Management of Primary and Recurrent Clostridium difficile Infection: An Update

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Abstract: Background: Clostridium difficile infection (CDI) is one of the most common healthcare-associated infections (HAI) in the United States and Canada, and incidence rates have increased worldwide in recent decades. Currently, antibiotics are the mainstay treatments for both primary and recurrent CDI, but their efficacy is limited, prompting further therapies to be developed. Aim: This review summarizes current and emerging therapies in CDI management including antibiotics, fecal microbiota transplantation, monoclonal antibodies, spore-based therapies, and vaccinations.

Keywords: Clostridium difficile infection; healthcare-associated infections; antibiotics; fecal microbiota transplantation

1. Introduction

Clostridium difficile infection (CDI) incidence rates have increased worldwide in recent decades. CDI is the most common healthcare-associated infection (HAI) in the United States (US) [1] and costs an estimated $4.8 billion in acute care facilities alone [2]. This figure does not include the increasing incidence of community-acquired CDI, which has nearly doubled in the past decade [3]. The incidence of multiple recurrent CDI has increased by 188.8% between 2001 and 2012 [4]. Antibiotics are mainstay treatments for both primary and recurrent CDI, with a recent trend toward vancomycin and fidaxomicin over metronidazole [5]. Given the limited efficacy of these antibiotics [6], however, further therapies have been pursued. These include fecal microbiota transplantation (FMT), monoclonal antibodies, newer antibiotics, spore-based therapies, and vaccinations. This article updates our 2015 article and highlights key changes in CDI management [7].

2. Vancomycin

Vancomycin is a glycopeptide antibiotic that requires oral ingestion to exert bacteriostatic effects against C. difficile via inhibition of bacterial cell wall synthesis [8]. It has long been a standard of care for both primary and recurrent CDI, and the Infectious Diseases Society of America (IDSA) now recommends vancomycin or fidaxomicin over metronidazole for primary and recurrent CDI [5]. This change is based on two large, multicentre randomized controlled trials (RCT) that investigated the clinical success of vancomycin 125 mg four times daily (81.1%; n = 259), metronidazole 250 mg four times daily (72.7%; n = 278), and tolevamer (44.2%; n = 534) (p = 0.02) in CDI [9]. Vancomycin was statistically superior to metronidazole in mild, moderate, and severe CDI, with more notable
superiority in patients with severe disease (78.5% vs. 66.3%), although this finding was not statistically significant ($p = 0.059$). Both studies also reported fewer CDI recurrences for patients treated with vancomycin, but these findings were not statistically significant. Superiority for vancomycin was previously limited to severe CDI based on an older RCT ($n = 172$) [10].

The recommended dosing regimen of vancomycin depends on the number of recurrences. For an initial nonsevere (WBC $\leq 15,000$ cells/mL and serum creatinine $< 1.5$ mg/dL) or severe CDI episode (WBC $\geq 15,000$ cells/mL and serum creatinine $> 1.5$ mg/dL), vancomycin 125 mg four times daily for 10 days is recommended. However, fulminant CDI may require up to 2 g per day with intravenous metronidazole. Further recurrences require pulsed and tapered vancomycin, which was found in one study to result in fewer recurrences compared to the standard 10-day regimen [11]. (The definitions of severe and complicated/fulminant CDI vary between guidelines, and the above definitions are based on IDSA guidelines [5,12,13]).

3. Fidaxomicin

Fidaxomicin is a macrocyclic lactone antibiotic that exerts its bactericidal effect against C. difficile via inhibition of bacterial RNA polymerase [14]. Its first-line treatment of primary and recurrent nonfulminant CDI is supported by two double-blinded RCTs ($n = 1164$) comparing fidaxomicin 200 mg twice daily to vancomycin 125 mg four times daily for 10 days [15]. A meta-analysis of these two studies demonstrated noninferiority of fidaxomicin in clinical cure rates compared to vancomycin, although—based on an intention-to-treat (ITT) analysis—fidaxomicin may have improved efficacy in reducing persistent diarrhea or death compared to vancomycin (37% reduction; 95% CI, 2–60; $p = 0.037$). However, modified ITT (mITT) and per-protocol analysis for this finding was not statistically significant [15]. Fidaxomicin was also found to be superior for reducing recurrence rates, persistent diarrhea, and death at day 40 by 40% (95% CI, 26–51; $p < 0.0001$) compared to vancomycin. Fidaxomicin has bactericidal effects and prolonged postantibiotic efficacy compared to vancomycin’s bacteriostatic effects [16].

4. Metronidazole

Oral metronidazole has been relegated to alternative therapy in primary, nonsevere CDI (WBC $\leq 15,000$ cells/mL and serum creatinine $< 1.5$ mg/dL) if vancomycin or fidaxomicin are contraindicated or unavailable. However, it is still recommended as an intravenous antibiotic in fulminant CDI (hypotension or shock, ileus, megacolon) as an adjunctive therapy to oral or rectal vancomycin, especially in setting of ileus [17]. Metronidazole can be neurotoxic, potentially causing cerebellar syndrome, encephalopathy, or peripheral neuropathy, especially when administered chronically over weeks to months [18,19]. Therefore, it should be limited to primary CDI treatment until other agents can be used [5].

5. Surotomycin

Surotomycin is a novel, oral lipopeptide antibiotic that has demonstrated bactericidal effects in vitro against C. difficile [20,21]. An initial, double-blinded Phase 2 RCT ($n = 209$) showed clinical cure rates of surotomycin 125 mg twice daily (92.4%), surotomycin 250 mg twice daily (86.6%), and vancomycin 125 mg four times daily (89.4%) for 10 days. The recurrences were also lower for surotomycin (17.2% for 250 mg; 27.9% for 125 mg) compared to vancomycin (35.6%) [22]. This trial was followed by two parallel, double-blinded Phase 3 RCTs, one of which ($n = 577$) demonstrated that the clinical cure rate of surotomycin 250 mg twice daily (83.4%) was noninferior to vancomycin 125 mg four times daily (82.1%) at Day 10 [23]. In contrast, the parallel Phase 3 study ($n = 570$) failed to show noninferiority at the same doses [24]. In addition, neither study met the criteria for superiority of surotomycin over vancomycin, although recurrence rates were found to be lower for surotomycin compared to vancomycin (14.0% vs. 50.4%; 95% CI, −54.1 to −15.1) [23]. In all three
studies, adverse events were similar among treated groups. Further studies are needed to characterize the role of surotomycin.

6. Ridinilazole (SMT 19969)

Ridinilazole is a novel, oral antibiotic with minimal systemic absorption that exerts bactericidal effects and reduces both toxin production and cell division of C. difficile [25]. A double-blinded, Phase 2 RCT (n = 100) showed it to be statistically superior to vancomycin for sustained clinical response rates 30 days after the end of treatment (66.7% vs. 42.4%; p = 0.0004) [26] and noninferior to vancomycin for initial cure rate at end of treatment (77.8% vs. 69.7%; 90% CI, −9.3 to 25.8). Adverse events were similar between the two groups. These promising results will need to be corroborated with further trials.

7. Fecal Microbiota Transplantation

Fecal microbiota transplantation (FMT) is a type of bacteriotherapy that introduces normal gut bacterial microbes from healthy, screened donors into recipients with dysregulated gut microbes in order to re-establish protective gut flora [27]. FMT has consistently demonstrated efficacy rates of 80 to 90% for clinical remission of recurrent CDI, regardless of the route of administration (such as nasoduodenal, colonoscopy, and enema) [28–31]. The first RCT of FMT for recurrent CDI patients in 2013 demonstrated significant superiority of a single FMT treatment via nasoduodenal route (81%) over vancomycin (31%) and catalyzed further research. Four more RCTs have investigated the efficacy of FMT vs. vancomycin, placebo, or autologous stool. One reported 90% clinical resolution rates with FMT via colonoscopy compared to 26% with vancomycin, paralleling findings in the initial RCT [29]. Other RCTs showed enema FMT vs. placebo (88.6% vs. 45.5%) and colonoscopy FMT vs. autologous stool (90.9% vs. 62.5%), although the clinical cure rates were variable among the 2 sites in the latter study [30,31]. In contrast, a small RCT (n = 30) comparing vancomycin followed by enema FMT and tapered vancomycin found comparable efficacy (43.8% vs. 58.3%) between the groups [32]. This finding is likely attributable to a single administration of FMT, increased efficacy of tapered vancomycin over regimens in previous FMT trials, and possible residual vancomycin affecting FMT efficacy, and the small number of patients. While many studies use multiple administrations of FMT in determining efficacy rates, single administrations of FMT may have lower CDI resolution rates with reported values of 62%, 65%, and 70% in 3 different RCTs [29,33,34]. A recent meta-analysis of these 5 RCTs (n = 284) reported that FMT was significantly more effective than its comparators (vancomycin, placebo, autologous stool) in providing a clinical cure of CDI with an RR 0.41 (95% CI, 0.22–0.74; p = 0.004) and had an NNT of 3 (95% CI, 2–7), although heterogeneity was noted and attributed to the location of the trial (Europe vs. North America) and the route of administration [35].

The best route of FMT administration has not been established. The previous meta-analysis determined that nasoduodenal and colonoscopy may be more effective than enema [35]. However, another meta-analysis determined that the clinical resolution rate was higher for lower gastrointestinal (GI) delivery including colonoscopy/enema (91.2%) compared to upper GI delivery (80.6%) including nasogastric/nasojejunal/gastroscopy [36]. One RCT (n = 20) showed not statistically significant differences between nasogastric and colonoscopy (60% vs. 80%; p = 0.628), while another (n = 116) randomized FMT by oral capsule or colonoscopy and found comparable clinical cure rates (96.2% vs. 96.2%, p < 0.001) [34,37]. Further studies are needed to characterize the best route, particularly for oral-encapsulated FMT, which would allow for commercialization and more widespread use of FMT therapy. The preparation of FMT—whether fresh, frozen, or lyophilized—can also expand use. Frozen FMT has been found to be noninferior to fresh FMT in 2 RCTs [33,38], and is more convenient and cost-effective. However, lyophilized FMT was found to be inferior to fresh in one small RCT [38].

The adverse effects of FMT are limited to postprocedural symptoms that resolve within a few hours and include bloating, abdominal pain, flatulence, diarrhea, and constipation [39]. Transmission of pathogens from donor to recipient has not been found in the literature beyond 2 cases of norovirus infection [40]. Long-term efficacy data on FMT is sparse, although there have been reports describing
the development of autoimmune diseases (Sjögren’s syndrome, rheumatoid arthritis, idiopathic thrombocytopenia), GI disorders (ulcerative colitis flares, microscopic colitis), peripheral neuropathy, and weight gain following FMT [41–43]. In comparison to vancomycin and placebo treatments, FMT did not differ in number of serious adverse events (death, hospitalizations) with an RR 0.64 (95% CI, 0.26–1.61) [35].

Guidelines on CDI management have been updated to include FMT. The Infectious Disease Society of America (IDSA) recommends using FMT after antibiotic failure for at least two CDI recurrences (three total episodes) [5]. The American College of Gastroenterology recommends at least three CDI recurrences (four total episodes) following a pulsed vancomycin treatment course [13]. The European Society of Clinical Microbiology and Infectious Disease (ECSMID) Guidelines recommend FMT after “multiple, recurrent CDI unresponsive to repeated antibiotic treatment” and in combination with oral antibiotics [12]. It remains unclear when FMT should be administered during recurrence. The delay between first onset of CDI and FMT treatment and its use in combination with antibiotics can be explained by how the treatment has been studied in clinical trials. Current barriers to FMT use include aesthetics, the invasive nature of delivery, and the need for personnel to administer it. The advent of oral-encapsulated FMT would remove these barriers.

8. Spore-Based Therapy

Nontoxigenic Clostridium difficile (NTCD) spore, M3, is derived from the bacterium that does not possess the genes for toxin production [44]. A double-blinded, Phase 2 RCT (n = 168) showed that it reduced recurrences of CDI from 30% with placebo to 11% with NTCD-M3 (OR 0.28; 95% CI, 0.11–0.69; p = 0.006) [44]. Colonization of NTCD-M3 was initially achieved in 69% of patients; however, after week 22, this strain was not detected.

SER-109 is another spore-based therapy comprising of around 50 species of Firmicutes spores isolated from healthy stool donors [45]. An open-label study (n = 30) reported efficacy of 86.7% in clinical cure rates for up to 8 weeks [45]. Colonization with spores was detected at week 24. A Phase III study (ECOSPOR III) on SER-109 vs. placebo is currently being conducted [46]. Further research is required to determine the role for spore-based therapy in CDI management.

9. Passive Immunization: Bezlotoxumab and Actoxumab

Bezlotoxumab is a monoclonal antibody for passive immunization against C. difficile toxin B by inhibiting the binding of toxin B to host cells [47]. MODIFY I and MODIFY II, two multisite Phase 3 studies including 2655 patients, reported recurrence rates in bezlotoxumab vs. placebo groups of 17% vs. 28% (95% CI, −15.9 to −4.3; p < 0.001) and 16% vs. 26% (95% CI, −15.5 to −4.3; p < 0.001), respectively [48]. This data has resulted in approval by the FDA in 2016 for its use as an adjunctive to antibiotics in reducing CDI recurrences. Actoxumab is also a monoclonal antibody, but against C. difficile toxin A. In combination with bezlotoxumab, it has been shown to further decrease recurrence rates in MODIFY I (16% vs. 28%; p < 0.001) and in MODIFY II (15% vs. 26%; p < 0.001). At 12 weeks, sustained clinical cure rates were 64% for bezlotoxumab alone, 58% for the combination, and 54% for placebo. It is unclear whether this therapy reduces the severity or duration of CDI symptoms in patients who have recurrences.

10. Active Immunization: Vaccines

Vaccines against C. difficile have the potential to allow a long-term, adaptive immune response against toxins A and B [49]. A few vaccines are being tested in clinical trials including a Phase 3 trial (Clover; Pfizer) [50], although results have not yet been published. Another Phase 3 trial for C. difficile vaccines (Diffense; Sanofi Pasteur) has been terminated due to low likelihood of attaining its primary outcome [51]. A phase 2 study (VLA84; Valneva) showed promising results, although Valneva has not yet continued the study [52].
11. Conclusions

Recent advances in *C. difficile* management for primary and recurrent CDI have improved current practices. Although antibiotic therapy with vancomycin and fidaxomicin remains first-line, alternative treatments have an important role, especially in treating recurrent CDI. Further research is underway to determine the efficacy of newer antibiotics for primary episodes of CDI and prevention of future recurrences along with vaccines and antibiotic-sparing therapies for CDI management.

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**References**

1. Magill, S.S.; Edwards, J.R.; Bamberg, W.; Beldavs, Z.G.; Dumyati, G.; Kainer, M.A.; Lynfield, R.; Maloney, M.; McAllister-Hollod, L.; Nadle, J.; et al. Multistate Point-Prevalence Survey of Health Care-Associated Infections. *N. Engl. J. Med.* 2014, 370, 1198–1208. [CrossRef] [PubMed]

2. Dubberke, E.R.; Olsen, M.A. Burden of Clostridium difficile on the Healthcare System. *Clin. Infect. Dis.* 2012, 55 (Suppl. 2), S88–S92. [CrossRef] [PubMed]

3. Ofori, E.; Ramai, D.; Dhaban, M.; Mustafa, F.; Gasperino, J.; Reddy, M. Community-acquired Clostridium difficile: Epidemiology, ribotype, risk factors, hospital and intensive care unit outcomes, and current and emerging therapies. *J. Hosp. Infect.* 2018. [CrossRef] [PubMed]

4. Ma, G.K.; Brensinger, C.M.; Wu, Q.; Lewis, J.D. Increasing Incidence of Multiply Recurrent Clostridium difficile Infection in the United States: A Cohort Study. *Ann. Intern. Med.* 2017, 167, 152–158. [CrossRef] [PubMed]

5. McDonald, L.C.; Gerding, D.N.; Johnson, S.; Bakken, J.S.; Carroll, K.C.; Coffin, S.E.; Dubberke, E.R.; Garey, K.W.; Gould, C.V.; Kelly, C.; et al. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin. Infect. Dis.* 2018, 66, e1–e48. [CrossRef] [PubMed]

6. Peng, Z.; Jin, D.; Kim, H.B.; Stratton, C.W.; Wu, B.; Tang, Y.W.; Sun, X. Update on Antimicrobial Resistance in Clostridium difficile: Resistance Mechanisms and Antimicrobial Susceptibility Testing. *J. Clin. Microbiol.* 2017, 55, 1998–2008. [CrossRef] [PubMed]

7. Vincent, Y.; Manji, A.; Gregory-Miller, K.; Lee, C. A Review of Management of Clostridium difficile Infection: Primary and Recurrence. *Antibiotics* 2015, 4, 411–423. [CrossRef] [PubMed]

8. Reynolds, P.E. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *Eur. J. Clin. Microbiol. Infect. Dis.* 1989, 8, 943–950. [CrossRef] [PubMed]

9. Johnson, S.; Louie, T.J.; Gerding, D.N.; Cornely, O.A.; Chasan-Taber, S.; Fitts, D.; Gelone, S.P.; Broom, C.; Davidson, D.M.; Polymer Alternative for CDI Treatment (PACT) Investigators. Vancomycin, Metronidazole, or Tolevamer for Clostridium difficile Infection: Results from Two Multinational, Randomized, Controlled Trials. *Clin. Infect. Dis.* 2014, 59, 345–354. [CrossRef] [PubMed]

10. Zar, F.A.; Bakkanagari, S.R.; Moorthy, 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, 302–307. [CrossRef] [PubMed]

11. McFarland, L.V.; Elmer, G.W.; Surawicz, C.M. Breaking the cycle: Treatment strategies for 163 cases of recurrent Clostridium difficile disease. *Am. J. Gastroenterol.* 2002, 97, 1769–1775. [CrossRef] [PubMed]

12. Debast, S.B.; Bauer, M.P.; Kuiper, E.J. European Society of Clinical Microbiology and Infectious Diseases: Update of the Treatment Guidance Document for Clostridium difficile Infection. *Clin. Microbiol. Infect.* 2014, 20, 1–26. [CrossRef] [PubMed]

13. 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, 478–498. [CrossRef] [PubMed]

14. Zhanel, G.G.; Walkty, A.J.; Karlovsky, J.A. Fidaxomicin: A Novel Agent for the Treatment of Clostridium difficile Infection. *Can. J. Infect. Dis. Med. Microbiol.* 2015, 26, 305–312. [CrossRef] [PubMed]
15. Crook, D.W.; Walker, A.S.; Kean, Y.; Weiss, K.; Cornely, O.A.; Miller, M.A.; Esposito, R.; Louie, T.J.; Stoeßer, N.E.; Young, B.C.; et al. Fidaxomicin Versus Vancomycin for Clostridium difficile Infection: Meta-analysis of Pivotal Randomized Controlled Trials. Clin. Infect. Dis. 2012, 55 (Suppl. 2), S93–S103. [CrossRef] [PubMed]
16. Babakhani, F.; Gomez, A.; Robert, N.; Sears, P. Postantibiotic Effect of Fidaxomicin and Its Major Metabolite, OP-1118, against Clostridium difficile. Antimicrob. Agents Chemother. 2011, 55, 4427–4429. [CrossRef] [PubMed]
17. Rokas, K.E.E.; Johnson, J.W.; Beardsley, J.R.; Ohl, C.A.; Luther, V.P.; Williamson, J.C. The Addition of Intravenous Metronidazole to Oral Vancomycin is Associated with Improved Mortality in Critically Ill Patients with Clostridium difficile Infection. Clin. Infect. Dis. 2015, 61, 934–941. [CrossRef] [PubMed]
18. Sarna, J.R.; Furtado, S.; Brownell, A.K.W. Neurologic Complications of Metronidazole. Can. J. Neurol. Sci. 2013, 40, 768–776. [CrossRef] [PubMed]
19. Kuriyama, A.; Jackson, J.L.; Doi, A.; Kamiya, T. Metronidazole-Induced Central Nervous System Toxicity: A Systematic Review. Clin. Neuropharmacol. 2011, 34, 241–247. [CrossRef] [PubMed]
20. Mascio, C.T.M.; Chesnel, L.; Thorne, G.; Silverman, J.A. Surotomycin Demonstrates Low In Vitro Frequency of Resistance and Rapid Bactericidal Activity in Clostridium difficile, Enterococcus faecalis, and Enterococcus faecium. Antimicrob. Agents Chemother. 2014, 58, 3976–3982. [CrossRef] [PubMed]
21. Bouillaut, L.; McBride, S.; Sorg, J.A.; Schmidt, D.J.; Suarez, J.M.; Tzipori, S.; Mascio, C.T.M.; Chesnel, L.; Sonenshein, A.L. Effects of Surotomycin on Clostridium difficile Viability and Toxin Production In Vitro. Antimicrob. Agents Chemother. 2015, 59, 4199–4205. [CrossRef] [PubMed]
22. Lee, C.H.; Patino, H.; Stevens, C.; Rege, S.; Chesnel, L.; Louie, T.; Mullane, K.M. Surotomycin versus vancomycin for Clostridium difficile infection: Phase 2, randomized, controlled, double-blind, non-inferiority, multicentre trial. J. Antimicrob. Chemother. 2016, 71, 2964–2971. [CrossRef] [PubMed]
23. Daley, P.; Louie, T.; Lutz, J.E.; Khanna, S.; Stoutenburgh, U.; Jin, M.; Adedoyin, A.; Chesnel, L.; Guris, D.; Larson, K.B.; et al. Surotomycin versus vancomycin in adults with Clostridium difficile infection: Primary clinical outcomes from the second pivotal, randomized, double-blind, Phase 3 trial. J. Antimicrob. Chemother. 2017, 72, 3462–3470. [CrossRef] [PubMed]
24. Boix, V.; Fedorak, R.N.; Mullane, K.M.; Pesant, Y.; Stoutenburgh, U.; Jin, M.; Adedoyin, A.; Chesnel, L.; Guris, D.; Larson, K.B.; et al. Primary Outcomes from a Phase 3, Randomized, Double-Blind, Active-Controlled Trial of Surotomycin in Subjects with Clostridium difficile Infection. Open Forum Infect. Dis. 2017, 4. [CrossRef] [PubMed]
25. Basseres, E.; Endres, B.T.; Khaleduzzaman, M.; Mirafabti, F.; Alam, M.J.; Vickers, R.J.; Carey, K.W. 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, 1245–1251. [CrossRef] [PubMed]
26. Vickers, R.J.; Tillotson, G.S.; Nathan, R.; Hazan, S.; Pullman, J.; Lucasti, C.; Deck, K.; Yacyshyn, B.; Malaakkal, B.; Pesant, Y.; et al. Efficacy and safety of ridinilazole compared with vancomycin for the treatment of Clostridium difficile infection: A phase 2, randomised, double-blind, active-controlled, non-inferiority study. Lancet Infect. Dis. 2017, 17, 735–744. [CrossRef]
27. Shahinas, D.; Silverman, M.; Sittler, T.; Chiu, C.; Kim, P.; Allen-Vercoe, E.; Weese, S.; Wong, A.; Low, D.E.; Pillai, D.R. Toward an understanding of changes in diversity associated with fecal microbiome transplantation based on 16S rRNA gene deep sequencing. mBio 2012, 3. [CrossRef] [PubMed]
28. van Nood, E.; Dijkstra, M.G.W.; Keller, J.J. Duodenal infusion of feces for recurrent Clostridium difficile. N. Engl. J. Med. 2013, 368, 2145. [CrossRef] [PubMed]
29. Cammarota, G.; Masucci, L.; Ianiro, G.; Bibbò, S.; Dinoi, G.; Costamagna, G.; Sanguinetti, M.; Gasbarrini, A. Randomised clinical trial: Faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. Aliment. Pharmacol. Ther. 2015, 41, 835–843. [CrossRef] [PubMed]
30. Kelly, C.R.; Khoruts, A.; Staley, C.; Sadowsky, M.J.; Abd, M.; Alani, M.; Bakow, B.; Curran, P.; McKenney, J.; Tisch, A.; et al. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. Ann. Intern. Med. 2016, 165, 609–616. [CrossRef] [PubMed]
31. Orenstein, R.; Dubberke, E.; Lee, C.H. RBX2660, a microbiota-based drug for the prevention of recurrent Clostridium difficile infection, is safe and effective: Results from a randomised, double-blinded, placebo-controlled trial (abstract LB08). In Proceedings of the United European Gastroenterology Week, Vienna Austria, 15–19 October 2016; Volume 4, p. 802.

32. Hota, S.S.; Sales, V.; Tomlinson, G.; Salpeter, M.J.; Geiger, A.; Coburn, B.; Gutman, D.S.; Low, D.E.; Poutanen, S.M. Oral Vancomycin Followed by Fecal Transplantation Versus Tapering Oral Vancomycin Treatment for Recurrent Clostridium difficile Infection: An Open-Label, Randomized Controlled Trial (abstract LB08). In Proceedings of the United European Gastroenterology Week, Vienna Austria, 15–19 October 2016; Volume 4, p. 802.

33. Lee, C.H.; Steiner, T.; Petrof, E.O.; Smieja, M.; Roscoe, D.; Nematallah, A.; Weese, J.S.; Collins, S.; Moayyedi, P.; Crowther, M.; et al. Frozen vs. Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients with Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. [CrossRef] [PubMed]

34. Youngster, I.; Saub, J.; Pindar, C.; Wilson, R.G.; Kaplan, J.L.; Smith, M.B.; Alm, E.J.; Gevers, D.; Russell, G.H.; Hohmann, E.L. Fecal Microbiota Transplant for Relapsing Clostridium difficile Infection Using a Frozen Inoculum from Unrelated Donors: A Randomized, Open-Label, Controlled Pilot Study. [CrossRef] [PubMed]

35. Moayyedi, P.; Yuan, Y.; Baharath, H.; Ford, A.C. Faecal microbiota transplantation for Clostridium difficile-associated diarrhoea: a systematic review of randomised controlled trials. [CrossRef] [PubMed]

36. Kassam, Z.; Lee, C.H.; Yuan, Y.; Hunt, R.H. Fecal Microbiota Transplantation for Clostridium difficile Infection: Systematic Review and Meta-Analysis. [CrossRef] [PubMed]

37. Kao, D.; Roach, B.; Silva, M.; Beck, P.; Rious, K.; Kaplan, G.G.; Chang, H.J.; Coward, S.; Goodman, K.J.; Xu, H.; et al. Effect of Oral Capsule- vs. Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. [CrossRef] [PubMed]

38. Jiang, Z.D.; Ajami, N.J.; Petrosino, J.F.; Jun, G.; Hanis, C.L.; Shah, M.; Hochman, L.; Ankoma-Sey, V.; DuPont, A.W.; Wong, M.C.; et al. Randomised clinical trial: Faecal microbiota transplantation for recurrent Clostridium difficile infection—Fresh, or frozen, or lyophilised microbiota from a small pool of healthy donors delivered by colonoscopy. [CrossRef] [PubMed]

39. Liubakka, A.; Vaughn, B.P. Clostridium difficile Infection and Fecal Microbiota Transplant. [CrossRef] [PubMed]

40. Schwartz, M.; Gluck, M.; Koon, S. Norovirus Gastroenteritis after Fecal Microbiota Transplantation for Treatment of Clostridium difficile Infection Despite Asymptomatic Donors and Lack of Sick Contacts. [CrossRef] [PubMed]

41. Brandt, L.J.; Aroniadis, O.C.; Mellow, M.; Kanatzar, A.; Park, T.; Stollman, N.; Rohlke, F.; Surawicz, C. Long-Term Follow-Up of Colonoscopic Fecal Microbiota Transplant for Recurrent Clostridium difficile Infection. [CrossRef] [PubMed]

42. Alang, N.; Kelly, C.R. Weight Gain after Fecal Microbiota Transplantation. Open Forum Infect. Dis. 2015, 2, ofv004. [CrossRef] [PubMed]

43. Tariq, R.; Smyrk, T.; Pardi, D.S.; Tremaine, W.J.; Khanna, S. New-Onset Microscopic Colitis in an Ulcerative Colitis Patient after Fecal Microbiota Transplantation. Am. J. Gastroenterol. 2016, 111, 751–752. [CrossRef] [PubMed]

44. Gerdin, D.N.; Meyer, T.; Lee, C.; Cohen, S.H.; Murthy, U.K.; Poirier, A.; Van Schooneveld, T.C.; Pardi, D.S.; Ramos, A.; Barron, M.A.; et al. Administration of Spores of Nontoxigenic Clostridium difficile Strain M3 for Prevention of Recurrent C. difficile Infection: A Randomized Clinical Trial. [CrossRef] [PubMed]

45. Khanna, S.; Pardi, D.S.; Kelly, C.R.; Kraft, C.S.; Dhure, T.; Henn, M.R.; Lombardo, M.J.; Vulic, M.; Osuomi, T.; Winkler, J.; et al. A Novel Microbiome Therapeutic Increases Gut Microbial Diversity and Prevents Recurrent Clostridium difficile Infection. J. Infect. Dis. 2016, 214, 173–181. [CrossRef] [PubMed]

46. ECOSPOR III—SER-109 versus Placebo in the Treatment of Adults with Recurrent Clostridium Difficile. Available online: https://clinicaltrials.gov/ct2/show/NCT03183126 (accessed on 22 June 2018).
47. Orth, P.; Xiao, L.; Hernandez, L.D.; Reichert, P.; Sheth, P.R.; Beaumont, M.; Yang, X.; Murgolo, N.; Ermakov, G.; DiNunzio, E.; et al. Mechanism of action and epitopes of Clostridium difficile toxin B-neutralizing antibody bezlotoxumab revealed by X-ray crystallography. *J. Biol. Chem.* **2014**, *289*, 18008–18021. [CrossRef] [PubMed]

48. Wilcox, M.H.; Gerding, D.N.; Poxton, I.R.; Kelly, C.; Nathan, R.; Birch, T.; Cornely, O.A.; Rahav, G.; Bouza, E.; Lee, C.; et al. Bezlotoxumab for Prevention of Recurrent *Clostridium difficile* Infection. *N. Engl. J. Med.* **2017**, *376*, 305–317. [CrossRef] [PubMed]

49. De Bruyn, G.; Saleh, J.; Workman, D.; Pollak, R.; Elinoff, V.; Fraser, N.J.; Lefebvre, G.; Martens, M.; Mills, R.E.; Nathan, R.; et al. Defining the optimal formulation and schedule of a candidate toxoid vaccine against Clostridium difficile infection: A randomized Phase 2 clinical trial. *Vaccine* **2016**, *34*, 2170–2178. [CrossRef] [PubMed]

50. Clostridium Difficile Vaccine Efficacy Trial (Clover). Available online: https://clinicaltrials.gov/ct2/show/NCT03090191 (accessed on 22 April 2018).

51. Sanofi Ends Development of Clostridium Difficile Vaccine (Press Release). 1 December 2017. Available online: http://mediaroom.sanofi.com/sanofi-ends-development-of-clostridium-difficile-vaccine/ (accessed on 22 April 2018).

52. Valneva’s Clostridium Difficile Vaccine Candidate—VLA84. Available online: http://www.valneva.com/en/rd/vla84 (accessed on 22 June 2018).