Fecal Microbiota Transplantation (FMT) after treatment for primary *Clostridium difficile* infection

ClinicalTrials.gov number: **NCT03795233**
Protocol Version Number: 1.7
Protocol Version Date: February 18, 2020
Funding Mechanism: This project was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through **BU-CTSI Grant Number 1UL1TR001430**.
IND / IDE
Sponsor: Tamar F Barlam MD
IND / IDE number: 18616
Principal Investigator: Tamar Barlam
Phone: 617-414-5190
E-mail: Tamar.Barlam@bmc.org

Statistical Plan pages 16-18

TABLE OF CONTENTS

1 List of Abbreviations

Abbreviation	Abbreviation definition
FMT	Fecal Microbiota Transplantation
BMC	Boston Medical Center
CDI	*Clostridium difficile* infection
CTU	Clinical Trials Unit
OTU	Operational taxonomic units

2 Protocol Summary

Title:	Fecal Microbiota Transplant (FMT) after treatment for primary *Clostridium difficile* Infection (CDI)
Population:	Patients >= 18 years hospitalized at Boston Medical Center (BMC) with a first documented episode of CDI.
Intervention:	30 FMT capsules administered orally under direct observation within 7 days of completion of 10-14 day treatment of oral vancomycin for CDI.
Objectives:	1. To characterize the microbial diversity in stool samples from subjects with a primary episode of CDI before and after oral vancomycin and determine the impact of FMT after completion of oral vancomycin course. 2. To characterize the feasibility and tolerability of FMT after completion of a course of oral vancomycin therapy for primary CDI, and to describe 30-day hospital readmission and gastrointestinal symptomatology and/or CDI recurrence during 60-day and 6 month follow-up.
Design/Methodology:	15 subjects will be enrolled who are diagnosed at BMC for a primary episode of CDI. A discard aliquot from baseline stool samples obtained clinically for diagnosis will be frozen. Subjects will receive the standard of care treatment (oral vancomycin for 10-14 days) and within 7 days following completion will receive oral FMT during a 2 hour visit in the ID Clinical Trials Unit. An additional 5 subjects will be enrolled as controls. Stool samples will be collected at time of CDI diagnosis and again 3 weeks after FMT for intervention group and 4 weeks after completion of oral vancomycin treatment for control subjects. The post-treatment samples will be obtained by the patient using RNAlater kits and mailed to the BMC CTU where it will aliquoted, centrifuged and frozen. Samples will be processed at a collaborating lab at Tufts for DNA extraction, 16SrDNA amplicon generation, and MiSeq sequencing will characterize the fecal microbiome pre- and post oral FMT. Study personnel will contact participants via telephone 60 days and 6 months after oral vancomycin completion to administer a follow-

	up survey (including questions on residual symptoms. CDI recurrence, re-hospitalization, adverse events and FMT acceptability). Subjects will be compensated for study participation with $100 gift cards provided for attendance of the clinic visit for FMT and $25 gift card after receipt of fecal sample post-treatment.
Total Study Duration:	Anticipated time: 12 months
Subject Participation Duration:	We anticipate a period of 1) 1-2 hours while an inpatient for the screening and consent process or 2) 1-2 hours if you are screened and consented as an outpatient, plus transit time to the visit, 2 hours for the CTU visit for FMT and 20-30 minutes responding to a follow up telephone survey. Total time in the study from enrollment to completion of follow-up will be approximately 6 months and will include 10-14 days of CDI treatment with oral vancomycin (per standard of care treatment), the FMT administration and a 60-day and 6-month follow up.

3 Background/Rationale & Purpose

3.1 Background Information

Clostridium difficile infection (CDI) is one of the most urgent health threats in the U.S. associated with antibiotic use, according to the Centers for Disease Control and Prevention [1]. An antibiotic course can affect the gut microbiome for years [2], and patients with CDI have additional dysbiosis of their gut flora for similarly prolonged periods [3,4]. After an episode of CDI, the risk of serious complications, including recurrent CDI, and mortality are high for 3-6 months, particularly in patients who are older or have comorbid illness [5-8]. Preventing CDI recurrence and restoring the microbiome is a high public health priority. Oral vancomycin, an effective therapy for CDI, perturbs the gut microbiome further [9]. CDI recurrences and relapses can occur in 20-30% of people treated with oral vancomycin [10]. In contrast, fidaxomicin preserves the intestinal microbiome during and after treatment of CDI, minimizing additional dysbiosis. Likely as a result, fidaxomicin has been shown to reduce recurrence of CDI when compared with standard treatment with oral vancomycin, 15.4% vs 25.3%, respectively [10].
Use of fidaxomicin as primary treatment for CDI has been advocated. However the agent is costly and there is still a significant risk of recurrence after disease.

Fecal Microbiota Transplant (FMT) has been studied as an effective treatment for recurrent CDI [11]. Konijeti et al performed a decision analysis comparing metronidazole, oral vancomycin, fidaxomicin and FMT for first-line treatment of recurrent CDI and found FMT colonoscopy was the most cost effective [12]. FMT has had very limited study for a primary episode of CDI and an endoscopic procedure for all those cases would be invasive, costly and associated with risk. However, FMT is now available via frozen oral capsules and has been shown to be non-inferior to FMT via colonoscopy in randomized controlled trials [13].

The FDA allows FMT without an IND only for treatment of recurrent episodes of CDI. At this time, FMT is not indicated for primary CDI therapy. We propose oral FMT following standard of care treatment for a first episode of CDI as a way to prevent recurrences and improve symptoms post infection. Potential benefits include reduced re-admissions from recurrent CDI, improved quality of life and resolution of

symptoms sooner.

This study will be conducted in compliance with the protocol, applicable regulatory requirements, and BMC/BU Medical Campus Human Research Protection policies and procedures.

3.2 Rationale and Purpose

Our study is based on the current understanding of the long-term impacts of antibiotic use and CDI. Medications such as antibiotics or proton-pump inhibitors, CDI itself, and the antibiotic treatment of CDI all adversely impact the diversity of the gut microbiome. Recurrent courses of CDI treated with antibiotics alone leads to progressive decreased microbial diversity in the gut [14]. The competition with and presence of other organisms in the gut are key to mucosal integrity and overall gut health [11]. Gut dysbiosis has not only been associated with recurrent CDI [15] but also increased risk of chronic gastrointestinal (GI) disorders, obesity, and non-*C. difficile* enteric infections [16] which FMT has been shown can improve [17]. Recent data suggests that antibiotic-resistant flora after a course of antibiotics can be reversed with FMT [18]. A regimen of CDI therapy followed by FMT may reduce recurrence rates beyond current available treatments as well as provide other health benefits. Fidaxomicin and bezlotoxumab, a human monoclonal antibody directed against *C. difficile* toxin B both have been shown to reduce CDI recurrence compared with oral vancomycin [10,19], but neither reverse dysbiosis or provide other potential health benefits.

There has been sparse study of FMT for primary CDI. Because of the potential health benefits, this cost-effective approach deserves further study. Our pilot study examines the effect of FMT on gut microbial diversity after CDI and oral vancomycin compared with gut microbial diversity in those subjects with CDI who receive oral vancomycin alone. Evidence of reduction of dysbiosis as well as information on GI symptomatology (e.g., diarrhea, abdominal pain, bloating), CDI recurrence and healthcare utilization, would provide preliminary data to support a randomized controlled, multicenter clinical trial to test this approach.

4 Objectives

4.1 Study Objectives

Objective 1: To characterize the microbial diversity in stool samples from subjects with a primary episode of CDI before and after oral vancomycin and determine the impact of FMT after completion of oral vancomycin course.
Objective 2: To characterize the feasibility and tolerability of FMT after completion of a course of oral vancomycin therapy for primary CDI, and to describe 30-day hospital readmission and gastrointestinal symptomatology and/or CDI recurrence during 60-day follow-up.

Study Outcome Measures

4.1.1 Primary Outcome Measures

Microbial community abundance and patterns of the stool dysbiosis will be compared before and after treatment for primary CDI with or without end of therapy FMT. Primary analyses will consist of direct

comparisons of the abundance of individual microbiomes, often represented by operational taxonomic units (OTUs), between conditions. We will also identify differences between samples pre- and post-treatment in FMT-treated subjects and controls using measures of biodiversity (α- and β-diversity, i.e., Shannon index, Jenson-Shannon Distance), summary meta-statistics such as principal components and coordinate analysis, and the PICRUSt software will be used to generate predicted pathway-level profiles for comparison between conditions.

4.1.2 Secondary Outcome Measures

- At ~60 days after completion of oral vancomycin treatment, secondary outcomes measured by telephone survey are:
    - Feasibility and tolerability of administering FMT after completion of a course of oral vancomycin therapy
    - Post CDI, post-treatment gastrointestinal symptomatology and response to therapy
- CDI recurrence within 60 days, defined as retreatment for CDI with vancomycin or fidaxomicin, and hospital admission within 30-day after treatment as determined by chart review.
- At ~6 months after completion of oral vancomycin treatment, secondary outcomes measured by telephone survey are:
    - CDI recurrence or hospital admission
    - Post CDI, post treatment gastrointestinal symptomatology and response to therapy
    - Any new onset chronic medical conditions that may represent theoretical association with prior FMT

5 Study Design

We propose an open label pilot study examining the effectiveness of oral FMT at restoring the microbial diversity following a primary episode of CDI.

Subjects who are diagnosed with CDI at Boston Medical Center via normal clinical processes (i.e., providers have a clinical concern for CDI, send a stool sample for diagnosis which is positive) will be screened for inclusion in the study until 10 subjects are enrolled in the FMT-treatment arm and 5 subjects are enrolled as controls. The assignment is not randomized for this pilot study to expedite recruitment and because the primary outcome, analysis of the gut microbiome diversity for each subject pre- and post CDI and FMT treatment, uses each subject as their own control. If the patient has a preference, they will be allowed to choose the treatment or control group if there are still slots open in the group preferred. If the patient does not have a preference, they will be assigned in a 2:1 ratio (2 patient receiving FMT for each control subject). All subjects will receive the standard of care for CDI treatment – oral vancomycin 125 mg orally for 10-14 days. The ten subjects in the FMT-treatment arm will receive oral FMT capsules within one week of oral vancomycin completion. Oral FMT frozen capsules DE (OpenBiome, Somerville, MA), a formulation designed to deliver the product to the large intestine, is the product currently used by BMC for recurrent CDI and will be used for this study. FMT will be stored and dispensed by the Investigational Pharmacy Service and administered in an outpatient setting supervised by the staff of the Clinical Trials Unit (CTU) within BMC's Center for Infectious Diseases. Study participants who receive FMT will attend one clinic visit (within 7 days of completion of

initial CDI therapy) where they will be administered 30 FMT capsules over ~2 hours, under direct observation.

Subjects will complete a baseline survey after completion of oral vancomycin. The survey will take 10-15 minutes and include questions on residual or recurrent symptoms, any new antibiotics, probiotics or other medications and any new or additional hospital admissions. Subjects in the treatment arm will complete the survey at the time of their FMT visit. Controls will be contacted by phone to complete a similar survey within a week of completing oral vancomycin. Study personnel will contact all participants via telephone 60 days and 6 months after the initial survey to administer a follow-up survey which will take 15-20 minutes. The 18-question survey was adapted from the validated Rome III questionnaire [17] and can be found in the Appendix. Subjects will be compensated for study participation with $100 gift cards for the CTU visit for FMT and with a $25 gift card after the post treatment fecal sample is received.

Sample processing and analysis: two stool samples will be analyzed. The first sample is collected in all subjects as part of routine clinical care and the microbiology laboratory will provide a discard aliquot for the study. The second sample will be collected by the subject at home 30 days after completion of oral vancomycin treatment, and mailed to investigators using a collection kit subjects have been provided. All stool samples will be aliquoted into 2 tubes, centrifuged and the pellets stored at -80^{o}C until all samples are received. Those samples will then be sent to Dr. Anne Kane at Tufts. Her laboratory will extract DNA from the stool samples and the V4 region of the 16S rRNA will be amplified and sequenced using an Ilumina MiSeq [16]. Sequences will be de-multiplexed, pre-processed for quality control, and aligned to the Greengenes database to generate a table of OTU abundances and taxonomic lineages. That data will then be analyzed by study investigator, Dr. W. Evan Johnson in BU Division of Computational Biomedicine. Microbial community abundance and patterns will be assessed between the treatment and control groups using: (i) direct comparisons of individual OTUs, (ii) measures of biodiversity (α- and β-diversity, i.e., Shannon index, Jenson-Shannon Distance), (iii) summary meta-statistics such as principal components and coordinate analysis, and (iv) the PICRUSt software to generate predicted pathway-level profiles.

Please see the Appendix for a schematic of the study design.

6 Potential Risks and Benefits

6.1 Risks

There will be 10 subjects who receive FMT and 5 controls.
For controls, care will be standard clinical care with no additional risks. However, they will be asked to submit a stool sample (a mailer will be provided so this can be done from home) and may feel some discomfort. Potential risk of confidentiality violation will be avoided because investigators will follow accepted procedures to prevent this.

For subjects who receive FMT, in addition to the above risks, they will be given oral capsules that may have side effects. Only capsules for subjects who are alert and can swallow will be given.
Common, mild adverse events: transient diarrhea (70%), transient abdominal cramps/discomfort (20%) and nausea (<5%) in the 24 hours post FMT. Transient fever, abdominal distension/bloating, belching, vomiting, dehydration, borborygmus have also been reported. Constipation (20%) and excess flatulence (25%) have been
reported in follow- up. There is also a theoretical risk of small intestinal bacterial overgrowth.
Rare, serious adverse events: The following risks should be considered:

Infection: Although this material has been screened for bacteria, viruses, fungi and parasites there is a risk of transmission of known and unknown infectious organisms contained in the donor stool. Post-FMT bacteremia (e.g. E. coli), sepsis and fatal events may rarely occur.
Inflammatory bowel disease (IBD) flare in those with underlying IBD (excluded from our study population but previously undiagnosed subjects may inadvertently be enrolled).
Allergy/Anaphylaxis to antigens in donor stool;
Non-infectious disease transmission: There is a theoretical risk of developing disease that may be related to donor gut microbiota. These include obesity, metabolic syndrome, cardiovascular disease, autoimmune conditions, allergic/atopic disorders, neurologic disorders, psychiatric conditions and malignancy. Persons with these known conditions are excluded from donating stool.
Aspiration: Capsule administration carries a risk of aspiration

Additional potential risks or discomfort include the extra visit to the hospital which will take ~2 hours that would not be necessary if they were not study participants. They will also have to submit stool samples and participate in a phone interview as controls and have same potential risk or discomfort.

Patients receiving FMT will be required to fast, which may risk hypoglycemia in diabetic patients. Fasting may also result in lightheadedness, dizziness or fainting.

6.2 Potential Benefits

For controls, there are no benefits to the patient other than satisfaction for participating in a study to improve patient care.

For subjects receiving FMT, potential benefit includes possible restoration of gut microbiome which has been linked to reduced recurrence of CDI; microbiome restoration may reduce the risk of post-infectious irritable bowel syndrome described after CDI. The recurrence rate for primary CDI is high (20-30%) after treatment with oral vancomycin and FMT may reduce that risk. Improved quality of life, fewer missed days of work and psychological upset due to recurrent CDI are potential benefits of oral FMT following primary CDI.

6.3 Analysis of Risks in Relation to Benefits

The recurrence rate of CDI following treatment with oral vancomycin (the current standard of care) is 20-30%, and the risks of oral FMT via frozen capsules are primarily temporary diarrhea, cramping/bloating and nausea. If we can decrease that with one time oral FMT then the benefits of avoiding recurrent CDI (and the morbidity, mortality and economic impacts therein) would outweigh the risks.

7 Study Subject Selection

7.1 Subject Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

Inclusion

- Adult (18 years and older) hospitalized patients with primary *C. difficile* infection for which oral vancomycin 125 mg every 6 hours for 10-14 days is standard of care therapy (CDI that is non-severe or severe e.g., leukocytosis ≥15,000 cells/mL and serum creatinine >1.5 mg/dl).

7.2 Subject Exclusion Criteria

Exclusion

An individual who meets any of the following criteria will be excluded from participation in this study:

- Previous history of CDI
- Patients <18 years of age
- History of ongoing antibiotic use (e.g., nitrofurantoin for UTI prophylaxis)
- Diagnosis of cancer not in remission and/or actively being treated
- Personal or family history of Inflammatory Bowel Disease or unexplained GI illness
- History of irritable bowel syndrome, excessive gas, bloating, lymphocytic colitis, idiopathic chronic constipation, chronic use of laxatives or chronic diarrhea
- Use of probiotics or any over the counter aids to regulate digestion
- Major immunosuppressive medications, e.g., calcineurin inhibitors, exogenous glucocorticoids, biologic agents, etc.
- Systemic anti-neoplastic agents
- Pregnant and breastfeeding women
- History of dysphagia: oropharyngeal, esophageal, functional, neuromuscular (e.g. stroke, multiple sclerosis, ALS), or patient shows evidence of dysphagia when the 'safety test' capsule is administered
- History of aspiration, intestinal obstruction or gastroparesis
- History of severe food allergies
- History of allergy to sodium chloride, glycerol, theobroma oil, hide bovine gelatin, sodium lauryl sulfate, colorants FD&C, or titanium dioxide
- Hospitalized at the time oral vancomycin therapy is completed
- Fulminant CDI (hypotension, ileus, and/or megacolon)
- Non-English speaking

8 Study Intervention

The main study intervention will be a one-time course of 30 FMT capsules after treatment with oral vancomycin for primary CDI.

9 Study Procedures

See the Appendix for the schedule of events and procedures.

1. Patient admitted to BMC has a positive stool PCR test for C. difficile. (The stool is sent as part of clinical care).
    a. Per BMC protocol, the microbiology laboratory reports the positive result in the electronic medical record for Infection control review in real time.
    b. A member of Infection Control, reviews this list daily, Monday through Friday and notifies study investigator of possible case.

c. The study investigator (under IRB-approved HIPAA waiver) reviews the patient chart to determine if this is a primary episode of CDI. She will also determine if there are study exclusions already recorded in the chart.
d. If the patient has primary CDI and no documented exclusions, a member of the study team will contact the patient's provider to ask permission to discuss the study with the patient if the patient is hospitalized at the time; outpatients will be contacted directly via the contact number listed in the electronic medical record.
e. A study team member either visits the patient if inpatient or calls the patient if outpatient, explains briefly the purpose of the visit, and reviews the Brief Screening Agreement. If the patient agrees, they are formally screened for inclusion/exclusion criteria. No identifying information will be kept after screening, consent will be obtained as soon as possible after screening. If consent is delayed, the screening data will not be kept in an identifiable manner.
f. If eligible and interested, the patient undergoes the informed consent process.
   i. Patients who are recruited in the outpatient setting will agree by phone to receive the informed consent documents via email (preferred) or mail. If interested and eligible, a study visit will be arranged in the CTU to complete the consent process.
   ii. Consent will be obtained during the hospital stay for inpatients. They will be given up to 48 hours to decide whether to participate and consent.
   iii. Patients who consent will be designated either a control or treatment subject. If the patient has a preference, they will be allowed to choose the treatment or control group as long as there are still slots open in the group preferred. If not, they will be assigned in a 2:1 ratio (2 patient receiving FMT for each control subject).
g. Subjects in the treatment group who are females of childbearing potential will have a pregnancy test that will be reviewed and negative before enrollment. Those subjects must agree to use an effective form of contraception from the time of enrollment through at least 30 days after FMT.
h. The patient will receive the standard of care treatment for CDI, vancomycin 125 mg po q6h for 10-14 days, per their physician.
i. The laboratory will provide a discard aliquot of the stored stool (on which the initial PCR test was done), which will be labeled by study ID number and stored at -80^{o}C by the CTU. There will be adequate stool sample remaining for any additional clinical testing the laboratory might need to do.
j. At outpatient enrollment or prior to hospital discharge, subjects will be provided an RNALater kit for stool collection with instructions on how to obtain the sample. They will be provided a prepaid, addressed mailer. For treatment subjects, an appointment for CTU will be confirmed for 3-7 days after completion of oral vancomycin is planned.
k. Subjects will be given written instructions related to FMT treatment (clear liquid diet after midnight the day of the appointment, fasting 2 hours prior to arrival to appointment)
l. All subjects will be given instructions on how to clean their bathroom at home.

2. Subjects with worsening CDI infection during oral vancomycin therapy who require escalation of care will be withdrawn from the study. All medical management will be by the subject's primary provider.
3. Subjects in the FMT-group will receive a phone call reminder two days prior to CRU appointment and instructions (i.e., clear liquid diet and fasting prior to visit) will be reviewed.

4. Phone call to controls within 7 days of completion of CDI therapy for a survey of symptoms that should take 10-15 minutes to complete.
5. The Investigational Pharmacy will handle storage and labeling for research of all FMT capsules. 10 courses of 30 capsules will be obtained from OpenBiome. OpenBiome will also provide either a small aliquot of stool for each treatment course, or genetic analysis of the microbiome, of the donor stool. As the microbiome can vary for each treatment, even for a single donor, this information is necessary for analysis. The oral FMT capsules are stored in a tamper evident plastic bottle inside packaging on dry ice, they are stored at -20 degrees C and removed from freezer and must be consumed within 90 minutes. OpenBiome FMT DE capsules contain: Frozen human fecal microbiota (filtered to 330 microns), theobroma oil, glycerol, hide bovine gelatin, sodium lauryl sulfate, colorants FD&C and titanium dioxide. Per OpenBiome's product information: "FMT Capsule DE is physically stable at room temperature for more than 30 days. However to preserve microbial viability, providers should strictly maintain the cold chain without allowing freeze-thaw cycles. Material should be delivered within 90 minutes of removal from frozen storage." Prior to swallowing the 30 capsules that constitutes oral FMT the patient will swallow an empty "placebo" capsule to confirm they can swallow the capsule without difficulty [20].
6. At CTU appointment (within 7 days after completion of oral vancomycin):
    a. Confirm fasting and liquid diet compliance
    b. In person survey on symptoms completed with patient by CTU personnel.
    c. CTU personnel goes through check list (reviewing contraindications and exclusion criteria)
    d. For subjects who are females of childbearing potential, a pregnancy test will be administered, reviewed and negative prior to administration of FMT. If positive, the subject will be excluded from the study.
    e. One test placebo capsule administered. If patient is able to swallow without a problem, administration proceeds.
    f. 30 pills FMT removed from freezer and given to patient (protocol) over 90 minutes (Study specific). If patient vomits, the procedure will be aborted.
    g. Patient instructed to fast for 1 hour post FMT. Patient given post-FMT instruction sheet and number to call to report any concerns or adverse events.
    h. Patient will be given a diary to record daily symptoms for a total of 7 days after FMT. Patients will be given an addressed, stamped envelope to return the diary to the investigators. Patients who cannot read the diary will have someone complete the form with them or the investigators will arrange to phone daily for one week to complete the form.
    i. The patient will receive a phone call to determine if there had been any adverse events after the FMT within 48-72 hours. Investigators will confirm that the patient is completing the symptom diary and will do so for a total of 7 days.
    j. Patient reminded to submit stool sample in 20 days using RNALater kit and instructions reviewed again. If patient has misplaced RNALater kit, a new one will be provided.
7. Any serious, life-threatening adverse event that is unexpected and possibly related to the research will result in suspension of the trial. All adverse events will be reported to OpenBiome using their adverse event reporting form: www.OpenBiome.org/adverse-events, as well as to the IRB.
8. Control subjects will provide stool sample ~30 days post completion of oral vancomycin; FMT-treated subjects will provided stool sample ~20 days after oral FMT administration.

   a. A study team member will phone 2 days prior to planned date for stool submission
   b. A study team member will phone 2 days after stool submission was planned to confirm it had been sent
   c. All stool samples will be collected at home per manufacturer's protocol, mailed to PI. A study team member will aliquot the sample, centrifuge, decant the RNALater media, and the pellets will be stored by CTU at -80°C till further processing. All baseline and follow-up stool samples will be shipped to Tufts together for batch processing.
9. Sixty days after initial survey (± 1 week) - Phone survey of cases and controls that will take 15-20 minutes and include questions on residual symptoms. CDI recurrence, 30 day readmission, adverse events as well as their impression of burden and acceptability of FMT post-vancomycin treatment.
10. Six months after the initial survey (± 1 week) - Phone survey of cases and controls that will take 15-20 minutes and include questions on residual symptoms. CDI recurrence, 30 day readmission, adverse events. They will also be questioned about any serious adverse events and/or new onset of chronic medical conditions that may represent theoretical risks of FMT (e.g., immune-mediated diseases, obesity, metabolic syndrome, gastrointestinal disorders, cardiovascular disease, neuropsychiatric disorders, and malignancies).
11. Frozen stool samples, all labeled only by study ID number, and sent to Tufts for batch processing and analyses. The V4 region of the 16S rRNA will be amplified from DNA extracted from the stool samples and sequenced using an Ilumina MiSeq [16] under the direction of Dr. Anne Kane. Sequences will be de-multiplexed, pre-processed for quality control, and aligned to the Greengenes database to generate a table of OTU abundances and taxonomic lineages.
12. Statistical analyses: BU Computational Biomedicine (Dr. Johnson's laboratory) will perform these analyses. Microbial community abundance and patterns will be assessed between the treatment and control groups using: (i) direct comparisons of individual OTUs, (ii) measures of biodiversity (α- and β-diversity, i.e., Shannon index, Jenson-Shannon Distance), (iii) summary meta-statistics such as principal components and coordinate analysis, and (iv) the PICRUSt software to generate predicted pathway-level profiles.

For each patient total time involved should be less than 180 days:

1-3 days for screening and informed consent process
10-14 days for CDI treatment
Oral FMT within 7 days of completion of oral vancomycin
60 days follow-up after completion of oral vancomycin course
6 month follow-up after completion of oral vancomycin course

10 Assessment of Safety and Data Safety Monitoring Plan (DSMP)

10.1 Definitions

The following definitions will be used in the assessment of safety:

Adverse Event (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

AEs will be evaluated for relationship to study intervention per the physician investigator who will determine the relationship as definite, probable, possible, and not related to the intervention.
This is defined as:
Definitely related: The adverse event is clearly related to the FMT material – i.e., an event that follows a reasonable temporal sequence from administration of the FMT material, follows a known or expected response pattern to the FMT material, that is confirmed by improvement on stopping and reappearance of the event on repeated exposure and that ***could not*** be reasonably explained by the known characteristics of the patient's clinical state.

Probably related: The adverse event is likely related to the FMT material – i.e., an event that follows a reasonable temporal sequence from administration of the FMT material, follows a known or expected response pattern to the FMT material, that is confirmed by improvement on stopping and reappearance of the event on repeated exposure and that is ***unlikely to be*** explained by the known characteristics of the patient's clinical state.

Possibly related: An adverse event that follows a reasonable temporal sequence from administration of the FMT material, follows a known or expected response pattern to the FMT material, but that could readily have been produced by a number of other factors.

Not related: The adverse event is clearly not related to the FMT material, i.e. another cause of the event is most plausible; and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the study intervention and/or causal relationship is considered biologically impossible.

AEs will be graded by severity as:
1- Mild AE (not requiring treatment)
2- Moderate AE (resolved with treatment)
3- Severe AE (inability to carry on normal activities/required professional medical attention)
4- Life threatening or disabling AE
5- Death

Serious Adverse Event (SAE) is any adverse event that

(1) results in death;
(2) is life-threatening;
(3) results in inpatient hospitalization or prolongation of existing hospitalization;
(4) results in a persistent or significant disability/incapacity;
(5) results in a congenital anomaly/birth defect; or
(6) based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Life-threatening means that the event places the subject at immediate risk of death from the event as it occurred.

Unanticipated Problem is defined as an event, experience or outcome that meets all three of the following criteria:

- is unexpected; AND
- is related or possibly related to participation in the research; AND
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research

Unexpected means the nature, severity, or frequency of the event is not consistent with either:

- the known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol–related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts; or
- the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

In addition, any adverse events will be reported to the FDA, as appropriate.

10.2 Safety Review

Both the risks listed in Section 6.1 and unknown risks will be monitored as follows:
Events:

- Study participants will be monitored during FMT test dose, the 90 minutes of FMT administration, and 30 minutes after FMT administration to ensure no reactions occur such as nausea or vomiting, abdominal pain or diarrhea.
- Following administration of oral FMT study participants will receive a handout with contact information to report any adverse symptoms or concerns.
- Subjects will be contacted within 48-72 hours after FMT to check on patient status.
- Study participants will be contacted at 60 days following their initial survey at which point a detailed survey will be administered on symptoms, side effects and any re-admissions.
- Physicians will go over any significant adverse events to determine if they are related, possibly related or unrelated to oral FMT administration
- Patients will be contacted six months after FMT to determine if there were any further serious adverse events and/or new onset of chronic medical conditions that may represent theoretical risks of FMT (e.g., immune-mediated diseases, obesity, metabolic syndrome, gastrointestinal disorders, cardiovascular disease, neuropsychiatric disorders, and malignancies).

10.3 Reporting Plans

The Principal Investigator at BMC/BU Medical Campus will report Unanticipated Problems, safety monitors' reports, and Adverse Events to the BMC/BU Medical Center IRB in accordance with IRB policies:

- Unanticipated Problems occurring at BMC/BU Medical Campus involving a fatal or life-threatening event will be reported to the IRB within 2 days of the investigator learning of the event.
- Unanticipated Problems occurring at BMC/BU Medical Campus not involving a fatal or life-threatening event will be reported to the IRB within 7 days of the investigator learning of the event.
- Adverse Events (including Serious Adverse Events) will be reported in summary at the time of continuing review, along with a statement that the pattern of adverse events, in total, does not suggest that the research places subjects or others at a greater risk of harm than was previously known.
- OpenBiome will receive serious adverse event reports within 24 hours and review with investigators to assess whether it needs to be reported to the FDA. The IRB will be informed.

10.4 Stopping Rules

- The study will be suspended if one or more serious or life-threatening adverse event that is unexpected and possibly related to the research occur in the study population. In addition, the following events would trigger a study pause and safety review: Any single new potentially transmitted infection assessed as at least possibly related to FMT, i.e., infection cannot be assessed as unrelated to FMT with certainty in the judgment of the investigator alone or in consultation with OpenBiome.
- Two or more FMT recipients develop a new medical condition or immune-mediated disease assessed by the investigator and/or OpenBiome as definitely related to FMT.
- Three or more FMT recipients experience a non-serious Grade 3 (severe) AE of the same type assessed as definitely related to FMT (e.g., vomiting, dehydration, abdominal pain, fever).

11 Data Handling and Record Keeping

11.1 Confidentiality

- The information that will be obtained from and/or about subjects and potential subjects is the minimum necessary to conduct the study; and
- If any interventions and interactions occur with subjects and potential subjects, they will take place in private settings.
- All patient data will be linked to a study ID and the master list of the study ID will be kept in a separate, password-protected file and stored within a locked BMC office in a HIPAA-compliant BMC-controlled computer that meets BMC's standard for storage of PHI.
  Clinical specimens (two stool samples) will be labeled with the study ID only and sample number only.
- Microbiome analyses will be labeled with the study ID only.
- Study participants will be identified by a unique Study ID linked to subject identifiers via a master code list as described, only available to study investigators and research staff. No other persons will have access to the master code list.
- The master code list will be kept for 7 years to allow the option for further follow-up of the subjects, for which additional IRB approval would be obtained. After 7 years and/or when no further changes need to be made to the underlining raw data, the master code list will be destroyed.

11.2 Source Documents

Data generated by the methods described in the protocol will be recorded in the subjects' medical records if part of routine patient care only. Otherwise, all records will be in each subjects Case Report Forms and/or study progress notes. Data may be transcribed legibly on CRFs supplied for each subject or directly inputted into an electronic system or any combination thereof.

11.3 Case Report Forms

The study Case Report Form (CRF) will be the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data will be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, "N/D" will be written. If the item is not applicable to the individual case, "N/A" will be written. All entries will be printed legibly in black ink. If any entry error has been made, to correct such an error, a single straight line will be drawn through the incorrect entry and the correct data will be entered above it. All such changes will be initialed and dated. There will be no erasures or white-out on CRFs. For clarification of illegible or uncertain entries, the clarification will be printed above the item, then initialed and dated. The following source data will be recorded directly on the CRFs: brief screening agreement, eligibility check list, informed consent, phone survey interviews, telephone contact documentation with reminders, Adverse event tracking, subject deviation/violation/withdrawal from the study. Duration of oral vancomycin and date of completion; antibiotic administration (other than for CDI) and dates; date of oral FMT administration; dates of stool specimens; patient diary; and any unscheduled contacts with subject will be recorded/included in the CRF folder for each subject.

See the Appendix for templates for the CRFs including documentation of eligibility and consent, documentation for the CTU visit, and adverse event tracking.

11.4 Study Records Retention

Study records will be retained by the PI in a locked BMC office, identified by Study ID only. The master list of the study ID will be kept in a in a HIPAA-compliant BMC-controlled computer that meets BMC's standard for storage of PHI for at least 7 years. Documentation of informed consent of subjects be retained for at least seven years after the study is closed electronically in a password-protected file on a HIPAA compliant BMC computer accessible only to the investigators and available for inspection and copying by authorized individuals.

Drug/Biologics:

- FDA requirements, records and reports will be kept for 2 years after a marketing application is approved for the drug; or if an application is not approved for drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA so notified.

12 Statistical Plan

12.1 Study Hypotheses

Objective 1: To characterize the microbial diversity in stool samples from subjects with a primary episode of CDI before and after oral vancomycin and determine the impact of FMT after completion of oral vancomycin course.

Hypothesis 1a: At the time of the CDI diagnosis (baseline), there will be dysbiosis of the gut microbiome compared to published microbiome data of healthy patients.

Hypothesis 1b: One month after completion of oral vancomycin therapy, the microbiome will be similar to baseline in subjects who do not receive FMT.

Hypothesis 1c: There will be a significant reduction in dysbiosis following FMT compared to subjects who do not receive FMT.

Objective 2 : To characterize the feasibility and tolerability of FMT after completion of a course of oral vancomycin therapy for primary CDI, and to describe 30-day hospital readmission and gastrointestinal symptomatology and/or CDI recurrence during 60-day follow-up.

Hypothesis 1a: FMT after completion of oral vancomycin treatment will not be unduly burdensome for subjects and will be well tolerated.

Hypothesis 1b: GI symptomatology will be improved in subjects who receive FMT compared to those subjects who do not, and other outcomes may show improvement.

Study Outcome Measures

12.1.1 Primary Outcome Measures

Microbial community abundance and patterns of the stool dysbiosis will be compared before and after treatment for primary CDI with or without end of therapy FMT. Primary analyses will consist of direct comparisons of the abundance of individual microbiomes, often represented by operational taxonomic units (OTUs), between conditions. We will also identify differences between samples pre- and post-treatment in FMT-treated subjects and controls using measures of biodiversity (α- and β-diversity, i.e., Shannon index, Jenson-Shannon Distance), summary meta-statistics such as principal components and coordinate analysis, and the PICRUSt software will be used to generate predicted pathway-level profiles for comparison between conditions.

12.1.2 Secondary Outcome Measures

- Descriptive data using survey responses and comparing control and treatment groups re:
  - Feasibility and tolerability of administering FMT after completion of a course of oral vancomycin therapy
  - Post CDI, post-treatment gastrointestinal symptomatology and response to therapy
  - CDI recurrence within 60 days
- 30-day hospital readmission after treatment – percent of subjects readmitted within 30 days of treatment per patient history and by chart review.

Secondary outcomes will be compared between treatment and control groups using Fisher's exact testing for small sample sizes.

12.2 Sample Size Determination

This is a pilot study for proof of concept i.e. that this approach is feasible and acceptable to subjects and that FMT after oral vancomycin therapy improves dysbiosis. We will likely lack statistical power for our secondary outcome analyses, given the small sample size. Further there will be no matching of controls

to intervention. Boston Medical Center had over 150 cases of primary CDI in 2017 which should be ample number to recruit 15 subjects.

12.3 Statistical Methods

Quality control of sequencing reads will be performed with the FastQC software [21]. Dr. Johnson's PathoScope and PathoStat software frameworks [22-25] will be used to preprocess the data, align the reads to the Greengenes database [26], and analyze the microbial composition of RNA-seq data analysis. This will generate a table of OTU-level relative abundances with associated taxonomic lineage, microbial function, gene coverage and expression, etc. The microbial communities will be characterized at multiple taxonomic levels (e.g., phylum, genus, and species). PathoStat [27] will be used for diversity estimates (alpha and beta), dimension reduction using PCA and PCoA, and differential abundance and expression using DeSeq2 [28] and other tools implemented into our PathoStat tool. Expression differences for microbial genes will be assessed also with DeSeq2, including relevant sets of pathways available in the KEGG database using PICRUSt, enrichment analysis and ASSIGN profiling.

Sample size: Since our sample size proposed here is small, we recognize that sample size will limit our ability to develop confirmed biomarkers for FMT treatment efficacy, so our study will be focused on exploration and to justify larger studies to make more concrete conclusions. However, our previously published work and microbiome work from others [29,30], have demonstrated that small sample sizes can yield significant results for discriminating diseased samples from controls. Our group size should be more than sufficient for our explorations and justify a larger study with larger sample size. We can estimate detectable effect sizes for the microbiome and host expression data based on a combination of the Human Microbiome Project (HMP) [31] and our previous work [29]. In our previous study, we observed an average of 8 species per sample with relative species abundance >1%, with corresponding standard deviation quartiles of [0.081, 0.10, 0.15] in the HMP, and another 47 rarer species our dataset (>0.01% relative abundance), with corresponding HMP standard deviation quartiles of [0.016, 0.027, 0.058]. Using the human microbiome project data as a baseline, we anticipate to have 80% power to detect a ~0.07-unit difference in rare taxon abundance and a ~0.25-unit difference in common taxa abundance, using a Bonferroni correction (0.05/300=0.00017). The corresponding detection limit for ~250 metabolic pathways allows for power of 80% for a ~0.25-unit difference in relative abundance, again employing a Bonferroni false positive cutoff. Analyses that evaluate diversity of organisms and pathways will be powered similarly as described above for common taxa abundances. This although our sample sizes are relatively small in each group, it is clear that we will have sufficient power to detect any large species-, higher level taxa-, and pathway-level differences present between the treatment and control groups.

13 Ethics/Protection of Human Subjects

This study is to be conducted according to applicable US federal regulations and institutional policies (which are based in federal regulations, guidance, and ICH Good Clinical Practice guidelines).

This protocol and any amendments will be submitted to the Boston Medical Center and Boston University Medical Campus IRB, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator. A copy of the initial IRB approval letter will be provided to the sponsor before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB. The consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. Consent will be documented as required by the IRB.

14 Literature References

References

1. Centers for Disease Control and Prevention (CDC), "Antibiotic Resistance Threats in the United States. 2013," *available at* http://www.cdc.gov/drugresistance/threat-report-2013/ (cited March 29, 2018).
2. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci USA 2011; 108 Suppl 1:4554-61.
3. Perez-Cobas AE, Artacho A, Ott SJ, Moya A, Gosalbes MJ, Latorre A. StruCRUral and functional changes in the gut microbiota associated to *Clostridium difficile* infection. Front Microbiol 2014;5:335
4. Skraban J, Dzeroski S, Zenko B, Mongus D, Gangl S, Rupnik M. Gut microbiota patterns associated with colonization of different *Clostridium difficile* ribotypes. PLoS One 2013;8(2):e58005
5. Murphy CR, Avery TR, Dubberke ER, Huang SS. Frequent hospital readmissions for *Clostridium difficile* infection and the impact on estimates of hospital-associated *C. difficile* burden. Infect Control Hosp Epidemiol 2012;33 (1):20-8.
6. Rodriguez-Pardo D, Almirante B, Bartolome RM, et al. Epidemiology of *Clostridium difficile* infection and risk factors for unfavorable clinical outcomes: results of a hospital-based study in Barcelona, Spain. J Clin Microbiol 2013; 51 (5):1465-73.
7. Louie TJ, Miller MA, Crook DW, et al. Effect of age on treatment outcomes in *Clostridium difficile* infection. J Am Geriatr Soc 2013; 61 (2):222-30.
8. Abou Chakra CN, Pepin J, Sirard S, Valiquette L. Risk factors for recurrence, complications and mortality in *Clostridium difficile* infection: a systematic review. PLoS ONE 2014;9 (6):e98400.
9. Louie T, Cannon K, Byrne B, Emery J, Ward L, Eyben M, Krulicki W. Fidaxomicin preserves the intestinal microbiome during and after treatment of *Clostridium difficile* infection (CDI) and reduces both toxin re-expression and recurrence of CDI. Clin Infect Dis 2012;55 (suppl 2) S132-142.
10. Louie T, Miller, M, Mullane K et al. Fidaxomycin versus Vancomycin for *Clostridium difficile*. N Engl J Med 2011; 364(5);422-31.
11. Khoruts A, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. Nat Rev Gastroenterol and Hepatol 2016; 13 (9): 508-516.
12. Konijeti G, Sauk J, Shrime M, Gupta M, Ananthakrishnan A. Cost effectiveness of Competing Strategies for Management of Recurrent *Clostridium difficile* infection: A Decision Analysis. Clin Infect Dis 2014;58 (11): 1507-14.
13. Kao D, Roach B, Silva M, et al. Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent *Clostridium difficile* infection: a randomized clinical trial. JAMA 2017;318(2):1985-93
14. Cheng J et al Decreased diversity of fecal microbiome in recurrent Clostridium difficile associated diarrhea. J infect Dis. 2008 Feb 1;197(3) 435-8
15. Seekatz AM, Rao K, Santhosh K, Young VB. Dynamics of the fecal microbiome in patients with recurrent and nonrecurrent *Clostridium difficile* infection. Genome Med 2016;8:47.
16.DuPont AW, DuPont HL. The intestinal microbiota and chronic disorders of the gut. Nat Rev Gastroenterol Hepatol 2011;8:523-31.
17. Konturek PC, Haziri D, Brzozowski T et al. Emerging role of fecal microbiota therapy in the treatment of gastrointestinal and extra-gastrointestinal diseases. J Physiol Pharmacol 2015; 66(4):483-91.

18. Davido B, Batista R, Michelon H et al. Is faecal microbiota transplantation an option to eradicate highly drug-resistant enteric bacteria carriage? J Hosp Infect 2017;95(4):433-37.
19. Wilcox MH, Gerding DN, Poxton IR et al. Bezlotoxumab for prevention of recurrent Clostridium difficile infection. N Engl J Med 2017;376(4):305-317.
20. OpenBiome Oral FMT G3 handling instructions
21. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
22. Byrd AL, Perez-Rogers JF, Manimaran S., et al. Clinical PathoScope: rapid alignment and filtration for accurate pathogen identification ni clinical samples using unassembled dsequencing data. BMC Bioinformatics 2014; 15:262. Doi: 10.1186/1471-2105-15-262.
23. Francis, O., Bendall, M. & Manimaran, S. Pathoscope: Species identification and strain attribution with unassembled sequencing data. Genome 2013; 1–10. doi:10.1101/gr.150151.112.Freely
24. Hong, C. Manimaran S, Shen Y, et al.. PathoScope 2.0: a complete computational framework for strain identification in environmental or clinical sequencing samples. Microbiome 2014:2,:33.
25. Hong, C., Manimaran, S. & Johnson, WE. PathoQC: Computationally Efficient Read Preprocessing and Quality Control for High-Throughput Sequencing Data Sets. Cancer Informatics 2014; 13: 167–76.
26. http://greengenes.secondgenome.com/
27. Solaiappan Manimaran et al. PathoStat: PathoStat Statistical Microbiome Analysis Package. 2017. https://rdrr.io/bioc/PathoStat
28. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15, 550.
29. Castro-Nallar, E., Shen Y, Freishtat RJ, et al. Integrating microbial and host transcriptomics to characterize asthma-associated microbial communities. BMC Med. Genomics 2015; 8:50 (2015).
30. Botero, L. E. et al. Respiratory tract clinical sample selection for microbiota analysis in patients with pulmonary tuberculosis. Microbiome 2014;2:29.
31. Consortium, T. H. M. P. Structure, function and diversity of the healthy human microbiome. Nature 2012;486: 207.

15 Appendix

Schedule of Events for Overall Study

	Months 1-3	Months 4-8	Months 9-11	Months 11-12	Months 13-15
Protocol development and IRB application, Clinical trials.gov registration, IND submission	X				
Patient enrollment		XX			
Stool sample collection		X			
Fecal Microbiota Transplant Capsules Administration		X			
Symptom Survey			XX		
Complete 60 day follow-up			X		
Complete six-month follow-up				X	X
Stool sample processing and analysis			X		
Manuscript Preparation				XX	

1. Study design schematic
2. Telephone surveys – baseline and 60-day follow-up
3. Inclusion/exclusion criteria screening form
4. Case Report Forms

Schedule of Events for Individual Subject

	Day
Stool sample positive for C. difficile	0
Patient enrollment	0-3
Completion of oral vancomycin therapy	10-14
Baseline survey within 1 week of completing vancomycin therapy	10-21
Fecal Microbiota Transplant Capsules Administration for treatment group	14-21
Stool sample 30 days after completing vancomycin	40-45
Complete 60 day follow-up	70-90
Complete six-month follow-up	~180