Safety of fecal microbiota transplantation for *Clostridioides difficile* infection focusing on pathobionts and SARS-CoV-2

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**Abstract:** *Clostridioides difficile* infection (CDI) is a consequence of flagrant use of antibiotics, an aging population with increasing comorbidities, and increased hospitalizations. The treatment of choice for CDI is antibiotics (vancomycin or fidaxomicin), with a possibility of recurrent CDI despite lack of additional risk factors for CDI. For the last 10 years, fecal microbiota transplantation (FMT) has emerged as a promising therapy for recurrent CDI, with success rates of over 85% compared with less than 50% with antibiotics for multiple recurrent CDI. Along with the success of FMT, several adverse and serious adverse events with FMT have been reported. These range from self-limiting abdominal pain to death due to severe sepsis. This review focuses on the safety of FMT, emphasizing the reports of transmission of pathobionts like extended-spectrum beta lactamase *Escherichia coli* and Shiga toxin-producing *E. coli*. The severe acute respiratory syndrome coronavirus-2 is a potential pathogen that could be transmitted via FMT during the COVID-19 pandemic. The challenges faced by clinicians for donor screening, clinical trials, and other aspects of FMT during the pandemic are discussed.

**Keywords:** C difficile, microbiome, FMT, adverse events, infections, E. coli, COVID-19, SARS-CoV-2

Received: 3 November 2020; revised manuscript accepted: 23 March 2021.
are not available). For a first severe episode either vancomycin or fidaxomicin are recommended. For a fulminant infection, a combination of high-dose vancomycin and intravenous metronidazole are used. For a first recurrence, either fidaxomicin (if vancomycin was used for the initial infection) or vancomycin (if metronidazole was used for the initial infection) should be prescribed. Another option is a pulsed and tapered vancomycin regimen (Figure 1).

For multiple recurrent CDI (three or more episodes) or refractory CDI (nonresponsive to medications), fecal microbiota transplantation (FMT) is recommended. This therapeutic modality restores a patients’ dysbiotic gut microbiome by transferring microbes from a stool of a healthy donor to a patient with recurrent CDI. A clinical trial from Europe compared vancomycin followed by FMT to fidaxomicin or vancomycin as treatment for recurrent CDI and demonstrated 92% success rates with FMT compared with 42% with fidaxomicin and 19% with vancomycin. Another trial including patients with refractory CDI showed a 75% cure of refractory CDI with a single FMT infusion and 100% cure with multiple infusions. FMT is cost effective and increases quality of life years (QALY) (and reduction in cost/QALY ratio) in patients compared with antibiotic treatments. A systematic review and meta-analysis

Figure 1. Standard pharmacologic management of Clostridioides difficile infection. A suggested regimen for vancomycin taper is as follows: 125 mg orally four times daily for 10–14 days, 125 mg orally two times daily for 7 days, 125 mg orally once daily for 7 days, and 125 mg orally every 2 or 3 days for 2–8 weeks. A rifaximin chaser regimen involves vancomycin 125 mg orally four times daily for 10 days followed by rifaximin 400 mg three times daily for 10 days.

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of 7 randomized control trials and 30 case series showed resolution of symptoms in 92% of the patients who underwent FMT.12 A systematic review and meta-analysis which included all studies on FMT until November 2019 showed that at 8 weeks post-FMT, a single dose had a efficacy of 84% [95% confidence interval (CI): 80–88%, P = 86%]. Repeat FMT was 91% (95% CI: 89–94%, P = 53%) effective.13 It also concluded that delivery by lower gastrointestinal endoscopy was the most superior delivery method and repeat FMT significantly increased treatment efficacy. Another meta-analysis measuring the efficacy of oral FMT capsules showed 82.1% efficacy (95% CI: 76.2–87.4%) in preventing recurrent CDI.20 In addition, FMT has also been associated with reduced bloodstream infections, shorter hospital length of stay, and a higher survival compared with patients receiving antibiotics for CDI.21 The US Food and Drug Administration (FDA) classifies FMT as an experimental therapy.

It is important to ponder on the safety aspects of FMT. We have seen inadvertent consequences of advances in medicine such as hepatitis C transmission with blood transfusions. Newer therapies such as FMT should be carefully monitored for adverse events and potential long-term consequences. It is of the utmost necessity that FMT is carried out in a standardized manner with thorough donor screening and testing to prevent adverse events. The FDA has an enforcement discretion policy allowing the use of FMT to treat CDI not responsive to standard therapies (Table 1).22 Below, we summarize the recent safety challenges associated with FMT and the impact of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic on FMT for CDI.

**Adverse events after FMT**

Most commonly after FMT patients develop mild symptoms such as abdominal pain, cramping, bloating, nausea, mild fever, and constipation, which tend to be self-limited.23–30 There may be a risk of a disease flare post-FMT for CDI in patients with underlying inflammatory bowel diseases.26,28 A case of unintentional weight gain of 41 pounds in 36 months after receiving FMT from an overweight donor has been reported, but the causality between FMT and obesity was not proven in larger studies.31,32 Autoimmune diseases post-FMT have been reported.33 Reports of aspiration following sedation used during an FMT procedure have been described.26,34 Toxic megacolon after FMT has been reported.35,36 In a study performed on patients with liver cirrhosis undergoing FMT, five serious adverse events were documented and classified as possibly related to the FMT. These were Crohn’s disease flare, fecal urgency, dehydration with acute kidney injury, worsening hepatic encephalopathy, melena, and anemia in a patient of portal hypertensive gastropathy post-FMT.37 Another study from the FMT national registry showed two infections possibly related to FMT: *Bacteroides fragilis* bacteremia in one participant, enteropathogenic *Escherichia coli* on a multiplex polymerase chain reaction (PCR) stool panel in another.38 A detailed risk stratification of side effects from several studies is outlined in Table 2.

**Lack of standardization of FMT**

There have been case reports where improper knowledge and standardization of donor screening has led to side effects.40,41 Expert opinion and evidence-based donor screening protocols have been proposed to standardize donor screening processes.42,43 These protocols suggest that the screening should be a four-step process. The first step should consist of a prescreening survey (for assessing general health, risk of infectious diseases). It should be followed by a clinical assessment (to exclude risk factors for transmissible disease and potential microbiome-mediated conditions), stool and nasal screening [including carbapenem-resistant Enterobacteriaceae, extended-spectrum beta-lactamase-producing (ESBL) organisms, and methicillin-resistant *Staphylococcus aureus* besides other standard organisms], and serological screening (abnormal blood counts, liver tests, and infectious pathogens). In addition to this protocol,
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Table 2. Adverse effects and risk stratification of FMT.

| Sr no. | Adverse effects       | Research authors | Risk stratification: number of patients having the disease/total number of patients (percentage) |
|--------|-----------------------|------------------|-----------------------------------------------------------------------------------------------|
| 1      | Fever                 | Dutta et al.23    | 18.5 (n = 27)                                                                                   |
|        |                       | Kelly et al.26    | 3.75 (n = 80)                                                                                   |
| 2      | Bloating              | Satokari et al.29 | 8.7 (n = 23)                                                                                   |
|        |                       | Dutta et al.23    | 11.1 (n = 27)                                                                                   |
|        |                       | Kelly et al.26    | 3.75 (n = 80)                                                                                   |
|        |                       | Russell et al.28  | 30 (n = 10)                                                                                     |
|        |                       | Kelly et al.38    | 15.3 (n = 222)                                                                                  |
| 3      | Abdominal pain/cramps | Hirsch et al.25   | 26.3 (n = 19)                                                                                   |
|        |                       | Russell et al.28  | 50 (n = 10)                                                                                     |
|        |                       | Kelly et al.38    | 17.6 (n = 222)                                                                                  |
| 4      | Diarrhea              | Kelly et al.26    | 3.75 (n = 80)                                                                                   |
|        |                       | Russell et al.28  | 30 (n = 10)                                                                                     |
|        |                       | Saha et al.39     | 60 (n = 609) (patients with underlying IBD, dialysis-dependent kidney disease, and multiple FMTs had higher risk) |
|        |                       | Kelly et al.38    | 31.1 (n = 222)                                                                                  |
| 5      | Aspiration            | Kelly et al.26    | 1.23 (n = 80)                                                                                   |
|        |                       | Baxter et al.34   | Case report                                                                                     |
| 6      | IBD flare             | Kelly et al.26    | 3.75 (n = 80)                                                                                   |
| 7      | Toxic megacolon       | Khan et al.35     | 5 (n = 20)                                                                                      |
|        |                       | Solari et al.36   | Case report                                                                                     |
| 8      | Constipation          | Saha et al.39     | 33 (n = 609)                                                                                     |
|        |                       | Kelly et al.38    | 10.8 (n = 222)                                                                                  |

FMT, fecal microbiota transplantation; IBD, irritable bowel syndrome.

A systematic review of 85 studies demonstrated a significant heterogeneity in the reporting of different aspects of FMT. In this study, 83 of the 85 (98%) did not describe the material used for collecting stools or the preparation of donors for collecting stools. Most [n=76 (89%)] did not specify the methods of dilution and homogenization or filtration of stools. In 82 reports (96%), the authors did not describe under what conditions stools were prepared or methods used for conservation of stools. Overall, 68 (80%) did not describe the type of stools used for infusion (e.g. fresh or frozen), volume of preparation, the amount of stool used, or duration of conservation of stools. A total of 51 reports (60%) did not specify the preparation of patients for transplantation, mode of administration of infusion, or number of infusions per patient.44 Many preparations and methods of instillation of FMT, such as nasogastric, nasoduodenal, and colonoscopic, are used in clinical practice. One study carried out in 2013 used synthetic stool, that is, purified intestinal bacterial cultures derived from a single healthy donor stool. It was used to treat recurrent CDI in two patients who failed standard antibiotic treatment. This stool substitute preparation was given via colonoscopy. Both patients showed formed bowel movements within 1–3 days. They did not show any recurrence of CDI/loose stools at 24 weeks postprocedure even after receiving numerous antibiotics for recurrent urinary tract infections and skin infections within these 24 weeks.45 In addition, standardized therapies which are enema-based (RBX2660) or capsule-based therapies (SER-109, CP101, RBX7455, VE303) are being developed.11,14,33,46–53 Details on these novel microbiome-based therapies are outlined in Table 3.

Serious infection transmission associated with FMT

In 2019, two immunocompromised patients were reported to have contracted ESBL E. coli infection after FMT from a common stool donor.41 The donor was not tested for the presence of ESBL E. coli and this screening was made standard practice after these instances. One patient with end-stage liver disease was administered FMT via oral capsules for hepatic encephalopathy as part of a clinical trial. The patient developed cough, fever, and chest infiltrates suggestive of pneumonia 17 days after FMT. Fluoroquinolone treatment led to no clinical improvement and blood cultures demonstrated Gram-negative bacilli identified as ESBL E. coli. He was managed with a carbapenem with eventual resolution.
The second patient had undergone hematopoietic stem-cell transplantation for myelodysplastic syndrome. The patient was given FMT as part of a clinical trial to prevent graft versus host disease. On day 8 after FMT, he developed fever, chills, and altered mental status progressing to hypoxia. Blood cultures demonstrated Gram-negative bacteremia with ESBL *E. coli*. Despite treatment with a carbapenem, he died 2 days later from severe sepsis. On further investigation, both bacterial isolates had a similar antibiotic resistance pattern and the same multilocus sequence type 131 and serotype O25:H4. The single nucleotide polymorphism (SNP) analysis of the bacterial samples from the patients and the donor stool sample showed SNP similarities suggesting transmission from the same donor. A few other patients who received capsules derived from this donor’s

| Table 3. Novel microbiome restoration therapies. |
|-----------------------------------------------|
| Sr no. | Therapy | Formulation details | Clinical trial results |
|--------|---------|---------------------|------------------------|
| 1      | SER109⁵⁴,⁵⁵ | Capsule-based preparation of purified spores of multiple Firmicutes species derived from healthy donors. | Phase III clinical trial: SER109 met the primary endpoint by showing a decrease in recurrence of CDI infection by 30.2% compared with placebo. |
| 2      | RBX2660⁵¹ | Microbiota-based suspension derived from donor stool administered via enema. | Phase II clinical trial: prevented CDI recurrence at 8 weeks with a success rate of 78.8% compared with 51.8% \(p < 0.001\). Phase III studies are under way [PUNCH CD 3]. [Clinicaltrials.gov NCT03244644] |
| 3      | RBX7455⁵⁴ | Lyophilized broad-spectrum gut microbiota formulation administered as a capsule. It is non-frozen, room temperature stable. | Single center phase I clinical trial of RBX7455 has been completed. [Clinicaltrials.gov NCT02981316] |
| 4      | VE303⁵⁷  | Live bacterial consortium stored in capsule in powdered form. It is administered orally as a capsule. | Data from the studies not available up to now. |
| 5      | CP101⁵⁸  | Oral capsule containing full-spectrum microbiota. | Phase II trial [PRISM3], CP101 met the primary efficacy endpoint, with 74.5% of recurrent CDI by week 8 (61.5% in the placebo group who received standard-of-care antibiotic therapy alone) \(p < 0.05\). |
| 6      | VE707⁵⁹  | Preclinical discovery program for prevention of colonization of multiple drug-resistant organisms. | Received a US$5.8 million grant in December 2019 from CARB-X company to advance VE707 program. |
| 7      | SER262⁶⁰,⁶¹ | SER262 contains bacterial spores from 12 strains, isolated from clades prevalent in healthy individuals. Spores are isolated via fermentation (not requiring human donor material) and formulated in a capsule. | Phase I b study: SER262 + vancomycin showed a 6.25% recurrence rate of CDI compared with 28.6% in vancomycin-only group. |
| 8      | MET-2⁶²  | Microbial ecosystem therapeutic-2 (MET-2) is a defined microbial community derived from a healthy donor stool administered in capsule form or via colonoscopy. | A phase I, open-label, single-center study on its safety and efficacy in treating recurrent CDI was started on 27 October 2017 and the primary completion date was 17 March 2020. [ClinicalTrials.gov Identifier: NCT02865616] |
stool showed ESBL *E. coli* in their stools but they did not suffer from bacteremia despite some being immunocompromised. The FDA issued a safety alert in June 2019 regarding the use of FMT and risk of serious adverse reactions due to transmission of multidrug-resistant organisms.\(^6^3\)

After the consultation with the FDA on 10 March 2020, OpenBiome (a non-profit stool bank in USA) started testing all donor stool prospectively for Shiga toxin type 1 and type 2 producing genes (*stx1/2*) by nucleic acid amplification testing rather than enzyme immunoassay.\(^6^4\) This was a result of adverse events due to infectious transmission.\(^4^0\) In February 2020, OpenBiome received reports of two patients developing abdominal pain and diarrhea after FMT who had received stool from the same donor. Stool from a stored aliquot of this donor was positive for the *stx1/2* genes (the genes responsible for producing Shiga toxin type 1 and type 2). Six serious adverse events (diarrhea and abdominal pain) and one non-serious adverse event were reported. There were two deaths but these were eventually determined to be unrelated to FMT.\(^4^0\) All stored stool samples from this donor underwent both PCR and enzyme immunoassay (EIA) testing. Out of 20 samples tested, 11 were positive via PCR but were negative via EIA (which was used as the initial mode of screening by OpenBiome), suggesting that PCR has higher sensitivity than EIA in this setting. The donor and one available patient isolates were assessed for clonality and isolates were deemed to belong to the same serotype H:7:O117 and were clonal (0–1 SNP differences between the two donor isolates and 0–6 SNPs between the donor and the patient isolate). Isolates with a distance of less than 10 SNPs are considered to be a part of a transmission cluster.\(^6^5–6^7\) This event emphasizes the importance of using a method of high-sensitivity testing rather than low-sensitivity testing for donor screening as donors are asymptomatic. This event suggests the importance of strong surveillance and quality measures to establish causality.

A 19-year-old man with primary immunoglobulin A deficiency status underwent FMT via colonoscopy for recurrent CDI with stool sourced from a commercial stool bank. At 10 days after FMT, he presented with explosive bloody diarrhea with 20 bowel movements a day, leukocytopenia, fever, chills, and night sweats. Stool was examined and found positive for enteropathogenic *E. coli*, enterotoxigenic *E. coli*, and Shiga toxin-producing *E. coli*.\(^6^8\) These events emphasize the need to practice caution whenever FMT is carried out in an immunocompromised patient.

A study investigating fecal specimens from 66 recipients and the RBX2660 product in a multicenter, randomized, double-blind, placebo-controlled phase Ib study showed that RBX2660 not only shifted the taxonomic structure of the intestinal microbiome in the recipient group, but it also lead to dynamic changes in their antibiotic resistance patterns.\(^6^9\) Besides decreasing the overall antibiotic resistance in the microbiome, it also simultaneously introduced RBX2660-origin antibiotic-resistant genes (ARGs) in a dose-dependent manner. This emphasizes the importance of screening fecal transplant samples for medically important ARGs via highly sensitive molecular methods.

Considering unintentional pathogen transmission via FMT and the lack of protocols to test asymptomatic recipients, it is indubitably true that the donor screening is still an imperfect science which will continue to be iterative over time. The COVID-19 pandemic increases the complexity of donor screening. There have been reports of SARS-CoV-2 shedding in stools of asymptomatic patients and in post-COVID recovery patients (who are negative on respiratory testing).\(^7^0,7^1\) There are no validated stool assays available for asymptomatic or symptomatic people. According to the recent guidance from the FDA for studies performing FMT under an investigational new drug application, donor stool obtained and banked before 1 December 2019 must be stored separately from the stool donated and stored after 1 December 2019.\(^7^2\) In this era, a more cautious approach is warranted. In order to consider a person to be a stool donor, travel history, contact with a recent symptomatic individual, past history of COVID-19 infection, and symptoms suggestive of COVID-19 should be added in the screening questionnaire. Donors donating stool after December 2019 should be screened for COVID-19 symptoms and exposure. Donors who pass the screening should undergo COVID-19 testing along with the usual donor screening. Rescue FMT should be used in fulminant patients with no response to initial antibiotic treatment. If the symptoms improve, the antibiotic dosage should be tapered to the lowest effective dosage and should be continued until the COVID-19 pandemic is
Considering the heavy impact COVID-19 had on our healthcare and economy, an improved approach should be in place for managing patients with recurrent CDI during the COVID-19 era. The largest stool bank in the USA, OpenBiome, has ceased operations as of the time of writing this manuscript. If FMT is not available, prolonged courses of antibiotics such as vancomycin at their lowest effective dosage may be used as the treatment of choice in those with multiple recurrent CDI and FMT performed once available.

**Challenges and opportunities in 2020 and beyond**

The future of microbiome restoration therapies is exciting. Besides patients with CDI, FMT has been effective in a different spectrum of patients. There is emerging evidence which supports the association of dysbiosis and autoimmunity, metabolic diseases, etc., suggesting a role of microbiota restoration to treat diseases beyond FMT. FMT is being explored for obesity, nonalcoholic steatohepatitis, primary sclerosing cholangitis, and autism. Methodologies for stool treatment to eliminate viral or bacterial pathogens and synthetically grown defined microbial consortia would avoid the risk of transmitting pathogens, including SARS-CoV-2.

The Infectious Disease Society of America/Society of Healthcare Epidemiology of America 2017 guidelines recommended FMT for multiple recurrent CDI despite “moderate quality” evidence, reflecting the great unmet need. Recently, two large phase III studies of standardized microbiome-based therapies have shown positive initial results and final results are awaited. Availability of these products will help further streamline access to FMT and help minimize side effects. FMT-maintained remission of CDI larger treatment trials are needed to prove its efficacy in treating an acute onset CDI. Designing and rationalizing trials for evaluating FMT’s efficacy in these diseases would be a challenge. These Covid-19 times provokes us to sharpen our imperfect donor screening so that pathogenic organism transmission could be prevented.

There remain diagnostic challenges for CDI in 2020. Stool EIA (rather than PCR) should be used to include or exclude patients in clinical trials as PCR has high rates of false positivity for recurrent CDI. Using PCR for diagnosis raises the question of whether the patient was really suffering from recurrent CDI or the diagnosis was a false positive.

In conclusion, CDI is a very rampant disease in hospital settings and FMT helps prevent recurrent CDI. It has previously been thought to have an excellent safety profile. More recently, transmission of Shiga toxin-producing *E. coli* and ESBL-producing *E. coli* emphasizes the importance of a comprehensive and universal screening protocol and standardization of FMT to mitigate adverse events in the future. As FMT is considered an experimental therapy and donor screening is an imperfect science, protocols should be in place to back trace the stool aliquots so that causalities can be established in future. The COVID-19 pandemic has made FMT increasingly challenging. The availability of standardized microbiome-based therapies will help to mitigate risks from FMT even further.

**Conflict of interest statement**

SK has received research grants from Rebiotix Inc. and consulting fees from Shire, Premier Inc., Facile Therapeutics, and Probiotech International, outside of the submitted work.

**Funding**

The authors received no financial support for the research, authorship, and/or publication of this article.

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**References**

1. Balsells E, Shi T, Leese C, et al. Global burden of *Clostridium difficile* infections: a systematic review and meta-analysis. *J Glob Health* 2019; 9: 010407.

2. Magill SS, O’Leary E, Janelle SJ, et al. Changes in prevalence of health care-associated infections in U.S. Hospitals. *N Engl J Med* 2018; 379: 1732–1744.

3. Antiobiotic Resistance Threats in United States. Centers for disease control. Atlanta, GA: United States Department of Health and Human Services, 2019.

4. Debast SB, Bauer MP and Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance
document for Clostridium difficile infection. Clin Microbiol Infect 2014; 20(Suppl. 2): 1–26.

5. Surawicz CM, Brandt LJ, Binion DG, et al. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol 2013; 108: 478–498; quiz 499.

6. Hu MY, Katchar K, Kyne L, et al. Prospective derivation and validation of a clinical prediction rule for recurrent Clostridium difficile infection. Gastroenterology 2009; 136: 1206–1214.

7. Khanna S, Pardi DS, Aronson SL, et al. The epidemiology of community-acquired Clostridium difficile infection: a population-based study. Am J Gastroenterol 2012; 107: 89–95.

8. Ma GK and Lewis JD. Increasing incidence of multiply recurrent Clostridium difficile infection. Ann Intern Med 2018; 168: 308.

9. Wadhwa A, Al Nahhas MF, Dierkhising RA, et al. High risk of post-infectious irritable bowel syndrome in patients with Clostridium difficile infection. Aliment Pharmacol Ther 2016; 44: 576–582.

10. Gupta A and Khanna S. Repeat Clostridium difficile testing. jAMA 2016; 316: 2422–2423.

11. Cho JM, Pardi DS and Khanna S. Update on treatment of Clostridioides difficile infection. Mayo Clin Proc 2020; 95: 758–769.

12. Quraishi MN, Widlak M, Bhala N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. Aliment Pharmacol Ther 2017; 46: 479–493.

13. McDonald LC, Gerdin DN, Johnson S, 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.

14. Hvas CL, Jørgensen SMD, Jørgensen SP, et al. Fecal microbiota transplantation is superior to fidaxomycin for treatment of recurrent Clostridium difficile infection. Gastroenterology 2019; 156: 1324–1332.

15. Ianiro G, Masucci L, Quaranta G, 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: 152–159.

16. Arbel LT, Hsu E and McNally K. Cost-effectiveness of faecal microbiota transplantation in the treatment of recurrent Clostridium difficile infection: a literature review. Cureus 2017; 9: e1599.

17. Varier RU, Biltaji E, Smith KJ, et al. Cost-effectiveness analysis of fecal microbiota transplantation for recurrent Clostridium difficile infection. Infect Control Hosp Epidemiol 2015; 36: 438–444.

18. Zowall H, Brewer C and Deutsch A. Cost-effectiveness of fecal microbiota transplant in treating Clostridium difficile infection in Canada. Value Health 2014; 17: A676.

19. Baunwall SMD, Lee MM, Eriksen MK, et al. Fecal microbiota transplantation for recurrent Clostridioides difficile infection: an updated systematic review and meta-analysis. EClinicalMedicine 2020; 29–30: 100642.

20. Du C, Luo Y, Walsh S, et al. Oral fecal microbiota transplant capsules are safe and effective for recurrent Clostridioides difficile infection: a systematic review and meta-analysis. J Clin Gastroenterol 2021; 55: 300–308.

21. Ianiro G, Murri R, Sciume GD, et al. Incidence of bloodstream infections, length of hospital stay, and survival in patients with recurrent Clostridioides difficile infection treated with fecal microbiota transplantation or antibiotics: a prospective cohort study. Ann Intern Med 2019; 171: 695–702.

22. Food and Drug Administration. Enforcement policy regarding investigational new drug requirements for use of fecal microbiota for transplantation to treat Clostridioides difficile infection not responsive to standard therapies. Rockville, MD: Food and Drug Administration, 2016, p.6.

23. Dutta SK, Girotra M, Garg S, et al. Efficacy of combined jejunal and colonic fecal microbiota transplantation for recurrent Clostridium difficile infection. Clin Gastroenterol Hepatol 2014; 12: 1572–1576.

24. Emanuelsson F, Claesson BEB, Ljungström L, et al. Faecal microbiota transplantation and bacteriotherapy for recurrent Clostridium difficile infection: a retrospective evaluation of 31 patients. Scand J Infect Dis 2014; 46: 89–97.

25. Hirsch BE, Saraiya N, Poeth K, et al. Effectiveness of fecal-derived microbiota transfer using orally administered capsules for recurrent Clostridium difficile infection. BMC Infect Dis 2015; 15: 191.

26. Kelly CR, Ilunnah C, Fischer M, et al. Fecal microbiota transplant for treatment of Clostridium difficile infection in
immunocompromised patients. *Am J Gastroenterol* 2014; 109: 1065–1071.

27. Ray A, Smith R and Breaux J. Fecal microbiota transplantation for Clostridium difficile infection: the Ochsner experience. *Ochsner J* 2014; 14: 538–544.

28. Russell GH, Kaplan JL, Youngster I, et al. Fecal transplant for recurrent Clostridium difficile infection in children with and without inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2014; 58: 588–592.

29. Satokari R, Mattila E, Kainulainen V, et al. Simple faecal preparation and efficacy of frozen inoculum in faecal microbiota transplantation for recurrent Clostridium difficile infection – an observational cohort study. *Aliment Pharmacol Ther* 2015; 41: 46–53.

30. Youngster I, Russell GH, Pindar C, et al. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. *JAMA* 2014; 312: 1772–1778.

31. Alang N and Kelly CR. Weight gain after fecal microbiota transplantation. *Open Forum Infect Dis* 2015; 2: ofv004.

32. Fischer M, Kao D, Kassam Z, et al. Stool donor body mass index does not affect recipient weight after a single fecal microbiota transplantation for Clostridium difficile infection. *Clin Gastroenterol Hepatol* 2018; 16: 1351–1353.

33. Brandt LJ, Aroniadis OC, Mellow M, et al. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection. *Am J Gastroenterol* 2012; 107: 1079–1087.

34. Baxter M, Ahmad T, Colville A, et al. Fatal aspiration pneumonia as a complication of fecal microbiota transplant. *Clin Infect Dis* 2015; 61: 136–137.

35. Khan MA, Sofi AA, Ahmad U, et al. Efficacy and safety of, and patient satisfaction with, colonoscopic-administered fecal microbiota transplantation in relapsing and refractory community- and hospital-acquired Clostridium difficile infection. *Can J Gastroenterol Hepatol* 2014; 28: 434–438.

36. Solari PR, Fairchield PG, Noa LJ, et al. Tempered enthusiasm for fecal transplant. *Clin Infect Dis* 2014; 59: 319.

37. Cheng YW, Alhaffar D, Saha S, et al. Fecal microbiota transplantation is safe and effective in patients with Clostridioides difficile infection and cirrhosis. *Clin Gastroenterol Hepatol*. Epub ahead of print 6 July 2020. DOI: 10.1016/j.cgh.2020.06.051.

38. Kelly CR, Yen EF, Grinspan AM, et al. Fecal microbiota transplantation is highly effective in real-world practice: initial results from the FMT National Registry. *Gastroenterology* 2021; 160: 183–192.e183.

39. Saha S, Mara K, Pardi DS, et al. Long-term safety of fecal microbiota transplantation for recurrent Clostridioides difficile infection. *Gastroenterology*. Epub ahead of print 11 January 2021. DOI: 10.1053/j.gastro.2021.01.010.

40. Zellmer C, Sater MRA, Huntley MH, et al. Shiga toxin-producing *E. coli* transmission via fecal microbiota transplant. *Clin Infect Dis*. Epub ahead of print 29 September 2020. DOI: 10.1093/cid/ ciaa1486.

41. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N Engl J Med* 2019; 381: 2043–2050.

42. Kassam Z, Dubois N, Ramakrishna B, et al. Donor screening for fecal microbiota transplantation. *N Engl J Med* 2019; 381: 2070–2072.

43. Tariq R, Weatherly R, Kammer P, et al. Donor screening experience for fecal microbiota transplantation in patients with recurrent *C. difficile* infection. *J Clin Gastroenterol* 2018; 52: 146–150.

44. Bafeta A, Yavchitz A, Riveros C, et al. Methods and reporting studies assessing fecal microbiota transplantation: a systematic review. *Ann Intern Med* 2017; 167: 34–39.

45. Petrof EO, Gloor GB, Vanner SJ, et al. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: ‘RePOOPulating’ the gut. *Microbiome* 2013; 1: 3.

46. Bang BW, Park JS, Kim HK, et al. Fecal microbiota transplantation for refractory and recurrent *Clostridium difficile* infection: a case series of nine patients. *Korean J Gastroenterol* 2017; 69: 226–231.

47. Dubberke ER, Orenstein R, Lee C, et al. Microbiome profile is distinct in patients with successful response to microbiota-based drug RBX2660 relative to placebo responders. *Open Forum Infect Dis* 2017; 4(Suppl. 1): S538.

48. Kelly CR, de Leon L and Jasutkar N. Fecal microbiota transplantation for relapsing *Clostridium difficile* infection in 26 patients: methodology and results. *J Clin Gastroenterol* 2012; 46: 145–149.

49. Khanna S, Pardi DS, Kelly CR, et al. A novel microbiome therapeutic increases gut microbial
diversity and prevents recurrent Clostridium difficile infection. J Infect Dis 2016; 214: 173–181.

50. Orenstein R, Dubberke E, Hardi R, et al. Safety and durability of RBX2660 (microbiota suspension) for recurrent Clostridium difficile infection: results of the PUNCH CD study. Clin Infect Dis 2016; 62: 596–602.

51. Orenstein R, Dubberke ER, Khanna S, et al. RBX2660 is safe, superior to antibiotic-treated controls for preventing recurrent Clostridium difficile, and may rehabilitate patient microbiomes: open label trial results. Open Forum Infect Dis 2017; 4(Suppl. 1): S535.

52. Van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 2013; 368: 407–415.

53. Kelly CR, Khoruts A, Staley C, 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.

54. McGovern BH, Ford CB, Henn MR, et al. SER-109, an investigational microbiome drug to reduce recurrence after Clostridioides difficile infection: lessons learned from a phase 2 trial. Clin Infect Dis. Epub ahead of print 7 April 2020. DOI: 10.1093/cid/ciaa387.

55. Seres Therapeutics, Inc. Seres therapeutics announces positive topline results from SER-109 phase 3 ECOSPOR III study in recurrent C. difficile infection, https://ir.serestherapeutics.com/news-releases/news-release-details/seres-therapeutics-announces-positive-topline-results-ser-109 (2020, accessed 10 August 2020).

56. Khanna S, Pardi DS, Jones C, et al. RBX7455, a Room Temperature-Stable, Orally-Administered Investigational Live Biotherapeutic, is Safe, Effective, and Shifts Patients’ Microbiomes in a Phase 1 Study for Recurrent Clostridioides difficile Infections. Clin Infect Dis. Epub ahead of print 24 September 2020. doi:10.1093/cid/ciaa1430

57. Vedanta Biosciences. VE303 for high risk Clostridioides difficile infection, https://www.vedantabio.com/pipeline/ve303 (accessed 21 January 2021).

58. BioSpace. Finch therapeutics announces positive topline results from randomized controlled trial of CP101, an oral microbiome drug, for the prevention of recurrent C. difficile infection, https://www.biospace.com/article/releases/finch-therapeutics-announces-positive-topline-results-from-randomized-controlled-trial-of-cp101-an-oral-microbiome-drug-for-the-prevention-of-recurrent-c-difficile-infection/ (2020, accessed 19 June 2020).

59. Vedanta Biosciences. VE 707, https://www.vedantabio.com/pipeline/ve707. (2021, accessed 21 January 2021)

60. Seres Therapeutics, Inc. Seres therapeutics announces initiation of a phase 1b clinical trial of SER-262 for primary Clostridium difficile infection, https://www.nestlehealthscience.com/newsroom/press-releases/Seres-Therapeutics-Announces-Initiation-of-Clinical-Trial (2016, accessed 21 January 2021).

61. Ford C, Litcofsky K, McGovern B, et al. 1503. Engraftment of investigational microbiome drug, SER-262, in subjects receiving vancomycin is associated with reduced rates of recurrence after primary Clostridium Difficile Infection (CDI). Open Forum Infect Dis 2019; 6(Suppl. 2): S547–S548.

62. Kao D, Wong K, Franz R, et al. The effect of a microbial ecosystem therapeutic (MET-2) on recurrent Clostridioides difficile infection: a phase 1, open-label, single-group trial. Lancet Gastroenterol Hepatol 2021; 6: 282–291.

63. Food and Drug Administration. Important safety alert regarding use of fecal microbiota for transplantation and risk of serious adverse reactions due to transmission of multi-drug resistant organisms. Rockville, MD: Food and Drug Administration, 2019.

64. Food and Drug Administration. Safety alert regarding use of fecal microbiota for transplantation and risk of serious adverse events likely due to transmission of pathogenic organisms. Rockville, MD: Food and Drug Administration, 2020.

65. Grad YH, Lipsitch M, Feldgarden M, et al. Genomic epidemiology of the Escherichia coli O104:H4 outbreaks in Europe, 2011. Proc Natl Acad Sci U S A 2012; 109: 3065–3070.

66. Peacock SJ, Parkhill J and Brown NM. Changing the paradigm for hospital outbreak detection by leading with genomic surveillance of nosocomial pathogens. Microbiology 2018; 164: 1213–1219.

67. Schürch AC, Arredondo-Alonso S, Willems RJL, et al. Whole genome sequencing options for bacterial strain typing and epidemiologic analysis based on single nucleotide polymorphism versus gene-by-gene–based approaches. Clin Microbiol Infect 2018; 24: 350–354.

68. Glover SC, Burstin S, Jones D, et al. 2099 E. coli sepsis following FMT in an IgA deficient IBD subject. Am J Gastroenterol 2019; 114: S1170.
69. Kwak S, Choi J, Hink T, et al. Impact of investigational microbiota therapeutic RBX2660 on the gut microbiome and resistome revealed by a placebo-controlled clinical trial. *Microbiome* 2020; 8: 125.

70. Wu Y, Guo C, Tang L, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol* 2020; 5: 434–435.

71. Gupta S, Parker J, Smits S, et al. Persistent viral shedding of SARS-CoV-2 in faeces – a rapid review. *Colorectal Dis* 2020; 22: 611–620.

72. Food and Drug Administration. Safety alert regarding use of fecal microbiota for transplantation and additional safety protections pertaining to SARS-CoV-2 and COVID-19, https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/safety-alert-regarding-use-fecal-microbiota-transplantation-and-additional-safety-protections (accessed 15 September 2020).

73. Khanna S and Pardi D. Fecal microbiota transplantation for recurrent *Clostridioides difficile* infection: the COVID-19 era. *Am J Gastroenterol* 2020; 115: 971–974.

74. Cutler DM and Summers LH. The COVID-19 pandemic and the $16 trillion virus. *JAMA* 2020; 324: 1495–1496.

75. Allegretti JR, Kassam Z, Carrellas M, et al. Fecal microbiota transplantation in patients with primary sclerosing cholangitis: a pilot clinical trial. *Am J Gastroenterol* 2019; 114: 1071–1079.

76. Delaune V, Orci LA, Lacotte S, et al. Fecal microbiota transplantation: a promising strategy in preventing the progression of non-alcoholic steatohepatitis and improving the anti-cancer immune response. *Expert Opin Biol Ther* 2018; 18: 1061–1071.

77. Kang D-W, Adams JB, Gregory AC, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome* 2017; 5: 10.

78. Marotz CA and Zarrinpar A. Treating obesity and metabolic syndrome with fecal microbiota transplantation. *Yale J Biol Med* 2016; 89: 383–388.

79. Evrensel A and Ceylan ME. Fecal microbiota transplantation and its usage in neuropsychiatric disorders. *Clin Psychopharmacol Neurosci* 2016; 14: 231–237.

80. Xu M-Q, Cao H-L, Wang W-Q, et al. Fecal microbiota transplantation broadening its application beyond intestinal disorders. *World J Gastroenterol* 2015; 21: 102–111.

81. Lashner BA, Korman L, Kraft CS, et al. 8- and 12-week efficacy and safety data from ECOSPOR-III a phase 3 double-blind, placebo-controlled randomized trial of SER-109 an investigational microbiome therapeutic for the treatment of patients with recurrent *Clostridium difficile* infection (rCDI). Presented at the American Journal of Gastroenterology Virtual Meeting, 23–28 October 2020.

82. Ferring Pharmaceuticals. Rebiotix and Ferring announce world’s first with positive preliminary pivotal phase 3 data for investigational microbiome-based therapy RBX2660. Saint-Prex, Switzerland: Ferring Pharmaceuticals, 2020.

83. Wilcox MH, McGovern BH and Hecht GA. The efficacy and safety of fecal microbiota transplant for recurrent *Clostridium difficile* infection: current understanding and gap analysis. *Open Forum Infect Dis* 2020; 7: ofaa114.