Serial Fecal Microbiota Transplant Plus Fidaxomicin in the Treatment of Severe or Fulminant *Clostridioides Difficile* Infection

by

Lindsey Anne Russell

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Translational Medicine

Department of Medicine

University of Alberta

ABSTRACT

BACKGROUND

Severe and fulminant *Clostridioides Difficile* infection (CDI) is associated with high rates of mortality and morbidity. Current practice guidelines recommend high dose vancomycin with metronidazole for treatment. Emerging evidence suggests efficacy of sequential fecal microbiota transplantation (FMT) by colonoscopy combined with vancomycin in patients failing maximal medical therapy. Fidaxomicin is non-inferior to vancomycin in treating CDI; however, it has not been studied in severe/fulminant cases. This single center, prospective, open-label study aimed to determine the efficacy and safety of combined serial FMT by enema plus fidaxomicin to treat patients who have severe or fulminant CDI not responding to maximal medical therapy. Furthermore, little is known about host response to FMT in this context.

METHODS

Consecutive participants with severe or fulminant CDI who fulfilled study inclusion and exclusion criteria were recruited. Sequential cycles of FMT, administered by enema daily over three days (720cc followed by 360cc and 180cc), plus fidaxomicin 200mg orally twice daily were given. Clinical symptoms and inflammatory markers were monitored during the study. Serum and stool samples were taken at regular intervals to determine changes in bile acids, short chain fatty acids and *C difficile* antibody production in these patients. Primary outcome was resolution of diarrhea 2 weeks following final FMT. Secondary outcomes were 1) resolution of diarrhea 8 weeks following final FMT; 2) safety of proposed treatment; and 3) colectomy rate. Exploratory outcomes included changes in host and microbiome metabolomics with serum and

stool short chain fatty acids and serum bile acids in addition to host immune response through antibody production after treatment. Study samples were compared to a historical control who received FMT and vancomycin.

RESULTS

A total of three participants were enrolled in this study between from Jan 22, 2019 to Aug 8, 2019; two of them reached both primary and secondary outcomes. There were no adverse events reported during this study. Although one participant did not reach primary outcome, he was free of CDI symptoms on suppressive vancomycin. Changes in both bile acids and short chain fatty acids before and after treatment in a participant reaching the primary outcome found trends similar to prior literature for recurrent CDI patients.

CONCLUSIONS

This pilot study is the first to demonstrate efficacy and safety of combined FMT by enema and fidaxomicin in treating severe or fulminant CDI patients. Exploratory analysis sheds light on the intricacies of the host-microbiome interaction with CDI. Further studies are needed to confirm these findings.

PREFACE

The clinical study, of which this thesis is a part of, obtained research ethics approval on Nov 19, 2018 from Health Research Ethics Board (Pro81229) at the University of Alberta, Health Canada (No Objection Letter control #220509) on Nov 1, 2018, and registered with clinicaltrials.gov (NCT03760484).

The literature review, designing the case record forms, participant information/consent forms, MERCK application for fidaxomicin and ethic approval application were my original work. In addition, I conducted the participant recruitment and consent, administered the FMT enemas, and collected daily information including clinical and inflammatory outcomes for each participant. The study design was my original work with the help of Dr. Kao.

Some of the research conducted for this thesis forms part of an international research collaboration led by Dr. Dina Kao at the University of Alberta with Dr. Tanya Monaghan, a Clinical Associate Professor at the University of Nottingham, and Dr. Benjamin Mullish, a Clinical Research Fellow in the Department of Metabolism, Digestion and Reproduction at Imperial College London. The analysis of stool and serum samples for bile acids and short chain fatty acid composition was done with the help of Dr. B Mullish. The immune anti-toxin reactivity was done with the help of Dr. T Monaghan. The interpretation and presentation of the results are my original work. No part of this thesis has been previously published.

ACKNOWLEDGEMENTS

Special thank you to Dr. Dina Kao and my supervisory committee Dr. Lynora Saxinger and Dr. Karen Wong for their guidance and advice through this process. Additional thanks for Dr. Eytan Wine for being an arm's length examiner.

Thanks to Brandi Roach, Matthew Emberg and Tracy Jordan for assisting with FMT preparation and participant follow up for this study. Additional thanks to Dr. Tanya Monaghan and Dr. Ben Mullish for their expertise and help with short chain fatty acids and bile acid analysis as well as immune anti-toxin reactivity.

Acknowledgement to MERCK Pharmaceuticals for providing the fidaxomicin for this research.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AAA	Abdominal aortic aneurysm
ALP	Alkaline phosphatase
ALT	Alanine transaminase
BID	Twice daily
BPH	Benign prostrate hypertrophy
CBC	Complete blood count
CDI	*Clostridioides Difficile* infection
CD3+ T cell	Cluster of differentiation 3 T Cell
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CT	Computed Tomography
DLD	Dyslipidemia
EIA	Enzyme immunoassay
FMT	Fecal microbiota transplant
GDH	Glutamate dehydrogenase
GPR3	G-couple protein receptor 3
HITT	Heparin induced thrombocytopenia
HIV	Human immunodeficiency virus
HTN	Hypertension
ICU	Intensive care unit
IFNγ	Interferon gamma
IL-6/17/18	Interleukin 6/17/18
INR	International normalized ratio
IV	Intravenous
MI	Myocardial infarction
MTBSTF	N-tert-Butyldimethylsilyl-Nmethyltrifluoroacetamide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NG	Nasogastric
NJ	Nasojejunal
PO	By mouth
PCR	Polymerase chain reaction/ nucleic acid amplification test
Q6H/Q8H	Every 6/8 hours
QID	Four times daily
RNA	Ribonucleic acid
SBO	Small bowel obstruction
SCFA	Short chain fatty acids
TBDMSCI	Tert-Butyldimethylchloro-silane
TNFα	Tumor necrosis factor- alpha
TLR	Toll-like receptor
Wk	Week

Chapter 1. INTRODUCTION

1.1 Background on Clostridioides difficile Infection

1.1.1 Epidemiology

Clostridioides difficile is a gram positive, spore-forming, anaerobic bacillus bacterium first discovered in 1978 as causing antibiotic-associated diarrhea and pseudomembranous colitis due to toxin production (1, 2). Risk factors for *Clostridioides difficile* infection (CDI) include antibiotic exposure, recent or prolonged hospitalizations, older age, infection with a higher virulent strain such as BI/NAP1/027, hypoalbuminemia and comorbidities such as malignancies, chronic kidney disease and inflammatory bowel disease (3, 4).

CDI is the leading cause of hospital-acquired infectious diarrhea, representing a significant healthcare burden worldwide. Severe CDI is associated with a high morbidity and mortality rate despite medical and/or surgical intervention (5). Around 8% of patients develop fulminant CDI resulting in toxic megacolon, multi-organ failure, and death (6). In a meta-analysis of primary studies published in 2002 and 2007, the annual cost of CDI in the United States adjusted to 2012 dollars was estimated at $1.5 billion (7). Recent surveillance data from Alberta Health Services found that Edmonton has the highest rate of healthcare-associated CDI and the highest CDI mortality in the province of Alberta (8). Traditionally CDI was thought to be only a hospital acquired infection, however more recent data has demonstrated a decrease in cases of hospital acquired CDI in Canadian provinces and an increase in community acquired CDI, up to 35% in British Columbia in 2017 (9, 10). Although the overall prevalence of CDI in Canada has decreased by 35.8% from 2009 to 2015, the incidence of severe outcomes such as ICU admission, colectomy, and death related to CDI have not changed (11).

1.1.2 Diagnosis

The diagnosis of CDI is critical to determine patients with active infection from asymptomatic carriers, as patients may have the bacterium detected in stool samples but not have

an active infection, typically due to negative toxin production (12, 13). There are numerous assays commercially available to determine CDI and each health care institution has different testing algorithm. One common assay is the nucleic acid amplification test (PCR), which detects *C difficile* toxin B genes. One concern is that a positive PCR test may not necessarily represent active infection, since *C difficile* toxins, responsible for pathogenesis of CDI, may not be produced (13). Other assays look at enzyme immunoassays (EIA), which detects stool for the presence of *C difficile* through glutamate dehydrogenase(GDH), an enzyme found in all *C difficile* organisms (12), with a reported sensitivity of 100% (95% CI, 0.79-0.10) (14). If the presence of *C difficile* through glutamate dehydrogenase is confirmed then *C difficile* toxin EIA is performed with a sensitivity of 73% (95% CI, 0.48-0.89) (12, 14). Other testing includes testing the amount of free toxin in stool through cell cytotoxic neutralization assays or through *C difficile* culture, which can be expensive and time consuming (15).

Proper interpretation of testing result is important to avoid the possibility of overdiagnosis and unnecessary treatment. Polage and colleagues determined in a prospective study that PCR positive but toxin negative patients had the same outcomes of diarrhea duration and lack of CDI complications as patients without CDI (13). Current testing algorithm for C difficile in Alberta involves using PCR assay first; if positive, GDH and toxin testing follow (12).

1.1.3 Treatment Options

Mild and moderate cases of CDI respond very well to vancomycin, which has become first-line therapy (1). Vancomycin is a tricyclic glycopeptide antibiotic that inhibits cell wall synthesis of both aerobic and anerobic gram positive bacteria and is considered to be bacteriostatic given the time to bacterial death (16). Unfortunately, vancomycin is not a narrow-spectrum antibiotic, and can also promote the development of vancomycin resistant *Enterococcus* (17). Fidaxomicin is a newer antibiotic in the macrolide family that inhibits the RNA polymerase of anaerobic gram-positive bacteria. Unlike vancomycin, fidaxomicin is bactericidal and kills bacteria *in vitro* at a quicker rate (16), and has been deemed more narrow spectrum as it has less of an effect on majority of gut flora (18, 19). Fidaxomicin is noninferior to vancomycin in clinical efficacy in the treatment of mild to moderate CDI (20) and has the advantage of reducing CDI recurrence (20). However,

the high cost prohibits fidaxomicin as a first-line therapy. Furthermore, it has not been used in patients with severe or fulminant CDI. Other therapies for CDI include tigecycline, a broad-spectrum antibiotic of the glycylcycline class that has been shown to significantly reduce the toxin production in hypervirulent strains of *C difficile*; however, it can lead to further disruption to the gut microbiome (21). Bezlotoxumab, a monoclonal antibody against *C difficile* toxin B, has been shown to reduce CDI recurrence, with success rates of only 80% for initial cure rate, defined as no diarrhea for 2 days post treatment with standard of care antibiotics for less than 16 days, and with sustained cure rate of 64%, defined as no recurrence of CDI within 12 weeks of the bezlotoxumab infusion (22). However, it is not clear how bezlotoxumab will fit into the CDI treatment algorithm given the high treatment cost. Although bezlotoxumab has been approved by the FDA, it has not received approval in Canada.

Severe CDI is defined as having a white blood cell count > 15,000 cells/mm^3 or creatinine level >1.5mg/dL or 1.5x premorbid level. Fulminant CDI is defined as having any of the following attributable to CDI: hypotension or shock, ileus, and toxic megacolon (1). Severe and fulminant CDI requires combined metronidazole with vancomycin therapy and surgical intervention is indicated when medical therapy fails (1). Surgical intervention is high risk in this population given the prevalence of multiple comorbidities and advance age, with mortality rates of 34-57% for patients who underwent colectomy (23). Traditionally, the surgical approach was a total abdominal colectomy; however Neal and colleagues proposed diverting loop ileostomy and intraoperative colonic lavage, saving the patient from a total colectomy and the opportunity for reversal in the future (24). A more recent study compared these two surgical approaches and demonstrated a similar mortality rate between diverting loop ileostomy (25.98%) and total abdominal colectomy (31.18%; $p = 0.28$), emphasizing the high mortality following surgical interventions (25).

1.2 Fecal Microbiota Transplantation

Fecal microbiota transplantation (FMT), which restores intestinal microbiome, is highly efficacious in treating mild to moderate recurrent CDI. FMT is generally well tolerated and the most common adverse event is abdominal pain (26). FMT can be administered by different routes, including enema, nasal gastric/jejunal tube (NG/NJ), colonoscopy and by oral capsules (27, 28).

The preparation of the FMT from fresh or frozen donor stool does not appear to affect the efficacy in the treatment of recurrent CDI (29). In a randomized control trial, it was shown that FMT by oral capsules was non inferior to FMT by colonoscopy in the treatment of recurrent CDI, with a success rate of 96% (27). In a single center randomized control trial between combination therapy of vancomycin and FMT, by either colonoscopy or NG tube, compared to fidaxomicin and vancomycin alone for the treatment of recurrent CDI demonstrated that FMT plus vancomycin lead to a 92% clinical resolution of recurrent CDI regardless of how FMT was delivered (30). In addition, the study demonstrated that fidaxomicin or vancomycin alone successfully prevented CDI recurrence in only 42% and 19% of patients, respectively (30). A systematic review demonstrated the overall efficacy of FMT for the treatment of recurrent CDI at 92% (95% CI, 0.89-0.94) but found significantly increased success rate in resolution of CDI with lower GI delivery of FMT of 95% (95% CI 0.92-0.97) compared to upper GI delivery success rate of 88% (95%, CI 0.82-0.94) (31). Another review mentioned similar overall success rates of 93% (95% CI, 0.90-0.95) but did not find a significant difference between upper and lower GI delivery of FMT in meta-regression analysis (32). The review also demonstrated in subgroup analysis that the overall success of FMT delivery by multiple enemas was significantly higher than the efficacy rate of a single infusion enema of 56% (95% CI, 0.41-0.69) (32).

The mechanisms of action behind FMT efficacy in the context of CDI is not well understood. One potential mechanism is thought to be colonization resistance through engraftment of donor microbiota (33). In addition, restored metabolism of fecal bile acids (34) and short chain fatty acids (SCFA) (35) post FMT may be additional mechanisms (36). A known function of a normal microbiota is the metabolism of primary to secondary bile acids (34). It has been demonstrated that primary bile acids, such as cholic acid and chenodeoxycholic acid are in more abundance in mice fecal samples post antibiotic administration with lower concentrations of secondary bile acids such as deoxycholic and lithocholic acid, suggesting that antibiotics can alter levels of bile acids (37). In *in vitro* studies, it was demonstrated that taurocholic acid, a primary bile acid, was a potent germinator of *C difficile* spores (38, 39) whereas ursodeoxycholic acid, a secondary bile acid, was an inhibitor of *C difficile* growth (40). In patients with recurrent CDI, higher levels of primary bile acids (cholic and chenodeoxycholic acid) were found prior to FMT, which subsequently reduced following successful FMT and were associated with increased levels

of secondary bile acids (lithocolic acid, deoxycholic acid and isodeoxycholic acid) (34, 41). SCFAs are important microbial metabolites, produced by fermentation of dietary fiber by the microbiome (42). Mouse models have demonstrated different fecal concentrations of acetate, butyrate and succinate in healthy, antibiotic treated and CDI affected animals (43). Patients with CDI had an increase of stool concentrations of acetate, propionate and butyrate after successful FMT, while levels of succinate and lactate remained unchanged (35). Another study demonstrated that FMT did not restore stool SCFA to levels of the donors, especially with linoleic and oleic acid (41). However, none of these studies examined the role of bile acids and SCFA in serum in context of CDI. Finally another potential mechanism for FMT efficacy is bacteriophages, suggested by the positive result from a preliminary study in which all 5 patients with recurrent CDI were successfully treated with sterile fecal filtrates, composed of proteins and bacteriophages but no live bacteria (44). Post administration of fecal filtrate demonstrated a phageome, or composition of gut viruses, in the recipients similar to donors, which may suggest a role for the phageome in treating CDI (44).

There is emerging evidence that FMT may be effective and safe in severe or fulminant CDI failing medical therapy, a situation where the only alternative is surgical intervention (45-47). For example, our group has successfully treated a patient with fulminant CDI with serial FMTs plus vancomycin over the past year at the University of Alberta Hospital. This patient had avoided colectomy as a result. Weingarden and colleagues demonstrated resolution of severe CDI refractory to medical therapy by colonoscopy delivered FMT in 4 patients, but noted that antibiotics were necessary in addition to FMT to treat these severe CDI cases, as the clinical benefit for FMT was short lived without antibiotics and repeated administrations of FMT (46). In addition, Fischer and colleagues demonstrated 17/19 (91%) patients achieving clinical cure from CDI in these critically ill patients using combined serial FMT and vancomycin (48). In this protocol, Fischer described using colonoscopy delivered FMT to treat fulminant CDI after the failure of improvement of symptoms and inflammatory markers with at least 5 days of combined metronidazole and vancomycin. Fischer et al also suggested repeat colonoscopies to assess the presence of pseudomembranous colitis to determine whether to continue with subsequent FMTs (48). Aroniadis et al. reported a success rate of 88.2% with severe and 94.1% with fulminant CDI for long-term follow up in 17 patients who received both FMT and antibiotics (49). Zainah et al

found that NG administration of FMT was effective in treating 13 patients with severe CDI (50). In a randomized, single center study with 56 participants, Ianiro and colleagues found that in addition to vancomycin, the use of serial FMT was superior to a single FMT in the treatment of severe refractory CDI, with a success rate of 100% compared to 75% respectively (51). Hocquart and colleagues reported that early administration of FMT improved survival in patients with severe CDI compared to medical therapy alone in a retrospective cohort study of 111 patients; not surprisingly, the survival benefit of early FMT was not seen in non-severe CDI cases (52).

Despite these studies, it remains unknown how many FMTs each patient with severe or fulminant CDI requires, what the most convenient or safest way to administer the FMT is, what the best strategy for monitoring response is, and what the optimal frequency of FMT is. Governing bodies also influence the mode of delivery of FMT available for health care professionals for patient care, as Health Canada has not approved the use of FMT by oral capsules to be administered outside the context of a clinical trial (53). Finally, vancomycin is not a narrow-spectrum antibiotic, and may potentially reduce FMT efficacy (19, 48). The success of these studies thus also highlights the gaps in knowledge in severe or fulminant CDI. The recent report of ESBL *E. coli* transmission in 2 immunocompromised patients from their stool donor who was not screened for multidrug-resistant bacteria, leading to 1 death (54, 55) further emphasizes the need to understand the mechanism of FMT in order to develop more refined therapeutic options.

1.3 Immune Response in CDI

In recent years, the role of the host immune system in relation to the microbiome is starting to be defined. The Human Functional Genomics Project has shown how differences in composition and function of gut microbial community may contribute to inter-individual variation in cytokine responses to microbial stimulation in healthy individuals (56). It further demonstrated that TNFα and IFNγ production are associated with specific microbial metabolites (56). In addition, specific ligands produced by gut bacteria can influence receptors on host immune cells and these interactions are a dynamic process as the microbiome changes with time and with different stresses such as infection (57).

In the context of CDI, the virulence factors of *C. difficile* include toxin A and toxin B, which are glucosyltransferases that induce a major proinflammatory cytokine release and disruption of the host intestinal epithelial cells leading to colonic inflammation (58, 59). Prior *in vitro* and animal studies have established that both toxin A and B have a role in the virulence of *C difficile* (60, 61). Most strains of *C difficile* contain either combination of toxin A and B genes or toxin B genes alone (59, 60). What remains unclear is how endogenous antibodies against toxin A or B protects again CDI. Clinical studies have demonstrated that the levels of anti-toxin B IgG antibodies were higher in patients who only had one episode of CDI compared to patients who had recurrent CDI and suggest that higher anti-toxin B IgG antibodies decreased the risk of developing recurrent CDI (62). Another study found that at day 3 of active CDI, patients with one episode of CDI had increased levels of anti-toxin A and anti-toxin B, and non-toxic IgM antibodies compared to patients that went on to develop recurrent CDI (63). A larger prospective study of 204 patients did not demonstrate any significant influence of either anti-toxin A or anti-toxin B both IgM or IgG antibodies measured at day 3 or day 12 of CDI on poor clinical outcomes, defined as treatment failure or progression to severe complicated recurrent CDI (64). A randomized control trial demonstrated that administration of bezlotoxumab alone was beneficial for preventing recurrent CDI (22). However in the same trial the combination of bexlotoxumab with actoxumab (monoclonal antibody against *C difficile* toxin A) or actoxumab alone did not show efficacy (22). Of note this study did not measure possible endogenous anti-toxin A or anti-toxin B levels in patients (22). The inconsistencies in the literature is difficult to interpret as the presence of an antibody does not demonstrate the function or degree of neutralization for *C difficile* toxins, which may have more clinical value. CDI also leads to a distinct phenotype of circulatory host immune cells that differs for primary compared to recurrent CDI (65). Initial episodes were associated with a greater amount of non-CD3+ cells such as monocytes whereas recurrence had more of CD3+ T lymphocytes involved in pro-inflammatory pathways (65). Thus, these studies highlight the gaps in our understanding of host immune response in the context of CDI (57).

1.4 Study Rationale

Although previous studies that have demonstrated success of the treatment of severe CDI with FMT and vancomycin, there is limited evidence that FMT by enema is effective in this

population. In addition, fidaxomicin has not been studied in the severe/fulminant CDI population failing medical therapy, and may be more advantageous then vancomycin for reducing the total number of FMT required or time to clinical resolution since fidaxomicin is a narrow spectrum antibiotic. The ease of twice daily for fidaxomicin dosing compared to four times a day with vancomycin is also advantageous for patients who are clinically unwell. The choice to deliver FMT by enema was for practical and safety reasons, since it can be administered by any trained medical personnel (eg. nurses, residents, physicians), and safer compared to colonoscopy, which is not only more invasive with risks of iatrogenic perforations (66) but also costly, and require trained physicians (eg. gastroenterologists, surgeons). Clinical monitoring, with symptoms and biochemical response may replace colonoscopies to gauge response and determine the timing for the next FMT (48). The knowledge gained during this trial will help optimize a treatment algorithm for patients suffering from treatment refractory CDI.

In addition, longitudinal clinical samples collected during this study will help gain a better understanding of the complex interaction between microbiome and human host in this context. These will have the potential to stimulate further studies aimed at elucidating mechanisms of action of FMT in order to develop rational microbiome-based therapy.

Chapter 2. STUDY OBJECTIVES AND HYPOTHESIS

2.1 Study Objectives

In this single center, prospective, open-label study, we aimed to determine the efficacy and safety of combined serial FMT by enema plus fidaxomicin to treat participants who have severe or fulminant CDI not responding to maximal medical therapy.

Our objectives are:

1. To determine clinical efficacy of serial FMT plus fidaxomicin in severe or fulminant CDI
2. To evaluate safety of serial FMT plus fidaxomicin

3. To explore potential mechanisms of action of FMT.

2.2 Hypothesis

Our hypothesis is that the combination of serial FMT plus fidaxomicin is safe and efficacious, and may potentially reduce the number of FMTs required and the length of hospital stay compared to a historical control that received FMT plus vancomycin.

Chapter 3. METHODS

3.1 Study Design

Prospective, open-label study at the University of Alberta Hospital, Edmonton AB.

3.2 Setting

In-patients with severe or fulminant CDI who fulfill the study criteria (Section 3.3) were screened for potential enrollment into this study. As this is a pilot study with a unique patient population, the sample size is based on an estimate of patient volume seen previously at the University of Alberta Hospital, a major FMT referral center. We aim to enroll a total of 10 participants in this preliminary study.

3.3 Study Population

3.3.1 Inclusion Criteria

- Participants with age over 18 years with severe or fulminant CDI, without an adequate response to metronidazole IV 500 mg q8H and vancomycin 500 mg PO q6h for at least 2 days or after FMT

- An adequate response is defined as a decrease in stool frequency or inflammatory markers (White Blood Cells [WBC] or C reactive protein [CRP]) by 10% over 48 hours
- Severe CDI was defined as having a WBC > 15,000 cells/mm^3 or serum creatinine level >1.5mg/dL or 1.5x premorbid level (1)
- Fulminant CDI was defined as having any of the following attributable to CDI: hypotension, shock, ileus, or megacolon (1)

- Participants had to have the ability to provide informed consent or an alternative decisionmaker providing consent

3.3.2 Exclusion Criteria

- Participants who had a bowel perforation, colostomy or ileostomy, colonic strictures, significant ileus were excluded from the study
- Participants were excluded if they were planning to have a colectomy
- Participants taking chemotherapy or radiation treatment with an absolute neutrophil count of < 1000 cells/mm^3 were excluded

3.4 Intervention

Participants who fulfilled inclusion criteria with no exclusion criteria noted were enrolled. When consent was obtained, metronidazole and vancomycin were discontinued and the participant took 2 L of Go-Lyte on the night prior to the first FMT. Each participant received a large volume fecal slurry, consisting of 720cc or equivalent to 200g of donor stool, delivered by enema on day 1, followed by two more days of small volume FMT enema on day 2 (360 cc) and day 3 (180 cc) (Appendix A Sup Figure 1.0). In the event that a colonoscopy was indicated to rule out other pathology, then the scheduled enema was delivered at the time of colonoscopy. On day 1, the participant also started a 7-10 day course of fidaxomicin 200mg PO BID. This constitutes as cycle 1. Each subsequent cycle of treatment consisted of 3 consecutive days of FMT enema in combination with fidaxomicin.

During each cycle of treatment, careful clinical monitoring including vitals, the requirement for vasopressors (if applicable), abdominal pain and distension, stool frequency and inflammatory markers including WBC and CRP were performed daily (Appendix A Sup Figure 1.0). As long as the participant had an improvement in clinical parameters and inflammatory markers, careful monitoring continued. When these improvements reached a plateau without further changes over 24-48 hours after the first cycle, the second cycle of treatment was initiated. The FMT and fidaxomicin combined cycles continued until clinical resolution of diarrhea and/or return of inflammatory markers to baseline prior to CDI. At that point, a final FMT enema (180cc) was administered. The maximal number of cycles administered was 4. At any time when a participant's condition worsened, the study team referred the participant for surgery. Each participant received FMT from a single donor, with multiple biospecimen (blood and stool) collections obtained over time as per study protocol (Appendix A Sup Figure 2.0).

3.4.1 Donor Selection

Three universal stool donors registered with the Edmonton FMT program provided the starting material, which was raw stool. The screening process can be found in Appendix C. Each donor provided a fresh stool specimen, weighing approximately 100g, as per donor stool collection protocol. The stool specimen is stored at 4-8 ^{0}C after collection and brought into the lab within 12 hours of collection. Donor stools had the appearance of type 2-5 on the Bristol Stool Scale, and were free of blood, mucus or urine contamination. No pooling of stools occurred. Once received by the lab, the stool sample was processed as per protocol for enema administration. Each donation of 100g of stool will produce approximately 360cc of fecal slurry, which was stored frozen as per our manufacturing protocol at -80 ^{0} C (See Appendix D).

3.5 Primary, Secondary, and Exploratory Outcomes

3.5.1 Primary Outcome

The primary outcome was CDI resolution, defined as < 3 unformed bowel movements over 24 hours or a return to baseline bowel habits, 2 weeks after final FMT treatment.

3.5.2 Secondary Outcomes

- Sustained CDI resolution defined as a lack of CDI recurrence 8 weeks after final FMT treatment without the need for additional anti-CDI therapy
- All serious adverse events up to week 8 after the final FMT. A serious adverse event entailed any event that results in any of the following: death, colonic perforation, proven infections related to FMT, and subsequent hospitalization due to CDI within the study period
- Colectomy due to CDI

3.5.3 Exploratory Outcomes

3.5.3.1 Serum Bile Acid Composition Analysis

Serum samples were thawed and centrifuged (9500 x*g*, 20 minutes), and the supernatant retained. A total of 75µl of serum samples were added to 225 µl of cold methanol and further centrifuged (9500 x *g*, 20 minutes) and a total of 120 µl of supernatant was loaded into test vials. Bile acids were extracted by cold methanol and incubation at -20 ^{o}C for >2 hours. As per previous protocols, Quality Control samples were prepared with equal parts of serum or stool extracts and used to ensure adequate performance monitoring (67). The Quality Control samples were added to the mixtures of bile acid standards (Steraloids, Newport, RI, USA) to assess retention time and metabolic identification. This was done by injecting 10 times of known bile acid sample prior to the run, and repeating once every 10 injections as well as at the end to ensure stability and reproducibility. Blank samples were also run to ensure no impurities were present.

Bile acid analysis of extracts were performed using ACQUITY UPLC (Waters Ltd, Elstree, UK) coupled to a Xevo G2 Q-ToF mass spectrometer equipped with an electrospray ionization source operating in negative ion mode (ESI-), using the method described by Sarafian and colleagues (68). Waters raw data files were converted to NetCDF format and data was extracted using XCMS (v1.50) package with R (v3.1.1) software (69). XCMS is open-access software for the pre-processing of LC-MS software that allows the input of preferred thresholds for a number

of key variables (70). Probabilistic quotient normalization was used to correct for dilution effects and chromatographic features with coefficient of variation higher than 30% in the Quality Control samples was excluded from further analysis (71). The relative intensities of the features were corrected to the dry weight of the fecal samples. Identification was also performed using a bioinformatics tool names PeakPantheR (69).

3.5.3.2 Serum and Stool Short-Chain Fatty Acid Composition Analysis

A targeted gas chromatography mass spectrometry protocol was used for the detection, identification and quantification of SCFA via adaptation of a protocol as previously-described (72). The protocol used tert-butyl methyl ether (Sigma) for the extraction of volatile compounds from serum or stool. In addition, derivatisation was performed using MTBSTF + 1% TBDMSCI (N-tert-Butyldimethylsilyl-Nmethyltrifluoroacetamide with 1% tert-Butyldimethylchloro-silane) (Sigma). Again, Quality Control samples were also prepared and run as a performance monitor for the assay. Calibration curves were obtained via the analysis of SCFA standards (Sigma) of known concentrations in full scan mode to allow specific quantification of each SCFA. Tert-butyl methyl ether with 100 parts per million methyl stearate (Sigma) was used as an internal standard.

Samples were randomized and analysed on an Agilent 7890B GC system coupled to an Agilent 5977A mass selective detector (Agilent, Santa Clara, California). Data analysis was performed using MassHunter software (Agilent), using retention times as stated in Appendix B Supplementary Table 1. Extracted ion chromatograms of the target ion selected for each SCFA were integrated, and the peak area was normalized to the internal standard to correct for variability in the instrument response.

3.5.3.3 Serum Anti-Toxin A/B Profiling Neutralization Assay

Cultured VERO cells were seeded at $1x10^4$ per well in a 96 well plate in 50ul of Dulbecco's Modified Eagle Medium supplemented with 10% Fetal Bovine Serum. After 18-20 hours, serum samples were serially diluted 2 fold (1:4 to 1:512) in serum-free and phenol red-free Dulbecco's Modified Eagle Medium and mixed with an equal volume of toxin A and Toxin B at 200ng/ml and

1ng/ml respectively (73, 74). Serum/toxin mixes were left to incubate for 1 hour at 37 °C. After incubation the mixes were added to the VERO cells to give a total well volume of 100ul, and incubated for 18h at 37 °C. Final concentration of Toxin A and Toxin B in culture was 50ng/ml and 0.25ng/ml respectively. All combinations, including negative controls were carried out in triplicate.

All media containing serum and toxins were removed and to each well added 50ul of 0.5mg/ml solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in serum free and phenol red-free Dulbecco's Modified Eagle Medium similar to assays with anthrax toxin (75). Plates were incubated for 4 hours at 37 °C. MTT solution was removed and 75ul Dimethyl sulfoxide added to each well. Plates were read on a BMG Labtech CLARIOstar plate reader at 595nm with a reference reading at 655nm with 20 second shaking prior to read to ensure complete solubilisation of formazan crystals.

3.6 Data Collection

Baseline characteristics including age, sex, duration and response to CDI therapy, medical history (including inflammatory bowel disease, irritable bowel syndrome, baseline bowel habit, bowel resection, chemotherapy or radiation, number of CDI in the previous 12 months) and medication were collected at the screening visit (Appendix A Sup Figure 2.0). Blood work including CBC, electrolytes, creatinine, ALT, ALP, albumin, CRP, INR, HIV, and viral hepatitis serology were drawn at the screening visit (Appendix A Sup Figure 2.0). In addition, a baseline collection of blood and stool samples were collected. Repeat stool and blood samples were collected after each cycle of treatment, and again at weeks 1, 2, 4 and 8 after the final FMT. Clinical status, as well as monitoring for adverse events, were assessed daily up to 2 weeks after the final FMT while a participant was hospitalized, then weekly up to week 8 after the final FMT.

3.7 Data Analysis

The primary outcome, defined as CDI resolution at week 2 post FMT, and secondary outcome of sustained CDI resolution at week 8, were analyzed using intention to treat principles. Adverse events were tabulated in each predetermined outcome.

3.7.1 Historical Control

A patient previously treated at the University of Alberta Hospital with fulminant CDI was used as a historical control. This patient received metronidazole 500 mg IV every 8 hours plus vancomycin 500 mg PO QID as well as serial FMTs delivered by colonoscopy as described in Fischer's protocol (48). This patient had the samples sent for the same analysis highlighted for study patients and was used as a comparison patient to the current study protocol.

3.8 Ethics

Study approval for this study was obtained on Nov 19, 2018 from Health Research Ethics Board (Pro81229) at the University of Alberta, Health Canada (No Objection Letter control #220509) on Nov 1, 2018, and registered with clinicaltrials.gov (NCT03760484). All participants signed consents willingly and were provided with the option to withdrawal at any time without any impact on their care. An independent data safety monitoring board consisting of Dr. Christina Surawicz (University of Washington), Dr. Geoff Taylor (University of Alberta) and Dr. Alexander Khoruts (University of Minnesota) was appointed and will follow the project until completion. The members of the data safety monitoring board were not involved in this trial and did not have potential participants who were enrolled in this study.

Chapter 4. RESULTS

4.1 Demographics and Descriptive Details of Study Participants and Historical Control

A total of three participants were enrolled in the study between Jan 22, 2019 to Aug 8, 2019. The baseline characteristics of the participants as well as the historical control are listed in

Table 1.0. Each participant had multiple comorbidities and were over the age of 60 (Table 1.0). Of note EDM001 had 4 prior episodes of CDI and failed multiple FMTs after an inadequate response to vancomycin and metronidazole a month before enrollment. EDM002 had a prior episode of CDI which resolved with a course of oral vancomycin. EDM001 had fulminant CDI due to the requirement of vasopressors, EDM002 also had fulminant CDI due to megacolon, whereas EDM003 had severe CDI. All participants had documented pseudomembranous colitis.

Table 1.0- Summary of participant and historical control baseline characteristics

Participant ID	**EDM001**	**EDM002**	**EDM003**	**Historical Control**
Sex	Male	Female	Female	Male
Age	70	61	85	84
Comorbidities	Chronic pain, cirrhosis, bariatric surgery, COPD, depression, atrial fibrillation, hypothyroidism	Congenital blindness in left eye, anxiety, restless leg syndrome	HTN, DLD, Moderate aortic stenosis, osteoarthritis	Hypothyroidism, type 2 DM, HTN, MI, AAA, BPH, CKD, Prior laparotomy for diverticulosis and SBO
Pertinent Medications	Hydromorphone, Flomax, Furosemide, Breo-Ellipta, Synthroid, Apixaban	Temazepam, Citalopram, Gabapentin	Rosuvastatin, Perindopril, Hydrochlorothiazide	Lipitor, Fenofibrate, Synthroid, Lopressor, Flomax
Number of prior CDI	4	1	None	None
Prior FMT	>5	None	None	None
CDI severity	Fulminant	Fulminant	Severe	Severe

4.2 Primary, Secondary, and Exploratory Outcomes

4.2.1 Primary Outcome

Two (EDM002 and EDM003) of the three participants (67%) achieved the primary outcome of symptoms resolution up to 2 weeks post final FMT with resolution of CDI as per intention to treat analysis. However, 100% of the participants who completed the full protocol achieved the primary outcome. One participant (EDM001) was withdrawn from the study by investigators due to worsening CDI at day 12 post final FMT (Table 2.0). Prior to the decision to

withdraw EDM001, he received a total of 2 cycles of FMT and the final FMT enema, similar to the other two participants. During the second cycle of treatment, this participant was given piperacillin-tazobactam for a urinary tract infection. Once he was withdrawn from the study, he was given vancomycin, metronidazole and four more large volume FMTs over 26 days. Each attempt to discontinue vancomycin led to diarrhea recurrence. Of note he did have formed bowel movements (Table 2.0) and was discharged from the hospital on vancomycin suppression and likely will require vancomycin suppressive therapy indefinitely.

The other two participants (EDM002 and 003) achieved the primary outcome and required a total of two cycles of treatment. As in Table 2.0, the final WBC and CRP both came down to normal levels after the final enema FMT. Their symptom scores also improved with final FMT, which persisted at the 2-week follow up appointment (Table 3.0).

4.2.2 Secondary Outcomes

Two (EDM002 and EDM003) of the three participants (67%) achieved sustained response defined as resolution of CDI symptoms up to 8 weeks post final FMT by intention-to-treat analysis. Of note, none of the participants, including EDM001, required a colectomy. There were no adverse events related to FMT or fidaxomicin. Participant EDM002 had fulminant CDI with pseudomembranous colitis and megacolon, possibly contributed by narcotic use and required colonic decompression (abdominal computed tomography, Appendix A Sup. Figure 3.0). Of note, resolution of pseudomembranous colitis was noted at the time of colonic decompression on day 4 of the first cycle of treatment (Figure 1.0). As her colon remained distended following two attempts at colonic decompression, she received neostigmine, which finally led to the resolution of her abdominal distension. As a precautionary measure, EDM002 did receive 2 days of IV metronidazole over concern of poor colonic motility in the context of megacolon that fidaxomicin may not be delivered to the target site, which is a protocol deviation. EDM002 also developed heparin-induced thrombocytopenia and thrombosis (HITT) likely related to heparin use in the hospital. There are no case reports of fidaxomicin or CDI relating to HITT.

Table 2.0- Summary of FMT timelines and donors used within the study and historical control. Serum WBC, CRP and clinical status during each cycle and only day 1 post cycle are shown

Participant ID	FMT	Day from Enrollment	Donor ID	WBC	CRP	Clinical Status
EDM001	**Cycle 1**		24			Pseudomembranous colitis seen on flexible sigmoidoscopy and colonic edema. Initially required vasopressors in ICU at time of enrollment. Required a paracentesis on Day 3
	200g Flex Sig	2		25.3	-	
	100g Enema	3		24.9	27.3	
	100g Enema	4		29	35.6	
	50g Enema	5		20.1	17.6	
	Day 1 post Cycle 1	6		17.2	16.1	
	Cycle 2		28			Had albumin infusion twice daily for ascites and had resolution of diarrhea on day 1 post cycle 2
	200g Enema	8		19.7	28	
	100g Enema	9		15	22.9	
	50g Enema	10		16.7	26.6	
	Day 1 post Cycle 2	11		12.0	35.2	
	Final Enema	17		8.8	49.7	
	Day 1 post Final Enema	18		9.3	44.7	
EDM001 FMTs following withdrawal from study	400g Colonoscopy	37	28	11.6	10.1	Required subsequent vancomycin and IV metronidazole, Eventually symptoms resolved on suppressive vancomycin therapy
	200g Colonoscopy	44		9.2	13.3	
	200g Enema	47	31	13.1	19.9	
	200g Enema	54		7.3	45.6	
	Day 1 post last FMT	55		6.6	31,4	
EDM002	**Cycle 1**		24			Initial creatinine was 2X baseline at diagnosis. Pseudomembranous colitis seen on endoscopy initially. CT abdomen showed megacolon On Day 4 of cycle 1, required colonic decompression that showed healed mucosa. Also required neostigmine before resolution of megacolon and two days of IV metronidazole
	200g Colonoscopy	2		11.9	22.4	
	100g Enema	3		10.9	23.5	
	50g Enema	4		11.9	24.9	
	Day 1 post Cycle 1	5		11.3	43.2	
	Cycle 2		31			Clinically still having abdominal pain. Flexible sigmoidoscopy post cycle 2 showed healing mucosa
	200g Enema	20		9.5	43	
	100g Enema	21		10.7	30.6	
	50g Enema	22		10.3	27.7	
	Day 1 post Cycle 2	23		10	30.8	
	Final Enema	29		7.2	15	
	Day 1 post Final Enema	30		6.7	12.6	
EDM003	**Cycle 1**		24			Significant pseudomembranous colitis seen on colonoscopy with edematous mucosa Diarrhea every 2 hours with decrease rectal tone Low appetite clinically
	200g Colonoscopy	2		13.9	35	
	100g Enema	3		13.7	57.7	
	50g Enema	4		10.9	46.2	
	Day 1 post Cycle 1	5		8.4	38.7	
	Cycle 2					Cycle 2 was started on the basis of ongoing diarrhea, every 4 hours with similar symptoms of low appetite On last day of fidaxomicin at final FMT enema was having semi formed stools
	200g Enema	8		5.8	8.2	
	100g Enema	9		6.5	8.2	
	50g Enema	10		5.5	6.1	
	Day 1 post Cycle 2	11		5.1	4.6	
	Final Enema	13		6.7	5.8	
	Day 1 post Final Enema	14		3.8	7	
Historical Control	**FMT 1** 100g Colonoscopy	1	24	46.4	-	Significant pseudomembranous colitis on colonoscopy
	FMT 2 100g Colonoscopy	6	24	24	-	Significant pseudomembranous colitis on colonoscopy
	FMT 3 100 g Colonoscopy + 2 days of 18 capsules (50g)	12	24	14	-	Some improvement on pseudomembranous colitis on colonoscopy
	FMT 4 100g Colonoscopy	22	24	11.4	-	No pseudomembranous colitis on colonoscopy
	FMT 5 2 days of 17 capsules (50g)	26	24	9.1	-	Having formed bowel movements

Table 3.0- Summary of participant symptom scores and clinical data with comparison to historic control

Participant ID	EDM001	EDM002	EDM003	Control*
Initial WBC	28.9	14.2	15.8	55.2
Initial CRP	22.4	24.4	55.3	-
Initial Pain Score^	7/10	8/10	1/10	-
Initial Appetite Score#	2/10	9/10	2/10	-
Initial Fatigue Score**	8/10	8/10	8/10	-
Initial Bowel Movements##	Slightly More Loose (6/10)	Significantly More Loose (10/10)	Significantly More Loose (8/10)	-
Number of FMTs	2 Cycles + Enema Post Study 4 FMTs	2 Cycles + Enema	2 Cycles + Enema	8 Total days of FMT *
Final WBC	9.3	6.7	5.4	9.2
Final CRP	44.7	12.6	7	-
Final Pain Score^	6/10	3/10	1/10	-
Final Appetite Score#	7/10	9/10	6/10	-
Final Fatigue Score**	5/10	4/10	6/10	-
Final Bowel Movements##	Baseline (5/10)	Slightly More Constipated (2/10)	Baseline (5/10)	-
2 Wk Pain Score^	6/10	1/10	1/10	-
2 Wk Appetite Score#	6/10	9/10	8/10	-
2 Wk Fatigue Score**	3/10	3/10	4/10	-
2 Wk Bowel Movements##	Slightly more constipated (4/10)	Baseline (5/10)	Baseline (5/10)	-
Time to CDI Resolution from Diagnosis	64 days	46 days	22 days	37 days
Length of Stay	88 days	49 days	24 days	54 days

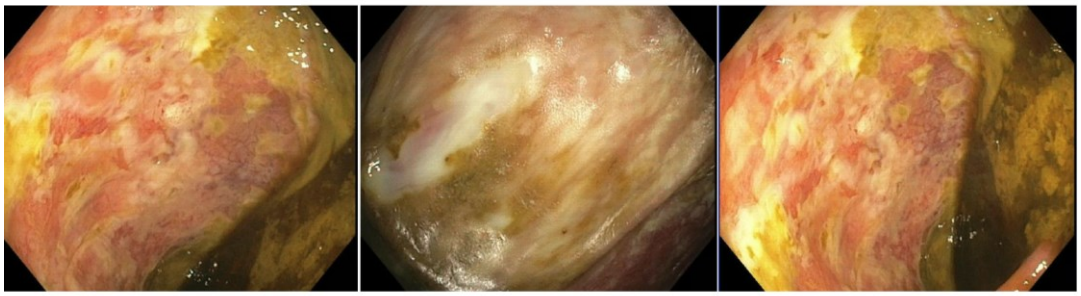
* The historical control had 5 FMTs over a total of 8 days. ^Pain: 1/10-no pain to 10/10-most severe pain. #Appetite: 1/10-no appetite to 10/10-great appetite. **Fatigue: 1/10-no fatigue to 10/10-most fatigue. ## Bowel movements: 1/10-more constipated then baseline, 5/10-baseline bowel movements, 10/10 - more loose then baseline

4.2.3 Exploratory Outcomes

Exploratory outcomes were analyzed for the historical control, EDM001, who achieved transient resolution of diarrhea but failed to meet primary outcome, and EDM002, who reached the primary outcome. Sampling timepoints and corresponding clinical symptoms are shown in

Appendix B Supplementary Table 2.0. Timepoints include initial assessment pre-treatment, after each treatment cycle, final FMT, as well as symptom resolution.

A. Study enrollment



B. Post 1 cycle of FMT and fidaxomicin

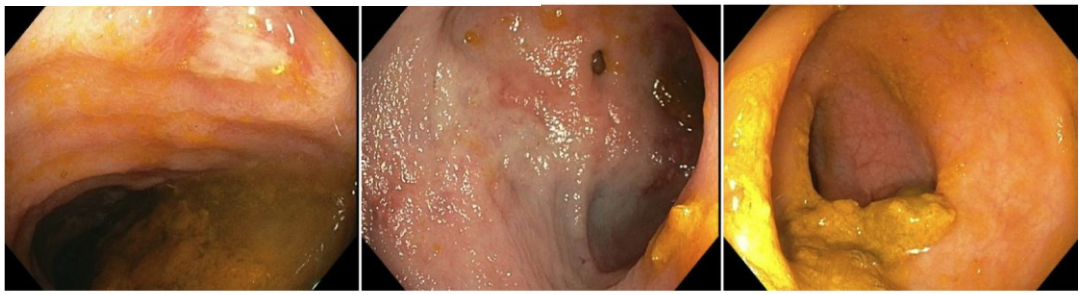


Figure 1.0- Endoscopic photos of EDM002 Colonoscopy at A. time of enrollment of study (Pre FMT) and B. Post Cycle 1 of FMT/Fidaxomicin

4.2.3.1 Serum Bile Acid Composition

Concentrations of primary, tauro-conjugated, and secondary bile acids were analyzed for donors and study participants (Figure 2.0). Exact serum concentrations of each bile acid can be found in Appendix B Supplementary Table 3.0.

Both the historical control and EDM001 had higher concentrations of cholic acid, a primary bile acid, compared to donors and EDM002 at symptom resolution. With chenodeoxycholic acid, highest levels were found in both EDM001 and EDM002 at symptom resolution compared to pre FMT. The tauro-conjugated bile acids were more abundant in the historic control with CDI resolution compared to EDM001 and EDM002. EDM002 had decreased levels in both tauro-conjugated bile acids post FMT, an effect not seen in EDM001. Both donors (#28 and #31) had higher serum secondary bile acid levels compared to study participants pre FMT. With CDI

resolution after treatment both the historic control and EDM002 had higher serum levels of secondary bile acids over time, although the effect was greater with EDM002. Of note EDM001 had a spike of ursodeoxycholic acid at time of final FMT prior to withdrawal of study, which may suggest transient restoration of secondary bile acid metabolism.

4.2.3.2 Serum Short-Chain Fatty Acid Composition

The five major SCFAs analyzed in serum; acetate, 2-hydroxybutyrate, butyrate, lactate, and propionate were compared between each participant at pre-determined timepoints as outlined above. Other SCFAs were analyzed at each time point and shown in Appendix B Supplementary Table 4.

Butyrate increased post FMT in both EDM001 and 002 but was not detected in either donors. The historic control subject had a decrease in 2-hydroxybutyrate and lactate with symptom resolution, and an inconsistent change with acetate and propionate. EDM001 had high levels of all SCFA except 2-hydrocybutytrate and lactate at symptom resolution compared to pre FMT. EDM002 had increased levels of propionate, butyrate and acetate post FMT compared to pre FMT samples.

4.2.3.3 Stool Short-Chain Fatty Acid Composition

Similar SCFA acids were analyzed in stool samples with the addition of Donor 24, who was the donor for the historic control (Appendix B Sup. Table 2.0). Lactate was unable to be reliably analyzed compared to quality control sample and therefore not reported.

The SCFA 2-hydroxybutyrate concentration decreased post FMT across all participants, similar to the donor level (Figure 4.0). The SCFAs acetate, butyrate and propionate had higher concentrations in the donors and subsequently increased post FMT in the historic control and EDM002 as compared to pre FMT (Figure 4.0). EDM001 had a similar pattern however acetate was lower at symptom resolution compared to pre FMT. The exact concentrations can be found in Appendix B Supplementary Table 5.0.

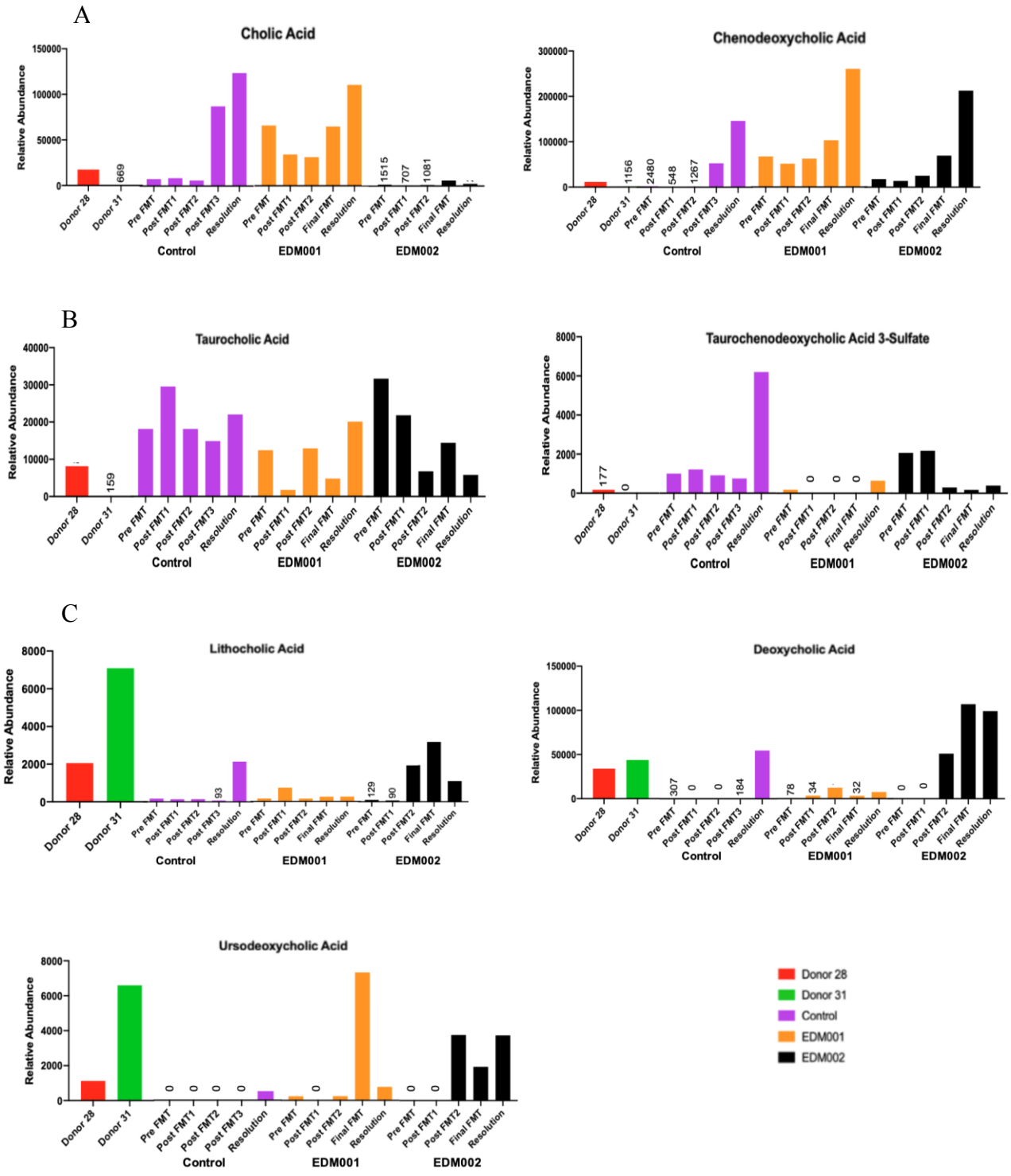


Figure 2.0 Comparison of relative abundance of major bile acids in serum between historic control, EDM001 and EDM002 at detailed timepoints**. A. Comparison of primary bile acids. B. Comparison of Tauro-conjugated bile acids. C. Comparison of secondary bile acids.

**Pre FMT denotes study enrollment. Post FMT 1 denotes post FMT 1 (control) or post FMT cycle 1 (EDM001/EDM002). Post FMT 2 denotes post FMT 2 (control) or post FMT cycle 2 (EDM001/EDM002). Post FMT 3 denotes third FMT for control. Final FMT is the final enema for EDM001/EDM002. Resolution denotes clinical and biochemical resolution

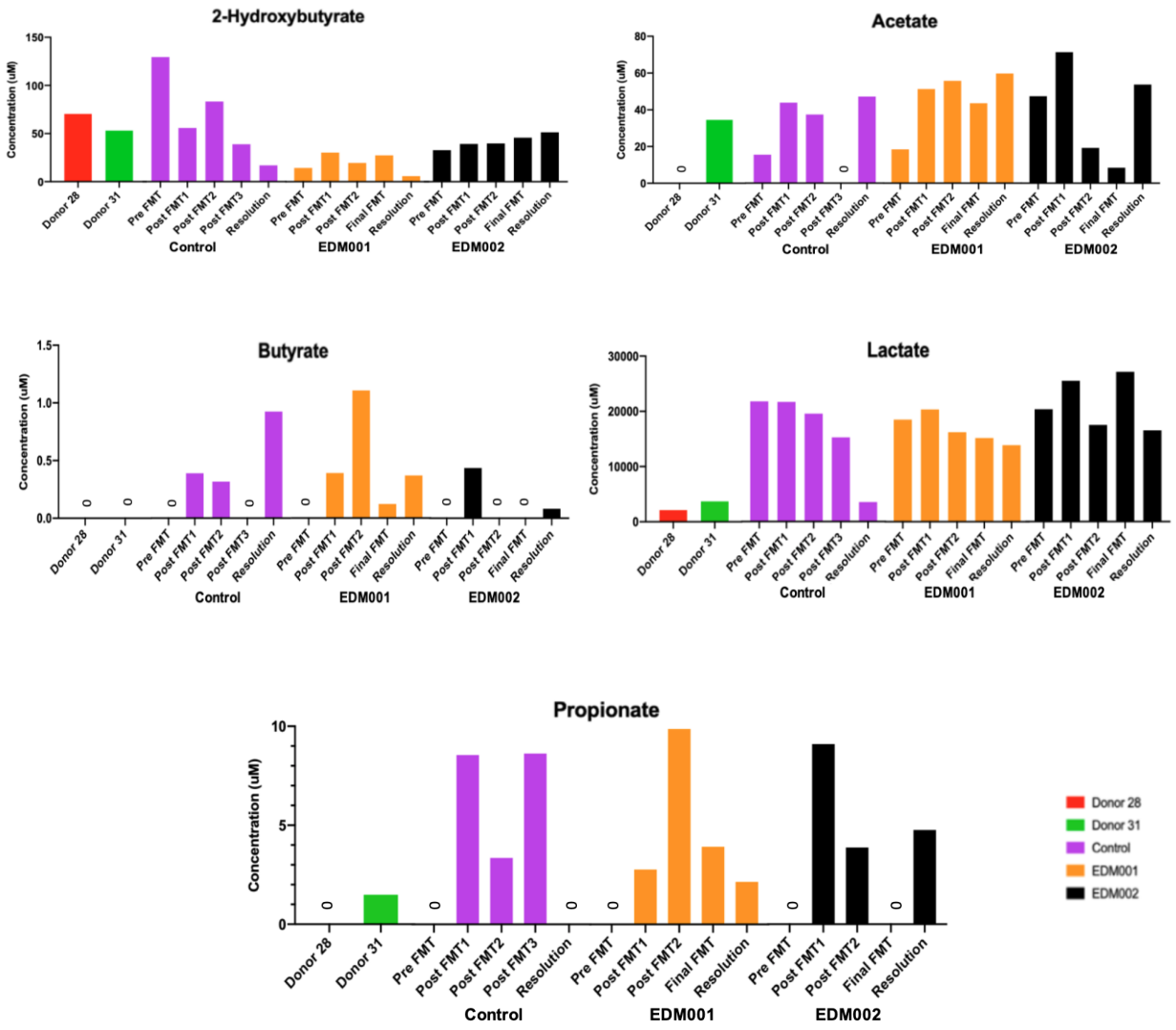


Figure 3.0 Comparison of five major Short Chain Fatty Acids (SCFA) in serum between control, EDM001 and EDM002 at detailed timepoints**

** Pre FMT denotes study enrollment. Post FMT 1 denotes post FMT 1 (control) or post FMT cycle 1 (EDM001/EDM002). Post FMT 2 denotes post FMT 2 (control) or post FMT cycle 2 (EDM001/EDM002). Post FMT 3 denotes third FMT for control. Final FMT is the final enema for EDM001/EDM002. Resolution denotes clinical and biochemical resolution

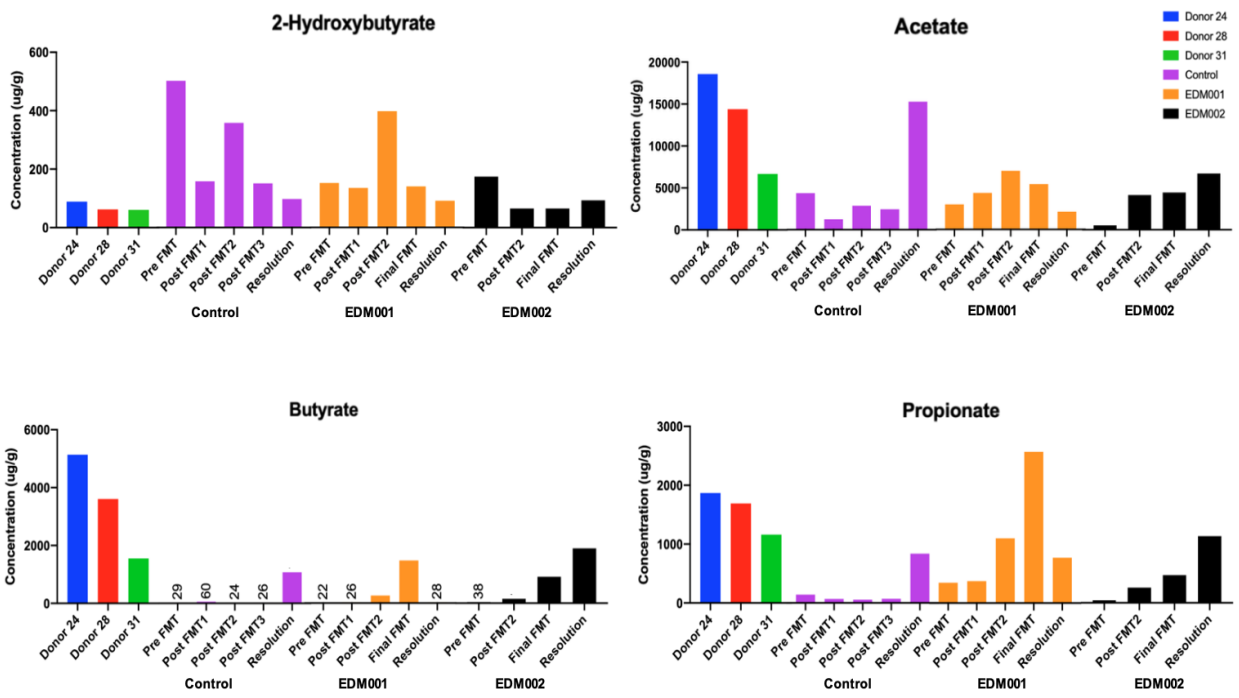


Figure 4.0 Comparison of five major short chain fatty acids (SCFA) in stool between control, EDM001 and EDM002 at detailed timepoints**

** Pre FMT denotes study enrollment. Post FMT 1 denotes post FMT 1 (control) or post FMT cycle 1 (EDM001/EDM002). Post FMT 2 denotes post FMT 2 (control) or post FMT cycle 2 (EDM001/EDM002). Post FMT 3 denotes third FMT for control. Final FMT is the final enema for EDM001/EDM002. Resolution denotes clinical and biochemical resolution

4.3.2.4 Serum Anti-Toxin A/B Profiling

For analysis of *C. difficile* anti-toxin neutralization, it was found that only EDM002, who reached the primary outcome, had anti-toxin B activity. The reactivity of anti-toxin B was higher at final FMT compared to pre FMT and noted to increase even at 2 weeks post final enema (Table 4.0). The control and EDM001 had no anti-toxin activity to either toxin A or toxin B.

Table 4.0- Summary of *C difficile* Toxin Neutralization activity of EDM002 at determined time points **. Of note both control and EDM001 had 0% neutralization for both Toxin A and B at any time point.

EDM 002	**Toxin A**	**Toxin B**		
Time Point **	**Percentage of Reactivity at Different Concentrations**			
Pre FMT	0	0		
Post FMT1	0	0		
Post FMT2	0	100% at 1:4	10% at 1:8	
Final FMT	0	100% at 1:4	50% at 1:8	~5-10% at 1:16
Symptom Resolution	0	100% at 1:4	60% at 1:8	10% at 1:16
2 Week Follow up	0	100% at 1:4	100% at 1:8	40% at 1:16
EDM001	0	0		
Historical Control	0	0		

** Pre FMT denotes study enrollment. Post FMT 1 denotes post FMT cycle 1. Post FMT 2 denotes post FMT cycle 2. Final FMT denotes the final enema. Resolution denotes clinical and biochemical resolution

Chapter 5. DISCUSSION

This proof-of-concept preliminary study demonstrated feasibility and efficacy of serial FMT by enema with fidaxomicin in treating severe or fulminant CDI. Both participants who reached the primary outcome required a total of 2 cycles of treatment. The requirement for multiple FMTs in context for severe or fulminant CDI is consistent with previous studies (51). The number of colonoscopy delivered FMT required was 3 to 4 in the study by Fischer and colleagues, similar to the number received in our historic control when combined with vancomycin (45). Two participants who reached the primary outcome in this study received a total of 7 FMTs by enema, which amounts to the similar donor stool volumes as colonoscopy delivered FMT. However the advantage with this protocol is the administration by enema, as colonoscopy is expensive and trained physicians are necessary to perform the procedure (66). Other studies have also used colonoscopy as a monitoring tool or a decision point to invoke more FMTs, and the success of this protocol with monitoring by symptoms and inflammatory markers suggests that repeat

colonoscopies may not be necessary. Of note, the predominant participant reported symptom differed between participants, highlighting the heterogeneity of this population. This is important when considering relying on clinical status and biochemical markers to monitor for CDI resolution.

The total length of hospital stay for the two participants reaching the primary outcome was reduced by an average of 30 days compared to the historic control, who received FMT and vancomycin. However there are multiple aspects of a hospital stay, such as psychosocial issues or rehabilitation need beyond CDI treatment and resolution. When examining length of stay from time of diagnosis of CDI and clinical resolution, the mean difference was 3 days shorter in the FMT/Fidaxomicin group compared to the historical control (Table 3.0). This difference could have been larger if EDM002 was referred for treatment earlier.

There were no adverse events noted, and none of the participants had a colectomy or died. These results support the safety of this study protocol and prevented these participants from undergoing a high-risk surgical procedure, which would have been their only option.

The study participant EDM001 had fulminant CDI and did not reach the primary outcome but had clinical resolution of CDI up to 12 days post final FMT prior to developing a urinary tract infection resulting in exposure to piperacillin-tazobactam and recurrence of diarrhea. Of note, this participant had 4 prior episodes of CDI in which he failed multiple FMTs with vancomycin and metronidazole. Interestingly there have been prior studies that fidaxomicin was less effective in patients that had 2 or more episodes of CDI, with effect in 82% of patients compared to 100% for patients with first episode of CDI (76). The fact that with subsequent FMTs and suppressive vancomycin therapy he achieved remission of CDI could suggest that the treatment prescribed in this protocol may have converted him from an antibiotic resistant state to an antibiotic sensitive state for CDI resolution.

Analysis of serum bile acids in the first two participants demonstrated that FMT led to a higher abundance of secondary bile acids compared to primary in EDM002 who reached primary outcome, which is consistent with previous literature analyzing fecal concentrations of bile acids (34). There is limited data on the serum levels of bile acids in this population, therefore it is difficult

to extrapolate with prior results on stool analysis; however similar trends were found. Both the historical control and EDM001 had higher abundance of primary bile acids at the time of final FMT and at symptom resolution than the donors. Medications can lead to alterations in microbiome metabolism of bile salts and both subjects were older patients with multiple comorbidities, which could be contributory factors to higher abundance of serum primary bile acids despite symptom resolution (77, 78). However, the high levels of primary bile acids may potentially suggest persistent dysbiosis, and a reason for subsequent CDI recurrence in EDM001. The decrease in serum tauro-conjugated post FMT noted in EDM002 is also similar with previous studies that found decreased tauro-conjugated bile concentrations post FMT in fecal samples (33, 35, 79, 80). The data on serum bile acids in this study showed similar trends seen in fecal analysis of bile acids in previous studies, which may suggest that serum levels can be used to monitor bile acid metabolism. A limitation of this however is the fact that bacteria can alter the metabolism of bile acids in the gut, which depending on reabsorption of bile acids in the gut can result in different serum levels and difficult to correlate with prior studies that analyzed stool samples (34).

This study was unique in analyzing SCFA in both serum and stool, which leads to a more comprehensive picture of SCFA metabolites in the context of CDI. Prior studies have found that FMT led to increased stool concentrations of acetate and butyrate in successfully treated patients (35). In addition a prior study examining patients with recurrent CDI and NJ administration of FMT had similar effects with increased butyrate and acetate post FMT (80). With EDM002, who reached primary outcome, there was an increase in all three SCFAs in both stool and serum at symptom resolution compared to pre FMT. Butyrate has been shown to decrease inflammation in the gut (81, 82). Acetate has been shown to be a key factor in colonic health, reducing inflammation (83) and prevention of enteropathogenic infections (84). Propionate has similar beneficial effects for colonocytes but to a lesser degree (42). Prior studies have demonstrated no difference in levels of propionate in stool samples between pre and post FMT compared to donor (80), whereas in this study EDM002 had higher levels of propionate at symptom resolution. Interestingly the pattern of SCFA in serum was similar to stool SCFA concentrations. However, the levels fluctuated at different timepoints and highlights the dynamic changes in these metabolites. SCFA are quickly absorbed by colonocytes, and SCFA metabolism are influenced by changes of environment, diet, and medications (85). Therefore future studies examining SCFA will need to these variables into

consideration (85). A prior study had noted that one patient who suffered from alcoholism did not have the expected increase of acetate and butyrate post FMT (80). Given the influences of multiple factors on the composition of metabolites in the gut, it is difficult to determine when the best time is for sampling for various analyses.

In regards to host immune response, EDM002 was the only one assessed to have high levels of Toxin B neutralizing antibodies activity against *C difficile* and reached the primary outcome (Table 4.0). It is difficult to determine whether it was the development of anti-toxin B antibodies that led to the positive clinical response in EDM002 or the combination of FMT and fidaxomicin. Certainly the greatest amount of toxin neutralisation occurred at 2-weeks follow-up, which suggests continued immune response post treatment and with clinical resolution. A recent study demonstrated that the presence of anti-toxin antibodies were not predictive of a positive or negative clinical outcome in active mild-moderate CDI, suggesting the presence of anti-toxin antibodies were present in patients who had a poor outcome, such as progression to severe CDI, treatment failure or development of recurrent CDI (64). It is difficult to assess whether different ribotyping of *C difficile* influenced the antibody production in the historical control or EDM001 (9). Certainly, it has been suggested that higher anti-toxin production is protective in recurrence of CDI, however EDM002 had a prior episode of mild CDI treated with vancomycin. Interestingly no toxin neutralization activity was present initially in EDM002 serum samples. A prior study demonstrated that patients who were asymptomatic *C difficile* carriers had higher levels of anti-Toxin A IgG antibodies compared to recurrent and symptomatic CDI patients (86). It could suggest that a different ribotype of *C difficile* or the severity of the recurrent infection led to increased toxin B neutralization compared to the prior episode of CDI. Overall the antibody neutralization assay done in this study was exploratory and certainly more investigations are needed to clarify influence of anti-toxin B activity in the protection and treatment for CDI. There is a possibility of FMT as an adjunct to anti-toxin derived therapy for the treatment of CDI, or that FMT helped with modulating the host immune response. More analysis in cytokine profiling and Toll-Like receptor data is ongoing and this study data highlights the importance of analyzing the host immune response in context of CDI (58) and may serve as potential biomarkers to prognosticate treatment outcome. Recent studies demonstrated that butyrate had the ability to induce IL-18 in mice colonocytes and affect T lymphocyte cell function, highlighting the intricacies of the gut

metabolites and the host immune system (81, 82). Signalling through the chemoattractant receptor, GPR3, on mice colonocytes by SCFA seems to be a direct pathway in which metabolites in the gut can influence the host immune system (83). More studies incorporating both immune response and microbiome metabolites such as bile acids and SCFA are needed to assess the multifaceted effect on CDI infection and resolution.

This study is the first to our knowledge to demonstrate that the combination of FMT by enema and fidaxomicin is a promising therapy in treating severe or fulminant CDI patients. In addition, this protocol demonstrated that following clinical status and inflammatory markers to determine when to administer subsequent FMTs can be done in lieu of repeat colonoscopies. There are limitations of this study however, including small sample size and the heterogeneity of this population in comorbidities. The analysis described include mainly observational data, as demonstrating statistical significance would be difficult in such a small sample size. Ultimately a randomized trial comparing serial FMT by enema plus fidaxomicin to serial FMT by colonoscopy plus vancomycin is the only way to demonstrate the differences between these two approaches, however this may be difficult given the low prevalence of severe or fulminant CDI failing maximal medical therapy. Despite these limitations, the changes in SCFA and bile acid metabolism and anti-toxin B activity highlight the complexities in the mechanisms of action of FMT. There is ongoing analysis on cytokine profiling, immune cell profiling and stool bile acids from participant samples. As technology to analyze the intricacies of host microbial interaction advances, the more targeted microbiome based therapies can be developed (87).

REFERENCES

1. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, 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). Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018.

2. Bartlett JG, Chang TW, Gurwith M, Gorbach SL, Onderdonk AB. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. The New England journal of medicine. 1978;298(10):531-4.

3. Khanna S, Pardi DS. Clostridium difficile infection: management strategies for a difficult disease. Therap Adv Gastroenterol. 2014;7(2):72-86.

4. Chilton CH, Pickering DS, Freeman J. Microbiologic factors affecting Clostridium difficile recurrence. Clin Microbiol Infect. 2018;24(5):476-82.

5. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. The American journal of gastroenterology. 2013;108(4):478-98; quiz 99.

6. Adams SD, Mercer DW. Fulminant Clostridium difficile colitis. Current opinion in critical care. 2007;13(4):450-5.

7. Zimlichman E, Henderson D, Tamir O, Franz C, Song P, Yamin CK, et al. Health care-associated infections: a meta-analysis of costs and financial impact on the US health care system. JAMA internal medicine. 2013;173(22):2039-46.

8. 2015/2016 AHSQPR. Hospital acquired clostridium difficile infections [Available from: https://www.albertahealthservices.ca/assets/about/publications/ahs-pub-pr-2016-17-q3-detail-hospital-acquired-infections.pdf.

9. Xia Y, Tunis MC, Frenette C, Katz K, Amaratunga K, Rose SR, et al. Epidemiology of Clostridioides difficile infection in Canada: A six-year review to support vaccine decision-making. Can Commun Dis Rep. 2019;45(7-8):191-211.

10. Brown KA, Khanafer N, Daneman N, Fisman DN. Meta-analysis of antibiotics and the risk of community-associated Clostridium difficile infection. Antimicrob Agents Chemother. 2013;57(5):2326-32.

11. Katz KC, Golding GR, Choi KB, Pelude L, Amaratunga KR, Taljaard M, et al. The evolving epidemiology of Clostridium difficile infection in Canadian hospitals during a postepidemic period (2009-2015). CMAJ. 2018;190(25):E758-E65.

12. AHS. ProvLab C. difficile Testing, Results and Comments 2019 [August 16, 2019:[Available from: https://www.albertahealthservices.ca/assets/wf/plab/wf-provlab-c-difficile-testing-results-and-comments.pdf.

13. Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, et al. Overdiagnosis of Clostridium difficile Infection in the Molecular Test Era. JAMA internal medicine. 2015;175(11):1792-801.

14. Swindells J, Brenwald N, Reading N, Oppenheim B. Evaluation of diagnostic tests for Clostridium difficile infection. J Clin Microbiol. 2010;48(2):606-8.

15. Planche TD, Davies KA, Coen PG, Finney JM, Monahan IM, Morris KA, et al. Differences in outcome according to Clostridium difficile testing method: a prospective multicentre diagnostic validation study of C difficile infection. Lancet Infect Dis. 2013;13(11):936-45.

16. Golan Y, Epstein L. Safety and efficacy of fidaxomicin in the treatment of Clostridium difficile-associated diarrhea. Therap Adv Gastroenterol. 2012;5(6):395-402.

17. Pepin J. Vancomycin for the treatment of Clostridium difficile Infection: for whom is this expensive bullet really magic? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;46(10):1493-8.

18. Venugopal AA, Johnson S. Fidaxomicin: a novel macrocyclic antibiotic approved for treatment of Clostridium difficile infection. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54(4):568-74.

19. Tannock GW, Munro K, Taylor C, Lawley B, Young W, Byrne B, et al. A new macrocyclic antibiotic, fidaxomicin (OPT-80), causes less alteration to the bowel microbiota of Clostridium difficile-infected patients than does vancomycin. Microbiology. 2010;156(Pt 11):3354-9.

20. Louie TJ, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, et al. Fidaxomicin versus vancomycin for Clostridium difficile infection. The New England journal of medicine. 2011;364(5):422-31.

21. Goyal H, Perisetti A, Rehman MR, Singla U. New and emerging therapies in treatment of Clostridium difficile infection. Eur J Gastroenterol Hepatol. 2018;30(6):589-97.

22. Wilcox MH, Gerding DN, Poxton IR, Kelly C, Nathan R, Birch T, et al. Bezlotoxumab for Prevention of Recurrent Clostridium difficile Infection. N Engl J Med. 2017;376(4):305-17.

23. Seltman AK. Surgical Management of Clostridium difficile Colitis. Clin Colon Rectal Surg. 2012;25(4):204-9.

24. Neal MD, Alverdy JC, Hall DE, Simmons RL, Zuckerbraun BS. Diverting loop ileostomy and colonic lavage: an alternative to total abdominal colectomy for the treatment of severe, complicated Clostridium difficile associated disease. Ann Surg. 2011;254(3):423-7; discussion 7-9.

25. Juo YY, Sanaiha Y, Jabaji Z, Benharash P. Trends in Diverting Loop Ileostomy vs Total Abdominal Colectomy as Surgical Management for Clostridium Difficile Colitis. JAMA Surg. 2019.

26. Wang S, Xu M, Wang W, Cao X, Piao M, Khan S, et al. Systematic Review: Adverse Events of Fecal Microbiota Transplantation. PLoS One. 2016;11(8):e0161174.

27. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, et al. Effect of Oral Capsule- vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. Jama. 2017;318(20):1985-93.

28. Allegretti JR, Mullish BH, Kelly C, Fischer M. The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. Lancet. 2019;394(10196):420-31.

29. Lee CH, Steiner T, Petrof EO, Smieja M, Roscoe D, Nematallah A, et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. Jama. 2016;315(2):142-9.

30. Hvas CL, Dahl Jorgensen SM, Jorgensen SP, Storgaard M, Lemming L, Hansen MM, et al. Fecal Microbiota Transplantation Is Superior to Fidaxomicin for Treatment of Recurrent Clostridium difficile Infection. Gastroenterology. 2019;156(5):1324-32 e3.

31. Quraishi MN, Widlak M, Bhala N, Moore D, Price M, Sharma N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. Alimentary pharmacology & therapeutics. 2017;46(5):479-93.

32. Ianiro G, Maida M, Burisch J, Simonelli C, Hold G, Ventimiglia M, et al. Efficacy of different faecal microbiota transplantation protocols for Clostridium difficile infection: A systematic review and meta-analysis. United European Gastroenterol J. 2018;6(8):1232-44.

33. Seekatz AM, Aas J, Gessert CE, Rubin TA, Saman DM, Bakken JS, et al. Recovery of the gut microbiome following fecal microbiota transplantation. MBio. 2014;5(3):e00893-14.

34. Weingarden AR, Chen C, Bobr A, Yao D, Lu Y, Nelson VM, et al. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. Am J Physiol Gastrointest Liver Physiol. 2014;306(4):G310-9.

35. Seekatz AM, Theriot CM, Rao K, Chang YM, Freeman AE, Kao JY, et al. Restoration of short chain fatty acid and bile acid metabolism following fecal microbiota transplantation in patients with recurrent Clostridium difficile infection. Anaerobe. 2018;53:64-73.

36. Allison SD, Martiny JB. Colloquium paper: resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci U S A. 2008;105 Suppl 1:11512-9.

37. Giel JL, Sorg JA, Sonenshein AL, Zhu J. Metabolism of bile salts in mice influences spore germination in Clostridium difficile. PLoS One. 2010;5(1):e8740.

38. Sorg JA, Sonenshein AL. Bile salts and glycine as cogerminants for Clostridium difficile spores. J Bacteriol. 2008;190(7):2505-12.

39. Wilson KH. Efficiency of various bile salt preparations for stimulation of Clostridium difficile spore germination. J Clin Microbiol. 1983;18(4):1017-9.

40. Sorg JA, Sonenshein AL. Inhibiting the initiation of Clostridium difficile spore germination using analogs of chenodeoxycholic acid, a bile acid. J Bacteriol. 2010;192(19):4983-90.

41. Brown JR, Flemer B, Joyce SA, Zulquernain A, Sheehan D, Shanahan F, et al. Changes in microbiota composition, bile and fatty acid metabolism, in successful faecal microbiota transplantation for Clostridioides difficile infection. BMC Gastroenterol. 2018;18(1):131.

42. van der Beek CM, Dejong CHC, Troost FJ, Masclee AAM, Lenaerts K. Role of short-chain fatty acids in colonic inflammation, carcinogenesis, and mucosal protection and healing. Nutr Rev. 2017;75(4):286-305.

43. Lawley TD, Clare S, Walker AW, Stares MD, Connor TR, Raisen C, et al. Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing Clostridium difficile disease in mice. PLoS Pathog. 2012;8(10):e1002995.

44. Ott SJ, Waetzig GH, Rehman A, Moltzau-Anderson J, Bharti R, Grasis JA, et al. Efficacy of Sterile Fecal Filtrate Transfer for Treating Patients With Clostridium difficile Infection. Gastroenterology. 2017;152(4):799-811.e7.

45. Fischer M, Sipe B, Cheng YW, Phelps E, Rogers N, Sagi S, et al. Fecal microbiota transplant in severe and severe-complicated Clostridium difficile: A promising treatment approach. Gut Microbes. 2017;8(3):289-302.

46. Weingarden AR, Hamilton MJ, Sadowsky MJ, Khoruts A. Resolution of severe Clostridium difficile infection following sequential fecal microbiota transplantation. Journal of clinical gastroenterology. 2013;47(8):735-7.

47. Brandt LJ, Borody TJ, Campbell J. Endoscopic fecal microbiota transplantation: "first-line" treatment for severe clostridium difficile infection? Journal of clinical gastroenterology. 2011;45(8):655-7.

48. Fischer M, Sipe BW, Rogers NA, Cook GK, Robb BW, Vuppalanchi R, et al. Faecal microbiota transplantation plus selected use of vancomycin for severe-complicated Clostridium difficile infection: description of a protocol with high success rate. Alimentary pharmacology & therapeutics. 2015;42(4):470-6.

49. Aroniadis OC, Brandt LJ, Greenberg A, Borody T, Kelly CR, Mellow M, et al. Long-term Follow-up Study of Fecal Microbiota Transplantation for Severe and/or Complicated Clostridium difficile Infection: A Multicenter Experience. Journal of clinical gastroenterology. 2016;50(5):398-402.

50. Zainah H, Hassan M, Shiekh-Sroujieh L, Hassan S, Alangaden G, Ramesh M. Intestinal microbiota transplantation, a simple and effective treatment for severe and refractory Clostridium difficile infection. Dig Dis Sci. 2015;60(1):181-5.

51. Ianiro G, Masucci L, Quaranta G, Simonelli C, Lopetuso LR, Sanguinetti M, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy plus vancomycin for the treatment of severe refractory Clostridium difficile infection-single versus multiple infusions. Alimentary pharmacology & therapeutics. 2018;48(2):152-9.

52. Hocquart M, Lagier JC, Cassir N, Saidani N, Eldin C, Kerbaj J, et al. Early Fecal Microbiota Transplantation Improves Survival in Severe Clostridium difficile Infections. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018;66(5):645-50.

53. Canada H. Guidance Document: Fecal Microbiota Therapy Used in the Treatment of Clostridium difficile Infection Not Responsive to Conventional Therapies. July 31, 2019 ed2019.

54. Miller S. Patient dies from fecal transplant containing drug-resistant bacteria. National Broadcasting Company News. 2019 June 13, 2019.

55. DeFilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, et al. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant. The New England journal of medicine. 2019.

56. Schirmer M, Smeekens SP, Vlamakis H, Jaeger M, Oosting M, Franzosa EA, et al. Linking the Human Gut Microbiome to Inflammatory Cytokine Production Capacity. Cell. 2016;167(4):1125-36 e8.

57. Karczewski J, Poniedzialek B, Adamski Z, Rzymski P. The effects of the microbiota on the host immune system. Autoimmunity. 2014;47(8):494-504.

58. Solomon K. The host immune response to Clostridium difficile infection. Ther Adv Infect Dis. 2013;1(1):19-35.

59. Voth DE, Ballard JD. Clostridium difficile toxins: mechanism of action and role in disease. Clin Microbiol Rev. 2005;18(2):247-63.

60. Kuehne SA, Cartman ST, Heap JT, Kelly ML, Cockayne A, Minton NP. The role of toxin A and toxin B in Clostridium difficile infection. Nature. 2010;467(7316):711-3.

61. Lyras D, O'Connor JR, Howarth PM, Sambol SP, Carter GP, Phumoonna T, et al. Toxin B is essential for virulence of Clostridium difficile. Nature. 2009;458(7242):1176-9.

62. Gupta SB, Mehta V, Dubberke ER, Zhao X, Dorr MB, Guris D, et al. Antibodies to Toxin B Are Protective Against Clostridium difficile Infection Recurrence. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;63(6):730-4.

63. Kyne L, Warny M, Qamar A, Kelly CP. Association between antibody response to toxin A and protection against recurrent Clostridium difficile diarrhoea. Lancet. 2001;357(9251):189-93.

64. Reigadas E, Alcala L, Marin M, Martin A, Bouza E. Clinical, immunological and microbiological predictors of poor outcome in Clostridium difficile infection. Diagn Microbiol Infect Dis. 2017;88(4):330-4.

65. Yacyshyn MB, Reddy TN, Plageman LR, Wu J, Hollar AR, Yacyshyn BR. Clostridium difficile recurrence is characterized by pro-inflammatory peripheral blood mononuclear cell (PBMC) phenotype. J Med Microbiol. 2014;63(Pt 10):1260-73.

66. Farooq PD, Urrunaga NH, Tang DM, von Rosenvinge EC. Pseudomembranous colitis. Dis Mon. 2015;61(5):181-206.

67. Sangster T, Major H, Plumb R, Wilson AJ, Wilson ID. A pragmatic and readily implemented quality control strategy for HPLC-MS and GC-MS-based metabonomic analysis. Analyst. 2006;131(10):1075-8.

68. Sarafian MH, Lewis MR, Pechlivanis A, Ralphs S, McPhail MJ, Patel VC, et al. Bile acid profiling and quantification in biofluids using ultra-performance liquid chromatography tandem mass spectrometry. Anal Chem. 2015;87(19):9662-70.

69. Wolfer AM CG, Pearce JTM, Sands C. peakPantheR: Peak Picking and ANnoTation of High resolution Experiments in R (Version v1.2.3): Zenodo; 2018, Sept 20 [

70. Smith RD. Future directions for electrospray ionization for biological analysis using mass spectrometry. Biotechniques. 2006;41(2):147-8.

71. Veselkov KA, Vingara LK, Masson P, Robinette SL, Want E, Li JV, et al. Optimized preprocessing of ultra-performance liquid chromatography/mass spectrometry urinary metabolic profiles for improved information recovery. Anal Chem. 2011;83(15):5864-72.

72. Garcia-Villalba R, Gimenez-Bastida JA, Garcia-Conesa MT, Tomas-Barberan FA, Carlos Espin J, Larrosa M. Alternative method for gas chromatography-mass spectrometry analysis of short-chain fatty acids in faecal samples. J Sep Sci. 2012;35(15):1906-13.

73. Maynard-Smith M, Ahern H, McGlashan J, Nugent P, Ling R, Denton H, et al. Recombinant antigens based on toxins A and B of Clostridium difficile that evoke a potent toxin-neutralising immune response. Vaccine. 2014;32(6):700-5.

74. Anosova NG, Cole LE, Li L, Zhang J, Brown AM, Mundle S, et al. A Combination of Three Fully Human Toxin A- and Toxin B-Specific Monoclonal Antibodies Protects against Challenge with Highly Virulent Epidemic Strains of Clostridium difficile in the Hamster Model. Clin Vaccine Immunol. 2015;22(7):711-25.

75. Ngundi MM, Meade BD, Lin TL, Tang WJ, Burns DL. Comparison of three anthrax toxin neutralization assays. Clin Vaccine Immunol. 2010;17(6):895-903.

76. Spiceland CM, Khanna S, Pardi DS. Outcomes With Fidaxomicin Therapy in Clostridium difficile Infection. Journal of clinical gastroenterology. 2018;52(2):151-4.

77. Debelius J, Song SJ, Vazquez-Baeza Y, Xu ZZ, Gonzalez A, Knight R. Tiny microbes, enormous impacts: what matters in gut microbiome studies? Genome Biol. 2016;17(1):217.

78. Wahlstrom A, Sayin SI, Marschall HU, Backhed F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. Cell metabolism. 2016;24(1):41-50.

79. Mullish BH, McDonald JAK, Pechlivanis A, Allegretti JR, Kao D, Barker GF, et al. Microbial bile salt hydrolases mediate the efficacy of faecal microbiota transplant in the treatment of recurrent Clostridioides difficile infection. Gut. 2019;68(10):1791-800.

80. Kellingray L, Gall GL, Defernez M, Beales ILP, Franslem-Elumogo N, Narbad A. Microbial taxonomic and metabolic alterations during faecal microbiota transplantation to treat Clostridium difficile infection. J Infect. 2018;77(2):107-18.

81. Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity. 2014;40(1):128-39.

82. Zimmerman MA, Singh N, Martin PM, Thangaraju M, Ganapathy V, Waller JL, et al. Butyrate suppresses colonic inflammation through HDAC1-dependent Fas upregulation and Fas-mediated apoptosis of T cells. Am J Physiol Gastrointest Liver Physiol. 2012;302(12):G1405-15.

83. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009;461(7268):1282-6.

84. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature. 2011;469(7331):543-7.

85. Hills RD, Jr., Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut Microbiome: Profound Implications for Diet and Disease. Nutrients. 2019;11(7).

86. Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. The New England journal of medicine. 2000;342(6):390-7.

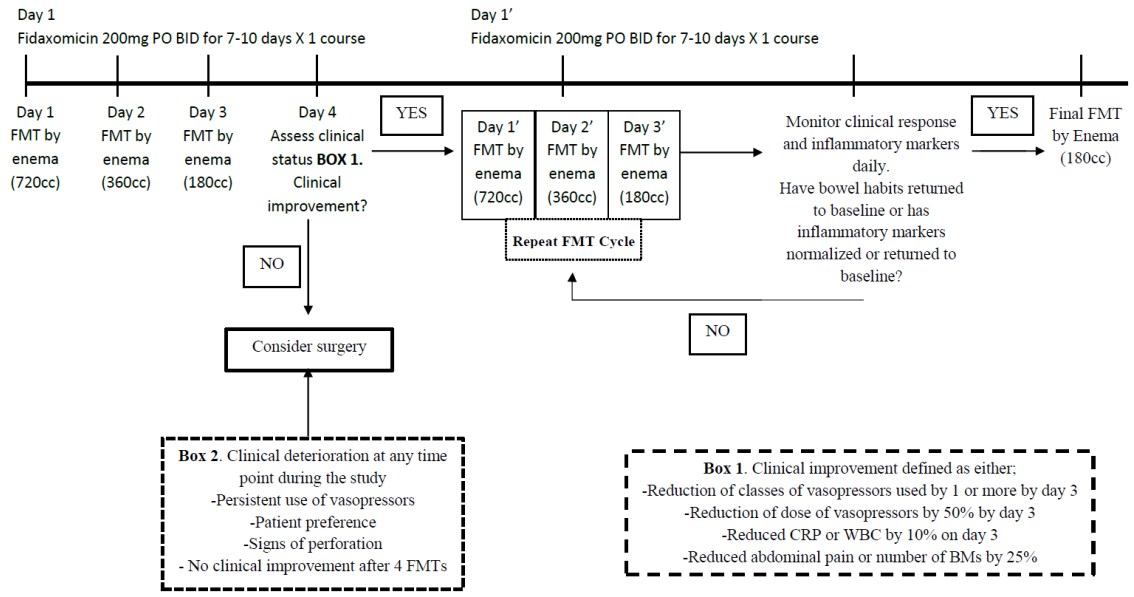
87. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. Nat Med. 2018;24(4):392-400.

88. Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. The American journal of gastroenterology. 2012;107(5):761-7.

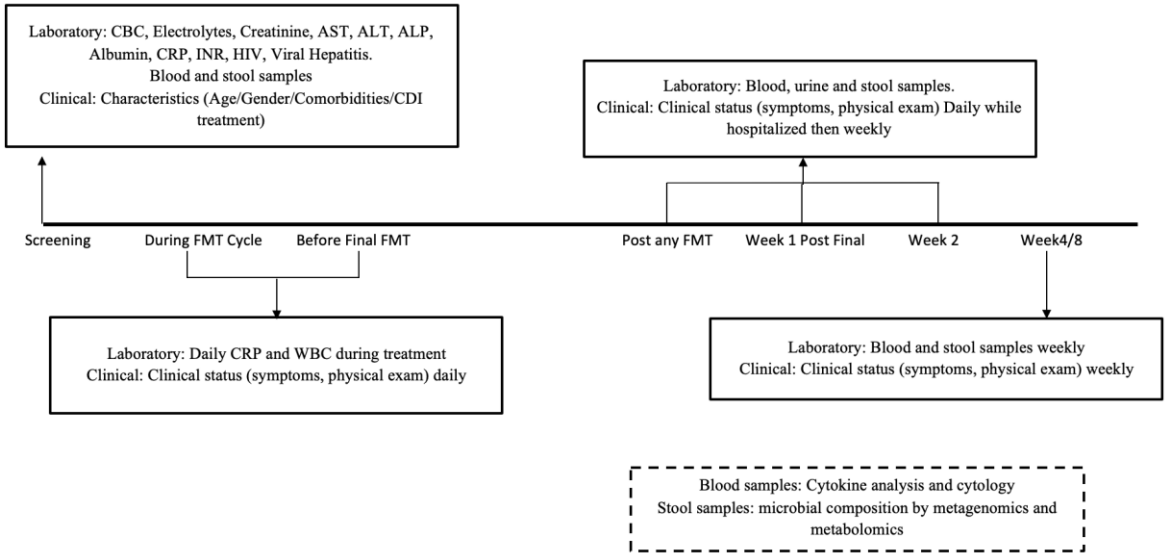
APPENDICES

Appendix A- Supplemental Figures

Sup Figure 1.0- Sequential fecal microbiota transplant protocol used

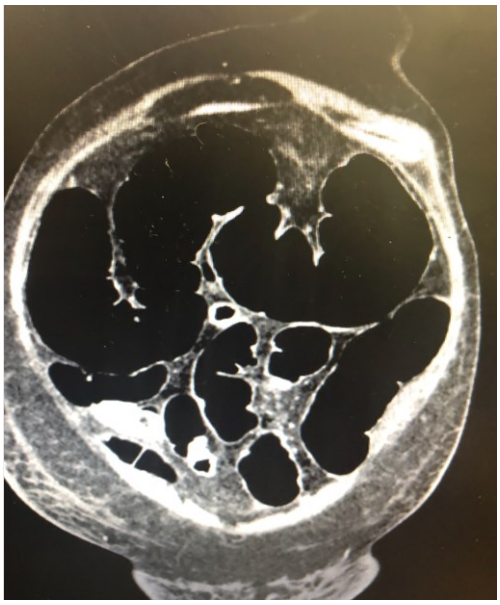


Sup Figure 2.0 - Proposed sampling for each participant

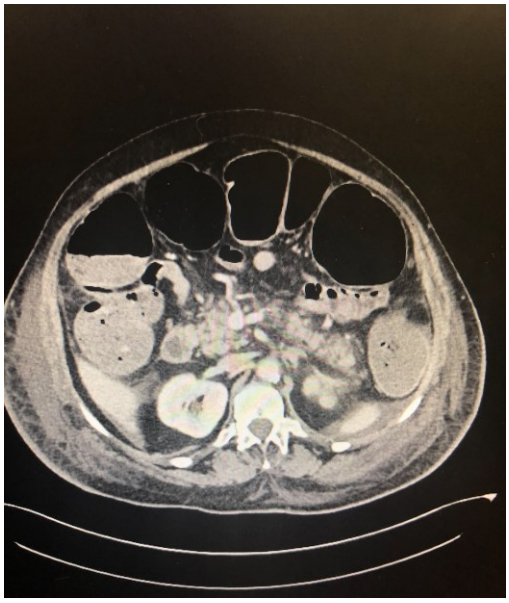


Sup Figure 3.0 – EDM002 with CT abdomen and X-ray showing right colonic distension consistent with Ogilvie's Syndrome. A) Coronal view B) Transverse View C) X ray upright view

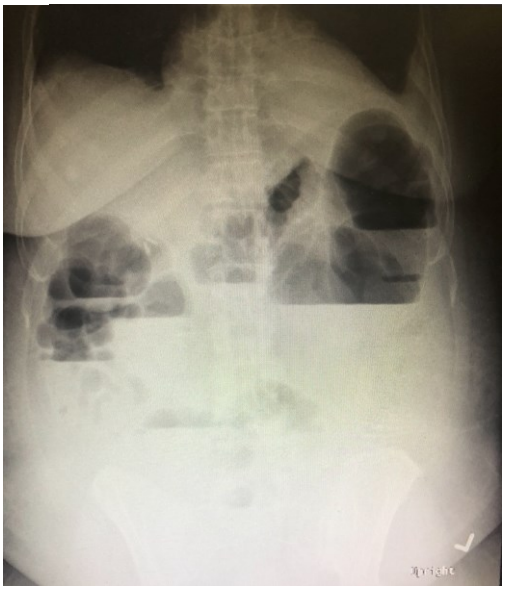
A



B



C



Appendix B- Supplemental Tables

Sup Table 1.0- Internalized retention times used for the identification of spectral peaks of different SCFAs in the Mass Spectrometry Protocol

Metabolite	Retention time (minutes)
Short chain fatty acid:	
Acetate	2.50
Propionate	3.07
Isobutyrate	3.20
Butyrate	3.40
2-methylbutyrate	3.70
Isovalerate	3.80
Valerate	4.20
Caproate	4.90
Lactate	6.14
2-hydroxybutyrate	6.40
Internal standard:	
Methyl stearate	7.88

Sup. Table 2.0- Timelines of samples and clinical correlation of historical control, EDM001, and EDM002 used for Bile acids, SCFA, and immunotyping data

Historical Control										
Time Point	**Date**	**Collection ID**	**Clinical Status**	**FMT Status**	**WBC**	**CRP**	**FMT composition**		**Donor**	
1	17-Nov	7500	Diarrhea	preFMT	46.4	-	FMT 1	100g	24	FMT 1
2	21-Nov	100186	Pseudomembranes on colonoscopy	Day 4 Post FMT1/ pre FMT2	30.9	-	FMT2	100g	24	FMT2
3	27-Nov	7503	improving pseudomembranes	Day 5 post FMT2/ Pre FMT3	14.9	-	FMT 3	100g D1/ D2+D3 50g oral	24	FMT 3
4	06-Dec	7499	3 bowel movements	Day 6 Post FMT 3/ Pre FMT4	12.5	-	FMT 4	100g	24	FMT 4
5	10-Jan	100191	Resolution of symptoms	Post FMT 5	9.3 (week prior)	-	FMT5	D1/D2 50g oral capsules	24	FMT5
EDM001										
Time Point	**Date**	**Collection ID**	**Clinical Status**	**FMT Status**	**WBC**	**CRP**	**FMT composition**			
1	22-Jan	001_01-22	Pseudomembranes on colonoscopy. Failed flagy/Vanco	start of study	25.3	(Jan 21 22.4)	FMT cycle	200g/100g/50g over 3 days	24	Cycle 1
2	25-Jan	001_01-25	Diarrhea	Post Cycle FMT 1	20.1	27.3	Final Enema	50g	28	cycle 2
3	30-Jan	001_01-30	resolution of diarrhea	Post Cycle FMT 2	16.7	26.6	4 FMTs		28	final FMT
4	04-Feb	001_02-04	enema for constipation	final FMT enema	8.2	50	1	400cc	28	400cc
5	15-Feb	001_02-15	Resolution of diarrhea	9 days post Final FMT enema	12.3	33	2	200cc	28	200cc
6	25-Feb	001_02-25	diarrhea, Cdif+ Feb 18 with hypotension	Pre 400cc FMT 3	10.5	12.1	3	200cc	31	200cc
7	27-Mar	001_03-27	resolution of diarrhea	Post FMT Cycles 1/2 and final enema and 4 further FMTs	7.5	23.9	4	200cc	31	200cc
EDM002										
Time Point	**Date**	**Collection ID**	**Clinical Status**	**FMT Status**	**WBC**	**CRP**	**FMT composition**			
1	15-Feb	002_02-15	start of study. +diarrhea, pseudomembranes seen on scop	start of study	11.9	22.4	FMT cycle 1	200g/100g/50g/200g over 4 days	24	cycle 1
2	19-Feb	002_02-19	abdominal distension, abdominal pain	post FMT cycle 1	11.1	73.3	FMT cycle 2	200g/100g/50g over 3 days	31	cycle 2
3	07-Mar	002_03-07	diarrhea	Post FMT cycle 2	10.3	27.7	Final Enema	50g	31	final enema
4	14-Mar	002_03-14	slowing of symtpoms. No abdominal distaneion	Final FMT enema	7.2	15				
5	19-Mar	002_03-19	formed BMs, resolution of symptoms	5 days post final FMT Enema	March 18 7	March 18 8.8				
6	27-Mar	002_03-27	1 week follow up. Doing well	2 week post final FMT Enema	-	-				

Sup. Table 3.0- Bile Acids in Serum Concentrations of the primary, tauro-conjugated and secondary Bile acids analyzed

			Clinical_data	Cholic acid	Chenodeoxycholic Acid	Taurocholic Acid	Taurochenodeoxycholic Acid-3-Sulfate	Lithocholic acid	Deoxycholic Acid	Ursodeoxycholic acid
		Original_ID								
Control										
Time Point	**Date**	**Collection ID**								
1	17-Nov	7500	Pre FMT	7725.122178	2479.871867	18151.68798	1002.968397	221.6322969	306.8421876	0
2	21-Nov	100186	Post FMT 1	8671.12875	547.9339709	29554.37974	1214.966913	182.6798042	0	0
3	27-Nov	7503	Post FMT 2	6249.658362	1266.738595	18136.92009	915.7778413	188.3514085	0	0
4	06-Dec	7499	Post FMT 3	87546.47098	55780.46257	14883.96177	758.6142214	93.49475921	184.3424505	0
5	10-Jan	100191	Symptom Resolution	124115.3506	152184.1741	22052.23897	6204.164121	2784.302388	54658.08651	521.607151
EDM001										
Time Point	**Date**	**Collection ID**								
1	22-Jan	001_01-22	Pre FMT	66238.8582	67585.34856	12444.59975	184.8240799	205.1603369	77.90944125	257.8324268
2	25-Jan	001_01-25	Post FMT 1	34462.82468	51740.57177	1783.126618	0	941.1307722	347.2534985	0
3	30-Jan	001_01-30	Post FMT 2	31405.7833	62998.84837	12906.28481	0	199.1244735	1238.727676	266.5121953
4	04-Feb	001_02-04	Final FMT	65093.87051	103686.4227	4807.302473	0	332.4651106	329.3223211	7931.480935
5	15-Feb	001_02-15	Post Final FMT	38860.395	246990.8571	1691.05752	0	0	597.8176541	17651.85243
6	25-Feb	001_02-25	Pre 400cc FMT 3	18040.35469	33406.72758	3039.535746	0	289.8148882	1083.642058	0
7	27-Mar	001_03-27	Symptom Resolution	110638.0439	261326.1189	20123.06106	641.9550948	339.6202864	756.06287	837.289617
EDM002										
Time Point	**Date**	**Collection ID**								
1	15-Feb	002_02-15	Pre FMT	1514.877547	1785.690286	31681.26222	2068.47476	128.893085	0	0
2	19-Feb	002_02-19	Post FMT 1	706.6852062	1389.403662	21834.62876	2186.373943	90.07062648	0	0
3	07-Mar	002_03-07	Post FMT 2	1081.136531	2545.863626	6775.686878	298.8173308	2505.581058	51236.11045	4062.484184
4	14-Mar	002_03-14	Final FMT	6465.475325	6986.936568	14454.13562	175.4883072	4217.651625	107267.2683	2088.424168
5	19-Mar	002_03-19	Symptom Resolution	2393.206883	21373.79998	5816.083146	397.0740704	1415.347287	99465.29692	4036.445606
6	27-Mar	002_03-27	2 Week Follo up	384.1632926	1838.579741	10582.52902	868.0033561	1974.086411	55161.64736	2950.744927
Donors										
			DN28_s	17407.53875	11402.38033	8153.938849	177.1484557	2053.45474	33980.18822	1121.166213
			DN31_s	668.954401	1155.896509	158.7348864	0	7086.682754	43787.78326	6595.893612

Sup. Table 4.0- Total SCFAs of each serum sample including Donors

Historical Control												
Time Point	**Date**	**Collection ID**	**2-hydroxybutyrate**	**2-methylbutyrate**	**Acetate**	**Butyrate**	**Caproate**	**Isobutyrate**	**Isovalerate**	**Lactate**	**Propionate**	**Valerate**
1	17-Nov	7500	129.577322	0	15.46860761	0	0.394573447	0	0.348782279	21772.09667	0	0
2	21-Nov	100186	55.77681017	0.029714044	43.8306481	0.389852827	0.746112865	0	0.307037384	21657.53632	8.556237296	0
3	27-Nov	7503	83.37034616	0.043522136	37.36299446	0.317340157	0.716187267	0	0	19541.00364	3.355337638	0
4	06-Dec	7499	38.98022029	0	0	0	0.300818375	0	0.053527701	15237.8954	8.627164611	0
5	10-Jan	100191	16.98930892	0.122412281	47.19335079	0.92383938	0.349236347	0	0.192963747	3546.372207	0.00768945	0
EDM001												
Time Point	**Date**	**Collection ID**										
1	22-Jan	001_01-22	14.28778563	0.021982661	18.44116647	0	0.408692652	0	0.030367327	18483.87505	0	0
2	25-Jan	001_01-25	30.29836972	0.050356924	51.28038434	0.390971398	0.380224411	0	0	20331.35141	2.770101875	0
3	30-Jan	001_01-30	19.61091038	0.154047642	55.79187852	1.107062363	0.449011758	0.230896335	0.125927412	16204.75209	9.86747715	0
4	04-Feb	001_02-04	27.45633978	0.077877382	43.59759995	0.122005641	0.264904593	0	0.185755372	15171.68971	3.922506806	0
5	15-Feb	001_02-15	9.018198345	0.096539219	52.51921586	0	0.331422163	0	0.09324734	6854.464952	7.602845292	0
6	25-Feb	001_02-25	22.07790413	0	27.31801681	0	0.465649937	0	0	16177.80851	0	0
7	27-Mar	001_03-27	5.796610914	0.058149819	59.77163915	0.369487128	0.334732146	0	0.016490578	13862.68936	2.145797489	0
EDM002												
Time Point	**Date**	**Collection ID**										
1	15-Feb	002_02-15	32.85744881	0.029364573	47.45413495	0	0.445189292	0	0.003443943	20362.23333	0	0
2	19-Feb	002_02-19	39.34785668	0.106547516	71.42008156	0.436123427	0.394879063	0	0.042980126	25502.98886	9.105795382	0
3	07-Mar	002_03-07	39.79435674	0.116535313	19.25631746	0	0.280642809	0	0.089869894	17515.49907	3.881773796	0
4	14-Mar	002_03-14	45.82664826	0.020779805	8.451030295	0	0.148824589	0	0.012142221	27130.44734	0	0
5	19-Mar	002_03-19	51.47604053	0.151846723	53.74843076	0.081985505	0.297036285	0	0.1128897	16544.86945	4.763877423	0
6	27-Mar	002_03-27	12.15387212	0.063834142	38.13998679	0	0.122345306	0	0.102908203	14234.3857	0	0
Donors												
DN28_s	Donor	N/A	70.39464231	0	0	0	0.186339834	0	0.222396457	2111.726177	0	0
DN31_s	Donor	N/A	53.00757175	0.039095344	34.53193318	0	0.480375888	0	0.216077444	3692.858133	1.485842398	0

Sup. Table 5.0- Total SCFAs of each stool sample including Donors that were analyzed. The weight based values were used in the Figure

					2-hydroxybutyrate			Acetate			Butyrate			Propionate		
		Timepoint	**Original_ID**	**Stool_weight**	**Relative_response**	**Calc. Conc.**	**ug/g**	**Relative_response**	**Calc. Conc.**	**ug/g**	**Relative_response**	**Calc. Conc.**	**ug/g**	**Relative_response**	**Calc. Conc.**	**ug/g**
Control																
Time Point	**Date**															
1	17-Nov	Pre FMT	2430-2	79	8838.568434	4.812383	500.99072	342165.8673	72.526994	4355.391	4566.180297	0.3342781	29.453242	26394.74479	3.8793179	283.46564
2	21-Nov	Post FMT 1	2432-2	72	2206.013629	1.5136632	157.57915	91606.80672	20.610711	1237.7144	7328.466045	0.6787269	59.802625	12730.37201	1.8813396	137.47137
3	27-Nov	Post FMT 2	2438-2	118	7208.108848	3.4337814	357.47209	256342.2071	47.630178	2860.2874	4080.827534	0.2726559	24.023711	12970.17623	1.5636569	114.25797
4	06-Dec	Post FMT 3	2449-2	106	2181.531974	1.448239	150.76819	167943.8337	40.572904	2436.484	3433.949119	0.3000237	26.435089	12300.53769	1.9827391	144.88073
5	10-Jan															
5a	15-Jan	Symptom Resolution	2486-2	93	1458.710881	0.932322	97.058916	1152857.465	254.01361	15254.025	128260.6523	12.161237	1071.5266	139204.2969	22.91256	1674.2436
EDM001																
Time Point	**Date**															
1	22-Jan	Pre FMT	2872-1	128	2257.564538	1.4613363	152.13169	204985.617	49.709375	2985.1474	2839.992974	0.245885	21.664928	26871.6668	4.7540316	347.38185
2	25-Jan	Post FMT 1	2882-1	126	2134.012884	1.2991488	135.24723	319875.4161	72.603159	4359.9649	3564.290371	0.29548	26.034745	30982.80631	5.1102461	373.41079
3	30-Jan	Post FMT 2	2898-1	101	7655.531323	3.8211663	397.80061	590820.652	116.38307	6989.0362	35438.24627	2.969886	261.67665	102408.5535	15.065462	1100.8484
4	04-Feb	Final FMT	2907-1	75	1978.568989	1.3489109	140.4277	379030.2597	90.170798	5414.9368	161308.7022	16.782989	1478.7492	194379.0622	35.196499	2571.8434
5	15-Feb	Post Final FMT	2924-1	125	1005.041911	0.6164179	64.171876	73446.88711	14.051606	843.82706	45153.5611	3.974652	350.20659	105351.5565	16.217575	1185.0344
6	25-Feb	Pre 400cc FMT 3	2941-1	111	871.7727192	0.7200228	74.957609	30058.77372	7.1272806	428.00746	3543.469271	0.3634332	32.022099	7292.394704	1.2281069	89.739001
7	27-Mar	Symptom Resolution	3022-1	115	1257.56689	0.8776877	91.371236	146911.8923	35.257747	2117.2982	3539.921747	0.3151134	27.764643	57798.65541	10.543549	770.42768
EDM002																
Time Point	**Date**															
1	15-Feb	Pre FMT	2922-1	97	2565.378263	1.6727352	174.13926	41214.15193	8.6382646	518.74507	4758.096253	0.4296897	37.859959	8275.043164	1.1725011	85.675829
2	19-Feb	Post FMT 1														
3	07-Mar	Post FMT 2	2972-1	106	829.0810869	0.6244441	65.007444	287245.3556	69.085691	4148.7339	16785.65582	1.7035808	150.1025	40038.01768	7.0785508	517.23678
4	14-Mar	Final FMT	2992-1	103	807.1656839	0.6286124	65.441382	298857.3467	73.925952	4439.4013	96088.56487	10.359122	912.74223	69714.81271	12.923722	944.34927
5	19-Mar	Symptom Resolution	3007-1	81	1139.818392	0.8956494	93.241131	432798.4048	111.62448	6703.2734	191345.226	21.500374	1894.398	159166.4791	31.054955	2269.2166
6	27-Mar	2 Week Follow up	3017-1	78	1080.80578	0.7506729	78.148432	724508.9063	156.19755	9379.9755	215922.6521	20.186952	1778.6723	156821.2713	25.37302	1854.032
Donors																
DN24_s	Donor		2818-1	92	1345.253011	0.8481363	88.294809	1452866.467	309.21326	18568.875	633832.2358	58.286599	5135.6323	160762.3158	25.572442	1868.6039
DN28_s	Donor		2843-1	106	818.203206	0.5989634	62.354784	1022624.489	239.67188	14392.776	404411.1861	40.965388	3609.4604	131880.8056	23.12982	1690.119
DN31_s	Donor		2801-1	112	905.8528926	0.5846976	60.869652	536082.5098	111.08089	6670.6295	195871.4065	17.612456	1551.8335	102328.9116	15.87678	1160.1322

Appendix C- Universal stool donor testing/screening SOP

Universal stool donors will provide the raw material for FMT. They are screened at baseline and then every 4 months. They underwent initial detailed history and physical exam, and had been screened with a donor questionnaire, which did not identify any high risk behaviors. They tested negative for all the following potential infections as listed below. They have provided stools for over 300 patients since 2012, and none of the recipients have developed any known infectious complications. All donors are personally known to the investigators. It is simply not practical or cost effective to keep testing them each and every time when there is a scheduled FMT. Since there is no consensus on the mandatory required tests for stool donor, we have chosen the recommendations published by Khoruts et al in the American Journal of Gastroenterology in 2012 (88).
These universal donors have been in the same positions for years are unlikely to move away or stop donating. We have not encountered a situation when a universal donor could not donate on a day when HBT was scheduled to treat patients with recurrent *Clostridioides difficile* infections since Oct 2012. In the unlikely event that he or she can no longer donate, a second donor will be assigned to a particular patient.

On initial history, the donors must fulfill the following inclusion and exclusion criteria:
Donor inclusion criteria:
1. Able to provide and sign informed consent.
2. Able to complete and sign the blood donor questionnaire.
3. Able to adhere to fecal transplantation stool collection standard operating procedure.

Donor exclusion criteria:
1. History of any type of active cancer or autoimmune disease (eg multiple sclerosis, connective tissue disease), metabolic syndrome, chronic pain syndrome, and atopic diseases.
2. History of risk factors for acquisition of HIV, syphilis, Hepatitis B, Hepatitis C, prion or any neurological disease as determined by the blood donor questionnaire.
3. Gastrointestinal comorbidities, e.g., inflammatory bowel disease, irritable bowel syndrome, chronic constipation or diarrhea, gastrointestinal malignancy or known polyposis.
4. Tattoo or body piercing within 6 months of stool donation.
5. Incarceration or history of incarceration.
6. Receipt of blood transfusion from a country other than Canada in preceding 6 months.
7. Antibiotic use, systemic immunosuppressive or biological agents, systemic antineoplastic agents and exogenous glucocorticoids in the preceding 3 months prior to stool donation.
8. Receipt of any type of live vaccine within 3 months prior to stool donation.
9. Ingestion of nut or shellfish 3 days preceding donation if the recipient has known allergies to these food.
10. Any current or previous medical or psychosocial condition or behaviors which in the opinion of the investigator may pose risk to the recipients or the donor.
11. Travel to areas of the world where diarrheal illnesses or BSE/TSE are endemic (within 6 months of stool donation).
12. High risk of multi-drug resistant organisms, including healthcare workers, recent hospitalization and medical tourism.

Initial blood work and stool testing, which will be repeated every 4 months are as follows:
Blood:

- CBC, electrolytes, creatinine, AST, ALT and ALP
- Human Immunodeficiency virus (HIV) 1/2
- hepatitis A IgM Ab
- hepatitis B: HBVsAg, HBVsAb, HBVc Ab (IgM and IgG)
- hepatitis C antibody
- RPR (syphilis)
- human T- lymphotrophic virus (HTLV) I/II

Stool:

- enteric pathogens: Salmonella, Shigella, E.coli O157 H7, Yersinia, Campylobacter
- C. difficile toxin
- ova and parasites

Appendix D- FMT Manufacturing Protocol

Once received by the processing laboratory, stool will be held in a cold room at 2ºC-8ºC, for no longer than 30 min, until visual inspection and HBT processing occur.

Each batch of stool for FMT is obtained from a single donor, and processed within 8 hours of collection. No pooling of stools will occur. Only one donor stool specimen from a single individual will be processed at a time. Each processed batch will contain approximately 100 g of stool mixed with 200 cc of 0.9% normal saline (NS) as a diluent. This will provide approximately 200 cc of filtrate, which will have 20 cc of 100% glycerol added and be frozen at -70^0C as a single dose. The thawed dose will be diluted with approximately 160cc of 0.9% NS and a total volume of 360 cc will be administered by colonoscopy as a single dose.

Disinfect the all inner surfaces of the class 2 biological safety cabinet (BSC) following sterilization SOP before and after each processing. The total stool inspection and processing time is less than 30 minutes.
The inspection and entire processing takes place within a biosafety cabinet:

1. Each stool collection is weighed, and only 100g of stool is retained. The specimen is visually inspected to ensure it contains no urine, mucus or blood. Discard if there is contamination with blood, mucus or urine. Ensure the stools have consistency between Bristol Stool Scale type 2-5 (the most common stool consistency of healthy, asymptomatic individuals, and unlikely to represent an infectious process), otherwise discard. Collect a quality control sample by taking approximately a 1 mL aliquot and storing it in a 1.5 mL Eppendorf tube at -70^0C.
2. One hundred grams of stool is then placed into a stomacher bag (7"x12", VWR CA89085-572) on one side of the filter mesh, to which 200 cc of 0.9% NS (for irrigation) is added.
3. Gently remove most of the air in the stomacher bag by draping the top of the bag over the heat sealer (Fisher Scientific, 14816237). Alternatively, use a reusable bag clamp (Fisher Scientific, 0100262) instead of heat-sealing to seal the bag for homogenization and filtration.
4. Close the heat sealer (set to 8 heat setting) across the open end of bag and wait for 1 second then release. The bag should be well sealed with no leaks and few air bubbles.
5. Mash and squish the bag with hands until liquid is homogeneous (3 to 5 minutes).
6. Find the side of the bag that has the filtered liquid.
7. Place filtered side up and pinch the outside plastic (using plastic clamping forceps) to create an air pocket in which to slice a hole with a disposable sterile blade. The hole should be 2 cm in diameter.
8. Allow the liquid stool slurry to drain into clean cups (16oz Eco-cup; Real Canadian Wholesale club, 18770800027).
9. Visually inspect the filtrate to ensure it maintains the usual brown color spectrum of stool filtrate.
10. Collect a quality control sample by taking approximately a 1 mL aliquot and storing it in a 1.5 mL Eppendorf tube at -70^0C.

11. To the slurry (should be approximately 200cc), mix in 20ml of sterile 100% glycerol and transfer the solution to 250ml storage bottles (Fisher Scientific, 02-896-1D) or non-filtered stomacher bag (VWR, 11216-900) for freezing at -70^{0}C for up to 2 months. Leave air space for expansion in either freezing vessel. Label the storage bottle or stomacher bag with the Lot Number created according to the date of specimen (dd/mm/yy) preparation and the 2 initials of donor (first and last names), followed by the time of processing (hour:minute). For example, if processing occurs on May 1, 2014 at 9:15 am and the donor's initials are JB, the Lot Number will be 050114JB915.
12. When required, thaw the bottles or sealed bags at 2-8^{0}C overnight. Check the production date to ensure that the specimen has not expired, ie greater than 2 months of storage.
13. Once thawed, add approximately 160ml of 0.9% NS to bring the total volume to 360 cc.
14. The HBT is then aspirated into sterile 60 cc slip-tip syringes (VWR CAB309653; components: polypropolene, polyethylene, synthetic isoprolene), which are then sealed with caps (VWR CAB305819). Each batch should contain approximately 360mL, or 6 syringes.
15. Label syringes with the Lot Number listed in step 11, followed by the date and time of frozen-and-thawed processing (dd/mm/yy/hour/minute). For example, if the initial manufacturing occurs on May 1, 2014 at 9:15 am, the donor's initials are JB, and the sample has been frozen till June 2 and thawed out on June 3 when the final processing occurs at 10:30, the Lot Number will be 050114JB915:0602141030.
16. Discard each batch if not used within 2 months of processing.
17. None of the supplies other than the heat sealer and weigh scale are reused. Discard all supplies in a biohazard waste bin and sterilize the heat sealer and scale with 10% Bleach for 10 min. Heat sealer and weigh scale are exclusive use for HBT processing.

Once processed, each batch will be kept with an ice pack in the sealed plastic tool box during transport to the endoscopy unit, to maintain temperature of 2-8^{0}C. The transport process from the lab to the University of Alberta Hospital endoscopy unit takes no more than 15 minutes. The transport process from the lab to the Royal Alexandria Hospital takes no more than 30 minutes.

Appendix E- Information Sheet and Consent Form

Study Name: Serial fecal microbiota transplant (FMT) plus fidaxomicin in the treatment of severe or fulminant *Clostridium difficile* infection, with detailed characterization in microbiota, metabolomics and host immune response

Study Doctors: Dr. Dina Kao, Dr. Lindsey Russell, Dr. Karen Wong, Dr.Haili Wang, Dr. Wendy Sligl,
Dr. Ryan Snelgrove, Dr. Lynora Saxinger

Study Coordinartor: Brandi Roach (780-248-1342)

In the case of third party consent, 'you' always refers to the research participant. The pronouns 'you' and 'your' should be read as referring to the participant rather than the parent/guardian/next-of-kin who is signing the consent for the participant.

Purpose of study

You are being asked to voluntarily participate in this study because you have a severe *Clostridium difficile* infection.

Before you make a decision one of the researchers will go over this form with you. You are encouraged to ask questions if you feel anything needs to be made clearer. You will be given a copy of this form for your records.

Clostridium difficile (C diff) is an infection that results when the healthy bacterial population in the colon is substantially altered by antibiotic treatment. The decrease in the normal, or good, bacteria allows for the overgrowth of the *C. difficile* bacteria. C diff makes a toxin that can make a person sick with diarrhea and abdominal pain. Treatment for mild infections can be done with antibiotics called vancomycin or metronidazole. A small portion of patients can become very ill, where they do not respond to antibiotic treatment and ultimately will need surgery to remove the large intestine. Despite surgery, 5% of affected patients can still die, and many patients are not fit for surgery. Therefore, better treatments are needed to help these sicker patients.

Fecal microbiota transplantation (FMT), also known as stool transplant, is when stool from a healthy person is transplanted into the bowel of sick patients to help restore the balance of normal bacteria in the colon. FMT has been very effective in treating patients when mild C diff infection keeps coming back. However, it is unclear if FMT will help patients with a more severe C diff infection. A previous study used both FMT with an antibiotic called vancomycin to treat a small number of patients with severe C diff infection and found that they were able to cure these patients with a success rate of 91% with several cycles of FMT plus vancomycin. In our own experience, we've learned that FMT alone is not sufficient to treat severe C diff infection in the initial stage, and that an antibiotic against C diff would be necessary to suppress C diff. In addition, multiple cycles of FMT treatments plus an antibiotic to kill C diff are usually need to manage this problem. However, the use of antibiotic to kill C diff can also kill good bacteria that we implant with FMT.

There is another new antibiotic approved by Health Canada for C diff called fidaxomicin, which is very specific for C diff and has been shown to be effective in treating mild or moderate C diff infection. We believe it is a better option than vancomycin and metronidazole in this situation because vancomycin and metronidazole not only kill C diff but will also kill good bacteria in the gut. Fidaxomicin has not been studied yet to be used for treating severe C diff infection. That is why we are doing this study to see if using FMT with fidaxomicin can potentially reduce the number of treatment cycles we need to manage this problem. The only other option is surgery. This is a pilot study, meaning the data collected will be used to plan future studies.

For this study, we have assembled a team of specialists from different fields: gastroenterology, infectious diseases, general surgery, intensive care unit, and scientists. We plan to use a combination of FMT and fidaxomicin and then monitor the clinical response very closely. FMT will be given by enema, which is a solution that will be given through a tube inserted into the rectum. We will also collect blood and stool samples, which will be analyzed to understand how FMT works. This will allow us to ultimately design a better treatment protocol and develop more targeted options for patients with this severe infection.

Currently Health Canada only allows FMT to be provided in a liquid form. This means we cannot provide FMT in a pill form unless patients are in a research study. In this study, we are using enema because sometimes participants can get very sick that they cannot take anything by mouth.

We will need to have your permission or consent to begin the study. In the event that you are not able to make your own medical decision, a substitute decision maker can make such a decision for you.

Description of the study, procedures to be used, and how long it will last
Approximately 10 participants are expected to participate in this trial, which will take place in Edmonton.

Each participant will be treated with multiple FMTs and fidaxomicin. After a bowel prep, which is where you are given a medication or 2L of a laxative to clean out your bowel of stool, you will be given FMT by enema for 3 days in a row. You will also receive fidaxomicin at the same time for 7-10 days depending on how you respond to the treatment. This completes cycle 1. The study doctors will perform daily physical exams and blood work to thoroughly track your response to treatment. If you do not respond or if your condition worsens during this time, we would recommend that you undergo surgery. However, if you do have a good response but the diarrhea has not cleared up, you will be given a second cycle (multiple FMT enemas + fidaxomicin) and your response will be monitored. If at the end of cycle 2 your diarrhea still has not cleared up, you will be given a 3rd cycle. When your diarrhea clears up, you will receive a final FMT enema. If by the fourth cycle you have not cleared up, then we will consider surgery to help you. For this study we think that you may need at least 2 cycles of treatment.

Your time in the study will be approximately 20 weeks.

If you choose to participate, the following tests and/or procedures will be done as part of this study:

At the beginning of the study you will have a medical and medication history taken. You will answer questions about your overall health. A physical exam will be performed. Blood (15 mL or about 3 teaspoons) stool samples will be collected from you. The blood sample is to look at your blood count, liver and kidney function, HIV and hepatitis B and C status. Of note, any positive finding will be reported to the

provincial health authority as required by Alberta Law. All the samples collected will be analyzed immediately. There will not be any genetic testing performed with the blood sample. Only the study doctors have access to the samples.

You will likely already be taking either vancomycin and metronidazole prior to the beginning of the study for the severe C diff infection. You will continue to those antibiotics until the time of the first FMT. You will also need to take 2 liters of a laxative the night before the FMT to clean out your intestine. Once the FMT cycle starts, then you will start taking the fidaxomicin as well.

What will happen for enema delivery FMT:
The procedure will take place in the hospital at the bedside. FMT liquid will be injected into your large intestine through a tube that is inserted in the rectum (day 1). You will be asked to stay in bed for an hour and keep the liquid inside for as long as possible. After the first large volume enema of 720cc, it will be followed by two days of smaller volume enemas of 360cc and 180cc to complete the first FMT cycle.

In the event that you need to have a colonoscopy to make sure that there is not another condition causing your diarrhea during this study, then FMT can be delivered at the time of colonoscopy.

While you are participating in this study:
The research team will track your bowel movements and ask questions about how you are feeling. You will be monitored by the study team closely to make sure there are no concerns and to follow up on how you are feeling after each intervention. If at any point your condition gets worse during the trial, then we will adjust your treatment plan accordingly to provide the best clinical care, which may include surgery.

We will also be collecting blood and stool samples at various points during the study, especially before and after a FMT. We expect to collect a total of about 8 batches of blood/stool sample from each participant. This will help us create a complete analysis of what is going on in your body while using this method to treat severe CDI. All samples will be promptly used for analysis. Only the study doctors have access to the samples.

Once you have the final FMT treatment, we will continue to monitor you to see how you are doing and to take blood and stool samples and monitor at 1, 2, 4 and 8 weeks afterwards.

Termination
You can decide to withdraw from this study at any time for any of the following reasons:

- You develop side effects that are considered dangerous
- You do not follow the study instructions given to you by the study doctors
- Your treating physician decides that it is not in your best interest to continue in the study
- You no longer want to be in the study

Your care will not be affected by withdrawing from the study.

What will I be asked to do during this study
If you choose to be a part of this study, your main role will be to allow us to take the blood and stool samples needed and to check on your clinical status through history and physical exams throughout the study at various times. If you want to be in this study you will agree to the proposed treatments that involve multiple FMTs and courses of the fidaxomicin antibiotic as given by the study investigators.

If you choose to stop your participation in the study for any reason, tell your study doctor immediately so that the final clinical evaluations and laboratory tests as described above can be performed and an alternative plan can be made for your care.

Risks

Risks of blood Tests. There may be some discomfort, swelling, or bruising around the vein from which blood is drawn. Some persons may become lightheaded or faint when blood is being drawn. Rarely, infections can occur at the blood drawing site. If a specific condition is identified during this process, we will make sure appropriate referral is made to further deal with the problem.

Risks Associated with Fidaxomicin. In previous trials many participants did not have much trouble taking fidaxomicin for C diff infections. Side effects could include minor nausea, occurring in 10% of participants, vomiting (6%), headache (7%), dizziness (4%), and rash (3%). However, no participants stopped taking the medication.

Risks Associated with FMT. The FMT will be made using the stool of a healthy donor who has been fully screened to ensure there is no HIV, syphilis, viral hepatitis and other viruses which can cause a chronic infection. Also, the donor's stool has been examined to rule out underlying parasites and bacterial infection. These volunteer donors go through the same rigorous screening process as they would if they are blood or organ donors. However, we can never guarantee 0% chance of some rare infection, just as with blood transfusion or with organ transplantation. If a blood born infection happens, people can have fever, chills and possibly low blood pressure; however, to date there has not been any report of such a problem related to FMT. Some of the potential side effects may include nausea, transient loose stools, constipation, abdominal discomfort and bloating. There is also the possible risk of developing disease which may be related to donor gut bacteria (obesity/metabolic syndrome, autoimmune conditions, allergic/atopic disorders, neurologic disorders, and malignancy) however there have not been good data demonstrating this risk. There is a very small risk of bowel perforation (tear) at a chance of 1 in 10,000 with enemas.

You should report anything that is causing you concern.

Benefits

There may be no health benefit to you from being in this study. What we learn from these studies may benefit society by finding a treatment other than surgery for severe *C. difficile*.

Voluntary Participation

Being in this study is your choice. If you decide to be in the study, you can change your mind and stop being in the study at any time and it will in no way affect the care or treatment you are entitled to.

Alternatives

You do not have to participate in the study to receive treatment for your condition. Alternative therapies include surgery which is the way we normally treat severe CDI not responding to treatment. Your doctor will discuss which option is best for you if you do not want to participate in the study.

Payment for Participation

Participants will not be paid to participate in this study.

Costs to You

During the study, you will be provided with the stool transplant and fidaxomicin at no charge. No commitment is made to provide the study treatment or to pay the expenses for the study treatment following the termination of the study.

Research related injury

If you become ill or injured as a result of being in this study, you will receive necessary medical treatment, at no additional cost to you. By signing this consent form you are not releasing the investigator(s), and institution(s) from their legal and professional responsibilities.

New findings

You will be told in a timely manner of any significant new findings that develop during the course of your participation in this study and that may relate to your willingness to continue to participate.

Confidentiality

During the study we will be collecting health data about you. We will do everything we can to make sure that this data is kept private. No data relating to this study that includes your name will be released outside of the study doctor's office or published by the researchers. Sometimes, by law, we may have to release your information with your name so we cannot guarantee absolute privacy. However, we will make every legal effort to make sure that your health information is kept private.

The study doctor/study staff may need to look at your personal health records held at the study doctor's office, and/or kept by other health care providers that you may have seen in the past (i.e. your family doctor). Any personal health information that we get from these records will be only what is needed for the study. During research studies, it is important that the data we get is accurate. For this reason, your health data, including your name, may be looked at by people from the University of Alberta auditors and members of the Research Ethics Board, and/or Health Canada.

By signing this consent form you are giving permission for the study doctor/staff to collect, use and disclose information about you from your personal health records as described above.

After the study is done, we will still need to securely store your health data that was collected as part of the study. In Canada, the law says we have to keep the data stored for 25 years after the end of the study. If you leave the study, we will not collect new health information about you, but we will need to keep the data that we have already collected.

Contact information

If you have any questions about your participation in this research study or if you feel that you have experienced a research-related injury or reaction to the study treatment, contact:

Dr. Dina Kao at 780-492-8307 or please contact hospital switch board at 780-407-8822 and ask for the gastroenterologist on call.

If you have any questions regarding your rights as a research participant, you may contact the Health Research Ethics Board at 780-492-2615. This office is independent of the study investigator

Study Name: Serial fecal microbiota transplant (FMT) plus fidaxomicin in the treatment of severe or fulminant *Clostridium difficile* infection, with detailed characterization in microbiota, metabolomics and host immune response

Study Doctors: Dr. Dina Kao, Dr. Lindsey Russell, Dr. Karen Wong, Dr. Haili Wang, Dr. Wendy Sligl, Dr. Ryan Snelgrove, Dr. Lynora Saxinger

	Yes	No
Do you understand that you or your family member/friend have been asked to be in a research study?	☐	☐
Have you read the attached Information Sheet? You will receive a signed copy.	☐	☐
Do you understand the benefits and risks involved in taking part in this research study?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Do you understand that you are free to withdraw from the study at any time, without having to give a reason and without affecting your future medical care?	☐	☐
Has the issue of confidentiality been explained to you?	☐	☐
Do you understand who will have access to your records, including personally identifiable health information?	☐	☐
Do you want the investigator(s) to inform your family doctor that you are participating in this research study? If so, your doctor's name is: _____	☐	☐
Who explained this study to you? _____		

I agree to take part in this study:

Participant name	Participant signature	Date: (dd/month/yy)
Witness name	Witness signature	Date: (dd/month/yy)

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

Signature of the person who obtained consent	Date: (dd/month/yy)

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A SIGNED COPY GIVEN TO THE RESEARCH

Appendix F- Case Record Form

FMT + Fidaxomicin for Severe/Fulminant CDI

Subject initials ___ ___ ___ Subject Number _____

Date: _____/_____/20_____
Month Day
Year

Date of birth: _____/_____/_____
Female: ____ Male:____
Informed consent signed: _____/_____/20____ Time: _____
Month day year

Physician to complete

Inclusion criteria		**Yes**	**No**
1	Informed consent/assent obtained and signed		
2	Age ≥ 18 and ≤ 90 years at the time of Screening		
3	Diagnosis of severe[1] or fulminant[2] CDI, without an adequate response to metronidazole IV 500 mg q8H and vancomycin 500 mg PO q6h for at least 2 days. An adequate response is defined as a decrease in stool frequency or inflammatory markers (WBC or C reactive protein) by at least 10% over 48 hours [1]- Severe CDI defined as WBC > 15,000 cells/mm3 or serum creatinine level >1.5mg/dL or 1.5x premorbid level [2]- Fulminant CDI defined as defined as having any of the following attributable to CDI: Hypotension or shock, ileus, megacolon. An abdominal CT scan should be strongly considered to rule out perforation		
4	Those with ability to provide informed consent or have an alternative decision maker providing assent.		
Exclusion criteria		**Yes**	**No**
1	Those with bowel perforation		
2	Those taking chemotherapy or radiation treatment with absolute neutrophil count of < 1000 cells/mm^3		
3	Those with colonic strictures		
4	Those taking chemotherapy or radiation treatment.		
5	Those with significant ileus or small bowel obstruction.		
6	Those with subtotal colectomy or planning to have a colectomy		

All Inclusion and Exclusion criteria have been reviewed
Patient **is** eligible for the study: ☐ Patient **is not** eligible for the study: ☐

Reason:

Signature of Investigator: _____ Date: _____
Mon/Day/Year

Screening Visit
Age:
Gender:
Past medical history:
Physician to complete

- Gastrointestinal disorder
    - Diagnosis of *C difficile* infection as:
      □Severe
      □Fulminant
    - Altered stool frequency and/or consistency at baseline (ie constipation or diarrhea)
      □Yes
      □No
    - History of inflammatory bowel disease (IBD)
      □Yes
      Type of IBD: □ulcerative colitis □Crohns disease
      Status of IBD at the time of screening: □ Clinical remission □Flare
      Therapy for IBD at the time of screening: □5 ASA □steroid □immunosuppressant □biologic
      □No
    - History of colonic polyps
      □Yes
      □No
    - History of colon cancer
      □Yes
      □No

Antibiotic exposure prior to CDI:
□Yes Name _____
□No
Immunocompromised status:
□Yes Reason _____
□No
History of chronic PPI use, defined as daily PPI for at least 3 months prior to 1st CDI:
□Yes
□No

History of CDI:
RN to complete

Episode	C diff toxin	Rx	Duration of Rx	Hospital admission	ER visit
1st	□Positive on _____(month/date/year) □Not done	□Flagyl □Vancomycin □Other _____	From _____ to _____ (month/date/year)	□Yes: from _____ to _____ (month/date/year) □No	
2nd	□Positive on _____(month/date/year) □Not done	□Flagyl □Vancomycin □Other _____	From _____ to _____ (month/date/year)	□Yes: from _____ to _____ (month/date/year) □No	
3rd	□Positive on _____(month/date/year) □Not done	□Flagyl □Vancomycin □Other _____	From _____ to _____ (month/date/year)	□Yes: from _____ to _____ (month/date/year) □No	

Systemic symptoms at the time of screening for CDI:

Weight loss

□Yes; how much in _____ kg with CDI

□No

Pt Reported Fatigue:

□Yes

1_____2_____3_____4_____5_____6_____7_____8_____9_____10

None Severe

□No

Patient Reported Loss of appetite:

□Yes

1_____2_____3_____4_____5_____6_____7_____8_____9_____10

None Severe

□No

Patient Reported abdominal pain:

□Yes

1_____2_____3_____4_____5_____6_____7_____8_____9_____10

None Significant

□No

Stool frequency at the time of screening: # of unformed stools in the previous 24 hours _____

Physical Exam

BP (sitting): ____/____mm/Hg HR: _____ bpm T: _____ C Resp: _____ Weight: _____kg

Body part or system	Normal	Abnormal	Not Done	**Abnormal, specify the abnormalities:** □	If Abnormal	
					Not clinically significant	Clinically significant
General – Skin	☐	☐	☐		☐	☐
Head, Eyes, Ears, Nose, Mouth & Throat	☐	☐	☐		☐	☐
Neck/Thyroid	☐	☐	☐		☐	☐
Cardiovascular	☐	☐	☐		☐	☐
Respiratory	☐	☐	☐		☐	☐
Neurological	☐	☐	☐		☐	☐
Abdomen (liver and spleen)	☐	☐	☐		☐	☐
Musculoskeletal	☐	☐	☐		☐	☐
Other	☐	☐	☐		☐	☐

_____ **Date:** _____/ _____ / _____

(Physician's Signature) **Day** **Month** **Year**

☐ Requisition given for screening bloodwork
- ☐ CBC, Electrolytes, Creatinine, AST, ALT, Alk Phos, Albumin, CRP, INR
- ☐ HIV, Hepatitis B SAg, Hep B S AB, Hep B C AB, HCV Antibody, Hep A IgM

☐ Blood and stool samples collected for BioBank

☐CEGIIR notified (or notify FMT lab if CEGIIR participation declined)

Initial FMT
Date of Fidaxomicin start: _____
Date of FMT: _____
Lot number: _____

☐ Laboratory:
Physician to complete

Day 1:
☐ Clinical Status:
- ☐ Vasopressors: #1: _____ #2: _____ #3:_____

Site of FMT delivered: ☐ Enema ☐ Colonoscopy :
☐ Right Colon ☐ Left Colon
Volume of FMT delivered: _____
Duration of retention: _____
☐ CRP and WBC collected
☐ Clinical Status:

Pt reported fatigue:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
none severe

Pt reported appetite:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
poor good

Pt reported abdominal discomfort/bloating:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
None/little severe

Pt reported bowel function:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
More constipated than baseline back to baseline more loose stools than baseline

GI Symptom directed physical exam required: ☐ Yes ☐ No

BP (sitting): ____/____mm/Hg HR: _____ bpm T: _____C Resp: _____ Weight: _____kg

Body system	Normal	Abnormal	Not Done	**Abnormal, specify the abnormalities:**	If Abnormal: Not clinically significant	If Abnormal: Clinically significant
Abdomen (liver and spleen)	☐	☐	☐		☐	☐
Other: Specify	☐	☐	☐		☐	☐

Physician's initials: _____

Day 2:

Site of FMT delivered: ☐ Enema ☐ Colonoscopy :
☐ Right Colon ☐ Left Colon
Volume of FMT delivered: _____
Duration of retention: _____
☐ CRP and WBC collected
☐ Clinical Status:
Pt reported fatigue:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
none severe

Pt reported appetite:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
poor good

Pt reported abdominal discomfort/bloating:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
None/little severe

Pt reported bowel function:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
More constipated than baseline back to baseline more loose stools than baseline

GI Symptom directed physical exam required: ☐ Yes ☐ No

BP (sitting): ____/____ mm/Hg HR: _____ bpm T: _____ C Resp: _____ Weight: _____kg

Body system	Normal	Abnormal	Not Done	**Abnormal, specify the abnormalities:**	If Abnormal	
					Not clinically significant	Clinically significant
Abdomen (liver and spleen)	☐	☐	☐		☐	☐
Other: Specify	☐	☐	☐		☐	☐

Physician's initials: _____

Day 3:

Site of FMT delivered: ☐ Enema ☐ Colonoscopy :
☐ Right Colon ☐ Left Colon
Volume of FMT delivered: _____
Duration of retention: _____
☐ CRP and WBC collected
☐ Blood and Stool Collected for Bio Bank
☐ Clinical Status:
Pt reported fatigue:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
none severe

Pt reported appetite:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
poor good

Pt reported abdominal discomfort/bloating:

1_____2_____3_____4_____5_____6_____7_____8_____9_____10

None/little severe

Pt reported bowel function:

1_____2_____3_____4_____5_____6_____7_____8_____9_____10

More constipated than baseline back to baseline more loose stools than baseline

GI Symptom directed physical exam required: ☐ Yes ☐ No

BP (sitting): ____/_____mm/Hg HR: _____ bpm T: _____ C Resp: _____ Weight: _____kg

Body system	Normal	Abnormal	Not Done	**Abnormal, specify the abnormalities:**	If Abnormal	
					Not clinically significant	Clinically significant
Abdomen (liver and spleen)	☐	☐	☐		☐	☐
Other: Specify	☐	☐	☐		☐	☐

Physician's initials: _____

Day 4: Clinical Assessment

☐ CRP and WBC collected

☐ Clinical Status:

☐ Vasopressors: #1: _____ #2: _____ #3:_____

Symptoms:

Pt reported fatigue:

1_____2_____3_____4_____5_____6_____7_____8_____9_____10

none severe

Pt reported appetite:

1_____2_____3_____4_____5_____6_____7_____8_____9_____10

poor good

Pt reported abdominal discomfort/bloating:

1_____2_____3_____4_____5_____6_____7_____8_____9_____10

None/little severe

Pt reported bowel function:

1_____2_____3_____4_____5_____6_____7_____8_____9_____10

More constipated than baseline back to baseline more loose stools than baseline

GI Symptom directed physical exam required: ☐ Yes ☐ No

BP (sitting): ____/_____mm/Hg HR: _____ bpm T: _____ C Resp: _____ Weight: _____kg

Body system	Normal	Abnormal	Not Done	**Abnormal, specify the abnormalities:**	If Abnormal	
					Not clinically significant	Clinically significant
Abdomen (liver and spleen)	☐	☐	☐		☐	☐
Other: Specify	☐	☐	☐		☐	☐

Physician's initials: _____

Repeat FMT: Cycle Number: ☐ 2nd ☐ 3rd ☐ 4th

Date of Fidaxomicin start: _____

Date of FMT: _____
Lot number: _____

☐ Laboratory:

Final Day of Fidaxomicin:

Stop Date: _____

☐ Blood, Stool and Collected for Bio Bank
☐ CRP, WBC Collected

FINAL FMT:

Date of Fidaxomicin Ended: _____

Date of FMT: _____
Lot number: _____

☐ Laboratory:
Physician to complete

Final FMT:

Site of FMT delivered: ☐ Enema ☐ Colonoscopy :
☐ Right Colon ☐ Left Colon
Volume of FMT delivered: _____
Duration of retention: _____
☐ CRP and WBC collected
☐ Clinical Status:
Pt reported fatigue:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
none severe

Pt reported appetite:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
poor good

Pt reported abdominal discomfort/bloating:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
None/little severe

Pt reported bowel function:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
More constipated than baseline back to baseline more loose stools than baseline

GI Symptom directed physical exam required: ☐ Yes ☐ No

BP (sitting): ____/____mm/Hg HR: _____ bpm T: _____ C Resp: _____ Weight: _____kg

Body system	Normal	Abnormal	Not Done	**Abnormal, specify the abnormalities:**	If Abnormal	
					Not clinically significant	Clinically significant
Abdomen (liver and spleen)	☐	☐	☐		☐	☐
Other: Specify	☐	☐	☐		☐	☐

Physician's initials: _____

Post Final FMT- Continued Hospitalization

Date of Final FMT:

☐ Daily CRP and WBC collected
☐ Clinical Status:
Pt reported fatigue:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
none severe

Pt reported appetite:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
poor good

Pt reported abdominal discomfort/bloating:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
None/little severe

Pt reported bowel function:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
More constipated than baseline back to baseline more loose stools than baseline

GI Symptom directed physical exam required: ☐ Yes ☐ No
BP (sitting): ____/_____mm/Hg HR: _____ bpm T: _____ C Resp: _____ Weight: _____kg

Body system	Normal	Abnormal	Not Done	**Abnormal, specify the abnormalities:**	If Abnormal	
					Not clinically significant	Clinically significant
Abdomen (liver and spleen)	☐	☐	☐		☐	☐
Other: Specify	☐	☐	☐		☐	☐

Physician's initials: _____

Week Post Final FMT (+/- 2 days)

☐ Blood, stool samples collected for BioBank
☐ CEGIIR or FMT lab notified

☐ Started a new antibiotic? List:

Pt reported fatigue:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
none severe

Pt reported appetite:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
poor good

Pt reported abdominal discomfort/bloating:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
None/little severe

Pt reported bowel function:
1_____2_____3_____4_____5_____6_____7_____8_____9_____10
More constipated than baseline back to baseline more loose stools than baseline

GI Symptom directed physical exam required: ☐ Yes ☐ No

BP (sitting): ____/_____mm/Hg HR: _____ bpm T: _____ C Resp: _____ Weight: _____kg

Body system	Normal	Abnormal	Not Done	**Abnormal, specify the abnormalities:**	If Abnormal	
					Not clinically significant	Clinically significant
Abdomen (liver and spleen)	☐	☐	☐		☐	☐
Other: Specifiy	☐	☐	☐		☐	☐

Physician's initials: _____