Project Title: A PHASE II RANDOMIZED, DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL TO DETERMINE IF FECAL MICROBIOTA TRANSPLANTATION IS EFFICATIOUS FOR HOSPITALIZED PATIENTS WITH *C. DIFFICILE* INFECTION HISTORY DURING ANTIBIOTIC TREATMENT

Principal Investigator: Nasia Safdar, MD, PhD
Department of Medicine
Division of Infectious Disease
University of Wisconsin-Madison
MFCB 5138
1685 Highland Ave.
Madison, WI 53705
(608) 213-4075
ns2@medicine.wisc.edu

Project Coordinator: Ashley Kates, PhD
Department of Medicine
Division of Infectious Disease
University of Wisconsin-Madison
akates@medicine.wisc.edu

Funding Source: AHRQ
(Finch Therapeutics and OpenBiome will manufacture and provide study drug capsules and placebos free of charge)

PROTOCOL REVISIONS AND AMENDMENTS

Protocol Version	Date	Change Initiated by (initials)	Brief description of protocol modifications/ actions requested, if any
Template	9/5/17	TNK, AK	Input from IRB, OCT, MARCH, IND/IDE services, PIs
Draft 1	4/10/17	AK, NS	First draft of the protocol from the template
Draft 2	4/13/17	TNK	Comments up to consent portion of the protocol
Draft 3	6/17/17	AK	Addressing TNK comments, formatting
Draft 4	6/25/17	AK	Responding to IRB comments
Draft 5	8/3/17	TO, AES, AR (ICTR)	ICTR DMC and SMS comments, formatting
Draft 6	8/7/2017	AK	Addressing DMC and SMS comments
Draft 7	8/25/2017	AK	Finalizing protocol
Draft 8	9/12/17	AK	Minor updates
Draft 9	3/23/18	AK	Updates to FMT product
Draft 10	5/22/18	AK	Minor editorial updates
Draft 11	7/31/2018	AK	Responses to FDA comments

Draft 12	9/14/18	AK	Changes requested by IRB
Draft 13	11/26/18	SZ	Changes requested by IRB
Draft 14	12/5/18	AK, IG	Updates to protocol – Inserted protocol number and clinicaltrials.gov number – Corrected typos – Clarified stool collection during intervention period regardless of hospitalization, for all groups – Updated contact information as needed – Clarified CRU nurse is considered a study nurse for the purpose of this study – Clarified fasting allows for clear liquids (not strict NPO) – Updated inclusion for new antibiotic to be started within 72 hours rather than three doses – Clarified 30-minute post-dose observation need only be done for first dose – Study test capsule and pregnancy test (when applicable) will be taken at intervention visit – Defined timeline for exclusion of bowel obstruction or gut motility issues – Clarified exclusion for neutropenia and how/when to evaluate – Further explained recruitment – Change from 19 days to 14 days for cutoff of antibiotic regimen concurrence – Explained withdrawal and early termination visit – Explained accountability recording (med diary) and potential drug return – Clarified concomitant medications, including certain long-term antibiotics – Removed Charlson's Comorbidity Index – Added option to collect perirectal swabs at baseline if needed – Removed phrase "safety assessment" - defined as AE evaluation, which is already listed – Clarified calls during treatment period will be once per week

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practices (GCP) as required by the following:

- United States Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312);
- International Conference on Harmonization (ICH) E6; 62 Federal Register 25691 (1997);

Compliance with these standards provides public assurance that the rights, safety and well-being of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.
All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Lead Principal Investigator:

Signed: _____ Date: _____

Name, Title

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1. LIST OF ABBREVIATIONS

ADL	Activities of Daily Living
AE	Adverse event
ANCOVA	Analysis of covariance
CAP	Community acquired pneumonia
CDI	*C. difficile* Infection
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CRF	Case Report Form
CRO	Contract Research Organization
CRU	Clinical Research Unit
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FFR	Federal Financial Report
FMT	Fecal Microbiome Transplant
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH E6	International Conference on Harmonisation Guidance for Industry, Good Clinical Practice: Consolidated Guidance
ICMJE	International Committee of Medical Journal Editors
ICU	Intensive Care Unit
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Investigational Review Board
ISO	International Organization for Standardization
MDRO	Multidrug Resistant Organism
MOP	Manual of Procedures
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSDS	Material Safety Data Sheet
NIH IC	NIH Institute & Center
NSAID	Non-steroidal Anti-inflammatory Drug
OHRP	Office for Human Research Protections
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PRC	Pharmaceutical Research Center
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SMS	Study Monitoring Service
TNF	Tumor necrosis factor
UTI	Urinary Tract Infection
UW-ICTR	University of Wisconsin Institute for Clinical and Translational Research

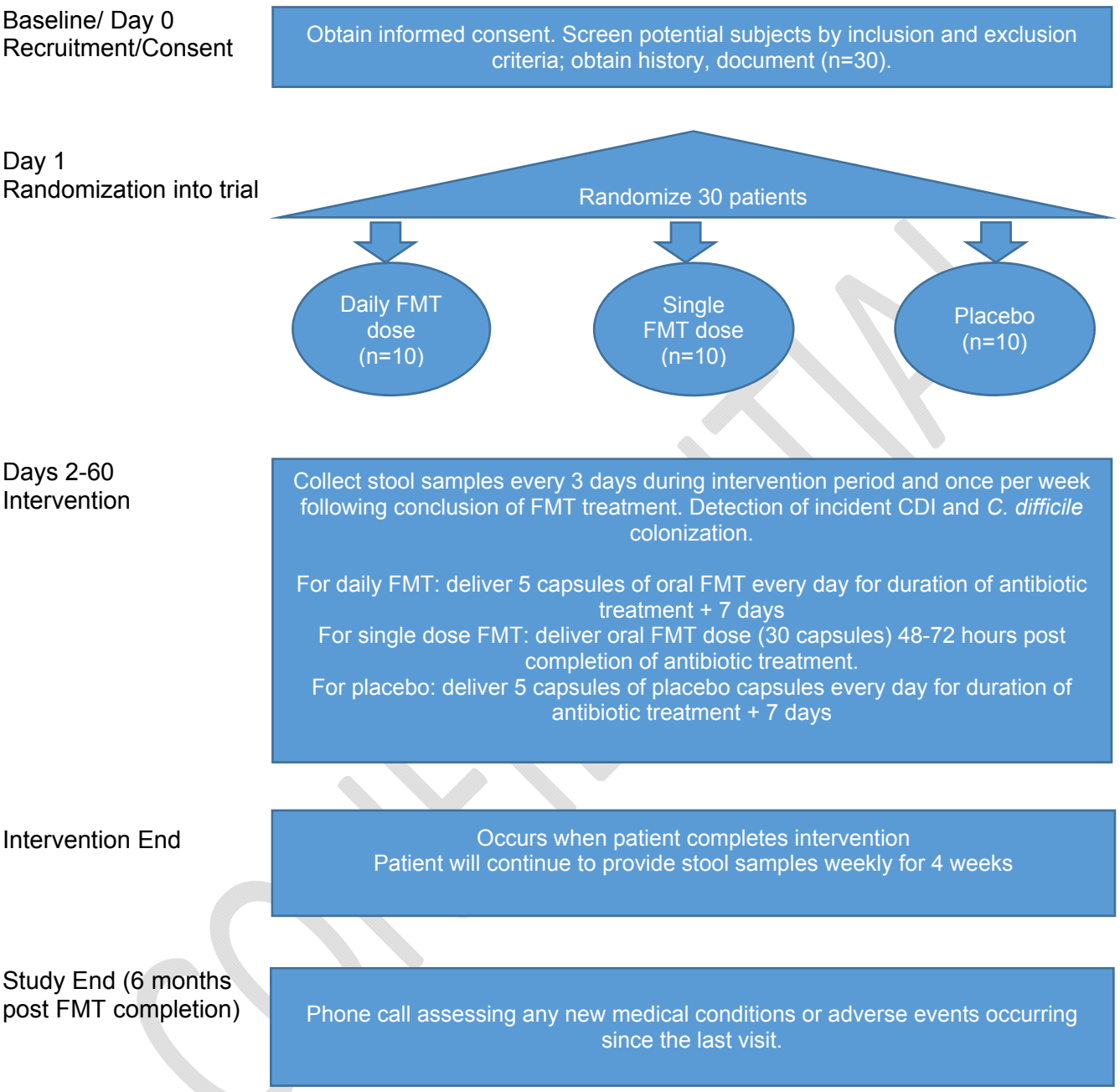
VRE	Vancomycin-resistant *Enterococcus*

2. STUDY SUMMARY

Title	A phase II randomized, double-blind placebo-controlled trial to determine if fecal microbiota transplantation is efficacious for hospitalized patients with *C. difficile* infection history during antibiotic treatment
Short Title and Precis	The GRAFT study
Protocol Number	2017-0789
ClinicalTrials.gov number	NCT03621657
Funding Sponsor	Agency for Healthcare Research and Quality (AHRQ) 5600 Fishers Ln, Rockville, MD 20857 301-427-1364
Study Product	OpenBiome fecal microbiota preparation capsule [FMT Capsule DE]
Phase	Phase II study
Methodology	Randomized, double-blind, placebo-controlled trial
Study Duration	1 year
Study Center(s)	Single-center, University of Wisconsin Hospitals and Clinics
Objectives	The primary objective of this study is to compare the gut microbiota and clinical outcomes of oral FMT during antibiotic treatment, immediately following antibiotic treatment, and placebo. The second objective is to assess the safety and feasibility of daily oral Fecal Microbiome Transplant (FMT) as a treatment option.
Number of Subjects	30 (1:1:1 randomization), 10 subjects per group
Diagnosis	Changes in the gut microbiota and *C. difficile* infection (CDI)
Main Inclusion Criteria	Receiving antibiotics for reasons other than CDI following a recent CDI episode (within last 90 days)
Main Exclusion Criteria	Bowel obstruction or gut motility issues, severely immunocompromised, or admitted to the ICU.
Study Product, Dose, Route, Regimen	FMT will be delivered through oral capsules prepared by OpenBiome. It will be administered in one of two ways depending on the arm of the trial. 1) A low dose of 5 capsules per day during antibiotic treatment and for seven days following conclusion of antibiotics. 2) A single dose of 30 capsules (22.5g) of oral FMT will be given 48-72 hours after antibiotic treatment conclusion.
FDA status of product	Encapsulated fecal microbiota preparation (FMT Capsule DE). Please refer to Finch Therapeutics BBMF # 17194. Sourced from human-derived microbes. Please refer to OpenBiome BBMF # 15543, Section F. IND: 18262

Duration of administration	Group 1: 5 capsules of oral FMT per day undergoing antibiotic treatment and for 7 days following conclusion of antibiotic treatment. Group 2: 30 capsules taken 48-72 hours following conclusion of antibiotic treatment
Reference therapy	Placebo: 5 capsules of oral placebo per day undergoing antibiotic treatment and for 7 days following conclusion of antibiotic treatment.
Statistical Methodology	All data will be analyzed using SAS and R. Patient characteristics will be summarized using descriptive statistics. To assess changes in the gut microbiota, ANCOVA and linear mixed models will be used for longitudinal measurements. Safety and adverse events will be summarized using descriptive statistics.

3. SCHEMATIC OF STUDY DESIGN



4. KEY ROLES

Principal Investigator:
Nasia Safdar MD, PhD
University of Wisconsin-Madison, Division of Infectious Diseases
UW Medicine Foundation Centennial Building
1685 Highland Ave, Madison, WI, 53705
Email: ns2@medicine.wisc.edu

Researchers:
Garret Suen, PhD
University of Wisconsin-Madison, Department of Bacteriology
5159 MSB, 1550 Linden Dr, Madison, WI 53706
Email: gsuen@wisc.edu

Ashley Kates, PhD
University of Wisconsin-Madison, Division of Infectious Diseases
5th Floor UW Medicine Foundation Centennial Building
1685 Highland Ave, Madison, WI, 53705
Email: akates@medicine.wisc.edu

Project Coordinator
Ilsa Gaulke
University of Wisconsin-Madison, Division of Infectious Diseases
H6/581 CSC
600 Highland Ave, Madison, WI 53792
Email: iagaulke@medicine.wisc.edu

Medical Monitor:
Nasia Safdar MD, PhD
University of Wisconsin-Madison, Division of Infectious Diseases
UW Medicine Foundation Centennial Building
1685 Highland Ave, Madison, WI, 53705
Email: ns2@medicine.wisc.edu

Biostatistician/Informatics:
KyungMann Kim, PhD
University of Wisconsin-Madison, Department of Biostatistics and Medical Informatics
600 Highland Ave, BX4675 CSC, Madison, WI 53705
Email: kmkim@biostat.wisc.edu

Data Monitoring Committee:
Contact: Amy Siedschlag
Data Monitoring Committee Protocol Manager
University of Wisconsin-Madison, UW Institute for Clinical & Translational Research
750 Highland Ave. 2112 Health Sciences Learning Center, Madison, WI 53705
Email: asiedschlag@wisc.edu

Study Monitoring Service:
Contact: Tracy Ohrt
Administrative Director, FDA Regulated Research Oversight Program
University of Wisconsin-Madison, UW Institute for Clinical & Translational Research
750 Highland Ave. 2112 Health Sciences Learning Center, Madison, WI 53705
Email: tracy.ohrt@wisc.edu

5. BACKGROUND AND INTRODUCTION

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

5.1. Background and Rationale

Antibiotics have been the leading treatment for bacterial infections over several decades. Unfortunately, antibiotic use in healthcare has led to microbial adaptation, resulting in increasing rates of antibiotic resistant organisms. *Clostridium difficile* is resistant to many antibiotics used to treat infections and therefore proliferates in the intestine of the host during antibiotic treatment. Recent data suggests that *C. difficile* has replaced other antibiotic resistant organisms, such as methicillin-resistant *Staphylococcus aureus*, as the most common etiologic agent for infections in acute care facilities. Of the 2-million people in the U.S. that acquire serious healthcare-acquired infections each year, half a million were due to *C. difficile* infection (CDI), claiming nearly 29,000 lives within 30 days of diagnosis. Additional analyses by the National Hospital Discharge Survey, Annual Files, found that rates of CDI among hospitalized patients aged ≥65 years increased 200%.

Gut microbiota disruption is fundamental to CDI pathogenesis. The gut microbiota is a symbiotic community of microorganisms that play an integral role in preventing pathogen colonization and infection, a feature termed colonization resistance. The pressures of antibiotic use in healthcare settings lead to alterations in native gut microbiota, resulting in loss of colonization resistance. This is especially relevant for acute care hospitals, where susceptible patients are exposed to heavy antibiotic pressure, diet changes, invasive procedures and *C. difficile*. The mitigation and prevention of CDI is a major challenge. Despite treatment with antibiotics metronidazole and vancomycin, recurrence occurs in 24 to 65% of patients. Approximately 12% of patients will experience an additional recurrence and 6% will experience more than two. Concurrent antibiotic use serves as a trigger for recurrent CDI.

5.2. Hypothesis

Specific aim 1: To demonstrate the effects of fecal microbiota transplantation (FMT) compared to placebo on the composition and function of the gut microbiota of hospitalized patients with recent history of CDI and who are currently receiving antibiotic treatment or just completed antibiotic treatment.

Hypothesis: Daily or at completion oral FMT will maintain the diversity and function of the gut microbiome in patients receiving antibiotic treatment.

Specific aim 2: To evaluate the feasibility and safety of concurrent oral FMT and antibiotic treatment in hospitalized patients at high risk for CDI recurrence in a phase II, randomized, controlled trial.

Hypothesis: Patients will be willing to take oral FMT daily or at the completion of antibiotic treatment and oral FMT will be safe for these patients.

5.3. Study Agent

The oral FMT formulation DE capsules will be provided by OpenBiome. These oral FMT capsules are considered an IND by the FDA (IND number: *PENDING*). These are encapsulated fecal microbiota. Refer to Finch Therapeutics BBMF #17192. The fecal microbiota are human-derived microbes. The human source generating the fecal microbes has been extensively screened for infectious pathogens and microbiome mediated diseases using a standardized process (refer to OpenBiome's BBMF#15542, Section F). Additionally, the capsule delivery vehicle enables *targeted deposition* of the investigational active pharmaceutical ingredient into the colon.

5.4. Summary of Clinical Data

FMT-instillation of stool from a healthy donor into the intestine of a CDI patient has been shown to be an effective approach for the treatment of recurrent CDI. A recent randomized controlled trial of FMT in patients with recurrent CDI found a 90% success rate. Its efficacy is thought to be due to restoration of gut microbial diversity and colonization resistance. In mouse models of CDI recurrence, FMT has been shown to be highly effective at reestablishing colonization resistance to *C. difficile* by restoring bacterial community structure in the gut. Current guidelines recommend that FMT be reserved for patients who have had several episodes of recurrent CDI and have exhausted other treatment options. Because of the high risk of recurrence with antibiotic use and lack of effective therapies available, novel and innovative approaches to prevent CDI recurrence in hospitalized patients receiving antibiotics would be a major advance in the field. This is a critical gap that our research group strives to fill**.**

Restoration of gut microbiota and prevention of antibiotic-associated diarrhea and CDI has been studied in several clinical trials using concurrent probiotics with antibiotic therapy with conflicting, although largely positive results. Given that FMT is comprised of entire bacterial communities rather than isolated taxa, FMT offers an ecological advantage. FMT, has been shown to be a highly effective and safe intervention for treatment of recurrent CDI. A recent systematic review and pooled analysis reports FMT was 83% effective in the treatment of recurrent CDI, with few reported adverse events.[13] Although not fully elucidated, the hypothesized mechanism of FMT for CDI is that normal colonic microbiota outcompete and competitively exclude *C. difficile*.[14]

Recently, Osman et al presented effectiveness and safety data from a 2,050 patient cohort among OpenBiome recipients. The data suggests that among the 42 suspected adverse events, 0 were definitely related to FMT material based on NIH criteria after a comprehensive investigation (Appendix 16.1).

5.5. Dose Rationale, Capsule Preparation, and Administration of Study Product

Thirty (30) DE capsules will be administered to the single dose FMT group. This dosing has been used in the past to successfully clear CDI. Five DE capsules or placebo will be administered to the daily groups every day of antibiotic treatment plus seven days post antibiotics. This dose was chosen to determine if lower doses of FMT treatment are effective at CDI prevention during antibiotic treatment and if they are well tolerated by the patient. Participants will undergo capsule administration in the following manner:

1. Document review: Confirmation/review of inclusion and exclusion criteria and contraindications
2. Safety capsules: Direct observed capsule test. Participants will ingest one inert, size 00 “safety” capsule under direct supervision of the study physician/nurse. In the CRU, for the purpose of this study, the CRU nurse will be considered a study nurse.
3. Dietary instructions: Patients in the one-time administration of 30 capsules group should maintain a clear liquid diet 8 hours prior to capsule ingestion and one hour post administration. Patients receiving the daily dosing should fast (clear liquids allowed) for a minimum of 2 hours prior to administration and one hour following administration (clear liquids allowed).
4. Capsule administration:
    a. One-time group: Patients should ingest 30 DE capsules under the direct observation of the study physician/nurse.

       Daily groups: Patients should ingest 5 DE capsules under the direct observation of the study physician/nurse while admitted to the hospital. Following discharge, patients will take 5 DE capsules daily at home. Outpatients will return to the clinic for their first dose of FMT and will ingest the first dose (five capsules) under direct observation of a study nurse/physician. There is currently no published data on home administration of DE capsules; however, this method of administration is currently being used in several studies being conducted by OpenBiome with good compliance and acceptance from the patient populations.

b. Patients should ingest the capsules within 90 minutes of extraction from the freezer. Patients receiving FMT at home will be provided a cooler and freezer packs, a method which has been used in prior studies by OpenBiome, to keep the capsules in good condition. Patients in the daily arms of the trial should ingest the capsules upon removal from the cooler or within 90 minutes. These patients will be asked to record when the capsules are consumed in a monitoring chart.
c. All patients should remain fasting (clear liquids allowed) for 1 hour following capsule ingestion after which they can return to a normal diet.

5.6. Potential Risks and Benefits to Subjects

There are risks associated with conducting this study; however, OpenBiome and its clinical affiliates possess a unique set of resources and expertise to mitigate these risks. To clarify, the risks described below are generalized to FMT therapies that are delivered endoscopically. There is no data available that is specific to FMT therapies that are delivered orally, which is the case for DE. It is expected that the risks of oral FMT therapy will be significantly reduced because many of the risks of FMT therapy delivered via endoscope are due to the procedure itself, not the drug that is delivered.

5.6.1. Known Potential Risks

In the first randomized controlled trial on FMT, mild diarrhea (94%), abdominal cramping (31%) and belching (19%) were observed on the day of colonoscopic infusion; however, these symptoms resolved within 3 hours. Of note, in follow-up, three patients who were treated with donor feces (19%) had constipation. No other adverse events related to the study treatment were reported.

There is a paucity of data on long term follow-up related to FMT. Brandt et al. conducted a retrospective multi-center study (n=77) with a follow up varying between 3 to 68 months and reported 7 deaths. However, none were related to FMT.[37] A recent systematic review on the safety of FMT suggests there are risks including: infection (fever, bacteremia), autoimmune disease (peripheral neuropathy, Sjogren's syndrome, idiopathic thrombocytopenic purpura and rheumatoid arthritis, inflammatory bowel disease flare among patients with ulcerative colitis) and other studies have suggested a link to metabolic syndrome. However, comprehensive screening of donors should decrease any potential future risk. OpenBiome donors are highly selected with only 2.8% of candidate donors qualifying to donate after screening both infectious disease and microbiome-mediated disease (Appendix 16.3).

While there is the potential for additional risks associated with FMT treatment due to the nature of capsules, OpenBiome donors are highly screened before selection to ensure safety. OpenBiome donors undergo a rigorous clinical evaluation with an internal medicine specialist as well as a wide range of screenings for infectious pathogens.

5.6.2. Protection Against Risks

To minimize risks, we will monitor all patients for fever and other signs of infection. All patients will have the phone number for the medical monitor to report any side effects. Subjects will be instructed to seek medical care emergency care if severe illness develops. If it is determined that a subject has a clinical infection due to the FMT, as determined by culture and strain-typing, standard medical care will be provided to the subject.

5.6.3. Potential Benefits to the Subjects

The potential benefit to the subjects is prevention of recurrent CDI. This benefit will only apply to the subjects receiving the FMT. If this study is able to demonstrate oral FMT is able to prevent recurrent CDI in patients at risk for CDI due to current antibiotic treatment, the community at large will benefit by gaining a preventative treatment for *C. difficile*. Additionally, the identification of a non-antibiotic treatment for infection prevention is a benefit to society.

Additionally, a number of case reports have reported decolonization of antibiotic resistant bacteria following FMT, including carbapenem-resistant *Enterobacteraciae* (CRE), extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) enteritis.[23-29] Participants enrolled in this study who have received an FMT may benefit from close health surveillance at follow up consultations in comparison to non-participants.

5.6.4. Risk Minimization

As stated in section 5.6.2, we will ensure patients know the signs of infection and know who to contact if they develop an illness during the study period. Additionally, we will administer the capsules under direct supervision while the patient is in the hospital. Subjects' tolerance for consuming capsules will be established at enrollment visit by giving them an inert size 00 capsule.

6. STUDY OBJECTIVES AND PURPOSE

The goal of this study is to assess the effect of daily oral FMT and a single dose FMT on the gut microbiota in patients currently undergoing antibiotic treatment.

- Primary Objective: To assess the efficacy of oral FMT on the composition and function of the gut microbiota compared to the placebo groups.
- Secondary Objective: To assess the safety and feasibility of both oral FMT regimens versus placebo.
- Secondary Objective: To determine the rate of CDI during oral FMT versus placebo.
- Secondary Objective: To determine the rate of *C. difficile* colonization during oral FMT compared to placebo.
- Secondary Objective: To evaluate time to CDI and/or colonization with *C. difficile*.

7. STUDY DESIGN AND ENDPOINTS

7.1. General Design

This is a phase II, double-blind, randomized, placebo controlled trial assessing the effects of either daily (group 1) or one-time (group 2) oral FMT on the composition and function of the gut microbiome compared to placebo (group 3) in a population of patients with a history of CDI currently on antibiotics for a reason other than current CDI. Participants will be 18 years of age or older, have 72 hours or less of an antibiotic and meet all inclusion criteria while having no exclusion criteria prior to randomization. Patients will be randomized at a 1:1:1 ratio using permuted blocks (block size=10). Randomization will be double-blinded. Each subject (n=30) will be followed for 6 months. Stool samples will be taken at baseline and every 3 days ± 1 day from all subjects regardless of treatment group while the patient is hospitalized. If/when a patient is discharged from the hospital, the patient will continue to provide stool samples every three days during FMT. Following completion of the FMT intervention, the patient will provide stool samples once weekly for the 1 month follow-up period. All patients will be asked to complete a patient diary to report diarrhea history.

Up to one gram of stool will be sent to Finch therapeutics for additional 16s rRNA sequencing.

Intervention: There are 2 intervention groups for this study. Both groups will receive oral FMT capsules from OpenBiome for the prevention of CDI during antibiotic treatment. All patients will fast (clear liquids only) for a minimum of 2 hours prior to administration and 1-hour post-administration for the daily groups and be fasting for 8 hours prior and 1 hour post FMT consumption for the single dose group. The capsules will be administered in the patient's room while admitted or in the ID clinic or CRU if the patient is an outpatient and the first dose will occur under direct observation. Patients admitted to the hospital as well as all patients in the single dose group will be observed for 30 minutes post-administration to ensure the capsules are safely consumed. Hospitalized patients need to be observed 30-minutes post-administration following only the first dose. As mentioned above, the oral FMT capsules will be administered in two ways:

Group 1: This group will receive daily oral FMT capsules. Five capsules will be administered each day of the

patient's antibiotic treatment plus seven days post-antibiotic treatment.

Group 2: This group will receive 30 oral FMT capsules one time 48-72 hours following completion of antibiotic treatment.

Oral FMT capsules: Capsules contain fecal microbiota sourced from healthy donors and screened for pathogens.

Control: The control is an oral placebo capsule identical to the FMT capsules given to the intervention groups and will be provided by OpenBiome. The placebo capsule consists of glycerol and saline and will be administered daily as five capsules per day during the duration of antibiotic treatment plus an additional seven days post-antibiotic treatment (group 3). Patients will be fasting (clear liquids allowed) for at least 2 hours prior to administration and at a minimum of 1-hr post-administration (Appendix 17.1)

7.2. Study Outcome Measures

7.2.1. Primary Study Outcome Measures

The primary outcome of the study is the microbial composition and function of the gut following oral FMT treatment during concurrent antibiotic treatment. Microbial composition of the stool will be assessed using 16s rRNA targeted amplicon sequencing at baseline, every three days (±1 day) during hospitalization and during the intervention, and weekly for one month following completion of the FMT intervention. At the same time points, function of the gut microbiome will be assessed using shotgun metagenomics.

7.2.2. Secondary Study Outcome Measures

1. Safety and feasibility. Safety endpoints are described in 7.2.3. For feasibility, are patients willing and able to take both daily oral FMT capsules or the one day FMT treatment? What are recruitment and retention rates for each group?
2. Recurrence of CDI: Does the patient develop a CDI during the course of the study, assessed through clinical symptoms and PCR for the *C. difficile* toxin in the stool samples collected as described in 7.2.1? If the patient develops a CDI during the study period, what is the time (in days) from randomization into the study to the CDI?
3. *C. difficile* colonization: Is the patient colonized or do they become colonized with *C. difficile* at any point during the study? *C. difficile* colonization will be detected by the presence of *C. difficile* via PCR. If the patient becomes colonized, what is the time (in days) from randomization into the study to colonization?

7.2.3. Primary Safety Outcome Measures

1. Proportion of participants with an adverse event (AE) occurring during any point of the study following randomization.
2. Proportion of participants with a severe adverse event (SAE) occurring during any point of the study following randomization.
3. Proportion of participants with newly acquired transmissible infectious diseases which are considered adverse events of special interest (AESI) after randomization.

8. STUDY SUBJECTS – ENROLLMENT AND WITHDRAWAL

8.1. Subject Population

We plan to enroll 30 subjects, 10 subjects per group, with the goal of having eight analyzable subjects who complete trial in each group. Children under 18 years of age will not be included in this study due to the uncertainty of the safety of the proposed intervention in those under 18 in this population. Participants must

meet inclusion criteria and have no exclusion criteria prior to randomization (described below). We expect the study population to be predominantly Caucasian due to the demographics of Dane County. We expect roughly 14% of the subjects to be minorities and half the subjects to be female. There are no study restrictions based on gender or race/ethnicity. Subjects will be patients at the University of Wisconsin Hospitals and Clinics (UWHC). Two groups of subjects from UWHC will be included in this study, inpatients and/or outpatients. Both study populations will have had a CDI within the last 90 days and are on an antibiotic for an unrelated reason and meeting all additional inclusion criteria listed in section 8.2. Both inpatients and outpatients will be identified through medical record review. Inpatients will always be contacted in a clinical setting while outpatients may be contacted by either mail/phone or in the clinic. Outpatients are included in this study as many infections treated with antibiotics are not severe enough to require hospitalization.

8.2. Inclusion Criteria

1. Cognitively intact and willing to provide informed consent.
2. Willing and able to comply with all study procedures for the duration of the study.
3. Able to take oral medications.
4. Age 18 or over.
5. Recent CDI episode occurring in the last 90 days with completion of therapy as confirmed by the electronic medical record (EMR)
6. Receiving antibiotics at enrollment for reasons other than CDI and having taken the antibiotics for no longer than 72 hours.
7. Women of childbearing potential in a sexual relationship with men must use an acceptable method of contraception (including, but not limited to, barriers with additional spermicidal foam or jelly, intrauterine devices, hormonal contraception started at least 30 days before enrollment into the study, or intercourse with men who underwent a vasectomy) for 4 weeks following completion of the study treatment.
8. Males must agree to avoid impregnation of women during and for 4 weeks following completion of the study treatment.
9. Able to take the test capsule successfully with no signs or symptoms of dysphagia.*

*For patients not receiving treatment during the enrollment visit, the ability to successfully swallow the test capsule can be confirmed the day the intervention is administered.

8.3. Exclusion Criteria

1. Admitted to an intensive care unit (ICU).
2. Females who are pregnant, lactating, or planning to become pregnant during the study. Female patients of childbearing potential will take a pregnancy test at the intervention visit and will be excluded if pregnant.
3. Inability (e.g. dysphagia) to or unwilling to swallow capsules.
4. Known or suspected toxic megacolon and/or known small bowel ileus.
5. Bowel obstruction or other gut motility issues occurring in the last two weeks that are unresolved as noted by the patient or in the EMR.
6. Major gastrointestinal surgery (e.g. significant bowel resection) within 3 months before enrollment not including appendectomy or cholecystectomy.
7. History of bariatric or colectomy surgery.

8. Concurrent intensive induction chemotherapy, radiation therapy, or biologic treatment for an active malignancy. Patients on maintenance chemotherapy may be enrolled after consultation with the medical monitor.
9. Expected life expectancy <6 months.
10. Patients with severe anaphylactic or anaphylactoid food allergy.
11. Solid organ transplant recipients ≤90 days post-transplant or on active treatment for rejection.
12. Neutropenia (≤ 500 neutrophils/mL) or other severe immunosuppression. Anti-TNF will be permitted. Patients on monoclonal antibodies to B and T cells, glucocorticoids, antimetabolites (azathioprine, 6-mercaptoputine, methotrexate), calcineurin inhibitors (tacrolimus, cyclosporine), and mycophenolate mofetil may only be enrolled after consultation with the medical monitor.*
13. At risk of CMV/EBV associated disease, negative IgG testing for cytomegalovirus (CMV) or Epstein Barr Virus (EBV).
14. Any other gastrointestinal illness including diarrhea.
15. On oral vancomycin or metronidazole.
16. Having been taking the currently prescribed antibiotic for over 72 hours.
17. On an antibiotic treatment anticipated to exceed 19 days.
18. Having received an FMT by any route in the 90 days prior to enrollment.
19. Any condition that would jeopardize the safety or rights of the patient, would make it unlikely for the patient to complete the study, or would confound the results of the study.

*Patients enrolled into the trial do not need to be checked for neutropenia. When considering whether a patient meets inclusion/exclusion criteria, conditions commonly causing neutropenia should be only enrolled at the discretion of the medical monitor. Such conditions include, but are not limited to, chemotherapy, chronic idiopathic neutropenia, Kostmann's syndrome, leukemia, myelofibriosis, and sepsis.

8.4. Subject Screening and Retention

8.4.1. Subject Identification

Potential subjects will be identified by the study team who has access to a list of patients admitted to UWHC for infectious diseases that is updated daily. The medical monitor will screen these patients' EMR to determine eligibility. Once potential subjects meeting the eligibility criteria are identified, the treatment team's physician or nurse will ask the patient if he/she is interested in hearing about the study and if so, will introduce the research team. The patient will be invited to hear about the study and, if interested and meeting all eligibility criteria, participate in the study. As patients with community acquired pneumonia (CAP) and urinary tract infections (UTIs) are likely to be on shorter antibiotic courses, initial recruitment will focus on these patients. We, however, do not plan to limit to just CAP and UTI patients and will consider patients admitted to other non-ICU acute care units or outpatients with anticipated short courses (2 weeks or fewer) of antibiotic treatment.

8.4.2. Recruitment and Retention

8.4.2.1. Recruitment

Adult inpatients that are on infectious control list secondary to a recent (90 day) CDI diagnosis and potentially meet the inclusion criteria will be invited to join the study. If patients have been discharged before recruitment, we will utilize the 'mailed letter option' signed by the study PI with clear study descriptions to reach them and schedule a day/time for a phone screening. We will follow-up this

letter with up to three phone calls. If the subject does not respond to the letter or any calls, they will be considered not interested in the study.

If the study team identifies an outpatient through screening that has not previously been contacted and appears to be potentially eligible a study team member will cold call them (using specific telephone script) to assess their interest in the study without mailing a letter first due to the 72-hour enrollment window. If they are interested and meet inclusion criteria they will be invited to a clinic visit for further screening and possible study enrollment.

Potentially eligible patients will also be recruited from ambulatory settings. Clinic providers will be given information on the study and eligibility criteria in order to assess the patient's interest in the study. Interested patients will be contacted by a study team member to further discuss the study and meet for the enrollment visit if indicated. Inpatients who have a diagnosis of CDI within the past 90 days will be approached by the study team to introduce the study and provide contact information for the study should the patients be prescribed antibiotics not targeting CDI.

The mailed letter option may also be utilized for those with known CDI within the past 90 days to alert them to the study with contact information should they be prescribed antibiotics and be interested in joining the study. One method research teams will use to identify potentially eligible patients from ambulatory settings is through viewing schedules of clinics that have a high percentage of potential subjects. These include Infectious Disease, Transplant and Primary Care clinics, and may expand as other focus areas are identified. Patients from these clinic lists will be prescreened for eligibility and approached as previously described. This prescreening process will help ensure only patients who are likely to meet eligibility criteria are approached.

If available, software (ex: Clinithink) may be used as screening aids provided they meet standards for data confidentiality as outlined in the protocol and IRB approval is obtained.

8.4.2.2. Retention

Stool samples will be taken from all subjects at baseline and every 3 days ± 1 day regardless of treatment group while the patient is hospitalized. If/when a patient is discharged from the hospital, the patient will continue to provide stool samples every three days during FMT and once weekly for 4 weeks following FMT. We will call or email the patient weekly until they have completed FMT and once weekly during follow-up to remind him/her to mail us a stool sample and discuss the patient diary. If the patient is receiving either the daily FMT or placebo, we will also remind him/her to be taking his/her capsules as well as ask him/her to count how many pills he/she currently has left for us. If a patient in the one-time FMT group is discharged prior to the FMT consumption time point (48-72 hours post antibiotic treatment completion), he/she will be asked to return to the ID clinic or CRU to receive his/her FMT capsules. Inpatients in the daily and placebo groups will have each dose of 5 capsules observed by a nurse/physician during admission and will be discharged with a cooler containing their FMT capsules and instructed on use. Outpatients in the daily and placebo groups will be asked to come to the ID clinic or CRU to have their first dose observed by a nurse or physician and be given the remaining FMT doses to take home. After discharge, all patients will receive a study brochure tailored to their study arm describing all post-discharge study procedures.

While we will target patients who are on antibiotic doses with a duration of less than 14 days, if a patient's antibiotic regimen exceeds 14 days will be handled in the following manner:

Group 1: Patients will be given the 30 capsule dose of FMT on day 16 or 17 (day 14 + 48 to 72 hours) of antibiotic treatment unless they meet one of the criteria for halting a patient's participation in the study (13.5.2). Participants who are not withdrawn from the study will continue follow-up the same as other patients.

Groups 2 and 3: At 14 days of antibiotics, we will discontinue the daily FMT treatments and patients will move to the weekly follow-up phase of the study unless they meet one of the criteria in section 13.5.2 in which case they will be withdrawn from the study.

8.4.2.3. Subject Capacity

We will ensure each subject's comprehension by asking the subject to explain the basics of the study, including his/her role and the possible risks and benefits after we have walked through the informed consent with him/her. This study will not enroll patients with impaired decision-making abilities.

8.5. Informed consent

The PI will be responsible for ensuring that valid consent is obtained and documented for all subjects. Consent will be obtained in-person during the enrollment/baseline visit. A member of the treatment team will introduce the research team and obtain permission from the potential subjects to discuss the study and provide information. During the enrollment visit, the research team member will explain the relevant aspects of the study (purpose, relevance, disclosure of risks and benefits) and the subject's role in the study.

8.5.1. Process of Consent

The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will adhere to the ICH Harmonised Tripartite Guideline for Good Clinical Practice. Informed consent will be conducted before any protocol-specified procedures or interventions are carried out. Informed consent will be obtained in accordance with 21 CFR 50.25 and 45 CFR 46. Information will be presented both orally and in written form.

An investigator or designee will describe the protocol to potential participants in person. The Participant Information and Consent Form may be read to the participants, but, in any event, the investigator shall give the participants ample opportunity to inquire about details of the study and ask any questions before the signing and dating the consent form.

Study staff must inform participants that the trial involves research, and explain the purpose of the trial, those aspects of the trial that are experimental, any expected benefits, all possible risks, the expected duration of the participant's participation in the trial, the procedures of the research study, and the probability for random assignment to treatment groups. Participants will be informed they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. They must also be informed of alternative procedures that may be available, and the important potential benefits and risks of these available alternative procedures. Participants and/or legal guardian must receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Participants and/or substitute decision maker/proxy guardian must be informed of the anticipated financial expenses, if any, to the participant for participating in the trial, as well as any anticipated prorated payments, if any, to the participant for participating in the trial. They must be informed of whom to contact (e.g., the PI or study physician/nurse practitioner) for answers to any questions relating to the research project. Information will also include the foreseeable circumstances and/or reasons under which the participant's participation in the trial may be terminated. The participants and/or substitute decision maker/proxy must be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the participant is otherwise entitled.

Neither the investigators, nor the trial staff, will coerce or unduly influence a participant to participate or continue to participate in the trial. The extent of the confidentiality of the participants' records must be defined, and participants must be informed that applicable data protection legislation will be followed. Participants and/or substitute decision maker/proxy must be informed that the monitor(s), auditors(s), IRB and regulatory authority(ies) will be granted direct access to the participant's medical records for

verification of clinical trial procedures and/or data without violating the confidentiality of the participant, to the extent permitted by the applicable laws and regulations, and that, by signing a written informed consent form, the participant is authorizing such access. Participants and/or substitute decision maker/proxy must be informed that records identifying the participant will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the participant's identity will remain confidential.

Consent forms are in a language fully comprehensible to the prospective participants. Informed consent will be documented by the use of a written consent form approved by the IRB and signed and dated by the participant or substitute decision maker/proxy and the person who conducted the informed consent discussion. The signature confirms that the consent is based on information that has been provided and all questions have been answered to the prospective participant's satisfaction. Each participant's signed informed consent form must be kept on file by the investigator for possible inspection by Regulatory Authorities and/or the sponsor and Regulatory Compliance persons. The participant should receive a copy of the signed and dated written informed consent form and any other written information provided to the participants, and should receive copies of any signed and dated consent form updates and any amendments to the written information provided to participants.

8.5.2. Consent Form

The consent form can be found in the supporting documents.

8.5.3. Future Use of Stored Specimens

Any leftover stool specimens will be stored and may be used for future research, under a future protocol, to learn more about DE. These specimens will be stored indefinitely after the study is completed. In the informed consent document, participants will be given an opportunity to choose whether or not their specimens are stored for future use. For participants who choose not to allow storage of their samples for future use, these samples will be destroyed at the end of the study. Data collected during the trial will be coded and stored on secure servers for future research as well. Stored specimens will be provided a unique coded identifier and only Dr. Safdar's research team will be allowed access to the linking data. Any specimens shared with other researchers are to be coded and no linking information shared.

There are no benefits to participants in the collection, storage and subsequent research use of specimens. Reports about future research done with participant's samples will NOT be kept in their health records, but participant's samples may be kept with the study records or in other secure areas. Participants can decide if they want their samples to be used for future research or have their samples destroyed at the end of the study. A participant's decision can be changed at any time before the end of the study by notifying the study doctors or nurses in writing. However, if a participant consents to future use and some of their stool has already been used for research purposes, the information from that research may still be used. If a participant decides they no longer want their specimens stored following the completion of the study, the research team will identify their specimens and destroy them. Any specimens already used for other research purposed or shared with other researchers may still be used.

Up to one gram of stool will be sent to Finch therapeutics for additional analysis. Samples will be de-identified.

8.5.4. HIPAA

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). As part of our consent form is a signed subject authorization form.

The HIPAA identifiers that will be collected for this study include:

- The subjects name
- Race
- Birthdate
- Medical record number

8.5.5. Revoking Consent

In the event a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, the research will ask permission to at least collect a final stool sample and/or ask final questions regarding the main outcomes of the study.

8.5.6. Costs to the Subject

The subject will incur no additional costs to their care by being in this study.

8.5.7. Payment to the Subjects

The subjects will be paid $50.00 upon completion of the study.

8.5.8. Exclusion of Women, Minorities, and Children (Special Populations)

Children are excluded for safety reasons.

8.6. Early Withdrawals of Subjects

8.6.1. Premature Termination of the Study

In the unlikely event that significant safety concerns arise, the principal investigator can terminate or halt the study pending review by the DMC. In addition, this study may be halted early based on the DMC charter or FDA recommendations. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, or data quality are addressed and satisfy the sponsor, IRB, DMC, and/or FDA.

8.6.2. When and How to Withdraw Subjects

All subjects will be informed during consent that consent is voluntary and they may discontinue participation at any time. If a subject wishes to withdraw from the study, we will ask him/her to contact the study coordinator whose number will be in the enrollment materials.

8.6.2.1. Data Collection and Follow-up for Withdrawn Subjects

If the subject is willing, the coordinator will ask to schedule a close-out study visit to collect a final stool sample and follow-up safety evaluation.

A subject may withdraw from the study at any time for any reason with no consequences. A participant

may be withdrawn from the study by the Investigator for the following reasons:

- Adverse event that may make it no longer in the best interest of the subject to continue to participate
- Recurrent *C.diff* infection
- Participant's choice
- Protocol violation/ non-compliance
- Pregnancy
- Lost to follow-up
- Other (must be noted)

The primary reason for withdrawal from the study will be recorded. Participants will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 11.4. Although participants are free to withdraw at any time, subjects will be encouraged to remain in the study for follow-up safety evaluation. Every attempt should be made to follow all AEs and SAEs ongoing at the time of early withdrawal to resolution or until stabilized. If possible, a final stool sample will be collected at the time of Early Termination. All subjects who are randomized will be included in the safety analysis.

8.7. Participant Confidentiality

Participant confidentiality is held strictly in trust by the PI, his/her staff, and his/her agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating participants.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval from the PI.

The PI or delegate and the FDA may inspect all documents and records required including, but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study.

9. STUDY AGENT

9.1. Description and Formulation

DE is encapsulated fecal microbiota sourced from human-derived microbes. The human source generating the fecal microbes has been extensively screened for infectious pathogens and microbiome mediated diseases using a standardized process (found in OpenBiome's BBMF # 15543, Section F). Additionally, the capsule delivery vehicle enables *targeted deposition* of the investigational active pharmaceutical ingredient into the colon.

DE capsules: The OpenBiome FMT Capsule DE contains frozen human fecal microbiota filtered to 330 microns, theobroma oil, glycerol, hide bovine gelatin, sodium lauryl sulfate, colorants FD&C, and titanium dioxide. The material is contained in a two capsule system: an interior gelatin capsule that houses the drug substance, and an exterior acid-resistant capsule for delivery of the drug substance past the stomach. The product should be stored at -20°C to -80°C. Product expires 6 months after freezing if stored at -20°C.

Placebo capsules: Placebo capsules will be identical in appearance to DE capsules but will not have human feces, the active pharmaceutical ingredient. The placebo capsules are filled with Sodium Chloride (0.9%, USP), Glycerol (12.5%, USP) and deionized water to the previously stated volume specification. Capsules are stored frozen at -80˚C before dispensing to local site storage. Placebo will be administered the same as the daily arm of the trial.

9.2. Packaging and Dissemination

OpenBiome will package the placebo and FMT capsules at their facility. Packaging will be identical and consist of the same saline and glycerol buffer used in FMT processing without the addition of stool. The capsules will be sent to the UW Pharmaceutical Research Center (PRC) to be dispensed.

An inventory will be performed and a drug receipt log filled out and signed by the person accepting the shipment. The designated study staff will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files.

The site principal investigator (PI) (or designee) will maintain an accurate record of the receipt of the investigational materials as shipped by OpenBiome, including the date received. One copy of this receipt will be returned to OpenBiome when the contents of the investigational materials shipment have been verified. In addition, the unblinded laboratory technician based at the PRC will maintain a log of all clinical trial materials (DE and placebo) dispensed. This clinical trial material accountability record will be available for inspection at any time.

9.3. Preparation, Administration and Storage of Study Drug

Produced material will be stored at -80°C and each unit will have a date of production printed on it for tracking purposes. Studies have been conducted to ensure long-term bacterial viability following the freezing process based on studies conducted by Hamilton et al. and Youngster et al.[40,41] Local site storage at -20°C will be permitted for up to 6 months. Discharged patients will be provided with a kit and instructions on proper at-home storage.

9.4. Route of Administration

Participants will undergo administration:

1. Document Review: Confirmation/review of exclusion criteria/contraindications as previously described at baseline.
2. Safety Capsules: Direct observed capsule test, where participants will ingest one inert, size 00 'safety' capsule under direct supervision of the study physician/nurse.
3. Dietary instructions: Patients should fast (clear liquids allowed) for a minimum of 2 hours prior to administration.
4. Capsule administration:
    a. Patient should ingest the capsules of either FMT or placebo (5 capsules daily or a one-time dose of 30 capsules depending on treatment group) under the direct observation of a physician/ nurse while they are admitted.
    b. Patient should ingest capsules after extraction from freezer, and no longer than 90 minutes after removal.
    c. Patients should remain fasting (clear liquids allowed) for 1 hour following ingestion but may then return to a full diet.

9.5. Prior and Concomitant Therapy/ Standard of Care

We will collect the following information on the patients care during their stay:

- Current medication list
- Antibiotic they are on and dose
- Vital signs at baseline and discharge (taken from medical record): temperature, height, weight, heart rate, blood pressure, O_2 saturation if on chronic oxygen therapy.
- Overall assessment of current health

9.6. Randomization and Blinding of Study Drug

Participants meeting the eligibility criteria and willing to consent to the study procedures will be randomized on a 1:1:1 basis with block sizes of 10 to receive one of the following treatments:

1. Daily FMT treatment with 5 capsules/day
2. One-time FMT treatment of 30 capsules
3. Daily placebo (5 capsules/day)

Randomization will be carried out by the UW PRC so that the research team and treatment team remains blinded to whether the patient is receiving FMT or placebo.

9.7. Accountability Procedures for the Study Intervention/Investigational Product

The site principal investigator (PI) (or designee) will maintain an accurate record of the receipt of the investigational materials as shipped, including the date received. One copy of this receipt will be returned to OpenBiome when the contents of the investigational materials shipment have been verified. In addition, the unblinded laboratory technician based at OpenBiome will maintain a log of all clinical trial materials (DE and placebo) dispensed. This clinical trial material accountability record will be available for inspection at any time.

Participants in the daily FMT or placebo groups will be given a diary to record when doses are taken and number of doses remaining. This diary will be collected after participants complete the treatment portion of the study. Study staff will attempt to collect any remaining study capsules from the participants in person or via mail as appropriate, and this accountability will be reviewed by the Infectious Disease Research Lab.

9.8. Modification of Study Intervention/Investigational Product for a Participant

If the participant is unable to complete the full dose of DE or placebo in one sitting the actual dose ingested will be recorded. Further study product administration, including the remainder of the incomplete dose and scheduled upcoming doses as applicable will be at the discretion of the treating physician. The remaining capsules scheduled to be administered may be offered to the participant on the same day they were scheduled. Intention-to-treat analysis will be utilized according to best practices in clinical trials.

9.9. Concomitant Medications/Treatments

Given our patient population is likely to have multiple comorbidities, all medications except those listed in the exclusion criteria will be accepted in this study, at the discretion of the treating physician. Though the exclusion criteria lists antibiotics used for more than 72 hours as an exclusionary medication, patients on long-term antibiotics – such as prophylactic Bactrim, azithromycin, or doxycycline – are eligible at the discretion of the study physician. This includes, but is not limited to:

1. Long-term antibiotics (one month or more, e.g. prophylactic Bactrim, azithromycin, or doxycycline)
2. Anti-hypertensive therapies
3. Heart failure medications
4. Diuretics
5. Laxatives
6. Topical medications
7. Anti-depressants
8. Statins
9. Diabetic medication

10. Non-steroidal Anti-inflammatory Drug (NSAID)
11. Acid-blockers
12. Anticoagulants
13. Opioids
14. Iron supplementation
15. Anti-TNF will be permitted. Patients on monoclonal antibodies to B and T cells, glucocorticoids, antimetabolites (azathioprine, 6-mercaptopurine, methotrexate), calcineurin inhibitors (tacrolimus, cyclosporine) and mycophenolate mofetil may be enrolled only after consultation with the medical monitor.

10. LABS

Tests will not be done in a CLIA-certified lab. Samples will be collected from the patient and brought to the lab while the subject is admitted. After discharge, the subject will mail the samples to the laboratory for processing. Samples will be labeled with only an ID number. We will not disclose any results to the patients. Data generated by the laboratory procedures will be used to determine the patient's colonization status with MDROs and *C. difficile* at baseline and to determine if colonization status changes during the course of the trial.

Laboratory procedures include:

- Bacterial DNA isolation from stool
- 16s rRNA sequencing of the v4 region
- Culture to identify MDROs, including but not limited to: MRSA, Vancomycin-resistant enterococcus (VRE), and *C. difficile*
- Polymerase Chain Reaction (PCR)

Data from laboratory procedures will also be used to determine if the FMT treatment has an impact on the patients gut microbiome. All stool remaining following the completion of laboratory procedures will be stored at -80°C and labeled with the sample ID and held for future analysis.

Up to one gram of stool will be sent to Finch therapeutics for additional 16s rRNA sequencing.

11. STUDY VISITS

11.1. Screening/Enrollment, and Baseline Visit

For some patients the screening, enrollment, and baseline visit should occur on the same day. However, the enrollment and baseline visit may be at a later time (though it must still be within the first 3 doses of the prescribed antibiotic window) if needed to meet the patient's needs.

Screening: Will take place in a clinical setting
Active screening. Any inpatient admitted to a non-ICU unit or outpatient presenting to any UWHC clinic (with a focus on patients presenting with CAP and UTI) meeting the enrollment criteria will be contacted about the study. Patients may also be identified through letters mailed to patients with a history of CDI in the last 90 days.

Enrollment: Will take place in a clinical setting
All patients with a history of CDI on anitbiotics for any reason other than CDI meeting the eligibility criteria will be eligible for enrollment. Eligibility will be confirmed during the enrollment visit. Patients admitting with CAP and UTIs will be preferred. A detailed consent process will be administered by study staff who are trained in consenting procedures. Potential participants who fulfill the eligibility criteria will undergo a detailed clinical assessment by the research team which will include demographics, medical history, dietary

information and current medication. Data from this assessment may be from the participant/substitute decision maker, healthcare team and/or medical record.

- Data points at enrollment study visit:
  - General parameters:
    - Date of birth
    - Sex
    - Smoking status
    - Race
    - Ethnicity
    - Highest level of education
  - Past medical history and allergies
    - A focused collection of past medical history (including common infections)
  - Current medication list
    - Current antibiotic use, including dose
  - Dietary history
  - Social history
    - Alcohol, smoking history
  - Recent hospitalizations
  - Exposure to farm animals
  - Recent international travel history
  - Examination findings
    - Vital signs: temperature, height, weight, heart rate, blood pressure, O2 saturation if on chronic oxygen therapy. (Vital signs measurements at all time points may be abstracted from medical records if available.)
    - Relevant review of symptoms
  - Overall assessment of current health
  - Stool consistency (Bristol Stool Scale) and frequency
- Sample collection
  - Stool sample collected using a stool collection kit/hat and provided with patient diary
- Formal aspects
  - Informed consent procedure
  - Verification of inclusion and exclusion criteria including administering 'test' capsule and documenting:
    - Safe to receive intervention (yes/no)
    - Able to swallow test capsule (yes/no)

Immunocompromised patients at risk for CMV/EBV will have blood drawn for the EBV and CMV antibody test done by the UWHC lab. The blood draw will done by a UWHC phlebotomist or their local lab if this is preferred by the patient. All costs will be covered by the study. Results will be released to the patient and included in their medical record.

Baseline (Day 0): Will take place in a clinical setting
After the baseline clinical asssessment and stool sample have been collected, subjects in groups 1 and 3 (daily FMT and daily placebo) are eligible to undergo adminstration of the intervention (DE or placebo) in

accordance with the randomization schedule. The intervention will be administered by the blinded treatment staff and the participant observed for 30 minutes following administration. Post-ingestion observation of inpatients will only occur after the first dosing; subsequent inpatient dosing will be administered by a study physician/nurse but do not require 30 minutes of observation. Pre-intervention vital signs (as previously described) will be collected or abstracted from the medical record prior to first dose. Participants will be provided with information regarding monitoring for minor and severe adverse event related to the intervention administration. Participants will be encouraged to report any concerning symptoms to staff. Oral nutrition may commence 1 hour post-capsules administration. Study staff do not need to collect a blood sample to check for neutropenia prior to dosing.

For all patients, a baseline stool sample will be collected and all patients should begin to fill out their patient diary starting on Day 0. If a patient is unable to provide a stool sample, peri-rectal swabs may be collected.

11.2. Follow-up

Follow up will be different based on the group the patient is in.

Group 1 (daily FMT) and Group 3 (daily placebo): Will occur in a clinical setting for inpatients.
Capsules will be delivered to the patients every day by a blinded member of the treatment team during the time they are admitted. If the patient is discharged or an outpatient, he/she will be provided with enough capsules to complete the study and will be asked to consume 5 capsules per day while on antibiotics as well as for 7 days post-antibiotic treatment completion. Outpatients and patients discharged during the follow-up period will be called or emailed (depending on patient preference) weekly to remind them to take their capsules as well as to send in their stool samples every three days (+/- 1 day). The researcher calling the subject will also ask the subject to count the number of pills he or she has remaining (recorded in the patient diary) and ask about any adverse events or changes to medications and diet. Study staff do not need to collect a blood sample to monitor for neutropenia. If the subject has any capsules remaining after completion of study intervention (e.g. missed doses, shortened antibiotic course after study intervention has been dispensed), the subject will be asked to return the remaining capsules to the Infectious Disease Research lab.

Group 2 (single dose FMT): Visit will occur in a clinical setting
Capsules will be delivered to the patient by a blinded member of the treatment team 48-72 hours following antibiotic treatment completion. If the patient is discharged he/she will be asked to return to the clinic 48-72 hours following completion of his/her antibiotics to consume the 30 capsules under supervision of a nurse or physician. Study staff do not need to collect a blood sample to monitor for neutropenia. During this visit the following will occur:

- Vitals collection
- Physical exam
- Clinical symptoms assessment
- Diet assessment
- Concomitant medications
- Stool sample
- FMT dosing
- Adverse events assessment
- 30 minute post-dose observation

All subjects:
All subjects will provide stool samples every three days after the baseline visit until their FMT intervention is complete and weekly for one month following conclusion of FMT treatment. Patients will also be asked to complete a patient diary daily from enrollment through the first month of follow-up. If a subject is discharged during the study period, he/she will be asked to complete the patient diary from home as well as provide stool samples to be mailed back to the lab for processing. A member of the study team will also call or email patients on a weekly basis during the intervention and follow-up period to remind to complete the stool sample

as well as record any adverse events. If a patient is discharged, vital signs, general health status, and medications at discharge will be assessed through the medical record.

At the end of the 1 month follow-up (last week of providing the weekly, post-FMT stool samples), patients will receive a call assessing the following:

- Clinical assessment by study nurse or physician, specifically evaluating adverse events related to the intervention.
- Data collection at visit:
  - Interim medical history with focus on infectious diseases
  - Concomitant medication
  - Significant changes in diet
  - Changes in stool consistency (Bristol Stool Scale) and frequency
  - General health status
  - Adverse events (NIH criteria)

A table of all study events is provided in Appendix II, 17.2 (Study Calendar).

11.3. Unscheduled Visits/Data Collection

If a patient develops CDI or related serious adverse event, an unscheduled visit will take place and the patient will be treated with standard recurrent CDI care by the treatment team. The development of a CDI will also result in early termination of the patient's study participation, and early termination procedures should be conducted. Other serious adverse events will be evaluated on a case-by-case basis for continuation versus termination of participation.

If a patient develops an infection (besides any he/she was admitted for) with a MDRO, the study team will track the duration of the infection. Any changes to the type of antibiotic, duration, and dosage will be noted as well.

Data points for infectious episode:

- Date
- Preliminary diagnosis
- Physical exam and vital signs
- Changes to diet
- Clinical symptoms questionnaire
- Method of testing for pathogen and pathogen(s) identified
- Sites from which organism was isolated and whether clinical specimens were collected from infection site
- Antibiotic(s)
  - Name
  - Dosage
  - Prescribed duration
  - Final (actual) duration of antibiotics
  - Change in antibiotics regimen
- Concomitant medication (Name, dose, route of administration, duration)
- Hospital admission (yes/no)
  - Name of facility
  - Department where participant was admitted (ward / step down/ ICU)

- Relevant medical procedures
  - Colonoscopy (including preparation)
  - Intestinal surgery
  - Dialysis
  - Other
- Final diagnosis

Data points collected at both the early termination and unscheduled visit only need to be recorded once to avoid duplication. This may include vitals, changes to diet, etc.

11.4. Early Termination

In the case of an early termination, all study procedures will stop. Study staff will attempt to collect patient diary and a final stool sample. The following will be assessed:

- Clinical assessment, including vital signs if visit is done in person
- Data collection
  - Changes to dietary history
  - Discharge diagnosis
  - Stool consistency and frequency
  - Changes to concomitant medications
  - Clinical symptoms
- Stool sample collection or peri-rectal swab (only if participant is unable to provide a stool sample at in-person visit)

Early termination procedures may be done over the phone or in person if the early termination is occurring during an unscheduled visit. Participants will be asked if study staff may follow participants passively using the medical record if clinical follow-up and/or stool collection is not viable.

11.5. Study End

All subjects will be called 6 months following completion of FMT treatment.

- Data collected during the final study visit call:
  - Changes to medical history
  - Changes in stool consistency (Bristol Stool Scale) and frequency
  - Adverse events (including SAEs, NIH criteria)

12. STUDY ANALYSIS

12.1. Sample Size

A total of 30 subjects – 10 per treatment group – will be recruited into this study. As this is a pilot study, sample size was not driven by a statistical test of hypotheses, instead it was driven by a pool of available patients eligible and willing to participate.

12.2. Subject Population for Analysis

We plan to enroll 10 patients per group with the goal of having 8 patients per group complete the treatment and be analyzed in the final analysis.

12.3. Final Analytic Plan

Categorical data will be described using descriptive statistics (proportions and percentages). Continuous data will be described using means and standard deviations (normally distributed data) or using medians and interquartile range (non-parametric data). Appropriate comparative statistical tests will be chosen based

in the variable types (categorical, dichotomous, continuous) and distribution (parametric, non-parametric) and will be used to describe significant differences between intervention and control groups. Where appropriate, point estimates and confidence intervals will be reported. The p-value will be two tailed with a significance level of 0.05.

The microbiome will be assessed using 16s rRNA targeted amplicon sequencing. Sequences will be cleaned and binned into operational taxonomic units and assigned taxonomy. Differences within individuals will be assessed using the Inverse Simpsons Diversity index and comparisons between study groups will be done using the Bray Curtis dissimilarity matrix and visualized using principal coordinates analysis.

12.4. Source Documents and Access to Source Data/Documents

We will maintain appropriate medical and research records for this trial, in compliance with ICH E6 GCP, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of participants. Forms for use as source documents will be derived from the electronic CRFs. Additional source data include records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, imaging, and participant files and records kept at the pharmacy, laboratories, and medico-technical departments involved in the clinical trial.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval from the PI.

The study monitor or other authorized representatives of the research team and FDA may inspect all documents and records required to be maintained by the Investigator, including, but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

All samples and forms will be encoded (labeled) only with a barcode and a unique tracking number to protect participant's confidentiality.

Up to one gram of stool from each collected sample will be sent to Finch therapeutics for additional 16s rRNA sequencing. Samples sent to Finch will be de-identified.

12.5. Quality Management System

The monitoring of subjects enrolled in this study includes both internal and external activities governing the administration of the investigational product. Internal monitoring is performed by members of the study team and external monitoring by a team of staff not involved in the conduct of study procedures with the appropriate clinical and technical expertise.

12.5.1. Study Team Training

The PI will ensure that members of the study team are appropriately qualified and trained on the protocol, and/or on study procedures applicable to their delegated roles/responsibilities. When the protocol and/or methods to conduct study procedures are updated, staff are retrained prior to performing any study-related activities described in the updated document. All training is documented and maintained in the study files.

Per University of Wisconsin-Madison policy, all personnel engaging in human subjects research must also complete Human Subjects Protection, Good Clinical Practice (GCP), and Health Insurance Portability and Accountability Act (HIPAA) training.

12.5.2. Standardized Procedures

A Manual of Procedures (MOP) has been created for this study to supplement the study protocol by providing additional details on the conduct of the study and study procedures. It is routinely updated to ensure consistent performance of study activities.

12.5.3. Internal Quality Monitoring

Members of the study team will conduct internal monitoring activities in real time when completing the study records and following each study visit. These activities will include, but are not limited to, periodic review of completed data collection forms, eligibility records, regulatory files and electronic data. Reviews will be performed by a different individual than those involved in data entry.

12.5.4. External Quality Monitoring

The purpose of external study monitoring is to ensure compliance with applicable regulations (21 CFR 312 and GCP), which require monitors to verify that:

- The rights and well-being of human subjects are protected.
- Reported study data are accurate, complete, and verifiable from source documentation.
- The conduct of the trial is in compliance with the currently approved protocol and all applicable regulatory requirements.

The sponsor-investigator has contracted with UW ICTR Study Monitoring Service. Refer to the ICTR SMS Monitoring Plan and Service Agreement for more information. The PI will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing, and inspection by local and regulatory authorities.

12.5.5. Database Quality Control (QC)

QC procedures regarding data entry will be implemented. Regular data quality control checks will be run on the database. Any missing data or data anomalies will be assessed for clarification and resolution.

12.6. Data Capture

12.6.1. Case Report Forms

The study case report form (CRF) is a data reporting instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, a single straight line is drawn through the incorrect entry and the correct data is entered above/near the correction. All such changes must be initialed and dated.

12.6.2. Data Collection Tools

Clinical data (including AEs, concomitant medications, and solicited events data) and clinical laboratory data will be entered into a secure, web-based Research Electronic Data Capture (REDCap) system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

12.7. Records Retention

Study files (except for future use consent forms) must be maintained for a minimum of two years after the

last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the lead PI or designate, if applicable. It is the responsibility of the lead PI or designate to inform the site PIs when these documents no longer need to be retained. Consent forms for future use will be maintained as long as the sample exists.

13. ASSESSMENT OF SAFETY

Safety will be assessed by the frequency and severity of adverse events (AE).

13.1. Specifications of Safety Parameters

13.1.1. Definition of Adverse Events (AE)

Adverse events (AEs) will be recorded at administration of the treatment and during phone calls once the patient is discharged, in the study patient record (source document) as well as on a specific AE case report form (CRF).

An AE is any untoward medical occurrence in a study patient or clinical investigation participant administered a pharmaceutical product. An AE does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product, e.g.:

- any new clinical diagnosis
- any symptom that requires medical clarification or leads to in-patient admission (surgery or accident)
- any suspected adverse drug reaction (ADR)
- any symptom, such as fever, that appears on the study patient's medical records
- any event related in time with the application of the study medication and affecting the health of the study patient (including laboratory value changes)

For the purposes of this study, baseline symptoms will be collected prior to the administration of study intervention. Only those symptoms identified as new or worsened compared to the baseline assessment will be recorded as an AE. In addition, only those abnormal laboratory values or physical examination assessments that are considered clinically significant will be recorded in the study documents.

If there is any doubt as to whether a clinical observation is an AE, the event will be recorded and assessed by the study medical monitor. AEs must be graded for severity and relationship to study product. Adverse events of special interest (AESI) will be defined as newly acquired transmissible infectious diseases.

13.1.2. Definition of Serious Adverse Events (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of the PI, it results in any of the following outcomes:

- Death
- Life-threatening adverse event*
- Inpatient hospitalization or prolongation of existing hospitalization
    - Subjects might be admitted to an inpatient unit to be eligible for the study; in these cases the initial hospitalization will not be considered an SAE in and of itself, rather it will only be considered an SAE if the planned hospitalization is prolonged
- A congenital anomaly/birth defect

- Persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life function
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered when, based upon appropriate medical judgement, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

*Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of the PI, its occurrence places the patient or participant at immediate risk of death. It does not include an adverse event which, had it occurred in a more severe form, might have caused death.

Any adverse event or suspected adverse reaction that meets the criteria for serious adverse event will be:

- recorded on the appropriate SAE CRF
- followed through resolution by a study clinician or up to 30 days post-treatment, whichever comes first
- reviewed and evaluated by a study clinician

13.1.3. Definition of Unanticipated Problems (UP)

On enrollment in the study, the study participants will be instructed to contact the medical monitor if an AE occurs. Patients will be given a patient diary with date, details and action taken to help with data collection. This diary will be taken to the site PI for evaluation if needed and patients instructed to seek immediate medical attention if indicated.

13.2. Classification of an Adverse Event

13.2.1. NIH Grading of Severity of Events

AEs will be assessed by the clinician using the NIH Common Terminology Criteria for Adverse Events (CTCAE) defined grading system (Appendix 17.3). Briefly, the criteria for estimating adverse event severity grade:

- **Grade 1, Mild:** Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Grade 2, Moderate:** Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).
- **Grade 3, Severe:** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- **Grade 4, Life threatening:** Places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.
- **Grade 5, Death**

13.2.2. Relationship to Study Agent

All adverse events, regardless of relatedness, should be reported. All adverse events should be evaluated for relatedness when reporting and documenting on the CRF.

The following guidelines of relatedness are used modified from the NIH guidelines:

Related: The adverse event is 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.

Not Related: The adverse event is 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 a causal relationship is considered biologically implausible.

13.2.3. Solicited Adverse Events after FMT

In addition to open-ended questions on adverse events meeting the above definitions, specific potential adverse events will be inquired about during the follow up period (following intervention through to 6 months after randomization):

Symptom that is clinically more severe than participant's baseline	**Severity**				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Fever*	38.0 - 39.0 degrees C (100.4 - 102.2 degrees F)	>39.0 - 40.0 degrees C (102.3 - 104.0 degrees F)	>40.0 degrees C (>104.0 degrees F) for <=24 hrs	>40.0 degrees C (>104.0 degrees F) for >24 hrs	Death
Diarrhea	Increase of <4 stools per day over baseline pre-FMT; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline; limiting instrumental ADL	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated	Death
Vomiting	1 - 2 episodes (separated by 5 minutes) in 24 hrs	3 - 5 episodes (separated by 5 minutes) in 24 hrs	>=6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death
Abdominal Pain	Mild pain	Moderate pain; limiting instrumental activities of daily life	Severe pain; limiting self-care activities of daily life	n/a	n/a
Bloating	No change in bowel function or oral intake	Systemic, decreased oral intake; change in bowel function	n/a	n/a	n/a

Symptom that is clinically more severe than participant's baseline	Severity				
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Constipation	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas indicated; limiting instrumental activities of daily life	Symptoms interfering with self-care activities of daily life; obstipation with manual evacuation indicated	Life-threatening consequences (e.g. obstruction, toxic megacolon); urgent intervention indicated	Death

13.3. Adverse Event Documentation and Reporting Procedures

13.3.1. Procedures to Document AEs

Study participants will be instructed to contact medical monitor if any serious or unexpected adverse event occurs. Study staff will inquire about AEs when treatment is administered and during the follow-up phone calls following the patients discharge. AE's will be recorded and documented in detail in an AE CRF.

AE information to be collected in the AE CRF:

- Nature of the event
- Date of onset
- Concomitant treatment: product (generic name), indication, dosage, dosage interval, presentation, mode of administration, administration regimen
- Duration of the AE
- Severity
- Seriousness
- Causality
- Outcome

The course and outcome of the adverse event will be commented on as follows:

- Recovered without sequelae
- Not yet recovered
- Recovered with sequelae
- Fatal

13.3.2. Procedures to Report AEs

Any SAE (including death, irrespective of the cause) occurring during the study will be immediately reviewed by the PI/medical monitor, the DMC, and the Health Sciences IRB (the IRB of record for this study).

If determined to be a SUSAR (suspected unexpected serious adverse reaction – SUSAR; Appendix II) by the PI/medical monitor, the event will be reviewed by the DMC. Regulatory filing to the FDA or local

IRB as per standard practices will occur. Any hospital related death due to a bowel obstruction or infection that could be related to FMT will be reviewed by the DMC to discuss a temporary suspension of the protocol until a full safety review is conducted.

In case of a SAE (non-SUSAR), the site PI will report the event to the study medical monitor and lead PI for review and compilation. The DMC will review all SAEs every 6 months or *ad hoc* depending on the clinical case at the discretion of the PI/medical monitor. A specific SAE CRF will be provided similar to Appendix 17.3. The report must contain a detailed description of the symptoms observed and the concomitant treatment administered. Furthermore, the report must comment on a possible causative relationship between the AE and the trial medication. Each SAE must be followed until it is resolved or can be explained satisfactorily.

For non-serious adverse reactions (definitely related, not related) the medical monitor/PI will complete a report. All non-serious adverse reactions will be reviewed by the DMC every 6 months or *ad hoc* depending on the clinical case at the discretion of the PI/medical monitor.

In accordance with safety requirements, the PI will inform the local IRB and will make sure that the involved persons will obtain adequate information. The following instructions must be heeded:

- In the case of an intolerable SAE, the study patient must, at the decision of the PI, be withdrawn from further treatment/placebo, and symptomatic treatment must be administered. The participant may opt to voluntarily provide samples for duration of study.
- The measures taken must be recorded on the CRF.
- In accordance with local legislation, the PI will submit copies of the final SAE-report to the Regulatory Authorities concerned, if necessary.

13.4. Follow-up after Adverse Events

AEs will be followed until resolution or stability even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Follow-up procedures, evaluations, and outcomes will be recorded on the participant's case report forms.

13.5. Study Halting Rules

13.5.1. Study Halting Rules

Enrollment and administration of study intervention will be suspended pending a safety review by the DSMB to determine whether the study will be terminated or re-initiated in the following situations:

- Three or more of the randomized participants in a study treatment group have a Grade 3 AE of the same organ system deemed related to the study intervention.
- Any serious adverse event of an enrolled participant related to the study intervention including transmission of a pathogen from donor to recipient.
- An overall pattern of symptomatic, clinical, or laboratory events that the Medical Monitor considers related to study product and that may appear minor in terms of individual events, but that may collectively represent a serious potential concern for safety.

The FDA and AHRQ will be notified of any study halt that occurs as a result of any of the above halting criteria.

13.5.2. Individual's Halting Rights

Participants who meet any of the following criteria must be assessed by the site PI to determine if it is in the participant's best interest to stop the study product(s):

- Participant choice (withdrawal of consent).
- Participant's non-compliance.
- Development of a significant medical condition and/or participation in the study is no longer in the best interest of the participant.
- Development of a severe or serious AE related to the FMT product.

13.6. Safety Oversight – Data Monitoring Committee (DMC)

UW-ICTR has established a Data Monitoring Committee (DMC) to provide a key resource for UW-Madison investigators conducting clinical research. This DMC will provide investigators services to ensure appropriate measures are in place to promote subject safety, research integrity and compliance with federal regulations and local policies for individual clinical research protocols in need of DMC review (as determined by the Principal Investigator (PI), the funding agency, the local Scientific Review Committee, or the local IRB, and for which no DMC exists). For these studies, the UW ICTR DMC will be the primary data and safety advisory group for the Principal Investigator.

The DMC is supported in its mission of safety and compliance by experienced ICTR staff to provide administrative assistance, experienced members representing a diversity of backgrounds, skills and knowledge, and the use of the Research Electronic Data Capture (REDCap) tool which provides data management functionality by allowing the development of eCRFs and surveys to support data capture. In providing oversight for the conduct of this study, the ICTR DMC will meet every 6 months during the 1-year study. Additional meetings may be scheduled as determined by the DMC or as requested by the PI. The DMC members will review protocol-specific reports created by statisticians that serve a non-voting member role on the DMC using data pulled from REDCap. These standard reports will include an overview of study objectives, a review of actual and projected accrual rates, an evaluation of patient demographics for balance of randomization, and a summary of the number and seriousness of adverse events. An interim analysis of study results may be performed and source documents may be reviewed to allow the DMC to independently judge whether the overall integrity and conduct of the protocol remain acceptable based on data provided and reported by the Principal Investigator. Additionally, the DMC may conduct a safety interim analysis after 50% of patients have enrolled in the daily treatment arm. The DMC will make recommendations to the Principal Investigator that could include actions of continuation, modification, suspension, or termination.

14. PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the participant, the PI, or the study staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, Section 5.1.1
- 5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the PI/study staff to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to the PI or designated personnel.

All protocol deviations, as defined above, must be addressed in study participant source documents. A completed copy of the Protocol Deviation Form must be maintained in the Regulatory File, as well as in the

participant's source document. Protocol deviations must be sent to the IRB/IEC per their guidelines. The PI/study staff is responsible for knowing and adhering to their IRB requirements.

15. REFERENCES

16. APPENDIX I

16.1. Osman et al. ID Week 2016

Osman et al. ID Week 2016, "Safety and efficacy of fecal microbiota transplantation for recurrent Clostridium difficile infection"

Abstract #59497
Title: Safety and efficacy of fecal microbiota transplantation for recurrent *Clostridium difficile* infection from an international public stool bank: Results from a 2,050 patient multi-center cohort
Original Submission Date: May 17, 2016
Last Edited Date: May 18, 2016
Authors: Majdi Osman, MD MPH[1], Kelsey O'Brien, MPH[2], Zachery Stoltzner, BS[2], Kelly Ling, BS[1], Emily Koelsch, RN BSN[1], Nancy Dubois, MSN MBA[3], Adila Khoiri, MD[4], Kanchana Amaratunga, MD, MPH[5], Mark Smith, PhD[1] and **Zain Kassam**, MD MPH[1], (1) OpenBiome, Medford, MA, (2)Clinical Safety, OpenBiome, Medford, MA, (3)William F. Connell School of Nursing, Boston College, Chestnut Hill, MA, (4)Sydney Children's Hospital, Sydney, Australia, (5)Infectious Disease, The Ottawa Hospital, Ottawa, ON, Canada

Background: *Clostridium difficile* infection (CDI) is a public health threat and fecal microbiota transplantation (FMT) appears to be an effective therapy. Recently, universal stool banks have emerged to enable safe and seamless access to FMT. However, there is a paucity of real-world safety and efficacy data from stool banks.

Methods: Quality assurance data on CDI classification, FMT delivery modality and clinical efficacy was consecutively collected from 482 healthcare facilities across 50 U.S states and 7 countries between January 16, 2014 and April 12, 2016. The primary outcome was physician-reported clinical cure as per standard of care follow-up. Safety data was assessed through mandatory adverse event (AE) reporting. Descriptive statistics and Chi-square analysis for binomial variables was conducted.

Results: Among consecutively collected reports, complete safety and efficacy data was returned for 2,050 patients, which were included in these analyses. Overall, the clinical cure rate from physician-reported data across all delivery modalities and CDI patient populations was 84.0%. The most common indication for FMT, recurrent CDI, had an 87.0% (1150/1322) clinical cure rate by lower gastrointestinal (GI) delivery (Figure 1). Across the entire cohort, 85.0% (n=1742) of patients were treated with 250mL lower GI delivery fecal microbiota preparation (FMP) and 15.0% used 30mL upper GI delivery FMP. FMT by colonoscopy (85.8% clinical cure, n=1441) was superior to upper endoscopy (74.1% clinical cure, n=201) ($p<0.01$). The relationship between fecal preparation type and efficacy was statistically significant ($p<0.05$), with 85.1% efficacy for 250mL preparations and 77.9% efficacy for 30mL preparations. CDI classification had a statistically significant impact on the rate of clinical efficacy (Figure 1). From a safety perspective, 42 AEs were reported; however, no AEs were determined to be "definitely related" to FMT, 3 were "possibly related" to FMT and 39 "not related" based on NIH criteria.

Conclusions: To our knowledge, this is the largest FMT study reported, and suggest in a large, real-world patient cohort that includes severe and refractory CDI patients, FMT from a public stool bank appears to be a safe and effective treatment for CDI not responsive to standard therapy.

Figure 1: Efficacy of FMT by *Clostridium difficile* infection classification and fecal microbiota preparation type

***Clostridium. difficile* infection Classification**	**Total**			**250 mL**			**30mL**		
	N	**Efficacy (%)**	**P-value**	**N**	**Efficacy (%)**	**P-value**	**N**	**Efficacy (%)**	**P-value**
Recurrent	1542	85.9	<0.001	1322	87.0	<0.001	220	79.5	0.278
Mixed (e.g. recurrent and severe)	259	79.2	0.021	229	80.0	0.047	30	66.7	0.118
Refractory	159	74.2	<0.001	126	75.4	<0.01	33	69.7	0.228
Severe	90	83.3	0.85	65	81.5	<0.01	25	88	0.205

16.2. Dubois et al. ID Week 2015

Title: Prospective Assessment of Donor Eligibility for Fecal Microbiota Transplantation at a Public Stool Bank: Results From the Evaluation of 1,387 Candidate Donors

Authors: Nancy E. Dubois MN, MBA[1], Kelly Ling[1], Majdi Osman MD, MPH[1], Laura Burns[1], Gina Mendolia[1], Dan Blackler, James Burgess[1], Carolyn Edelstein MPA[1], Andrew Noh[1], Elaine Vo PhD, Eric Alm PhD[1,2,3], Mark Smith PhD[1,2] **Zain Kassam MD, MPH[1,2]**

1. OpenBiome
2. Massachusetts Institute of Technology
3. Broad Institute

Character count: 1,948/1,950

Background: Recurrent *Clostridium difficile* infection is a major public health threat and fecal microbiota transplantation is a promising therapy. Public stool banks have emerged to meet increasing demand, supplied with fecal material from rigorously screened, universal donors. However, limited data exists regarding best practices for donor assessment. Accordingly, we aim to outline a donor screening framework, capture etiology of exclusion, and quantify the number of qualified stool donors.

Methods: Enrollment was conducted prospectively over a 1-year period. Candidates were directed to an online registry to complete a pre-screening survey to rule out common exclusion criteria. Eligible participants were invited for a 109-point, in-person clinical assessment by a nurse or physician and overseen by an internal medicine specialist to exclude risk factors for transmissible diseases and potential microbiome-mediated conditions. Candidate donors completed stool and serologic screening by a CLIA-approved laboratory (Figure 1).

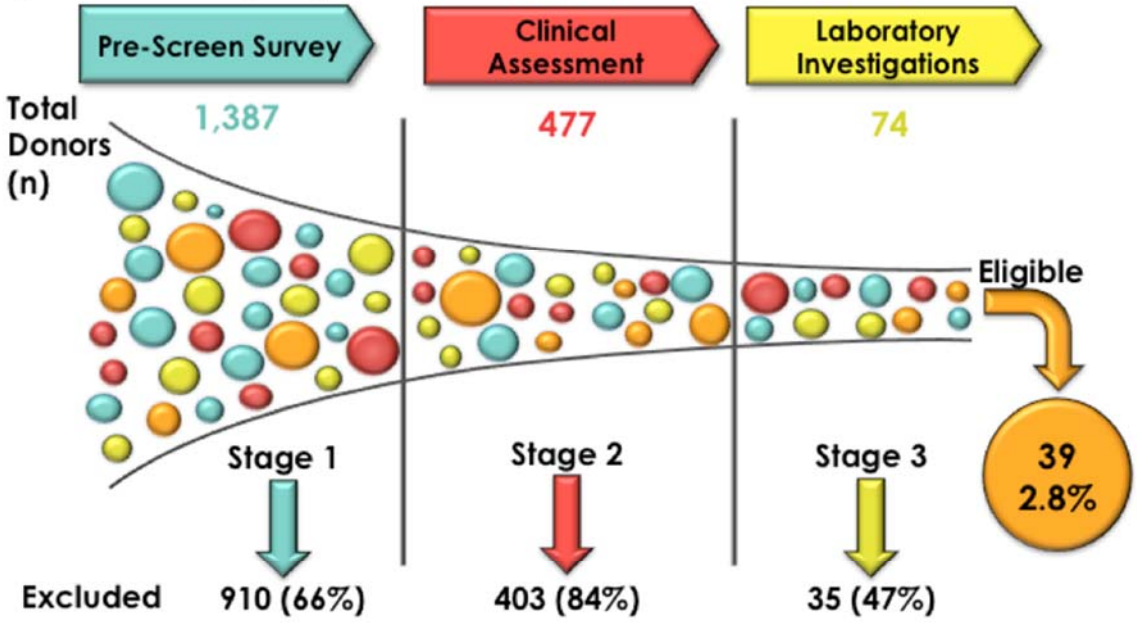
Results: Overall, 1,387 participants enrolled in the donor program. At Stage 1, candidates completed a pre-screen survey with 910 (66%) individuals excluded, commonly for abnormal body mass index, logistic constraints and recent antimicrobial use. At Stage 2, remaining participants underwent a clinical assessment with 403 (84%) participants excluded, most commonly for loss to follow-up with 235 (58%) candidates failing to attend an invited assessment. During the clinical interview, psychiatric illness, medications and infectious disease risk factors were identified as the most common reasons for exclusion. At Stage 3, remaining candidates underwent laboratory investigation with 35 (47%) candidates excluded, commonly for rotavirus and *C. difficile*. Overall, 39 participants qualified as stool donors resulting in a 2.8% acceptance rate (Figure 2).

Conclusions: Healthy, rigorously screened stool for use in FMT is rare with only 2.8% donors qualifying. An unanticipated, large number of asymptomatic potential donors were not eligible and logistics as well as loss to follow-up appear to be important factors for stool banks. Consensus-based guidelines are urgently needed to ensure safe standards for stool donors.

Figure 1. Stool and serologic screening panels

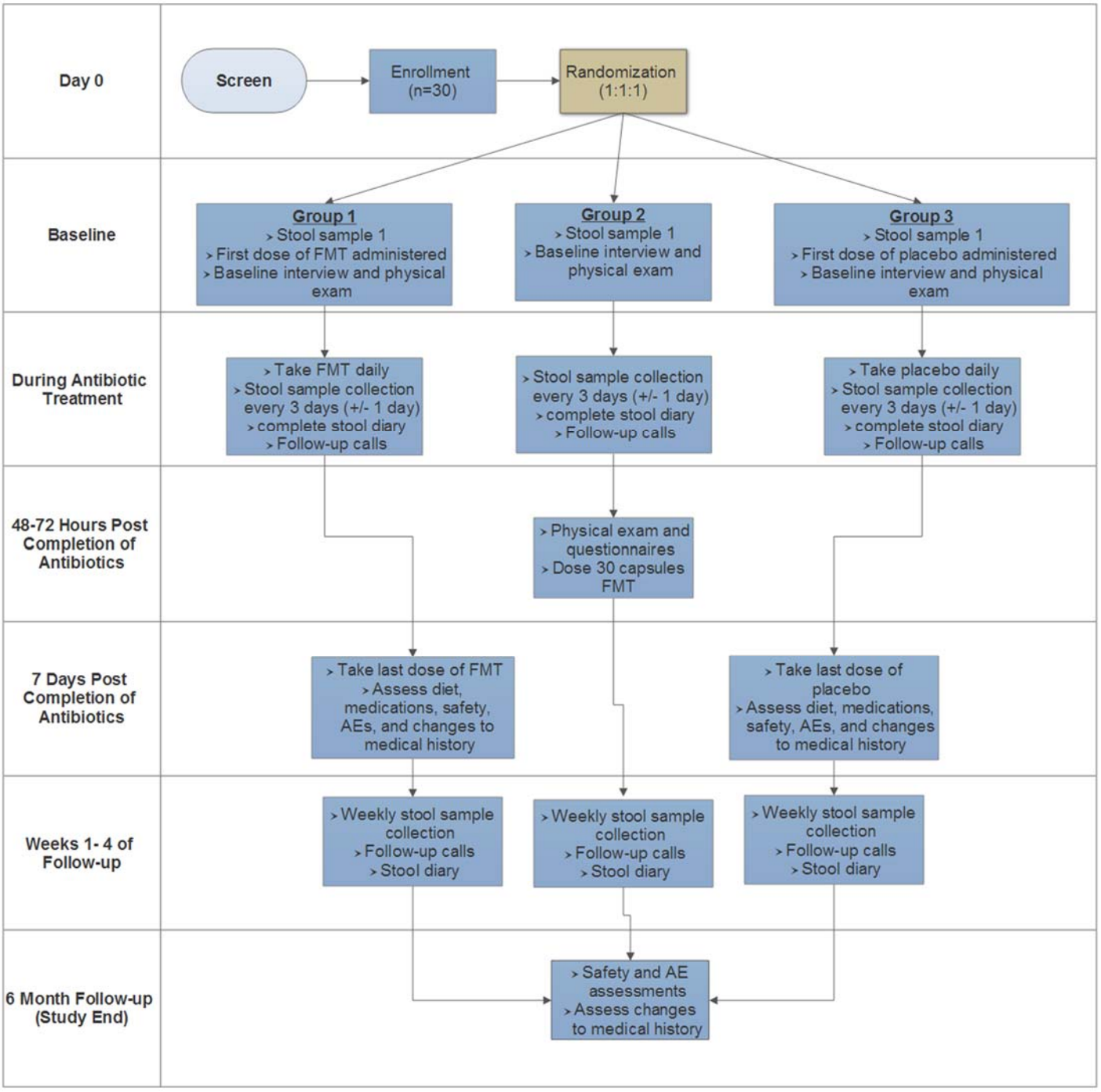
STOOL TESTING	SEROLOGIC TESTING
- PCR assay for *Clostridium difficile* toxin gene NAA - Culture-based assays for common enteric pathogens (including *Salmonella, Shigella, Campylobacter, Vibrio, E. coli* Shiga toxin EIA) - *Helicobacter pylori* fecal antigen EIA - Ova and parasites (including *Isospora*) - *Giardia lamblia* fecal antigen EIA - *Cryptosporidium* fecal antigen EIA - Acid-fast stain for *Cyclospora* - Microscopic exam for *Microsporidia* - Real-time PCR assay for fecal Norovirus - Rotavirus and Adenovirus (Type 40/41) fecal antigen EIA - Culture-based assay for fecal Vancomycin-Resistant Enterococcus (VRE)	- HIV antibody, type 1 and 2 - Hepatitis A (IgM) - Hepatitis B panel (HBsAg, anti-HBc [IgM and Total]) - Hepatitis C (HCV antibody) - *Treponema pallidum* (EIA with reflex to RPR) - HTLV 1 and 2 - Complete blood count with differential - Hepatic function panel (AST, ALT, ALP, bilirubin, albumin)

Figure 2. Donor eligibility workflow for an international public stool bank



17. APPENDIX II

17.1. Study Schematic



17.2. Study Calendar

	Screening	Enrollment/ Baseline Visit*	Daily for Duration of Antibiotic regimen +7 days	Every 3 days (+/-1 day) from Enrollment to intervention end	Follow-up Calls	48-72 hours post Antibiotic Completion*	7 Days Post Antibiotic Completion	Unscheduled Visit*	Weekly Post-FMT Follow-Up Sample Collection[1]	1 Month Post-FMT Call	6 month Post-FMT assessment
Verification of Inclusion and Exclusion Criteria	X	X									
Invited to participate	X										
Informed Consent		X									
Pregnancy test		D[2]				S[2]					
Test Capsule		D				S					
CMV/ EBV PCR testing		X[3]									
Vitals Collection		X				S		X			
Physical Exam		X				S		X			
Clinical Symptoms Assessment		X			X	S		X	X	X	
Dietary Assessment		X			X	S	D	X		X	
Risk Factor Assessment		X									
Demographics collection		X									
Medical History		X									X
Concomitant Medications		X			X	S	D	X	X	X	
FMT or Placebo Administration		D	D			S	D				
Stool Sample Collection		X		X	X	S	D		X		
Antibiotic Resistant Bacteria Testing		X		X		S	D		X		
Patient diary		X	X	X	X	X	X		X		
Post-ingestion Observation		D				S					
Adverse Events Assessments		D			X	S	D	X		X	X

X-Indicates all groups D-indicates daily dosing groups (Groups 1 and 3) only S-indicates single dose group (Group 2) only
*Indicates a clinic visit [1]Lasts a duration of 4 weeks [2]Women of childbearing potential only [3] Patients at risk of CMV/EBV associated disease only

17.3. NIH Common Terminology Criteria for Adverse Events (CTCAE)

Adverse Events Recording Form

Record of adverse events:				
System:	**Present**	**Grade**	**Attribute**	**Describe reaction (refer to appendix 9)**
Systemic				
Infection				
Injection site reaction				
Skin/dermatologic				
Cardiovascular				
Gastrointestinal				
Neurologic				
Respiratory				
Musculoskeletal				
Genitourinary				
Ocular/Visual				
Endocrine/metabolic				
Laboratory AE:				
Hematologic				
Chemistry				
Urinalysis				

NIH Adverse Event Severity Grading Scale*		
Scale		*Description*
1	Mild	Symptoms causing no or minimal interference with usual social and functional activities
2	Moderate	Symptoms causing greater than minimal interference with usual social and functional activities
3	Severe	Symptoms causing inability to perform usual social and functional activities
4	Potentially life-threatening	Symptoms causing inability to perform basic self-care functions OR medical and operative intervention indicated to prevent permanent impairment, persistent disability, or death
5	Death	Fatal event related to adverse event

NIH Adverse Event Relatedness *	
Likely	*Description*
Definitely related	The adverse event is 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.
Not related	The adverse event is 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 a causal relationship is considered biologically implausible.

*Source: NIH Adverse Event and Serious Adverse Event Guidelines, available online at https://www.nia.nih.gov/sites/default/files/niaaeandsaeguidelinesfinal011012_0.doc

Completed _____ Date _____

Name

17.4. Adverse Event Assessment Flow Chart