Review Article

Journal of Clinical & Experimental Immunology

Cultured Media Containing Bacterial Flora Could Be a Better Alternative to Fecal Transplantation in Treating Recurrent *Clostridium Difficile* Colitis

Jeff Mistroff MS IV, Gianna Guzzardo MS III*, Aaron Salem MS III and M. Cornelious Musara MD

Trinity School of Medicine, Class of 2018, 5755 North Point Pkwy #230, Alpharetta, GA 30022, USA

***Corresponding author**
Gianna Guzzardo, Trinity School of Medicine, Class of 2018, 5755 North Point Pkwy #230, Alpharetta, GA 30022, USA, Tel: (586) 453-4873; E-mail: giannaguzzardo@gmail.com.

Submitted: 16 Nov 2016; **Accepted:** 01 Dec 2016; **Published:** 06 Dec 2016

Abstract

Over the past few years, the mainstay of treatment for recurrent Clostridium difficile colitis has become fecal transplantation. Its efficacy over standard antibiotics therapy has been proven in multiple trails. However, there are inherent drawbacks in this treatment modality such as the transfer of unknown pathogens, the cost of testing and processing donor material, and the delay in onset of treatment. These obstacles may be circumvented by the clinical use of cultured media of bacterial isolates mimicking endogenous feces. We propose that such techniques have the potential to reduce the transfer of unknown pathogens to the patient, eliminating the cost of testing and processing the donor's stool, and by allowing for earlier onset of treatment. By eradicating these pitfalls in the current treatment, future patients could further benefit from treatment with cultured media when compared to fecal transplantation.

Keywords: *Clostridium difficile*, Recurrent *C. difficile* colitis, Cultured media, Fecal transplant alternative.

Introduction

Clostridium difficile (*C. difficile*) is the most common etiology of hospital acquired antibiotic-resistant colitis [1]. Between 2000 and 2005, the incidence of *C. difficile* infection (CDI) increased from 5.5 to 11.2 cases per 10,000 people [2]. Metronidazole and vancomycin are first line treatments of moderate to severe CDI [3]. While 90-98% of patients respond to oral vancomycin or metronidazole, the rate of recurrence is statistically significant [3,4]. Fidaxomicin and rifaximin are alternative antibiotics that have shown success in patients with recurrent disease [4,5]. Despite such advances in drug therapy, recurrence rates of *C. difficile* colitis remain at 30-65% [6].

In patients experiencing chronic relapsing CDI, fecal microbiota transplantation (FMT) has become an accepted form of therapy with trial evidence. FMT was designed for allogeneic transplantation of healthy colon microbiota from one individual to another. Promoting such indigenous microbiota growth within a compromised colon was theorized to be superior and more effective than antibiotic therapy. Lee et al. and Sharma et al. have discussed the advantage of FMT [7-9]. The benefits include vitamin production, fermentation of carbohydrates, metabolization of bile, growth and development of immune system, and competitive inhibition of pathogenic microbes [8,9].

Fecal transplantation involves harvesting stool from a healthy donor and implanting it in the GI tract of an infected recipient. Oral ingestion of capsules, fecal enema, colonoscopic implantation, and nasogastric/jejunal intubation and implantation are the different modes of administering a fecal transplant. Each route provides different advantages to transplantation. Enemas allow for direct transplantation into the rectum up to the splenic flexure [10-13]. *C.diff* colitis proximal to the splenic flexure is managed by endoscopic administration [10-13]. A colonoscopy allows for transplantation throughout the entire colon and the distal ileum.

Administration via nasogastric or nasojejunal tube allows for transplantation of the entire small intestine [10-13]. In a meta-analysis review, 317 patients in 27 case series and reports were treated with fecal transplantation [14]. The review showed a 92% rate of resolution of colitis after fecal transplantation [14].

Interestingly, different modes of administration had variable rates of resolution of CDI. Many studies have shown the efficacy of oral FMT. Youngster et al. looked at 20 patients with two or more episodes of severe *C. difficile* requiring hospitalization and also patients with three or more episodes of mild to moderate *C. difficile* who failed a six to eight week course of vancomycin taper therapy [15]. The patients were treated with thirty FMT capsules for two days [15]. Fourteen patients had complete resolution of their diarrhea [15]. The six patients who did not respond to the initial FMT capsule treatment were retreated with a second round of thirty FMT capsules for two days. The diarrhea resolved in four of the six patients on retreatment [15]. No major adverse effects were noted in the following six months [15].

Better results were shown in studies utilizing fecal enemas for fecal transplantation. The efficacy of FMT via retention enema was tested in a case series of 27 patients by Kassam et al. [16]. These patients had refractory or recurrent *C. difficile* and were treated with FMT via retention enema. The enema consisted of stool

from two healthy donors. This series resulted in 93% of patients having resolution of their symptoms, and 81% of the patients had their symptoms resolved within 24 hours [16]. Five of the patients failed initial therapy and received a second round of FMT.

Three of the five patients had resolution of their symptoms after their second treatment [16]. There were no major complications noted during the FMT therapy [16].

Van Nood et al. conducted a randomized control trial on 43 patients with recurrent *C. difficile* infection after at least one course of standard antibiotic therapy [17]. This trial compared a single nasogastric or nasojejunal infusion of donor feces to standard vancomycin regimen with or without bowel lavage. On interim analysis, donor feces treatment was shown to be significantly more effective where 81% of patients had resolution of their symptoms without relapse at 10 weeks when compared with standard vancomycin therapy with or without lavage [17]. Standard vancomycin therapy with or without lavage had a 23% and 31% resolution of patient's symptoms respectively after 10 weeks without relapse [17]. The study was discontinued due to the overwhelming evidence showing the advantage of nasogastric or nasojejunal infusions of donor feces over oral vancomycin therapy. Further analysis revealed an increase in diversity of their GI tract microbiota. Patients were found to have an increase in Bacteroides species and a decrease in Proteobacteria species [17]. The most common adverse effects found were mild diarrhea in 94% of patients, cramping in 31% of patients, and belching in 19% of patients [17]. All adverse effects resolved within three hours of the infusion. There have been no reported serious side effects associated with the fecal transplantation aside from the inherent risks of colonoscopy and nasogastric/jejunal tube placement.

Irrespective of the benefits, there are still concerns associated with the treatment of recurrent *C. difficile* with FMT, such as cost of testing and treatment, delay in on set of treatment, transfer of unknown pathogens in fecal material, and simply, an emotional aversion to the idea of receiving foreign fecal material. Pursuing alternative treatment modalities may help circumvent the concerns associated with standard FMT. We theorize that replacing endogenous human feces with cultured bacterial isolates has the potential to reduce associated risks involved in current fecal transplantation techniques.

Disadvantages of Donor Samples

Despite fecal microbiota therapy showing outstanding results, patients and physicians are still concerned about various factors associated with this therapy. A side from the aversion to ingesting or transplanting human feces, the transfer of undetected pathogens or transfer of unbalanced gut microbiota have been a concern. Physicians performing the fecal transplant have the responsibility of evaluating the donor for potential pathogens. The patient must undergo serological and fecal antigen testing. To date, there are only recommended guidelines for donor testing prior to transplantation. These include serological testing for Hepatitis A, B and C, HIV-1 and HIV-2, and syphilis. Stool cultures for bacteria, ova and parasites are also done. Stool antigen testing should be done for Giardia, Norovirus, Rotavirus, Helicobacter pylori and *C. difficile* [18].

Although these guidelines are in place to prevent the transfer of certain pathogens, this does not discount the transfer of untested pathogens or the transfer of fecal dysbiosis.

Donor testing adds an extra cost, which is typically incurred by the donor. Table 1 itemizes the screening tests recommended for the donated stool based on 2015-2017 LabCorp Fee Schedules [19]. The various pathogens listed in Table 1 show a total book price of $1,910.50 and a total net fee of $373.19 for stool and serological pathogen testing. In addition, the processing cost can vary depending on location or hospital due to lack of standardization. The cost of treating refractory or recurrent *C. difficile* continues to grow when adding in multiple doctor visits and processing of stool into an infusate.

LabCorp Net Fee Schedule VADMHMRSAS
September 1st, 2015 - August 31st, 2017

	CPT Code	Book Price	Net Fee	Short Description
1	86592	$ 27.50	$ 2.10	RPR
2	86593	$ 107.25	$ 6.98	RPR Qnt TP Ab
3	86704	$ 77.50	$ 4.77	Hep B Core Ab, Tot
4	86706	$ 70.25	$ 3.44	Hep B Surface Ab
5	86708	$ 73.50	$ 6.43	Hep A Ab, Total
6	86709	$ 77.25	$ 8.87	Hep A Ab, IgM
7	86780	$ 79.75	$ 4.88	Treponema pallidum Antibodies
8	86803	$ 88.00	$ 7.21	HCV Antibody
9	87045	$ 163.25	$ 32.14	Stool Culture
10	87046	$ 50.75	$ 16.80	Stool Culture, Yersinia Only /Campylobacter Culture
11	87177	$ 75.25	$ 14.69	Ova + Parasite Exam
12	87209	$ 75.00	$ 11.89	O+P Exam, PVA Only
13	87206	$ 75.75	$ 12.74	Cryptosporidium/Isospora Smear
14	87324	$ 107.00	$ 11.96	C difficile Toxins A+B, EIA
15	87328	$ 122.75	$ 21.19	Cryptosporidium EIA
16	87329	$ 90.00	$ 16.63	Giardia lamblia Ag, EIA
17	87338	$ 183.75	$ 41.57	H. pylori Stool Ag, EIA
18	87340	$ 59.50	$ 3.30	HBsAg Screen
19	86706	$ 113.75	$ 6.74	HBsAb+Ag
20	87493	$ 125.00	$ 125.00	C difficile Toxin Gene NAA
21	87075	$ 67.75	$ 13.86	C difficile Toxigenic Culture

Table 1: LabCorp Net Fee Schedule VADMHMRSAS: Itemized list of the screening tests recommended for the donated stool based off of LabCorp Fee Schedules from September 1st, 2015 through August 31st, 2017. Of note: CPT codes are provided for your convenience, but coding often varies from one carrier to another. These CPT codes listed here are to be used as general guidelines [19].

Delay in initiating treatment is a concern in FMT. Patients have to wait until they find a viable donor, who must be willing to undergo all the serological and stool testing, and then have the fecal matter processed into an infusate.

An additional weakness to FMT is patients' aversion to ingesting or transplanting another person's feces into their own body. Some Physicians find this method unappealing. In a Canadian Journal of Gastroenterology and Hepatology study that was published in 2014, researchers discussed their findings from an electronic survey sent to 135 gastroenterologists. The survey concluded that while 100% of the physicians reported treating patients with recurrent CDI, only 20% had treated a patient with fecal transplant, and most (65%) had neither offered fecal transplant nor referred

for fecal transplant [20]. Of note, 24% held the belief that patients would find the concept of fecal transplant too unappealing while 18% of physicians cited their own aversion to fecal transplant as a reason for not offering the procedure to patients [20].

The disadvantages of possible transfer of unknown pathogens (with some risk of resistance), cost of testing and processing donor material, and the delay in onset of treatment can be obviated with alternative treatment modalities, such as the utilization of a cultured media.

Combatting the Disadvantages

Cultured media, as an alternative to donor supplied FMT, would benefit patients and avoid the necessity for donors. Cultured media can become commercially produced after isolating beneficial and effective bacteria. These combinations can be packaged in varying profile spectrum contents. Commercialization of an effective alternative to fecal transplantation would lower the cost of therapy. Donors and patients would avoid the costs of multiple doctor visits, including stool and blood testing. In addition, with cultured media, patients would avoid delays in the initiation of therapy as samples would be readily available. This rapid onset of treatment can potentially decrease duration of symptoms with rapid resolution. The major concern of transferring donor fecal isolates with undetected bacteria is the risk of developing a super infection or transferring resistant pathogens. This would be eliminated with the use of cultured media. In addition, transfer of intestinal dysbiosis would be eradicated.

In one experiment, researchers administered a mixture of ten different cultured facultatively aerobic and anaerobic bacteria to five patients and one patient was selected to receive a fecal transplant. All five patients who received the cultured bacteria mixture tested negative for *C. diff* and its toxin during follow up analysis of stool samples [21]. This experiment by Tvede and Rask-Madsen showed the potential efficacy of cultured media as an alternative treatment for FMT in patients with recurrent *C. difficile*. Tvede and Rask-Madsen found bowel colonization of Bacteroides species, which were not present in pretreatment stool samples [21]. Additionally, they found that strains of Escherichia coli, C. bifermentans, and Peptostreptococcus species inhibited the growth of *C. difficile* in-vitro [21]. This data can direct research towards cultured media and its potential efficacy.

In an experiment, nicknamed repopulate, synthetic stool and human probiotic mixture was created based on the microbial diversity of a healthy, 41 year old female donor. Bacterial isolates were purified and identified based on 16s rRNA gene sequencing and antimicrobial sensitivity testing [22]. The final product consisted of thirty-three bacterial isolates and was based on a relative ratio. This relative ratio was created by metagenomic analysis and levels of abundance in a healthy individual [22]. Table 2 below lists the final thirty-three bacterial products in the stool substitute, repopulate. The repopulate solution was trialed in two patients. The first patient was a 74-year-old female, with six episodes of recurrent CDI over eighteen months [22]. The second patient was a 70 year old female, with three episodes of recurrent CDI [22]. Both patients were initially treated with standard treatment. The patients were required to have standard colon cleansing and all antibiotics were withheld for two days. The patients received 100 ml of repopulate solution via colonoscopy [22]. 50% of the solution was placed in the patient cecum and the other 50% was spread throughout the patient's transverse colon. They were placed in trendelenburg position for 60 minutes prior to being discharged.

Both patients were suggested to continue a high fiber diet upon discharge. No *C. difficile* toxin was found ten days post administration of repopulate in the first patient, and she was asymptomatic at 24 weeks post administration 72 hours post administration, the second patient reported relief of symptoms [22]. The second patient had many episodes of recurrent cellulitis requiring broad spectrum antibiotics before and after the repopulate treatment. 26 weeks after administration, the second patient continued to remain free of *C. difficile* symptoms and toxin, despite her recurrent broad spectrum antibiotic use after the repopulate treatment [22]. The repopulate study warrants further research and development in cultured media [22]. The proposed combinations of probiotics have proved to be effective on a small scale and continued research on a larger model is justified.

Closest species match, inferred by alignment of 16S rRNA sequence to GreenGenes database[a]	% identity to closest match	Relative abundance (by biomass) in RePOOPulate formulation
Acidaminococcus intestinalis	100	+++
Bacteroides ovatus	99.52	+
Bifidobacterium adolescentis (two different strains)	99.79	++
	99.79	++
Bifidobacterium longum (two different strains)	99.86	+++
	99.16	+++
Blautia producta	96.43	+
Clostridium cocleatum	91.92	+
Collinsella aerofaciens	98.73	+
Dorea longicatena (two different strains)	99.62	+
	99.60	+
Escherichia coli	99.80	+
Eubacterium desmolans	94.90	+
Eubacterium eligens	98.15	+++++
Eubacterium limosum	97.05	+
Eubacterium rectale (four different strains)	99.59	+++++
	99.60	+++++
	99.19	+++++
	99.53	+++++
Eubacterium ventriosum	100	++
Faecalibacterium prausnitzii	99.17	+++++
Lachnospira pectinoshiza	95.22	+
Lactobacillus casei/paracasei	99.47	+
Lactobacillus casei	99.74	+
Parabacteroides distasonis	99.45	++
Raoultella sp.	99.40	+
Roseburia faecalis	99.65	++
Roseburia intestinalis	100	++
Ruminococcus torques (two different strains)	99.15	+++
	99.29	+++
Ruminococcus obeum (two different strains)	94.89	+
	94.69	+
Streptococcus mitis[b]	99.79	+

Table 2: Composition of stool substitute (repopulate): List of culture isolates used by Petrof E, et al., from one health 41 year old female donor, with favorable antibiotic resistance profiles that were used in stool substitute preparation. Bacterial isolates were purified and identified based on 16s rRNA gene sequencing and antimicrobial sensitivity testing. The final product consisted of 33 bacterial isolates and was based on the relative ratio. This relative ratio was created by metagenomic analysis and levels of abundance in the healthy individual. Possible pertinent strains are indicated with "+", and increase with relative abundance by biomass. Of note: patient was found to be vancomycin and/or imipenem sensitive, with further sensitivity to other various commonly used antibiotics [22].

Discussion

Fecal transplantation is becoming the first line therapy in treating recurrent CDI. Many studies have shown the effectiveness and success of FMT with low risks. Emotional aversions from the recipient and treating physicians do exist and hinder the widespread use of FMT. The fee of pre-procedure screening on the donor adds cost and time to the process. The use of cultured medium would resolve the majority of these issues. It would also allow different cultured products to be available. This would address the fact that each individual has a unique microbiota composition and the option for various synthetic communities could further combat the resistance of *C. difficile* colitis in certain individuals. Thus providing a second and third line medias to be used for those who fail conventional therapy, with minimal additional workup or cost. Pre-manufacturing testing and the creation of different bacterial combinations to produce maximal effects would result in a line of highly efficacious culture isolates. The risk of transmitting resistant bacteria from the donor to recipient has not been studied but the potential is present especially with wide spread use of antibiotics. A carefully controlled isolate would remove this risk. Further research of cultured media to treat recurrent CDI is required. Large multicenter trials would show the efficacy and role of isolates in the treatment. We further propose that isolates in a tablet form can be utilized prophylactically in patients with a high risk of developing CDI. We strongly hold the belief that the gold standard of care for initial and recurrent CDI will be cultured stool substitute with antibiotics being demoted to backup or consigned to the storage shelves.

References

1. Nelson R (2007) Antibiotic treatment for Clostridium difficile-associated diarrhea in adults. Cochrane Database Syst Rev 18: CD004610.
2. Zilberberg MD, Shorr AF, Kollef MH (2008) Increase in Adult Clostridium difficile –related Hospitalizations and Case-Fatality Rate, United States, 2000-2005. Emerg Infect Dis 14: 929-931.
3. Zar FA, Bakkanagari SR, Moorthi KM, Davis MB (2007) A Comparison of Vancomycin and Metronidazole for the Treatment of Clostridium difficile-Associated Diarrhea, Stratified by Disease Severity. Clin Infect Dis 45: 302-307.
4. Louie TJ, Miller MA, Mullane KM, Weiss K, Lentnek A, et al. (2011) Fidaxomicin versus vancomycin for Clostridium difficile infection. N Engl J Med 364: 422-431.
5. Garey KW, Ghantoji SS, Shah DN, Habib M, Arora V, et al. (2011) A randomized, double-blind, placebo-controlled pilot study to assess the ability of rifaximin to prevent recurrent diarrhoea in patients with Clostridium difficile infection. J Antimicrob Chemother 66: 2850-2855.
6. Brandt LJ, Aroniadis OC, Mellow M, Kanatzar A, Kelly C, et al. (2012) Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection. Am J Gastroenterol 107: 1079-1087.
7. Robinson CJ, Bohannan BJ, Young VB (2010) From structure to function: the ecology of host-associated microbial communities. Microbiol Mol Biol Rev 74: 453-476.
8. Lee YK, Mazmanian SK (2010) Has the microbiota played a critical role in the evolution of the adaptive immune system? Science 330: 1768-1773.
9. Sharma R, Young C, Neu J (2010) Molecular modulation of intestinal epithelial barrier: contribution of microbiota. J Biomed Biotechnol 2010: 305879.
10. Khoruts A, Dicksved J, Jansson J, Sadowsky M (2009) Changes in the Composition of the Human Fecal Microbiome After Bacteriotherapy for Recurrent Clostridium Difficile-associated Diarrhea. J Clin Gastroenterol 44: 354-360.
11. Rohlke F, Surawicz C, Stollman N (2010) Fecal Flora Reconstitution for Recurrent Clostridium difficile Infection: Results and Methodology. J Clin Gastroenterol 44: 567-570.
12. Persky S (2000) Treatment of recurrent Clostridium difficile-associated diarrhea by administration of donated stool directly through a colonoscope. Am J Gastroenterol 95: 3283-3285.
13. Yoon S, Brandt L (2010) Treatment of Refractory/Recurrent *C. difficile*-associated Disease by Donated Stool Transplanted via Colonoscopy. J Clin Gastroenterol 44: 562-566.
14. Gough E, Shaikh H, Manges AR (2011) Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. Clin Infect Dis 53: 994-1002.
15. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, et al. (2014) Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. JAMA 312: 1772-1778.
16. Kassam Z, Hundal R, Marshall JK, Lee CH (2012) Fecal transplant via retention enema for refractory or recurrent Clostridium difficile infection. Arch Intern Med 172: 191-193.
17. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, et al. (2013) Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 368: 407-415.
18. Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, et al. (2011) Treating Clostridium difficile Infection With Fecal Microbiota Transplantation. Clin Gastroenterol Hepatol 9: 1044-1049.
19. http://www.dbhds.virginia.gov/library/administration/adm%20-%2004093%20labcorp%20pricing%20sheet%202015-2017.pdf
20. Zipursky JS, Sidorsky TI, Freedman CA, Sidorsky MN, Kirkland KB (2014) Physician Attitudes toward the Use of Fecal Microbiota Transplantation for the Treatment of Recurrent Clostridium difficile Infection. Can J Gastroenterol Hepatol 28: 319-324.
21. Tvede M, Rask-Madsen J (1989) Bacteriotherapy for chronic relapsing Clostridium difficile diarrhoea in six patients. Lancet 1: 1156-1160.
22. Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, et al. (2013) Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut. Microbiome 1: 3.