* **2009,** *63* (5), 972-976.

[146] Critchley, I. A.; Green, L. S.; Young, C. L.; Bullard, J. M.; Evans, R. J.; Price, M.; Jarvis, T. C.; Guiles, J. W.; Janjic, N.; Ochsner, U. A., Spectrum of activity and mode of action of REP3123, a new antibiotic to treat *Clostridium difficile* infections. *J. Antimicrob. Chemother.,* **2009,** *63* (5), 954-963.

[147] Vickers, R.; Tinsley, J.; Storer, R.; Wilson, F.; Dorgan, C.; Wren, S.; Wilcox, M.; Baines, S.; Freeman, J.; Morrissey, I.; Maher, K.; Knight, D., SMT19969 – a novel antibiotic for *C. difficile* infection *C. difficile* growth inhibition, spectrum of activity and resistance development. In *51st Interscience Conference on Antimicriobial Agents and Chemotherapy*, Chicago, IL, USA, September 17-20, **2011**, Abstract No: B-1194.

[148] Bhattacharya, S.; Chaudhuri, P., Metal-ion-mediated tuning of duplex DNA binding by bis(2-(2-pyridyl)-1H-benzimidazole). *Chem. Asian J.,* **2007,** *2* (5), 648-655.

[149] Chaudhuri, P.; Ganguly, B.; Bhattacharya, S., An experimental and computational analysis on the differential role of the positional isomers of symmetric bis-2-(pyridyl)-1H-benzimidazoles as DNA binding agents. *J. Org. Chem.,* **2007,** *72* (6), 1912-1923.

[150] Crowther, G. S.; Baines, S. D.; Todhunter, S. L.; Freeman, J.; Chilton, C. H.; Wilcox, M. H., Evaluation of NVB302 versus vancomycin activity in an *in vitro* human gut model of *Clostridium difficile* infection. *J. Antimicrob. Chemother.,* **2013,** *68* (1), 168-176.

[151] Farver, D. K.; Hedge, D. D.; Lee, S. C., Ramoplanin: a lipoglycodepsipeptide antibiotic. *Ann. Pharmacother.,* **2005,** *39* (5), 863-868.

[152] Fulco, P.; Wenzel, R. P., Ramoplanin: a topical lipoglycodepsipeptide antibacterial agent. *Expert Rev. Anti Infect. Ther.,* **2006,** *4* (6), 939-945.

[153] Freeman, J.; Baines, S. D.; Jabes, D.; Wilcox, M. H., Comparison of the efficacy of ramoplanin and vancomycin in both *in vitro* and *in vivo* models of clindamycin-induced *Clostridium difficile* infection. *J. Antimicrob. Chemother.,* **2005,** *56* (4), 717-725.

[154] Citron, D. M.; Tyrrell, K. L.; Merriam, C. V.; Goldstein, E. J., *In vitro* activities of CB-183,315, vancomycin, and metronidazole against 556 strains of *Clostridium difficile*, 445 other intestinal anaerobes, and 56 *Enterobacteriaceae* species. *Antimicrob. Agents Chemother.,* **2012,** *56* (3), 1613-1615.

[155] Snydman, D.; Jacobus, N.; McDermott, L., Activity of a novel cyclic lipopeptide, CB-183,315, against resistant *Clostridium difficile* and other gram-positive aerobic and anaerobic intestinal pathogens. *Antimicrob. Agents Chemother.,* **2012,** *56* (6), 3448-3452.

[156] LaMarche, M. J.; Leeds, J. A.; Amaral, A.; Brewer, J. T.; Bushell, S. M.; Deng, G.; Dewhurst, J. M.; Ding, J.; Dzink-Fox, J.; Gamber, G.; Jain, A.; Lee, K.; Lee, L.; Lister, T.; McKenney, D.; Mullin, S.; Osborne, C.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Sachdeva, M.; Shao, J.; Tiamfook, S.; Trzasko, A.; Whitehead, L.; Yifru, A.; Yu, D.; Yan, W.; Zhu, Q., Discovery of LFF571: An investigational agent for *Clostridium difficile* infection. *J. Med. Chem.,* **2012,** *55* (5), 2376-2387.

[157] Citron, D. M.; Tyrrell, K. L.; Merriam, C. V.; Goldstein, E. J. C., Comparative *in vitro* activities of LFF571 against *Clostridium difficile* and 630 other intestinal strains of aerobic and anaerobic bacteria. *Antimicrob. Agents Chemother.,* **2012,** *56* (5), 2493-2503.

[158] Trzasko, A.; Leeds, J. A.; Praestgaard, J.; LaMarche, M. J.; McKenney, D., Efficacy of LFF571 in a hamster model of *Clostridium difficile* infection. *Antimicrob. Agents Chemother.,* **2012,** *56* (8), 4459-4462.

[159] Ting, L. S. L.; Praestgaard, J.; Grunenberg, N.; Yang, J. C.; Leeds, J. A.; Pertel, P., A first-in-human, randomized, double-blind, placebo-controlled, single- and multiple-ascending oral dose study to assess the safety and tolerability of LFF571 in healthy volunteers. *Antimicrob. Agents Chemother.,* **2012,** *56* (11), 5946-5951.

[160] Bouza, E.; Burillo, A., Oritavancin: a novel lipoglycopeptide active against Gram-positive pathogens including multiresistant strains. *Int. J. Antimicrob. Agents,* **2010,** *36* (5), 401-407.

[161] Ambrose, P. G.; Drusano, G. L.; Craig, W. A., *In vivo* activity of oritavancin in animal infection models and rationale for a new dosing regimen in humans. *Clin. Infect. Dis.,* **2012,** *54* (Suppl. 3), S220-S228.

[162] Baines, S. D.; O'Connor, R.; Saxton, K.; Freeman, J.; Wilcox, M. H., Comparison of oritavancin versus vancomycin as treatments for clindamycin-induced *Clostridium difficile* PCR ribotype 027 infection in a human gut model. *J. Antimicrob. Chemother.,* **2008,** *62* (5), 1078-1085.

[163] Chilton, C.; Freeman, J.; Crowther, G.; Todhunter, S.; Wilcox, M., Effectiveness of a short (4 day) course of oritavancin in the treatment of simulated *Clostridium difficile* infection using a human gut model. *J. Antimicrob. Chemother.,* **2012,** *67* (10), 2434-2437.

[164] Chilton, C. H.; Freeman, J.; Baines, S. D.; Crowther, G. S.; Nicholson, S.; Wilcox, M. H., Evaluation of the effect of oritavancin on *Clostridium difficile* spore germination, outgrowth and recovery. *J. Antimicrob. Chemother.,* **2013**, *68*(9), 2078-2082.

[165] Freeman, J.; Marquis, M.; Crowther, G. S.; Todhunter, S. L.; Fawley, W. N.; Chilton, C. H.; Moeck, G.; Lehoux, D.; Wilcox, M. H., Oritavancin does not induce *Clostridium difficile* germination and toxin production in hamsters or a human gut model. *J. Antimicrob. Chemother.,* **2012,** *67* (12), 2919-2926.

[166] Baines, S. D.; Crowther, G. S.; Todhunter, S. L.; Freeman, J.; Wilcox, M. H., *In vitro* activity of cadazolid (ACT-179811) against *Clostridium difficile* and in an *in vitro* gut model of *C. difficile* infection. In *52nd Interscience Conference on Antimicrobial Agents and Chemotherapy*, San Francisco, CA, USA, September 9-12, **2012**, Abstract No: B-662.

[167] Locher, H. H.; Pfaff, P.; Schroeder, S.; Specklin, J. L.; Hubschwerlen, C.; Keck, W. In Cadazolid, a novel quinolonyl-oxazolidinone antibiotic with potent activity against *Clostridium difficile: in vitro* antibacterial activity and propensity for resistance development. In *52nd Interscience Conference on Antimicrobial Agents and Che-*

motherapy, San Francisco, CA, USA, September 9-12, **2012**, Abstract No: C1-1346.

[168] Baldoni, D.; Gutierrez, M.; Dingemanse, J.; Timmer, W., Cadazolid, a novel antibiotic with potent activity against *Clostridium difficile*: safety, tolerability, and pharmacokinetics in healthy subjects following single and multiple oral doses In *52nd Interscience Conference on Antimicrobial Agents and Chemotherapy*, San Francisco, CA, USA, September 9-12, **2012**, Abstract No:1273.

[169] Ochsner, U. A.; Bell, S. J.; O'Leary, A. L.; Hoang, T.; Stone, K. C.; Young, C. L.; Critchley, I. A.; Janjic, N., Inhibitory effect of REP3123 on toxin and spore formation in *Clostridium difficile*, and *in vivo* efficacy in a hamster gastrointestinal infection model. *J. Antimicrob. Chemother.,* **2009,** *63* (5), 964-971.

[170] Al-Moubarak, E.; Simons, C., A homology model for *Clostridium difficile* methionyl tRNA synthetase: active site analysis and docking interactions. *J. Mol. Model.,* **2011,** *17* (7), 1679-1693.

[171] Corbett, D.; Wise, A.; Payne, L. J.; Vickers, R.; Warn, P. A., SMT19969: *In vitro* pharmacodynamics against *Clostridium difficile*. In *23rd European Society of Clinical Microbiology and Infectious Diseases*, Berlin, Germany, April 27-30, **2013**, Abstract No: 1654

[172] Weiss, W. J.; Vickers, R.; Pulse, M.; Nguyen, P.; Renick, P.; Simecka, J. W., Efficacy of SMT19969 and SMT21829 in a hamster model of *Clostridium difficile* associated disease (CDAD). In *51st Interscience Conference on Antimicriobial Agents and Chemotherapy*, Chicago, IL, USA, September 17-20, **2011**, Abstract No: B-1195.

[173] Vickers, R.; Tinsley, J.; Wilson, F.; Wilcox, M.; Baines, S.; Freeman, J.; Morrissey, I.; Maher, K.; Weiss, W.; Pulse, M. SMT19969: in vitro and in vivo evaluation of a novel antibiotic for Clostridium difficile infection. In 23rd European Society of Clinical Microbiology and Infectious Diseases, Berlin, Germany, April 27-30, 2013, Abstract No: 1655 Berlin, Germany.

[174] Goldstein, E. J. C.; Citron, D. M.; Tyrrell, K. L.; Merriam, C. V., Comparative in-vitro activity of SMT19969, a new antimicrobial agent, against *Clostridium difficile* and 350 Gram-positive and negative aerobic and anaerobic intestinal flora isolates. *Antimicrob. Agents Chemother.,* **2013**, *57*(10), 4872-4876.

[175] Summit PLC programs-*C. difficile* infection. http://www.summitplc.com/programmes/c-difficile/ (accessed August 1, 2013).

[176] Shi, Y.; Bueno, A.; van der Donk, W. A., Heterologous production of the lantibiotic Ala(0)actagardine in *Escherichia coli*. *Chem. Commun.,* **2012,** *48* (89), 10966-10968.

[177] Novacta Biosystems Limited, Product Pipeline, *C. difficile*. http://www.novactabio.com/page.php?id=68 (accessed August 1, **2013**).

[178] Cowan, M. M., Plant products as antimicrobial agents. *Clin. Microbiol. Rev.,* **1999,** *12* (4), 564-582.

[179] Simoes, M.; Bennett, R. N.; Rosa, E. A., Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. *Nat. Prod. Rep.,* **2009,** *26* (6), 746-757.

[180] Cushnie, T. P.; Lamb, A. J., Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents,* **2005,** *26* (5), 343-356.

[181] Abreu, A. C.; McBain, A. J.; Simões, M., Plants as sources of new antimicrobials and resistance-modifying agents. *Nat. Prod. Rep.,* **2012,** *29* (9), 1007-1021.

[182] Rios, J.; Recio, M., Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.,* **2005,** *100* (1-2), 80-84.

[183] Daglia, M., Polyphenols as antimicrobial agents. *Curr. Opin. Biotechnol.,* **2012,** *23* (2), 174-181.

[184] Putnam, S. D.; Castanheira, M.; Moet, G. J.; Farrell, D. J.; Jones, R. N., CEM-101, a novel fluoroketolide: antimicrobial activity against a diverse collection of Gram-positive and Gram-negative bacteria. *Diagn. Microbiol. Infect. Dis.,* **2010,** *66* (4), 393-401.

[185] Putnam, S. D.; Sader, H. S.; Farrell, D. J.; Biedenbach, D. J.; Castanheira, M., Antimicrobial characterisation of solithromycin (CEM-101), a novel fluoroketolide: activity against staphylococci and enterococci. *Int. J. Antimicrob. Agents,* **2011,** *37* (1), 39-45.

[186] Llano-Sotelo, B.; Klepacki, D.; Vazquez-Laslop, N.; Mankin, A. S., Binding and action of CEM-101. In *48th Interscience Conference on Antimicrobial Agents and Chemotherapy* October 25-28, **2008**, Washington DC, USA, Abstract No: F1-3983.

[187] Rodgers, W.; Frazier, A. D.; Champney, W. S., Solithromycin inhibition of protein synthesis and ribosome biogenesis in *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*. *Antimicrob. Agents Chemother.,* **2013,** *57* (4), 1632-1637.

[188] Kumar, M.; Mathur, T.; Barman, T. K.; Ramkumar, G.; Bhati, A.; Shukla, G.; Kalia, V.; Pandya, M.; Raj, V. S.; Upadhyay, D. J.; Vaishnavi, C.; Chakrabarti, A.; Das, B.; Bhatnagar, P. K., *In vitro* and *in vivo* activities of the novel Ketolide RBx 14255 against *Clostridium difficile*. *Antimicrob. Agents Chemother.,* **2012,** *56* (11), 5986-5989.

[189] Anton, P. M.; O'brien, M.; Kokkotou, E.; Eisenstein, B.; Michaelis, A.; Rothstein, D.; Paraschos, S.; Kelly, C. P.; Pothoulakis, C., Rifalazil treats and prevents relapse of *Clostridium difficile*-associated diarrhea in hamsters. *Antimicrob. Agents Chemother.,* **2004,** *48* (10), 3975-3979.

[190] Garey, K. W.; Salazar, M.; Shah, D.; Rodrigue, R.; DuPont, H. L., Rifamycin antibiotics for treatment of *Clostridium difficile*-associated diarrhea. *Ann. Pharmacother.,* **2008,** *42* (6), 827-835.

[191] Rothstein, D. M.; Shalish, C.; Murphy, C. K.; Sternlicht, A.; Campbell, L. A., Development potential of rifalazil and other benzoxazinorifamycins. *Expert Opin. Investig. Drugs,* **2006,** *15* (6), 603-623.

[192] Tanaka, T.; Endo, T.; Shimazu, A.; Yoshida, R.; Suzuki, Y., A new antibiotic, multhiomycin. *J. Antibiot.,* **1970,** *23* (5), 231-237.

[193] Haste, N. M.; Thienphrapa, W.; Tran, D. N.; Loesgen, S.; Sun, P.; Nam, S. J.; Jensen, P. R.; Fenical, W.; Sakoulas, G.; Nizet, V.; Hensler, M. E., Activity of the thiopeptide antibiotic nosiheptide against contemporary strains of methicillin-resistant *Staphylococcus aureus*. *J. Antibiot.,* **2012,** *65* (12), 593-598.

[194] Cundliffe, E.; Thompson, J., The mode of action of nosiheptide (multhiomycin) and the mechanism of resistance in the producing organism. *J. Gen. Microbiol.,* **1981,** *126* (1), 185-192.

[195] Walsh, C. T.; Acker, M. G.; Bowers, A. A., Thiazolyl peptide antibiotic biosynthesis: a cascade of post-translational modifications on ribosomal nascent proteins. *J. Biol. Chem.,* **2010,** *285* (36), 27525-27531.

[196] LaMarche, M. J.; Leeds, J. A.; Dzink-Fox, J.; Gangl, E.; Krastel, P.; Neckermann, G.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Tiamfook, S.; Yu, D., Antibiotic optimization and chemical structure stabilization of thiomuracin A. *J. Med. Chem.,* **2012,** *55* (15), 6934-6941.

[197] LaMarche, M. J.; Leeds, J. A.; Dzink-Fox, J.; Gangl, E.; Krastel, P.; Neckermann, G.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Tiamfook, S.; Yu, D., Correction to antibiotic optimization and chemical structure stabilization of thiomuracin A. *J. Med. Chem.,* **2013,** *56* (3), 1360.

[198] Morris, R. P.; Leeds, J. A.; Naegeli, H. U.; Oberer, L.; Memmert, K.; Weber, E.; LaMarche, M. J.; Parker, C. N.; Burrer, N.; Esterow, S.; Hein, A. E.; Schmitt, E. K.; Krastel, P., Ribosomally synthesized thiopeptide antibiotics targeting elongation factor Tu. *J. Am. Chem. Soc.,* **2009,** *131* (16), 5946-5955.

[199] Zhang, S. J.; Yang, Q.; Xu, L.; Chang, J.; Sun, X., Synthesis and antibacterial activity against *Clostridium difficile* of novel demethylvancomycin derivatives. *Bioorg. Med. Chem. Lett.,* **2012,** *22* (15), 4942-4945.

[200] Boeck, L. D.; Mertz, F. P.; Wolter, R. K.; Higgens, C. E., N-demethylvancomycin, a novel antibiotic produced by a strain of *Nocardia orientalis.* Taxonomy and fermentation. *J. Antibiot.,* **1984,** *37* (5), 446-453.

[201] Goldstein, E. J. C.; Citron, D. M.; Merriam, C. V.; Warren, Y.; Tyrrell, K.; Fernandez, H. T., *In vitro* activities of dalbavancin and nine comparator agents against anaerobic gram-positive species and corynebacteria. *Antimicrob. Agents Chemother.,* **2003,** *47* (6), 1968-1971.

[202] Goldstein, E. J. C.; Citron, D. M.; Merriam, C. V.; Warren, Y. A.; Tyrrell, K. L.; Fernandez, H. T., *In vitro* activities of the new semisynthetic glycopeptide telavancin (TD-6424), vancomycin, daptomycin, linezolid, and four comparator agents against anaerobic gram-positive species and *Corynebacterium* spp. *Antimicrob. Agents Chemother.,* **2004,** *48* (6), 2149-2152.

[203] Hurdle, J. G.; O'Neill, A. J.; Chopra, I.; Lee, R. E., Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections. *Nat. Rev. Microbiol.,* **2010,** *9* (1), 62-75.

[204] Lao II, D.; Chiang, T.; Gomez, E., Refractory *Clostridium difficile* infection successfully treated with tigecycline, rifaximin, and vancomycin. *Case Rep. Med.,* **2012,** *2012*, Article ID 702910, 3 pages, doi:10.1155/2012/702910.

[205] Lu, C.-L.; Liu, C.-Y.; Liao, C.-H.; Huang, Y.-T.; Wang, H.-P.; Hsueh, P.-R., Severe and refractory *Clostridium difficile* infection

successfully treated with tigecycline and metronidazole. *Int. J. Antimicrob. Agents,* **2010,** *35* (3), 311-312.

[206] Herpers, B. L.; Vlaminckx, B.; Burkhardt, O.; Blom, H.; Biemond-Moeniralam, H. S.; Hornef, M.; Welte, T.; Kuijper, E. J., Intravenous tigecycline as adjunctive or alternative therapy for severe refractory *Clostridium difficile* infection. *Clin. Infect. Dis.,* **2009,** *48* (12), 1732-1735.

[207] Larson, K. C.; Belliveau, P. P.; Spooner, L. M., Tigecycline for the treatment of severe *Clostridium difficile* infection. *Ann. Pharmacother.,* **2011,** *45* (7-8), 1005-1010.

[208] McKeage, K.; Keating, G., Tigecycline. *Drugs,* **2008,** *68* (18), 2633-2644.

[209] Baines, S. D.; Saxton, K.; Freeman, J.; Wilcox, M. H., Tigecycline does not induce proliferation or cytotoxin production by epidemic *Clostridium difficile* strains in a human gut model. *J. Antimicrob. Chemother.,* **2006,** *58* (5), 1062-1065.

[210] Hawser, S. P., Activity of tigecycline against multidrug-resistant clinical isolates of *Clostridium* spp. from Europe. *Int. J. Antimicrob. Agents,* **2010,** *35* (3), 310-311.

[211] Nord, C. E.; Sillerström, E.; Wahlund, E., Effect of tigecycline on normal oropharyngeal and intestinal microflora. *Antimicrob. Agents Chemother.,* **2006,** *50* (10), 3375-3380.

[212] Kopterides, P.; Papageorgiou, C.; Antoniadou, A.; Papadomichelakis, E.; Tsangaris, I.; Dimopoulou, I.; Armaganidis, A., Failure of tigecycline to treat severe *Clostridium difficile* infection. *Anaesth. Intensive Care,* **2010,** *38* (4), 755-758.

[213] Safety and efficacy of tigecycline in the treatment of *C. Difficile* associated diarrhea (CDAD). http://www.clinicaltrials.gov/ct2/show/NCT01401023?term=01401023&rank=1 (accessed September 8, 2013).

[214] Slover, C. M.; Rodvold, K. A.; Danziger, L. H., Tigecycline: a novel broad-spectrum antimicrobial. *Ann. Pharmacother.,* **2007,** *41* (6), 965-972.

[215] Koga, T.; Masuda, N.; Kakuta, M.; Namba, E.; Sugihara, C.; Fukuoka, T., Potent *in vitro* activity of tomopenem (CS-023) against methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.,* **2008,** *52* (8), 2849-2854.

[216] Tanaka, K.; Mikamo, H.; Nakao, K.; Ichiishi, T.; Goto, T.; Yamagishi, Y.; Watanabe, K., *In vitro* activity of tomopenem (CS-023/RO4908463) against anaerobic bacteria. *Antimicrob. Agents Chemother.,* **2009,** *53* (1), 319-322.

[217] Zapun, A.; Contreras-Martel, C.; Vernet, T., Penicillin-binding proteins and β-lactam resistance. *FEMS Microbiol. Rev.,* **2008,** *32* (2), 361-385.

[218] Jeong, E.-Y.; Jeon, J.-H.; Lee, C.-H.; Lee, H.-S., Antimicrobial activity of catechol isolated from Diospyros kaki Thunb. roots and its derivatives toward intestinal bacteria. *Food Chem.,* **2009,** *115* (3), 1006-1010.

[219] Heipieper, H. J.; Keweloh, H.; Rehm, H. J., Influence of phenols on growth and membrane permeability of free and immobilized *Escherichia coli*. *Appl. Environ. Microbiol.,* **1991,** *57* (4), 1213-1217.

[220] Kim, M. G.; Lee, H. S., Growth-inhibiting activities of phenethyl isothiocyanate and its derivatives against intestinal bacteria. *J. Food Sci.,* **2009,** *74* (8), M467-M471.

[221] Hideki, M.; Woo, G.; Shin, I.; Kinae, N., Inhibitory effects of Gochoonangi (Wasabial japonica) against *Helicobacter pylori* and its urease activity. *Food Sci. Biotechnol.,* **2004,** *13* (2), 191-196.

[222] Abreu, A. C.; Borges, A.; Simoes, L. C.; Saavedra, M. J.; Simoes, M., Antibacterial activity of phenyl isothiocyanate on *Escherichia coli* and *Staphylococcus aureus*. *Med. Chem.,* **2012,** *9* (5), 756-761.

[223] Jeong, E.-Y.; Jeon, J.-H.; Kim, H.-W.; Kim, M.-G.; Lee, H.-S., Antimicrobial activity of leptospermone and its derivatives against human intestinal bacteria. *Food Chem.,* **2009,** *115* (4), 1401-1404.

[224] van Klink, J. W.; Larsen, L.; Perry, N. B.; Weavers, R. T.; Cook, G. M.; Bremer, P. J.; MacKenzie, A. D.; Kirikae, T., Triketones active against antibiotic-resistant bacteria: Synthesis, structure-activity relationships, and mode of action. *Bioorg. Med. Chem.,* **2005,** *13* (24), 6651-6662.

[225] Hurdle, J. G.; Heathcott, A. E.; Yang, L.; Yan, B.; Lee, R. E., Reutericyclin and related analogues kill stationary phase *Clostridium difficile* at achievable colonic concentrations. *J. Antimicrob. Chemother.,* **2011,** *66* (8), 1773-1776.

[226] Wu, X.; Cherian, P. T.; Lee, R. E.; Hurdle, J. G., The membrane as a target for controlling hypervirulent *Clostridium difficile* infections. *J. Antimicrob. Chemother.,* **2012,** *68* (4), 806-815.

[227] Gänzle, M. G.; Vogel, R. F., Studies on the mode of action of reutericyclin. *Appl. Environ. Microbiol.,* **2003,** *69* (2), 1305-1307.

[228] Canning, C.; Sun, S.; Ji, X.; Gupta, S.; Zhou, K., Antibacterial and cytotoxic activity of isoprenylated coumarin mammea A/AA isolated from *Mammea africana*. *J. Ethnopharmacol.,* **2013,** *147* (1), 259-262.

[229] Gellert, M.; O'Dea, M. H.; Itoh, T.; Tomizawa, J., Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. *Proc. Natl. Acad. Sci. U.S.A.,* **1976,** *73* (12), 4474-4478.

[230] Ali, J. A.; Jackson, A. P.; Howells, A. J.; Maxwell, A., The 43-kilodalton N-terminal fragment of the DNA gyrase B protein hydrolyzes ATP and binds coumarin drugs. *Biochemistry,* **1993,** *32* (10), 2717-2724.

[231] Lewis, R. J.; Tsai, F. T. F.; Wigley, D. B., Molecular mechanisms of drug inhibition of DNA gyrase. *Bioessays,* **1996,** *18* (8), 661-671.

[232] Maxwell, A., The interaction between coumarin drugs and DNA gyrase. *Mol. Microbiol.,* **1993,** *9* (4), 681-686.

[233] Sugino, A.; Higgins, N. P.; Brown, P. O.; Peebles, C. L.; Cozzarelli, N. R., Energy coupling in DNA gyrase and the mechanism of action of novobiocin. *Proc. Natl. Acad. Sci. U.S.A.,* **1978,** *75* (10), 4838-4842.

[234] Nijveldt, R. J.; van Nood, E.; van Hoorn, D. E.; Boelens, P. G.; van Norren, K.; van Leeuwen, P. A., Flavonoids: a review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr.,* **2001,** *74* (4), 418-425.

[235] Wu, X.; Alam, Z.; Feng, L.; Tsutsumi, L. S.; Sun, D.; Hurdle, J.G. Prospects for flavonoid and related phytochemicals as nature-inspired treatments for *Clostridium difficile* infection. *J. Appl. Microbiol.,* **2013**, in press (doi:10.1111/jam.12344).

[236] Jacoby, G. A.; Hooper, D. C., Review of the quinolone family. In *Antibiotic Discovery and Development*, Dougherty, T. J.; Pucci, M. J., Eds. Springer US: New York, **2012**; pp 119-146.

[237] Payne, D. J.; Gwynn, M. N.; Holmes, D. J.; Pompliano, D. L., Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat. Rev. Drug Discov.,* **2006,** *6* (1), 29-40.

[238] Fischbach, M. A.; Walsh, C. T., Antibiotics for emerging pathogens. *Science,* **2009,** *325* (5944), 1089-1093.

[239] Walsh, C., Where will new antibiotics come from? *Nat. Rev. Microbiol.,* **2003,** *1* (1), 65-69.

[240] Costello, C.; Karpanen, T.; Lambert, P. A.; Mistry, P.; Parker, K. J.; Rathbone, D. L.; Ren, J.; Wheeldon, L.; Worthington, T., Thiosemicarbazones active against *Clostridium difficile*. *Bioorg. Med. Chem. Lett.,* **2008,** *18* (5), 1708-1711.

[241] Raether, W.; Hänel, H., Nitroheterocyclic drugs with broad spectrum activity. *Parasitol. Res.,* **2003,** *90* (Suppl. 1), S19-39.

[242] Farrell, D. J.; Robbins, M.; Rhys-Williams, W.; Love, W. G., *In vitro* activity of XF-73, a novel antibacterial agent, against antibiotic-sensitive and -resistant Gram-positive and Gram-negative bacterial species. *Int. J. Antimicrob. Agents,* **2010,** *35* (6), 531-536.

[243] Ooi, N.; Miller, K.; Hobbs, J.; Rhys-Williams, W.; Love, W.; Chopra, I., XF-73, a novel antistaphylococcal membrane-active agent with rapid bactericidal activity. *J. Antimicrob. Chemother.,* **2009,** *64* (4), 735-740.

[244] Beveridge, T. J., Structures of gram-negative cell walls and their derived membrane vesicles. *J. Bacteriol.,* **1999,** *181* (16), 4725-4733.

[245] Butler, M. M.; Shinabarger, D. L.; Citron, D. M.; Kelly, C. P.; Dvoskin, S.; Wright, G. E.; Feng, H.; Tzipori, S.; Bowlin, T. L., MBX-500, a hybrid antibiotic with *in vitro* and *in vivo* efficacy against toxigenic *Clostridium difficile*. *Antimicrob. Agents Chemother.,* **2012,** *56* (9), 4786-4792.

[246] Steele, J.; Zhang, Q.; Beamer, G.; Butler, M.; Bowlin, T.; Tzipori, S., MBX-500 is effective for the treatment of *Clostridium difficile* infection in gnotobiotic piglets. *Antimicrob. Agents Chemother.,* **2013,** *57* (8), 4039-4041.

[247] Butler, M. M.; Williams, J. D.; Peet, N. P.; Moir, D. T.; Panchal, R. G.; Bavari, S.; Shinabarger, D. L.; Bowlin, T. L., Comparative *in vitro* activity profiles of novel bis-indole antibacterials against gram-positive and gram-negative clinical isolates. *Antimicrob. Agents Chemother.,* **2010,** *54* (9), 3974-3977.

[248] Brosh, R. M.; Karow, J. K.; White, E. J.; Shaw, N. D.; Hickson, I. D.; Bohr, V. A., Potent inhibition of Werner and Bloom helicases by DNA minor groove binding drugs. *Nucleic Acids Res.,* **2000,** *28* (12), 2420-2430.

[249] Panchal, R. G.; Ulrich, R. L.; Lane, D.; Butler, M. M.; Houseweart, C.; Opperman, T.; Williams, J. D.; Peet, N. P.; Moir, D. T.; Nguyen, T.; Gussio, R.; Bowlin, T.; Bavari, S., Novel broad-spectrum bis-(imidazolinylindole) derivatives with potent antibacterial activities against antibiotic-resistant strains. *Antimicrob. Agents Chemother.,* **2009,** *53* (10), 4283-4291.

[250] Ravic, M.; Suckling, C.; Gemmel, C.; Warn, P.; Sattar, A.; Saunders, M., Disposition of MGB-BP-3, a new class of antibacterial agent, after oral administration in a hamster model of severe *Clostridium difficile* associated diarrhoea (CDAD). In *52nd Interscience Conference on Antimicrobial Agents and Chemotherapy*, San Francisco, CA, USA, September 9-12, 2012, Abstract No: 1530.

[251] Dvoskin, S.; Xu, W. C.; Brown, N. C.; Yanachkov, I. B.; Yanachkova, M.; Wright, G. E., A novel agent effective against *Clostridium difficile* infection. *Antimicrob. Agents Chemother.*, **2012,** *56* (3), 1624-1626.

[252] Torti, A.; Lossani, A.; Savi, L.; Focher, F.; Wright, G. E.; Brown, N. C.; Xu, W.-C., *Clostridium difficile* DNA polymerase IIIC: basis for activity of antibacterial compounds. *Curr. Enzym. Inhib.,* **2011,** *7* (3), 147-153.

[253] Barman, T. K.; Pandya, M.; Mathur, T.; Bhadauriya, T.; Rao, M.; Khan, S.; Singhal, S.; Bhateja, P.; Sood, R.; Malhotra, S.; Das, B.; Paliwal, J.; Bhatnagar, P. K.; Upadhyay, D. J., Novel biaryl oxazolidinones: *in vitro* and *in vivo* activities with pharmacokinetics in an animal model. *Int. J. Antimicrob. Agents,* **2009,** *33* (3), 280-284.

[254] Mathur, T.; Kumar, M.; Barman, T. K.; Kumar, G. R.; Kalia, V.; Singhal, S.; Raj, V. S.; Upadhyay, D. J.; Das, B.; Bhatnagar, P. K., Activity of RBx 11760, a novel biaryl oxazolidinone, against *Clostridium difficile*. *J. Antimicrob. Chemother.,* **2011,** *66* (5), 1087-1095.

[255] Ackermann, G.; Adler, D.; Rodloff, A. C., *In vitro* activity of linezolid against *Clostridium difficile*. *J. Antimicrob. Chemother.,* **2003,** *51* (3), 743-745.

[256] Wise, R.; Andrews, J.; Boswell, F.; Ashby, J., The in-vitro activity of linezolid (U-100766) and tentative breakpoints. *J. Antimicrob. Chemother.,* **1998,** *42* (6), 721-728.

[257] Edlund, C.; Oh, H.; Nord, C. E., *In vitro* activity of linezolid and eperezolid against anaerobic bacteria. *Clin. Microbiol. Infect.,* **1999,** *5* (1), 51-53.

[258] Mathur, T.; Kalia, V.; Barman, T. K.; Singhal, S.; Khan, S.; Upadhyay, D. J.; Rattan, A.; Raj, V. S., Anti-anaerobic potential of ranbezolid: insight into its mechanism of action against *Bacteroides fragilis*. *Int. J. Antimicrob. Agents,* **2013,** *41* (1), 36-40.

[259] Kalia, V.; Miglani, R.; Purnapatre, K. P.; Mathur, T.; Singhal, S.; Khan, S.; Voleti, S. R.; Upadhyay, D. J.; Saini, K. S.; Rattan, A., Mode of action of ranbezolid against staphylococci and structural modeling studies of its interaction with ribosomes. *Antimicrob. Agents Chemother.,* **2009,** *53* (4), 1427-1433.

[260] Hemphill, A.; Mueller, J.; Esposito, M., Nitazoxanide, a broad-spectrum thiazolide anti-infective agent for the treatment of gastrointestinal infections. *Expert Opin. Pharmacother.*, **2006,** *7* (7), 953-964.

[261] Warren, C. A.; van Opstal, E.; Ballard, T. E.; Kennedy, A.; Wang, X.; Riggins, M.; Olekhnovich, I.; Warthan, M.; Kolling, G. L.; Guerrant, R. L.; Macdonald, T. L.; Hoffman, P. S., Amixicile, a novel inhibitor of pyruvate: ferredoxin oxidoreductase, shows efficacy against *Clostridium difficile* in a mouse infection model. *Antimicrob. Agents Chemother.*, **2012,** *56* (8), 4103-4111.

[262] Ballard, T. E.; Wang, X.; Olekhnovich, I.; Koerner, T.; Seymour, C.; Salamoun, J.; Warthan, M.; Hoffman, P. S.; Macdonald, T. L., Synthesis and antimicrobial evaluation of nitazoxanide-based analogues: identification of selective and broad spectrum activity. *Chem. Med. Chem.,* **2011,** *6* (2), 362-377.

[263] Ballard, T. E.; Wang, X.; Olekhnovich, I.; Koerner, T.; Seymour, C.; Hoffman, P. S.; Macdonald, T. L., Biological activity of modified and exchanged 2-amino-5-nitrothiazole amide analogues of nitazoxanide. *Bioorg. Med. Chem. Lett.,* **2010,** *20* (12), 3537-3539.

[264] Goldstein, E. J. C.; Citron, D. M.; Tyrrell, K. L.; Merriam, C. V., Comparative *in vitro* activities of GSK2251052, a novel boron-containing leucyl-tRNA synthetase inhibitor, against 916 anaerobic organisms. *Antimicrob. Agents Chemother.,* **2013,** *57* (5), 2401-2404.

[265] Bouchillon, S.; Hoban, D.; Hackel, M.; Butler, D.; Demarsch, P.; Alley, D., *In vitro* activities of AN3365: a novel boron containing protein synthesis inhibitor and other antimicrobial agents against anaerobes and *Neisseria gonorrhoeae*. In *50th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Boston, MA, USA, September 12-15, **2010**, Abstract No: F1-1640.

[266] Rock, F. L.; Mao, W.; Yaremchuk, A.; Tukalo, M.; Crépin, T.; Zhou, H.; Zhang, Y.-K.; Hernandez, V.; Akama, T.; Baker, S. J., An antifungal agent inhibits an aminoacyl-tRNA synthetase by trapping tRNA in the editing site. *Science,* **2007,** *316* (5832), 1759-1761.

[267] Richter, S. G.; Elli, D.; Kim, H. K.; Hendrickx, A. P. A.; Sorg, J. A.; Schneewind, O.; Missiakas, D., Small molecule inhibitor of lipoteichoic acid synthesis is an antibiotic for Gram-positive bacteria. *Proc. Natl. Acad. Sci. U.S.A.,* **2013,** *110* (9), 3531-3536.

[268] Kennedy, D. A.; Vembu, N.; Fronczek, F. R.; Devocelle, M., Synthesis of mutual azo prodrugs of anti-inflammatory agents and peptides facilitated by α-aminoisobutyric acid. *J. Org. Chem.,* **2011,** *76* (23), 9641-9647.

[269] Wade, D.; Silberring, J.; Soliymani, R.; Heikkinen, S.; Kilpeläinen, I.; Lankinen, H.; Kuusela, P., Antibacterial activities of temporin A analogs. *FEBS Lett.,* **2000,** *479* (1-2), 6-9.

[270] Rea, M. C.; Sit, C. S.; Clayton, E.; O'Connor, P. M.; Whittal, R. M.; Zheng, J.; Vederas, J. C.; Ross, R. P.; Hill, C., Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against *Clostridium difficile*. *Proc. Natl. Acad. Sci. U.S.A.,* **2010,** *107* (20), 9352-9357.

[271] Rea, M. C.; Dobson, A.; O'Sullivan, O.; Crispie, F.; Fouhy, F.; Cotter, P. D.; Shanahan, F.; Kiely, B.; Hill, C.; Ross, R. P., Effect of broad- and narrow-spectrum antimicrobials on *Clostridium difficile* and microbial diversity in a model of the distal colon. *Proc. Natl. Acad. Sci. U.S.A.,* **2011,** *108* (Suppl. 1), 4639-4644.

[272] Johnson, S.; Gerding, D. N.; Louie, T. J.; Ruiz, N. M.; Gorbach, S. L., Sustained clinical response as an endpoint in treatment trials of *Clostridium difficile*-associated diarrhea. *Antimicrob. Agents Chemother.,* **2012,** *56* (8), 4043-4045.

[273] Baines, S. D.; Freeman, J.; Wilcox, M. H., Tolevamer is not efficacious in the neutralization of cytotoxin in a human gut model of *Clostridium difficile* infection. *Antimicrob. Agents Chemother.,* **2009,** *53* (5), 2202-2204.

Received: August 09, 2013 Revised: September 06, 2013 Accepted: September 15, 2013