Ref: Ro J Infect Dis. 2022;25(2)
DOI: 10.37897/RJID.2022.2.6

Current and future approaches in Clostridioides Difficile management

George Sebastian Gherlan

"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
"Dr. Victor Babes" Clinical Hospital of Infectious and Tropical Diseases, Bucharest, Romania
"Dr. Victor Babes" Diagnostic and Treatment Center, Bucharest, Romania

ABSTRACT

Clostridioides Difficile is considered one of the main causes of healthcare associated infections worldwide and the main cause of antibiotic-associated diarrhea, with forms of clinical disease ranging from mild to deadly. Both 2021 IDSA and ESCMID guidelines recommend that for the first episode of CDI Fidaxomicin should be considered as the preferred regimen of treatment and Vancomycin becomes 2nd Standard of Care (SOC) line, an alternative to fidaxomicin. Monoclonal antibodies, fecal microbiota transplantation and surgery remain other recommendations in the guidelines.

New means of management of Clostridioides Difficile Infection are under development, these including prevention measures (vaccination, population with non-toxigenic strains of the colon, standardized fecal microbiota products) and new antibiotics.

Keywords: Clostridioides

INTRODUCTION

Pseudomembranous colitis was first described in 1893 [1], before the discovery of antibiotics, but it was not until the antibiotics era when this disease knew a substantial increase in cases. Lyncomicins (of which clindamycin was the most prominent representative) seemed to be especially linked with this situation and therefore, in 1974, FJ Tedesco started a prospective study that led to the identification of Clostridium Difficile and its toxins as the cause of the "Clindamycin associated colitis" [2]. No signs indicated at that time the magnitude this pandemic was about to achieve in the years after 2000. Today, Clostridioides Difficile is considered one of the main causes of healthcare associated infections worldwide and the main cause of antibiotic-associated diarrhea, with forms of clinical disease ranging from mild to deadly. It is worth mention that up to 3% percent of the patients can be colonized, and they develop no signs of illness [3].

DEFINITIONS

The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) released in 2021 an update of the previous (2014) guidelines for the treatment of Clostridioides Difficile infection (CDI) in adults [4]. The following definitions were set:

An **episode of CDI** is defined as clinical findings compatible with CDI and microbiological evidence of C. difficile free toxins by enzyme immunoassay without reasonable evidence of another cause of diarrhoea **OR** a clinical picture compatible with CDI and a positive nucleic acid amplification test (NAAT) preferably with a low cycle threshold (Ct) value, or positive toxigenic C. difficile culture **OR** pseudomembranous colitis as diagnosed during endos-

Corresponding author:
George Sebastian Gherlan
E-mail: gherlanus@gmail.com

Article History:
Received: 28 June 2022
Accepted: 30 June 2022

copy, after colectomy or on autopsy, in combination with a positive test for the presence of toxigenic C. difficile [4].

Treatment response is present when the patient has resolution of diarrhoea and has had a normal stool for that patient, with maintenance of resolution for the duration of therapy and at least 48 hours after the end of treatment, and no further requirement for CDI therapy, **AND** parameters of disease severity (clinical, laboratory, radiological) have improved.

Refractory CDI is CDI not responding to recommended CDI antibiotic treatment after 3-5 days of therapy.

Recurrence is present when CDI recurs within 8 weeks after a previous episode, provided the symptoms from the previous episode resolved after completion of initial treatment

Severe CDI is characterized by one of the following factors at presentation: fever, marked leucocytosis and rise in serum creatinine.

Current approach:

Both IDSA and ESCMID guidelines recommend that for the first episode of CDI Fidaxomicin should be considered as the preferred regimen of treatment (200 mg orally bid 10 days) and Vancomycin becomes 2nd Standard of Care (SOC) line, an alternative to fidaxomicin (at a dose of 125 mg orally qid for 10 days) [4,5]. This recommendation is based on the fact that fidaxomicin, as well as vancomycin is orally administered, has low systemic absorption and a narrower spectrum. Recurrences are fewer with fidaxomicin. For non-severe CDI, if fidaxomicin and/or vancomycin are not available, metronidazole can be used (500 mg orally tid for 10-14 days). Other general measures should be considered:

- Stopping any other antibiotic therapy if not necessary
- Stopping proton pump inhibitors
- Avoiding the use of anti-motility treatment
- Treating adequately the hidro-electrolitic imbalances.

In cases of recurrence, for the first recurrence, Fidaxomicin (200 mg orally bid 10 days) plus bezlotoxumab should be used if Fidaxomicin was used as first line, and if Vancomycin was used, then fidaxomicin alone (200 mg orally bid 10 days). For the second recurrence, and further episodes, fecal microbiota transplantation (FMT) should be considered or Fidaxomicin (200 mg orally bid 10 days) plus bezlotoxumab. Vancomycin taper and pulse is still recommended if the above alternatives for recurrences are not available: 2 weeks 125 mg qid, 1 week 125 mg bid, 1 week 125 mg qid, then 1 week 125 mg q48h, and finally 125 mg q72h. Vancomycin in higher doses than 125 mg qid (e.g. 500 mg qid) is no longer recommended. I.v. metronidazole or i.v. tigecycline are recommended as adjunctive therapies in severe and/or complicated cases [4,5]. Prolonged administration of fidaxomicin (extended fidaxomicin), i.e. 200 mg twice daily on days 1-5, and 200 mg once daily on alternate days on days 7-25 can be considered for an episode of CDI with increased risk of recurrence, especially in elderly hospitalized patients.

Bezlotoxumab is a monoclonal antibody directed against C. difficile toxin B. His addition to SOC CDI antibiotics resulted in 10% reduced risk of recurrences in the MODIFY-I and II trials and similar rates of cure. Most patients received treatment with vancomycin; if fidaxomicin is used for treatment of initial or recurrent CDI, the utility of bezlotoxumab is unknown [6]. Bezlotoxumab received US Food and Drug Administration approval in 2016 [7]. Bezlotoxumab is given as a one-time infusion, during administration of a standard treatment regimen and caution is recommended in patients with congestive heart failure [6]. Bezlotoxumab seems to be more efficient when 3 risk factors of age ≥65 years, history of severe CDI, immunocompromise, and infection with ribotype 027/078/244 are present, reducing the risk of recurrence by 25%, compared to 1 or 2 risk factors from the above, when the risk is reduced by 15% [8]. Therefore, the ESCMID guidelines recommend the addition of bezlotoxumab to SOC for an episode of CDI with increased risk of recurrence when fidaxomicin is not available or feasible [4].

Surgery is part of the treatment of C. Difficile infection, in severely complicated cases. Surgical consultation is recommended in patients with signs of severity like hypotension, high fever, ileus, peritonitis, high number of white blood cells (over 20.000/mmc), altered mental status, acidosis, MSOF, no longer improving after 5 days of maximal therapy [4,9,10]. Total abdominal colectomy (TAC) is the standard when surgery is needed [4]. If possible, some authors recommend partial colectomy or loop ileostomy if the situation permits as mortality seems to be significantly low in these cases [11,12]. Discordantly, others favor open total abdominal colectomy with end ileostomy rather than another procedure [13].

Fecal microbiota transplantation (FMT) refers to instillation of processed stool collected from a healthy donor into the intestinal tract of a patient with CDI. FMT has been made under various protocols that may differ largely from center to center [5,14]. In general, FMT protocols utilize donor stool suspended in normal saline prior to administration (via colonoscopy, enema, or nasoduodenal/nasojejunal tube). Stool is homogenized (using blender, manual effort, or other method) to liquid consistency and filtered (eg, gauze, coffee filter, strainer) to

remove particulate matter. This processed specimen is then either directly infused into the GI tract or further centrifuged and capsulized in gelatin capsules that can be administered orally. FMT has become an accepted treatment for multiple recurrent CDI [4,5]. FMT has a clear role in severe refractory CDI and its main virtue is that frequently can lead to avoidance of surgery in these cases [4,15].

Primary prophylaxis of a first episode of CDI is mainly based on the minimization of the use of antibiotics and proton pump inhibitors or other medication that lowers gastric acidity. Using oral vancomycin may be of benefit in patients at high risk for CDI [16]. The use of probiotics is not recommended by guidelines [4,5,14] and instead the risk of complications and delay in microbiome restitution are highlighted [17.18]. There are multiple studies of various probiotics for CDI prevention and the data are highly inconsistent. Secondary prevention can be obtained by the above measures or by using SOC for CDI antibiotics in patients that require other courses of antibiotics.

Future approaches for the prevention of CDI:

Vaccine studies in CDI domain were mainly based on toxin-based formulations until recent [19]. Unfortunately, the concept did not seem to function as these vaccines, even if they showed an effect in reducing disease episode as well as good safety profile, they did not prevent the infection. These vaccines promote toxin-neutralising serum antibodies but fail to confer protection from infection in the gut. Some studies showed even a non-favorable safety profile with reactions at the site of administration that led to early stop of the programs [20, 21]. Another proposed mechanism for the vaccination is via oral administration of an antigen (the colonization factor CD0873 and a fragment of toxin B (TcdB) in order to produce mucosal IgA and systemic IgG. This approach seems promising, but data are still needed to conclude [22]. Colonization with non-toxigenic strains of C. Difficile may prevent infection acting like an active vaccine or by substituting the toxin-producing strains. One of the most studied non-toxigenic strains is CCUG37785 that was engineered to express immunogenic fragments of TcdA and TcdB [23]. No vaccine is still available, but many researches are in progress and there is hope that in the future an effective vaccine can be produced.

A novel approach for primary prevention of CDI is coadministration of a poorly absorbed beta-lactamase (ribaxamase, SYN004) when administering broad-spectrum antibiotics [24, 25].

Alternatives to classic FMT are also in development. RBX2660 - developed by Rebiotix MRT™ - is a formulation composed of filtered and cryopreserved stool derived from healthy donors. In phase III studies already, RBX2660 has already shown efficacy and safety in preventing recurrences [26]. In order to avoid the lack of regulation and uniformity in the domain of FMT, a group of researchers proposed a microbial consortium called RePOOPulate or Microbial Ecosystem Therapeutic 1 (MET – 1) formed of 33 bacterial strains from the stool of a healthy donor cultivated using a bioreactor and selected based on antimicrobial resistance profiles. MET – 1 showed that enhances barrier function in T84 human intestinal epithelial cells and protects against the disruption of F-actin by purified TcdA in T84 and Caco2 cells [27]. SER-109, developed by Seres Therapeutics is another formulation of almost 50 species of Firmicutes, that showed a promising result, with 29 of 30 patients achieving clinical improvement in a study, and also a phase 3 study showed a good effect on recurrence [28].

Future approaches for the treatment of CDI:

Some of the antibiotics already on the market have been used off-label for the treatment of CDI and showed efficacy.

Nitazoxanide, an antiparasitic medication already commercialized has shown results comparable with vancomycin [29]. It showed comparable results with metronidazole also [30]. Fusidic acid, an anti-staphylococcal medication excerpts also some effects on CDI [31]. The tetracyclines, have an increasing amount of evidence emerging to support their use for CDI. From this class, tigecycline is recommended by the guidelines as an additive in severe complicated forms of CDI. Other compounds have already been tested and showed activity against C. Difficile in in vivo or in vitro studies: eravacycline, omadacycline and aminomethycicline [32,33]. Because these tetracyclines have a broad spectrum, their use in CDI will be probably limited in the future as they can cause collateral damage.

A systematic review that included 8 studies on rifaximin in the treatment of CDI concluded that in the treatment of mild-moderate CDI, rifaximin is a viable alternative to existing therapies, but large rates of resistance and the risk of selecting more resistant strains are discouraging [34]. Clofazimine was approved by FDA in 1986 and is currently tested for CDI.

New antibiotics are being developed for the treatment of CDI, some of them with quite promising results.

Ridinilazole is an orally administered antibiotic whose mechanism of action is incompletely understood [35]. Phase III studies are currently ongoing and results are promising, with results superior to vancomycin (sustained clinical response 66.7% versus 42.4%) and no significant adverse events that led to discontinuation of the therapy [36].

Nisin is a bacteriocin produced by a group of Gram-positive bacteria that belongs to Lactococcus

and Streptococcus species. Nisin Z and A inhibit the growth of C. difficile in vitro. Nisin A also affects C. difficile spores, decreasing the spore viability by 40–50% [37].

LFF571 inhibits bacterial growth by binding to protein synthesis elongation factor Tu (EF-Tu) [38]. Novartis has finished phase II studies in 2015 and promising results were published, the substance showing high potency with narrow spectrum against CDI, still no news regarding phase III studies have occurred yet [39].

Ramoplanin is a glycolipodepsipeptide antibiotic. Like LFF571, finished phase 1 and 2 studies with good results, but are no sign of continuation of the development [39]. It affects gut flora at the same extent as vancomycin, but what seemed promising was the fact that it affected spores of C. Difficile [40].

DNV3837 is an intravenously administered oxa-zolidinone–quinolone hybrid prodrug as well as ca-dazolid [39]. It decreases populations of C. Diff, Bifidobacterium spp., Lactobacillus spp., and Enterococcus spp. and increases Escherichia coli while not affecting Bacteroides spp. Counts [39].

Other drugs in more incipient phases of development are *Ibezapolstat* an oral antibiotic that inhibits the DNA polymerase IIIC of C. difficile, *CRS3123*, a fully synthetic diaryldiamine antibiotic that acts by inhibiting type 1 methionyl tRNA synthetase, a factor involved in bacterial protein synthesis, *DS-2969b*, that acts by inhibiting DNA gyrase B and can be administered orally or intravenously [39].

Gold nanoclusters developed with the help of nanotechnology are a solution under development [41].

As seen above, because of the existence of refractory forms, of severe and complicated forms, of relatively frequent recurrences, the need for new treatments in CDI is still high and therefore many studies are currently in development with promising compounds in the pipeline.

Conflict of interest: none declared
Financial support: none declared

REFERENCES

1. Finney JMT. Gastroenterostomy for cicatrizing ucler of the pylorus. *Johns Hopkins Med J.* 1893;4:53–55.
2. Tedesco FJ, Barton RW, Alpers DH. Clindamycin associated colitis. A prospective study. *Ann Intern Med.* 1974;81:429–433.
3. Padua D, Pothoulakis C. Novel approaches to treating clostridium difficile-associated colitis. *Expert Rev Gastroenterol Hepatology.* 2016;10(2):193–204. doi: 10.1586/17474124.2016.1109444
4. van Prehn J, Reigadas E, Vogelzang EH, Bouza E, Hristea A, Guery B, Krutova M, et al; Guideline Committee of the European Study Group on Clostridioides difficile. European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for Clostridioides difficile infection in adults. *Clin Microbiol Infect.* 2021 Dec;27(2):S1-S21. doi: 10.1016/j.cmi.2021.09.038. Epub 2021 Oct 20. PMID: 34678515.
5. Johnson S, Lavergne V, Skinner AM, Gonzales-Luna AJ, Garey KW, Kelly CP, Wilcox MH. Clinical Practice Guideline by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA): 2021 Focused Update Guidelines on Management of Clostridioides difficile Infection in Adults. *Clin Infect Dis.* 2021 Sep 7;73(5):e1029-e1044. doi: 10.1093/cid/ciab549. PMID: 34164674.
6. Wilcox MH, Gerding DN, Poxton IR, Kelly C, Nathan R, Birch T, et al. Bezlotoxumab for prevention of recurrent Clostridium difficile infection. *N Engl J Med* 2017;376:305-17
7. Johnson S, Gerding DN. Bezlotoxumab. *Clin Infect Dis*. 2019 Feb 1;68(4):699-704. doi: 10.1093/cid/ciy577. PMID: 30020417.
8. Gerding DN, Kelly CP, Rahav G, Lee C, Dubberke ER, Kumar PN, Yacyshyn B, Kao D, Eves K, Ellison MC, Hanson ME, Guris D, Dorr MB. Bezlotoxumab for Prevention of Recurrent Clostridium difficile Infection in Patients at Increased Risk for Recurrence. *Clin Infect Dis.* 2018 Aug 16;67(5):649-656. doi: 10.1093/cid/ciy171. PMID: 29538686; PMCID: PMC6093994.
9. Bhangu A, Nepogodiev D, Gupta A, Torrance A, Singh P; West Midlands Research Collaborative. Systematic review and meta-analysis of outcomes following emergency surgery for Clostridium difficile colitis. *Br J Surg.* 2012 Nov;99(11):1501-13. doi: 10.1002/bjs.8868. Epub 2012 Sep 13. PMID: 22972525.
10. van der Wilden GM, Velmahos GC, Chang Y, Bajwa E, O'Donnell WJ, Finn K, Harris NS, Yeh DD, King DR, de Moya MA, Fagenholz PJ. Effects of a New Hospital-Wide Surgical Consultation Protocol in Patients with Clostridium difficile Colitis. *Surg Infect (Larchmt).* 2017 Jul;18(5):563-569. doi: 10.1089/sur.2016.041. Epub 2017 May 30. PMID: 28557651.
11. Neal MD, Alverdy JC, Hall DE, Simmons RL, Zuckerbraun BS. Diverting loop ileostomy and colonic lavage: an alternative to total abdominal colectomy for the treatment of severe, complicated Clostridium difficile associated disease. *Ann Surg* 2011;254:423e7; discussion 7-9.
12. Ferrada P, Callcut R, Zielinski MD, Bruns B, Yeh DD, Zakrison TL, et al. Loop ileostomy versus total colectomy as surgical treatment for Clostridium difficile-associated disease: an Eastern Association for the Surgery of Trauma multicenter trial. *J Trauma Acute Care Surg* 2017;83:36e40.
13. Kautza B, Zuckerbraun BS. The Surgical Management of Complicated Clostridium Difficile Infection: Alternatives to Colectomy. *Surg Infect (Larchmt).* 2016 Jun;17(3):337-42. doi: 10.1089/sur.2016.006. PMID: 27206241.
14. Kelly CR, Fischer M, Allegretti JR, LaPlante K, Stewart DB, Limketkai BN, Stollman NH. ACG Clinical Guidelines: Prevention, Diagnosis, and Treatment of Clostridioides difficile Infections. *Am J Gastroenterol.* 2021 Jun 1;116(6):1124-1147. doi: 10.14309/ajg.0000000000001278. Erratum in: *Am J Gastroenterol.* 2022 Feb 1;117(2):358. PMID: 34003176.
15. Ianiro G, Masucci L, Quaranta G, Simonelli C, Lopetuso LR, Sanguinetti M, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy plus vancomycin for the treatment of severe refractory Clostridium difficile infection-single versus multiple infusions. *Aliment Pharmacol Ther* 2018;48:152e9.
16. Johnson SW, Brown SV, Priest DH. Effectiveness of Oral Vancomycin for Prevention of Healthcare Facility-Onset Clostridioides difficile Infection in Targeted Patients During Systemic Antibiotic Exposure. *Clin Infect Dis.* 2020 Aug 22;71(5):1133-1139. doi: 10.1093/cid/ciz966. PMID: 31560051.
17. Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, et al. Probiotic prophylaxis in predicted

severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008;371:651e9.
18. Suez J, Zmora N, Zilberman-Schapira G, Mor U, Dori-Bachash M, Bashiardes S, et al. Post-Antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. Cell 2018;174:1406e1423 e16.
19. Karyal C, Hughes J, Kelly ML, Luckett JC, Kaye PV, Cockayne A, Minton NP, Griffin R. Colonisation Factor CD0873, an Attractive Oral Vaccine Candidate against Clostridioides difficile. *Microorganisms.* 2021 Feb 2;9(2):306. doi: 10.3390/microorganisms9020306. PMID: 33540694; PMCID: PMC7913071.
20. de Bruyn G, Gordon DL, Steiner T, Tambyah P, Cosgrove C, Martens M, Bassily E, et al. Safety, immunogenicity, and efficacy of a Clostridioides difficile toxoid vaccine candidate: a phase 3 multicentre, observer-blind, randomised, controlled trial. *Lancet Infect Dis.* 2021 Feb;21(2):252-262. doi: 10.1016/S1473-3099(20)30331-5. Epub 2020 Sep 15. PMID: 32946836.
21. Lawrence J, Kitchin N, Anderson AS, Pride MW, Jansen KU, Gruber WC, Peng Y, et al. Safety and immunogenicity of different Clostridioides (Clostridium) difficile vaccine formulations in two early phase randomized studies of healthy adults aged 50-85 years. *Vaccine.* 2021 Sep 24;39(40):5991-6003. doi: 10.1016/j.vaccine.2021.05.028. Epub 2021 Sep 3. PMID: 34483022.
22. Karyal C, Hughes J, Kelly ML, Luckett JC, Kaye PV, Cockayne A, et al. Colonisation Factor CD0873, an Attractive Oral Vaccine Candidate against Clostridioides difficile. *Microorganisms.* 2021 Feb 2;9(2):306. doi: 10.3390/microorganisms9020306. PMID: 33540694; PMCID: PMC7913071.
23. Wang S, Heuler J, Wickramage I, Sun X. Genomic and Phenotypic Characterization of the Nontoxigenic Clostridioides difficile Strain CCUG37785 and Demonstration of Its Therapeutic Potential for the Prevention of C. difficile Infection. *Microbiol Spectr.* 2022 Apr 27;10(2):e0178821. doi: 10.1128/spectrum.01788-21. Epub 2022 Mar 22. PMID: 35315695; PMCID: PMC9045287.
24. Kokai-Kun JF, Roberts T, Coughlin O, Le C, Whalen H, Stevenson R, et al. Use of ribaxamase (SYN-004), a beta-lactamase, to prevent Clostridium difficile infection in beta-lactam-treated patients: a double-blind, phase 2b, randomized placebo-controlled trial. *Lancet Infect Dis* 2019;19:487e96.
25. Kokai-Kun JF, Le C, Trout K, Cope JL, Ajami NJ, Degar AJ, Connelly S. Ribaxamase, an Orally Administered β-Lactamase, Diminishes Changes to Acquired Antimicrobial Resistance of the Gut Resistome in Patients Treated with Ceftriaxone. *Infect Drug Resist.* 2020 Jul 22;13:2521-2535. doi: 10.2147/IDR.S260258. PMID: 32801790; PMCID: PMC7383106.
26. Khanna S, Assi M, Lee C, Yoho D, Louie T, Knapple W, Aguilar H, Garcia-Diaz J, Wang GP, Berry SM, Marion J, Su X, Braun T, Bancke L, Feuerstadt P. Efficacy and Safety of RBX2660 in PUNCH CD3, a Phase III, Randomized, Double-Blind, Placebo-Controlled Trial with a Bayesian Primary Analysis for the Prevention of Recurrent Clostridioides difficile Infection. *Drugs.* 2022 Oct;82(15):1527-1538. doi: 10.1007/s40265-022-01797-x. Epub 2022 Oct 26. Erratum in: Drugs. 2022 Nov 7. PMID: 36287379; PMCID: PMC9607700.
27. Munoz S, Guzman-Rodriguez M, Sun J, Zhang YG, Noordhof C, He SM, Allen-Vercoe E, Claud EC, Petrof EO. Rebooting the microbiome. *Gut Microbes.* 2016 Jul 3;7(4):353-363. doi: 10.1080/19490976.2016.1188248. Epub 2016 May 13. PMID: 27176179; PMCID: PMC4988458.
28. Feuerstadt P, Louie TJ, Lashner B, Wang EEL, Diao L, Bryant JA, Sims M, Kraft CS, Cohen SH, Berenson CS, Korman LY, Ford CB, Litcofsky KD, Lombardo MJ, Wortman JR, Wu H, Auniņš JG, McChalicher CWJ, Winkler JA, McGovern BH, Trucksis M, Henn MR, von Moltke L. SER-109, an Oral Microbiome Therapy for Recurrent Clostridioides difficile Infection. *N Engl J Med.* 2022 Jan 20;386(3):220-229. doi: 10.1056/NEJMoa2106516. PMID: 35045228.
29. Musher DM, Logan N, Bressler AM, Johnson DP, Rossignol JF. Nitazoxanide versus vancomycin in Clostridium difficile infection: a randomized, double-blind study. *Clin Infect Dis*. 2009 Feb 15;48(4):e41-6. doi: 10.1086/596552. PMID: 19133801.
30. Musher DM, Logan N, Hamill RJ, Dupont HL, Lentnek A, Gupta A, Rossignol JF. Nitazoxanide for the treatment of Clostridium difficile colitis. *Clin Infect Dis.* 2006 Aug 15;43(4):421-7. doi: 10.1086/506351. Epub 2006 Jul 11. PMID: 16838229.
31. Cronberg S, Castor B, Thorén A. Fusidic acid for the treatment of antibiotic-associated colitis induced by Clostridium difficile. *Infection.* 1984 Jul-Aug;12(4):276-9. doi: 10.1007/BF01645961. PMID: 6490173.
32. Snydman DR, McDermott LA, Jacobus NV, Kerstein K, Grossman TH, Sutcliffe JA. Evaluation of the in vitro activity of eravacycline against a broad spectrum of recent clinical anaerobic isolates. *Antimicrob Agents Chemother.* 2018;62(5):e02206–17.
33. Stapert L, Wolfe C, Shinabarger D, Marra A, Pillar C. In vitro activities of omadacycline and comparators against anaerobic bacteria. *Antimicrob Agents Chemother.* 2018;62(4):e00047–18.
34. Ng QX, Loke W, Foo NX, Mo Y, Yeo WS, Soh AYS. A systematic review of the use of rifaximin for Clostridium difficile infections. *Anaerobe.* 2019 Feb;55:35-39. doi: 10.1016/j.anaerobe.2018.10.011. Epub 2018 Nov 2. PMID: 30391527.
35. Basséres E, Endres BT, Khaleduzzaman M, Miraftabi F, Alam MJ, Vickers RJ, et al. Impact on toxin production and cell morphology in Clostridium difficile by ridinilazole (SMT19969), a novel treatment for C. difficile infection. *J Antimicrob Chemother.* 2016;71:1245–51.
36. Cho JC, Crotty MP, Pardo J. Ridinilazole: a novel antimicrobial for Clostridium difficile infection. *Ann Gastroenterol.* 2019 Mar-Apr; 32(2):134-140. doi: 10.20524/aog.2018.0336. Epub 2018 Dec 13. PMID: 30837785; PMCID: PMC6394264.
37. Shin JM, Gwak JW, Kamarajan P, Fenno JC, Rickard AH, Kapila YL. Biomedical applications of nisin. *J Appl Microbiol.* 2016 Jun;120(6):1449-65. doi: 10.1111/jam.13033. Epub 2016 Feb 12. PMID: 26678028; PMCID: PMC4866897.
38. Leeds JA, Sachdeva M, Mullin S, Dzink-Fox J, LaMarche MJ. Mechanism of action of and mechanism of reduced susceptibility to the novel anti-Clostridium difficile compound LFF571. *Antimicrob Agents Chemother.* 2012;56(8):4463–5.
39. Carlson, T.J., Gonzales-Luna, A.J. Antibiotic Treatment Pipeline for Clostridioides difficile Infection (CDI): A Wide Array of Narrow-Spectrum Agents. *Curr Infect Dis Rep* 22, 20 (2020).
40. Kraus CN, Lyerly MW, Carman RJ. Ambush of Clostridium difficile spores by ramoplanin: activity in an in vitro model. *Antimicrob Agents Chemother.* 2015;59(5):2525–30.
41. Yang H, Cai R, Zhang Y, Chen Y, Gu B. Gold Nanoclusters as an Antibacterial Alternative Against Clostridium difficile. *Int J Nanomedicine.* 2020 Aug 25;15:6401-6408. doi: 10.2147/IJN.S268758. PMID: 32904597; PMCID: PMC7457724.