RACE:
Randomized controlled trial of Autologous microbiome reconstitution to prevent Colonization by antibiotic rEsistant bacteria

ClinicalTrials.gov Identifier: NCT03061097

Statement of Compliance

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

1. 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);
2. 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.

PRINCIPAL CLINICIAN SIGNATURE PAGE

Protocol Title: **Randomized controlled trial of Autologous microbiome reconstitution to prevent Colonization by antibiotic rEsistant bacteria**

Version Date: 17 September 2019

I acknowledge that I have read and understand the protocol named above and agree to conduct the study according to the protocol named above. I also agree and will adhere to terms and procedures in accordance with United States Food and Drug Administration (FDA)/International Council for Harmonisation (ICH) guidelines, including all federal and locally applicable regulations and laws.

I assure that the study drug supplied by OpenBiome will be used only as described in the protocol named above.

_____ _____

Signature Date

_____

Print Name

APPENDIX C: TABLE OF CONTENTS

C.1. List of Abbreviations

AE	Adverse event
ARB	Antibiotic resistant bacteria
BU-BMC	Boston University – Boston Medical Center
CDC	Centers for Disease Control and Prevention
CDI	*Clostridium difficile* Infection
CNA	Certified nurse assistant
CRE	Carbapenem-resistant Enterobacteriaceae
CRF	Case report form
DSMB	Data and safety monitoring board
eCRF	Electronic case report form
EIA	Enzyme Linked ImmunoAssay
ESBL	Extended spectrum beta-lactamase producing organisms
FMP	Fecal microbiota product
FMT	Fecal Microbiome Transplant
GCP	Good clinical practice
ICH	International Conference on Harmonisation
IND	Investigational new drug
IRB	Institutional review board
MDI	Microbiome disruption indices
MIT	Massachusetts Institute of Technology
NIH	National Institute of Health
NPO	Nil per os / fasting
OTU	Operational taxonomic unit
PCR	polymerase chain reaction
PI	Principal investigator
QA	Quality assurance
QC	Quality control
RACE	Randomized controlled trial of Autologous microbiome reconstitution to prevent Colonization by antibiotic rEsistant bacteria (RACE) trial
SAE	Severe adverse event
SOP	Standard operating procedure
VRE	Vancomycin-resistant enterococci

C.2. Protocol Summary

Title	Randomized controlled trial of Autologous microbiome reconstitution to prevent Colonization by antibiotic rEsistant bacteria (RACE) trial
Phase	I/II
Population	Stool collection from long-term care facility residents (n=180) Key inclusion criteria: 1. Long-term care residents associated with Boston University-Boston Medical Center nursing home consortium 2. Adults (18 years or older) Key exclusion criteria: • Colostomy • Hospice Target sample size of n= 20* (14 in the FMT group and 6 in the placebo group, randomized 2:1) participants who develop an infection requiring antibiotic therapy. * Based on antibiotic resistant bacteria (ARB) prevalence and care-facility infection rates.
Number of sites	Up to 4 long-term care facilities in greater Boston area
Study duration	18 months
Intervention	Autologous fecal microbiota preparation, collected at enrollment, administered by enema delivery vehicle
Study objectives	To investigate the safety, feasibility and the role of autologous fecal microbiota transplantation (FMT) for the prevention of antibiotic resistant bacteria (ARB) through microbiome restoration. Primary objective: To evaluate the safety and feasibility of autologous FMT administered by enema delivery in an elderly, long-term care population. Secondary objectives:

	1. Evaluate changes in microbial communities' pre-infection, post-antibiotic exposure and following FMT administration 2. To examine the effect of autologous FMT on ARB colonization and associated infection
Study endpoints	<u>Primary endpoint</u>: • Safety (short-term) at Day 7 defined as NIH Grade ≥2 adverse events <u>Secondary endpoints:</u> • Among patients with ARB colonization at Day 0 (post-antibiotics), rate of ARB clearance at Day 3, Day 7, and Day 28 o Carbapenem-resistant Enterobacteraciae (CRE) by PCR or culture-based assay o Extended spectrum beta-lactamase (ESBL)-producing organisms by PCR or culture-based-assay o Vancomycin-resistant enterococci (VRE) by PCR or culture-based assay o *Clostridium difficile* by PCR • Composite endpoint for presence of any ARB-associated clinical infection at Day 3, Day 7, Day 28, and Month 6. • Safety (intermediate at Day 28 and long-term at Month 6) defined as NIH Grade ≥2 adverse events. <u>Possible exploratory endpoint:</u> • Microbiome disruption indices (16S rRNA sequencing): MDI-community and MDI-species at baseline (pre-infection on the date of stool collection), post-antibiotics on the intervention/placebo date (Day 0, Day 3, Day 7, and Day 28). MDI will be applied to pre- and post-intervention communities in comparison to baseline and reference communities.

Study design	A pilot, randomized, double-blind, placebo-controlled trial to assess the safety and feasibility of autologous FMT, its effect on the intestinal microbiome and the prevention of ARB colonization and infection. In order to yield the target sample size of n=20 participants who develop an infection requiring antibiotics, a total of 180 participants will be enrolled for stool collection. After completion of the antibiotic course, participants (n=20) will be randomized (2:1) to placebo or autologous FMT enema if eligible. Once the antibiotics have finished and at least 72 hours have passed, a pre-FMT stool sample or rectal swabs will be collected. Both trial arms will have the treatment or placebo administered at least 72 hours post-antibiotics (± renal/hepatic adjustment at the discretion of the treating physician to ensure specific antibiotics has been cleared and will not impact FMT). Follow-up: Follow-up and sample collection will occur at Day 3 (±1 day), Day 7 (±2 days), and Day 28 (±5 days). Additional follow-up for safety will occur at Month 6 (±14 days) including a stool sample. All samples will be analyzed for 1) presence of ARBs using a PCR test and 2) 16S rRNA sequencing.

C.3. Key Roles

Lead Study Principal Investigator	Majdi Osman, MD, MPH OpenBiome
Site Principal Investigator [redacted]	Christine Liu, MD, MS Boston University-Boston Medical Center (will be responsible for direct oversight of study conduct and subject safety at each of the four sites)
Clinical Leads	Gabriel Brandeis, MD, CMD New Jewish Home and Icahn School of Medicine at Mt. Sinai Susan Frazier, NP (Lead NP) Boston University-Boston Medical Center
Medical Monitor	Shrish Budree, MD MBChB DCH FCPeds OpenBiome
Scientific Lead	Majdi Osman, MD, MPH OpenBiome Mark Smith, PhD Finch Therapeutics Group Eric Alm, PhD Massachusetts Institute of Technology
Study Advisors	Kanchana Amaratunga, MD, MPH, FRCPC University of Ottawa Jessica Allegretti, MD, MPH Brigham Woman's Hospital-Harvard Medical School
Data Coordinating Center	OpenBiome Chair: Shrish Budree, MD, MBChB, DCH, FCPeds Data Analyst: Scott Olesen, PhD

	Data Analyst: Pratik Panchal, MD, MPH

C.4. Background Information and Scientific Rationale

C.4.1. Background Information

Antibiotic-resistant bacteria (ARB) pose an urgent public health threat, with over 2 million Americans infected each year.[1] Over half of these infections are transmitted in healthcare settings, where the widespread use of antibiotics selects for resistance and creates a stable pool of vectors, enabling the transmission of resistant organisms.[1]

Elderly residents of long-term care facilities are particularly vulnerable to ARB colonization, and in turn, infection.[2] Geriatric populations are commonly frail, immunocompromised and often possess multiple co-morbidities that increase their risk of infections.[2] Furthermore, communal assisted living and frequent transfer between long-term residential facilities and acute care settings facilitate microbial transmission between high-ARB environments.[2]

Antibiotic exposure is one of the greatest risk factors for ARB acquisition.[3,4] While rates vary across facilities, up to 70% of nursing home residents receive at least one course of antibiotics per year, a rate approximately twice that of the general population.[5,6] Exposure to antibiotics results in an immediate and substantial perturbation of the gut microbiome with only partial recovery of original taxonomic composition.[7] A common working hypothesis is that intact microbial communities competitively exclude exogenous microbiota, providing 'colonization resistance.' This hypothesis is supported by research that indicates disruption of the resident microbiota with antibiotics decreases the minimum infective dose and increases the colonization potential of enteric pathogens.[8,9,10,11]

Efforts to decrease ARB transmission through infection control strategies and antimicrobial stewardship are underway; however, they have proven both expensive and ineffective.[5] Point-prevalence studies report up to 43% of nursing home residents are colonized with at least one ARB at any given time, contributing to a high potential for dissemination.[3] This reservoir of ARB in an already vulnerable population contributes to an estimated 23,000 ARB infection deaths and $20 billion in direct costs to the U.S. healthcare system annually.[1]

C.4.1.1 Fecal Microbiota Transplantation for the Treatment of Recurrent Clostridium difficile *Infection and Other ARB*

Allogeneic fecal microbiota transplantation (FMT), the introduction of colonic microbiota from a healthy donor into patients with gut dysbiosis, has been shown to be a highly effective and safe intervention for treatment of recurrent *Clostridium difficile* infection (CDI). A recent systematic review and pooled

analysis reports FMT as 83% effective in treatment of recurrent CDI, which commonly impacts the geriatric population, with few reported adverse events.[12,13] A recent large case series of 146 elderly recurrent CDI patients supports the relative safety of allogeneic FMT.[12,13] Although not fully elucidated, the hypothesized mechanism of FMT for CDI is that normal colonic microbiota outcompete and thereby competitively exclude *C. difficile*.[14]

A recent commentary by Halpin and McDonald of the Centers for Disease Control and Prevention (CDC) highlights the ecological principles and preliminary research suggesting FMT may also be effective for eliminating enteric colonization with ARB such as vancomycin-resistant enterococci (VRE).[15] Similar to CDI, VRE colonization is known to be induced by microbial dysbiosis.[16] Furthermore, vancomycin-resistance is known to carry a fitness defect for the organism carrying this gene, making it a prime target for microbial intervention such as FMT that introduces new microbes that may competitively exclude VRE. *In vitro* competition assays with enterococci have demonstrated a 4% fitness defect of VanA relative to sensitive enterococci.[17] Similarly, expression of VanB-type resistance results in reduced fitness *in vitro*, as well as reduced colonization ability and dissemination in gnotobiotic mice.[18] In another murine study, the transfer of healthy colonic microbiota from antibiotic-naive mice has been shown to reverse dysbiosis and eliminate VRE infection within 14 days.[19]

In addition to this translational work, a number of case reports have suggested that FMT may be able to decolonize patients colonized with ARB. Several case reports have reported de-colonization of ARB following FMT, including carbapenem-resistant Enterobacteraciae (CRE), extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) enteritis.[20,21,22,23,24] Our group has submitted work that supports the role of FMT for VRE decolonization (see Eysenbach et al. IDWeek 2016, Appendix A.2.). Our group performed a multicenter retrospective analysis was performed using stool samples from recurrent CDI patients treated with FMT (n=31) or autologous FMT as a control from a previous trial (n=18). VRE was assessed using a PCR-based assay targeting VanA (Acuitas® MDRO Gene Test). Colonization was defined as positive result at any dilution. VRE decolonization was defined as absence of VRE colonization post-FMT among a patient colonized with VRE pre-FMT. Among this cohort, 16 patients were VRE colonized; 9/9 (100%) in the FMT group tested VRE-negative compared to 3/7 (43%) in the control group (p=0.02, Fisher's Exact Test). Two of these patients were treated with a single dose of 30 FMT capsules, while the rest received FMT via traditional modalities.

C.4.1.2. Rationale

OpenBiome's universal donors have been rigorously screened for both infectious and potentially microbiome-mediated chronic disease resulting in a qualification of only 2.8% of candidate donors.[25] Additionally, among a large, multi-center cohort (n=2,050) who received allogeneic FMT from OpenBiome to treat *C. difficile* infection not responsive to standard therapy, only 42 adverse events (AE) were reported, none of which were determined to be "definitely related" to FMT. Three were "possibly related" to FMT and 39 cases were "not related" based on NIH criteria, suggesting a favorable short-term safety profile (See Osman et al. IDWeek 2016, Appendix A.1.). Despite this data, there remains important, yet theoretical, long-term risks associated with such a dramatic manipulation of the microbiome and the risk of transmitting currently unknown or untestable pathogens or other risk factors.[26] For critically ill patients or those without effective treatment alternatives, such a risk is justified, but clinical equipoise becomes more challenging if FMT is to be used as a prophylactic intervention, for example, to restore colonization resistance following antibiotic exposure as a novel infection control and public health tool.

Accordingly, the use of autologous FMT, using fecal material collected from a patient at a previous time of relative health may significantly reduce the risks of FMT by limiting the recipient's exposure to exogenous organisms and restoring their own healthy microbial community. The logistical feasibility of autologous FMT has been validated by OpenBiome's PersonalBiome program and an ongoing pilot among bone marrow transplant recipients.[27]

C.4.1.3. Potential Impact

By mitigating the potential risks of allogenic FMT from universal donors, autologous prophylactic FMT carries with it transformative possibilities in addressing the threat presented by ARB. Should this trial suggest safety and yield promising translational results, we would plan to follow up with a fully-powered randomized controlled trial that has the potential to suggest prophylactic autologous FMT as a novel public health tool for preventing enteric ARB infections among high-risk patients. We envision a future where autologous FMT emerges to enable microbiome reconstitution following antibiotics, reducing the patient, public health, and economic burdens of nosocomial infections, and mitigating the risk of antibiotic resistance.

C.4.2 Potential Risks and Benefits

C.4.2.1. Potential Risks

There are risks associated with conducting this study, including loss of confidentiality; however, our group possess a unique set of resources and

expertise to mitigate these risks. The long-term side effects of FMT, by enema form, are not currently well known. There may be unknown risks or discomforts involved.

C.4.2.2. Insufficient Enrollment Risk

[redacted], and [redacted] have a pooled population of 1,023 patients, requiring ~18% of all nursing home residents to enroll. Dr. Christine Liu and Dr. Gabriel Brandeis believe enrollment to be feasible based on preliminary discussions with nursing homes. Additionally, Dr. Brandeis recently published the results of a study on tuberculosis diagnosis across three BMC nursing homes that featured a 31.4% enrollment rate, suggesting the feasibility of enrolling for this study.[30] OpenBiome has also demonstrated success in driving patient adoption of FMT, having developed a patient education program and providing over 29,000 treatments to over 900 clinical partners demonstrating broad scale adoption.

C.4.2.3. Safety Risks

- Pathogens or diseases transmissible through stool: Autologous FMT offers a safety advantage over allogenic FMT by eliminating the risk of acquiring another individual's microbiome-mediated chronic disease. However, Trick and colleagues report 40% of patients colonized with an ARB. Accordingly, stool at the time collection could be positive for an ARB and a participant may re-infect themselves with their autologous FMT. To mitigate this risk, we will screen all biobanked stool (n=180) for the following three common ARBs: CDI by EIA, given latest 2016 CDI testing guidelines. We will also assay for CRE, VRE, and ESBL.[31] There is a still risk of transmission of known and unknown infectious organisms. This will be characterized by fever, chills and possibly low blood pressure. There have been two cases of a bacterial infection in the blood after FMT, but it is unclear if this was from the FMT, and the patient did well on antibiotics.
- Inflammatory bowel disease (IBD) flare in those with underlying IBD
- Enema delivery modality: Irritation of the perianal area, including exacerbation of any potential pressure ulcers in the perianal area, may occur during enema administration. To prevent this, we will ensure that any open wounds or ulcers in the perianal area are covered with a barrier cream and waterproof dressing prior to enema administration. To minimize any potential abdominal and rectal discomfort during the enema, only gentle pressure will be used to administer the enema, and all persons who administer the enema will be instructed to stop immediately if the subject has significant pain. As with any enema, bowel perforation

may due to the pressure required to administer the enema. To mitigate the risk of a perforation, only gentle pressure will be used to administer the enema. All persons who administer the enema will be instructed to stop if the patient begins to have severe abdominal pain or cramping during the enema, which would be a sign of possible perforation. In this case, we would immediately send the subject to the emergency room for further evaluation, as bowel perforation is a surgical emergency. Hemorrhoid bleeding may also occur due to the modest pressure required to administer the enema. To mitigate the risk of bleeding, only gentle pressure will be used to administer the enema. All persons who administer the enema will be instructed to stop if the patient begins to bleed during the enema to prevent further blood loss.

C.4.2.4. Baseline Geriatric Microbiome Risk

Compositional changes in the gut microbiota are associated with aging.[32] Mariat and colleagues demonstrated that the ratio of *Firmicutes* to *Bacteriodetes* evolves throughout an individual's lifetime, and that the elderly have a higher count of *E. coli* than the adult population.[33] Furthermore, Claesson and colleagues established that geriatric residents of long-term care facilities have lower gut microbial diversity than elderly individuals who live in the broader community.[32] Autologous FMT derived from an inadequately diverse baseline microbial community may fail to confer protective benefits against ARB. We intend to mitigate this risk by making eligibility criteria as permissive as possible, recognizing that physical frailty is associated with lower microbial biodiversity.[32] Furthermore, although likely underpowered, we plan on conducting an *a priori* sub-group analysis of patients with high versus low microbial diversity to determine if there is a correlation between study outcomes and baseline diversity.

C.4.2.5. Known Potential Benefits

Studies have suggested that FMT may be able to decolonize patients colonized with ARB. Several case reports have reported de-colonization of ARB following FMT, including carbapenem-resistant Enterobacteraciae (CRE), extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) enteritis.[20,21,22,23,24] 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.

C.5. Objectives

C.5.1 Study Objective

C.5.1.1. Study Objective

To investigate the safety and the role of autologous fecal microbiota transplantation (FMT) for the prevention of antibiotic resistant bacterial (ARB) colonization and infection through microbiome restoration.

C.5.1.2. Primary Objectives

To evaluate the safety and feasibility of autologous FMT administered by enema delivery vehicle in an elderly, long-term care patient population.

C.5.1.3. Secondary Objectives

- Evaluate changes in microbial communities' pre-infection, post-antibiotic exposure and following FMT administration.
- To examine the effect of autologous fecal microbiota transplantation (FMT) on antibiotic resistant bacteria.

C.5.2. Endpoints

C.5.2.1. Primary Endpoint

Safety (short-term) at day 7 defined as absence of NIH Grade ≥2 adverse events including IND safety reporting criteria (see C.11 and C.18.1, C.18.2).

C.5.2.2. Secondary Endpoints

- Clearance of ARB among patients colonized at Day 3, Day 7, and Day 28 by Polymerase Chain Reaction (PCR) assay ([redacted] MDRO test) or culture-based assay ([redacted] culture assay)
    - Carbapenem-resistant Enterobacteraciae (CRE) by PCR or culture assay
    - Extended spectrum beta-lactamase (ESBL)-producing organisms by PCR or culture assay
    - Vancomycin-resistant enterococci (VRE) by PCR or culture assay
    - *Clostridium difficile* by PCR
- Composite endpoint for presence of any ARB-associated clinical infection at Day 3, Day 7, Day 28, and Month 6. This is defined as CDI-associated diarrhea or ARB-associated bacteremia, urinary tract infection, wound infection or other clinical infection at the discretion of the treating physician.
- Safety (intermediate at Day 28 and long-term at Month 6) defined as NIH Grade ≥2 adverse events including IND safety reporting criteria.

C.5.2.3. Possible Exploratory Endpoints

- Microbiome disruption indices (16S rRNA sequencing): MDI-community and MDI-species at baseline (pre-infection on the date of enrollment stool collection), post-antibiotics on the intervention/placebo date (Day 0, Day 3, Day 7, Day 28 and Month 6). MDI will be applied to pre- and post-intervention communities in comparison to baseline and reference communities.

C.6. Study Design

This study will be a pilot, randomized, double-blind, placebo-controlled trial to assess the safety and feasibility of autologous FMT and to investigate the effect of FMT on the intestinal microbiome and the prevention of ARB colonization and infection. It will be performed in collaboration with Boston Medical Center with a geriatric patient population at 4 Boston-area nursing homes. A schematic of this study design can be found in C.19.1.

C.6.1. Study Population

Enrollment will occur at 4 nursing homes affiliated with Boston Medical Center: [redacted], [redacted], [redacted], and [redacted]. Eligible nursing home residents whose fecal sample is negative for ARB will have their healthy stool processed into FMT material. Only participants who receive a course of antibiotics will undergo 2:1 randomization to the intervention or placebo arms. A schematic of the patient enrollment pipeline can be found in C.19.2. Inclusion criteria is found in C.7.1 and exclusion criteria in C.7.2.

C.6.2. Intervention

14 participants will be randomized to the autologous FMT arm and will receive their own fecal material, collected before they were treated with antibiotics. These patients will receive a single autologous FMT enema a minimum of 72 hours (+/- renal/hepatic adjustment at the discretion of the treating physician to ensure specific antibiotics has been cleared and will not impact FMT) after the cessation of antibiotics.

C.6.2.1. Dosing Rationale

The dosing rationale is driven by previous successful VRE decolonization of 2 patients with recurrent CDI presented by our group (Appendix A).[30]

C.6.3. Control

6 participants will be randomized into the control arm. These patients will receive a single identical placebo enema, similar to other studies,[31] 72 hours (+/- renal/hepatic adjustment at the discretion of the treating physician to ensure specific antibiotics has been cleared and will not impact FMT) after the cessation of antibiotics. Placebos will consist of the same saline and glycerol buffer used in FMT processing without the addition of stool.

C.7. Inclusion and Exclusion Criteria

C.7.1. Inclusion Criteria

C.7.1.1. Inclusion Criteria for Study Enrollment

1. Long-term care residents associated with Boston University-Boston Medical Center nursing home consortium
2. Adults (18 years or older)

C.7.1.2. Inclusion Criteria for Randomization

1. Infection requiring antimicrobial treatment at the discretion of the treating physician

C.7.2. Exclusion Criteria

C.7.2.1. Exclusion Criteria for Study Enrollment

1. Pregnant. Participants of childbearing age will undergo urine pregnancy testing.
2. Participant or substitute decision maker unable to provide informed consent
3. Allergies to following ingredients generally recognized as safe: glycerol and sodium chloride
4. Current enrollment in hospice
5. Colostomy
6. Unable to adhere to protocol requirements
7. Any condition that the physician investigators deems unsafe, including other conditions or medications that the investigator determines puts the participant at greater risk from FMT
8. Recent travel (last six months) to high risk regions based on the International SOS Medical Risk Rating system
9. Recent exposure (last six months) to unsafe drinking water

C.7.2.2. Exclusion Criteria for Stool Collection

Enrollment stool sample will be tested for ARBs and processed into autologous FMT treatment (if of qualifying size). Sample will not be collected if any of the following are true:

1. Oral or intravenous antibiotic exposure within previous 6 weeks of stool collection date (topical antibiotic will be permitted)
2. Active gastrointestinal infection at stool collection
3. Fever at the time of stool collection
4. Currently ill or complaining of any of the following signs or symptoms of illness: fever, diarrhea, blood stools and/or vomiting
5. Participants with a history of gastrointestinal (GI) illness within the past 30 days prior to enrollment stool collection, that at the discretion of the site

investigator could reasonably be caused by one of the following pathogens 1) *Vibrio* spp. 2) Norovirus 3) Rotavirus 4) Adenovirus 5) Shiga toxin.

C.7.2.3. Exclusion Criteria for Randomization

- Colonized with CRE (assessed by PCR or culture assay during enrollment phase)
- Colonized with VRE (assessed by PCR or culture assay during enrollment phase)
- Colonized with ESBL (assessed by PCR or culture assay during enrollment phase)
- Colonized with CDI (assessed by EIA assay on stool collected at enrollment phase)
- Treatment with antibiotics that are active against MRSA (i.e. vancomycin or linezolid) prior to randomization to FMT intervention or placebo
- Stool culture positive for common enteric pathogens (*Salmonella spp., Shigella spp., Campylobacter spp.*)
    - Participants who develop a GI illness with symptoms such as (but not limited to) vomiting or diarrhea within 30 days **after** collection of enrollment stool will be evaluated by the site investigator. If the site investigator determines that the symptoms were most likely caused by 1)*Vibrio* spp. 2) Norovirus, 3) Rotavirus, 4) Adenovirus, or 5) Shiga toxin, the enrollment stool will be sent out to test for these organisms. If the culture is positive for any of these organisms, the participant will be excluded from randomization
- Any condition that the physician investigators deems unsafe, including other conditions or medications that the investigator determines puts the participant at greater risk from FMT
- Participants who become severely immunocompromised, as defined by the investigator or treating physician, will be excluded prior to receiving intervention

C.8. Treatment Assignment Procedures

C.8.1. Randomization Procedures

Participants who fulfil the criteria for randomization (infection requiring antibiotic treatment), will be randomized on a 2:1 basis with a random permuted block sizes of 3 or 6 to receive FMT or placebo. Participant's randomization will be carried out by the data coordination center chair using random number generation software to create a randomization schedule.

The randomized, blinded treatment assignment will be sent via email to the site by the data coordination center chair after site confirmation that the participant has been treated with antibiotics and is eligible for randomization. This assignment will inform the site, in a blinded manner, about which treatment (active or placebo) the participant will be administered.

C.8.2. Masking Procedure

This is a randomized, double blind, placebo-controlled trial where both the participant and treating physicians will be blinded. Autologous fecal microbiota product (auto-FMP) enemas or placebo will be dispensed by Finch Therapeutics thereby maintaining blinding of study physicians and nurse practitioners. Placebo enemas will be packaged identical to auto-FMPs and consist of the same saline and glycerol buffer used in auto-FMP processing without the addition of stool.

The data coordination center will maintain the randomization schedule and study participants and their health care proxies will remain blinded and not be provided any information until all participants have completed the trial and the database has been locked.

Under normal circumstances, the blind should not be broken. The blind should be broken only if specific emergency treatment would be dictated by providing the treatment the participant was receiving. In such cases, the site PI must contact the Medical Monitor to request that the blind be broken. For participants who require unblinding, this information will be captured in the case report form (CRF).

C.8.3. Interim Analysis

An interim analysis will be performed by the unblinded study's data coordination center chair after randomization of the first 10 participants. The purpose of this interim analysis is to assess safety and feasibility.

C.8.4. Participant Withdrawal

A participant may choose to withdraw from this study at any time for any reason, without consequence.

A participant may be withdrawn from the study by the site PI for any of the following reasons*:

- Severe or intolerable adverse event
- Lack of participant cooperation
    - Participants request to withdraw from study
    - Lack of compliance (fails to attend the follow-up visits as agreed)
    - Technical / logistical reasons (relocation)
- Inclusion criterion not fulfilled
- Other reasons (must be noted)

*Treated participants should be followed in the study through their 6-month safety visit as long as feasible or as long as they are willing.

C.8.5. Procedure for Handling Participant Withdrawal

The primary reason for participant withdrawal should be noted on the 'participant withdrawal' CRF. Participants will be encouraged to attend an Early Termination Visit where the final examination will be documented in the CRF. If possible, a final stool sample will be collected at this visit.

Participants who are withdrawn from the study after randomization, but before intervention or placebo will be replaced. Possible reasons for participant withdrawal after randomization include but are not limited to:

- Participants who are started on a new course of antibiotics after randomization and before intervention/placebo
- Unrelated participant death

Intention-to-treat analysis will be utilized according to best practices in clinical trials.

C.8.6. Study Termination

In the unlikely event that significant safety concerns arise, the PIs can terminate or halt the study pending review by the DSMB. In addition, this study may be halted early based on the DSMB charter or FDA recommendations.

C.9. Study Interventions / Investigational Drug

C.9.1. Interventional Product Description

C.9.1.1. Autologous Treatment Preparation

Participant's stool will be collected at the clinical site and transported to Finch Therapeutics' biomanufacturing facility for processing and storage. Each sample will be processed into an auto-FMP enema formulation. Stool will be barcoded, labeled and physically segregated to ensure traceability to each donor. The facility will be set-up for auto-FMP preparation, temporally segregating it from other operations, with full decontamination between each sample processed to prevent cross-contamination. Quality assurance measures will be deployed to ensure appropriate tracking, distribution and administration of product to ensure appropriate treatment.

C.9.1.2. Fecal Microbiota Transplant (FMT)

FMT is the process by which processed donor microbiota material is transplanted into recipients. The aim is to reconstitute the normal intestinal microbial flora in recipients.

C.9.2. Formulation

C.9.2.1. Auto-FMP Enema

Donor feces [redacted] filtered [redacted] suspended [redacted], to constitute a total volume of 125ml for enema administration.

C.9.2.2. Placebo Enema Preparation

The placebo enema preparation will comprise [redacted] to prevent unmasking of the trial arms.

C.9.3. Product Storage and Stability

C.9.3.1. Auto-FMP Enema Preparation

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 Young et. al.[34,35]

Once thawed, material may NOT be refrozen and can remain for up to 4 additional hours at room temperature (22°C±5) or 8 hours refrigerated (4°C±2) prior to use.

C.9.4. Dosage, Preparation and Administration of Study Intervention

C.9.4.1. Auto-FMP Enema

The study physician/nurse practitioner will evaluate participants prior to administration of the enema specifically focusing on any contraindications for an enema. The study physician/nurse practitioner will ensure that participants are clinically well prior to enema administration. The auto-FMP enema will be thawed over the course of 1 hour in a warm water bath (approximately 30°C) or at room temperature for 4.5 hours. After thawing, the clinician will swirl moderately for 10 seconds to resuspend particulates and using a 50ml sterile disposable syringe the thawed material will be transferred into an enema administration bottle. The auto-FMP enema (up to 125ml) will be slowly administered rectally by retention enema, in keeping with standard of care, with a target dwell time of 1 hour if feasible. Participants will lie in the left lateral decubitus position but if mobility permits will rotate to supine and right lateral decubitus position. A 125ml enema will be administered using an enema 'squeeze' bottle (similar to a sodium phosphate enema) but administration will be discontinued if there is any participant distress.

C.9.5. Accountability Procedures for the Study Intervention/Investigational Product(s)

The site PI (or designee) will maintain an accurate record of the receipt of the test materials, including the date received. The data coordination center chair or designee will maintain a log of all clinical trial materials (enema auto-FMP and placebo) dispensed. This clinical trial material accountability record will be available for inspection at any time.

C.9.6. Assessment of Participant Compliance with Study Intervention/Investigational Product

The retention enema will be administered by study staff and will be performed according to the enema administration SOP.

C.9.7. Concomitant Medications/Treatments

Given the patient population is elderly with multiple comorbidities, all medications will be accepted in this study at the discretion of the treating physician. This includes (but is not limited to):

- Anti-hypertensives
- Heart failure medications
- Diuretics
- Laxatives
- Topical medications
- Anti-depressants

- Statins
- Diabetic medication
- NSAID
- Anti-neoplastics
- Acid-blockers
- Anticoagulants
- Opioids
- Iron supplementation

Patients who receive antibiotics within the previous 6 weeks will be excluded from stool collection.

C.9.8. Screening Visit and Patient Information (Visit 1)
A diagrammatic representation of the study schedule can be found in C.19.1.

All residents living in participating long-term care facilities will be equally eligible for enrollment into this study, provided they fulfill the eligibility criteria. Awareness about the study will be raised by standard, approved recruitment techniques. A detailed consent process will be administered by study staff who are trained in consenting procedures.

Participants who agree to enroll in the study will complete the following:

- Informed consent will be obtained from the participant. If the participant is judged to be cognitively impaired, consent will be obtained from their healthcare proxy/substitute decision maker. The purpose of the study, outcomes and its contribution to advancing healthcare in the elderly population will be explained. Each participant or substitute decision maker/proxy must provide written signed consent before proceeding with any study procedures.

After informed consent is obtained, study staff will perform a detailed data collection via record review, which will include demographics, medical history, dietary information and current medications. Participants will also undergo a brief clinical assessment by a study physician/nurse practitioner.

Data points to be collected:

- Informed consent procedure
- Verification of inclusion and exclusion criteria
- General parameters:
    - Date of birth
    - Sex

    - Smoking status
    - Race
    - Ethnicity
- Past medical history and allergies
    - A focused collection of past medical history (list of common infectious and chronic inflammatory diseases)
    - Charlson Comorbidity Index
- Current medication list
- Dietary history will be collected using a structured institutional dietary record or similar tool
- Social history
    - Alcohol, substance abuse, smoking history
    - Country of birth, duration living in the U.S.
- Travel history
- Family history
- Level of mobility (ADL/IADL) and grip strength
- Examination findings
    - Vital signs: temperature, height (measured or abstracted from medical records), weight (measured or abstracted from medical records), heart rate, blood pressure, O2 saturation, and waist circumference
    - Relevant review of systems
    - Overall assessment of current health

C.9.9. Collection and Processing (Visit 2)

At the collection and processing visit, study staff will collect stool and a rectal swab from participants. The stool and rectal swab will be labelled and transported within 12 hours to the laboratory and subsequently tested for CRE, VRE, ESBL, *C. difficile* and common enteric pathogens (*Salmonella* spp., *Shigella* spp., *Campylobacter* spp.). Ideally the swab will be collected at the same time as the stool sample. However, to encourage collection, the swab may also be collected up to 12 hours post stool collection, if the participant is willing.

Only participants whose stool test negative for the above organisms will have their stool banked and processed into autologous enema. Any stool samples that test positive will be destroyed (although a research aliquot will be retained for future analysis and the site PI will be notified).

C.9.10. Surveillance for Infectious Episodes

Enrolled participants will undergo surveillance by study staff through bi-weekly review of patient medical records, review of facility logs or telephone/on-site/in-

person ward rounds aimed at identifying participants who develop infections requiring antibiotics. The study data team will provide study site staff with a weekly updated log of participants who are eligible for randomization.

- Study staff (nurse practitioners and physicians) based at the long-term care facilities will perform ongoing surveillance for development of new infections among study participants by monitoring nursing, hospital and long-term care facility logs. They will therefore be able to cross-reference all new infections with a list of study participants.

C.9.11. Infectious Episode (Visit 3)

Study staff will complete an infection CRF for each infection episode. This CRF will be used to track duration of the episode, dosage and duration of antibiotics.

- Data points for infectious episode:
    - Date
    - Preliminary diagnosis
    - Antibiotic(s)
        - Name
        - Formulation (IV, suspension, capsules)
        - Dosage
        - Prescribed duration
        - Final (actual) duration of antibiotics
        - Change in antibiotics regime
    - 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

C.9.12. Post-Antibiotic Stool Collection (Visit 4)

Once participants have completed their antibiotic course, the site PI or designee will be notified. Subsequently, a stool sample for microbial sequencing and ARB testing will be collected post-antibiotics, but prior to the FMT visit, which will occur after a minimum of 72 hours (+/- renal/hepatic adjustment at discretion of treating physician) after cessation of antibiotics.

If a stool sample is not available (for example due to constipation), then rectal swabs will be collected from the participant and processed, as per SOP, for microbiome sequence analysis and ARB testing.

C.9.13. FMT Study Visit (Visit 5): Day 0

At the FMT study visit, the participant will undergo a clinical examination by the study physician/nurse practitioner in order to confirm the participant is still eligible for randomization. Participants who become severely immunocompromised, as defined by the investigator/treating physician, will be excluded prior to receiving intervention. Once eligibility is confirmed, participants will receive their autologous FMT by auto-FMP enema or placebo enema. The FMT procedure will be conducted by the blinded study physician/nurse practitioner. Participants will be observed for 30 minutes following the FMT procedure. Participants and the nursing staff at the long-term care facility will be provided with information to assist with monitoring for adverse events.

Data collection at FMT visit

1. Confirm inclusion/exclusion criteria: each participant will be re-assessed for inclusion and exclusion criteria before administering treatment.
    - Interim medical history
    - The study physician/nurse practitioner will iterate through a list of common medical conditions in this patient population (See C.20).
2. Prior and concomitant medications
    - The study physician/nurse practitioner will confirm that the patient has not received treatment with an antibiotic active against MRSA (i.e. vancomycin, linezolid) after collection of autologous fecal transplant material
3. Clinical examination findings:
    - Vital signs: Temperature, heart rate, blood pressure, weight
    - Systemic examination
    - Overall health assessment
    - Safe to receive FMT (yes/no)
    - Immediate post FMT assessment
    - Adverse events (NIH criteria)

C.9.13.1. Pre-FMT Procedure

- A stool sample and rectal swabs will be collected just before the FMT administration for microbial sequencing and ARB testing, If a stool sample

is not available (for example due to constipation), then only the rectal swabs should be processed, as per SOP, for microbial sequencing and ARB testing
- Study physician/nurse practitioner will perform a clinical assessment prior to FMT administration

C.9.13.2. FMT Procedure
- Participants undergo FMT delivery by retention enema delivery at the discretion of the treating physician.
- Auto-FMP enemas (or placebo) will be administered according to the study's SOP.

C.9.13.3. Post- FMT Procedure
- All participants will be observed for 30 minutes following the FMT procedure for immediate adverse reactions. Oral nutrition may commence immediately after enema administration.
- Participants will be provided with information regarding monitoring for minor and severe adverse events related to the FMT procedure. Participants and their care team will be encouraged to report any concerning symptoms to staff at their long-term care facility.

C.9.14 Follow-Up (Visit 6, 7, 8, 9)

At the 3-day and 7-day post FMT visits, the study physician/nurse practitioner will perform a clinical assessment and administer a study questionnaire which will specifically inquire about solicited and unsolicited symptoms of AEs. Access to the medical records may be utilized to capture salient changes in healthcare status. Participants will have a stool sample and rectal swabs collected for microbial sequencing and ARB testing at their next available bowel movement within each follow-up window. If a stool sample is not available (for example due to constipation), then only the rectal swabs will be processed, as per SOP, for microbiome sequence analysis and ARB testing.

At the 28-day and 6-month study visits, participants will undergo a similarly structured study visit which includes a clinical assessment, an AE focused questionnaire administration and collection of a stool sample and rectal swabs for ARB and 16S rRNA sequencing and analysis.

C.9.14.1. Study Visit 6: Day 3 Post-FMT (+/- 1 Day)
- Clinical assessment by study nurse/physician, specifically enquiring about adverse events related to the FMT
- Data collection at visit:

    - Interim medical history (See C.19.3)
    - Concomitant medication
    - Significant changes in diet
    - Changes in stool consistency (Bristol Stool Scale) and frequency
    - Clinical evaluation using standard, structured assessment
    - Vital signs: Temperature, heart rate, blood pressure, weight
    - General health status
    - Adverse events (NIH criteria)
- Stool samples and rectal swabs will be collected for 16S sequencing and ARB testing. If a stool sample is not available (for example due to constipation), then only the rectal swabs will be processed, as per SOP, for microbiome sequence analysis and ARB testing

C.9.14.2. Study Visit 7: Day 7 post-FMT (+/- 2 Day)- Primary End Point

- Clinical assessment by study nurse / physician, specifically enquiring about adverse events related to the FMT.
- Data collection at visit:
    - Interim medical history (See C.19.3)
    - Concomitant medication
    - Significant changes in diet
    - Changes in stool consistency (Bristol Stool Scale) and frequency
    - Clinical assessment findings
    - Vital signs: Temperature, heart rate, blood pressure, weight
    - General health status
    - Adverse events (NIH criteria)
- Stool samples and rectal swabs will be collected for 16S sequencing and ARB testing. If a stool sample is not available (for example due to constipation), rectal swabs should be collected, as per SOP, from the participant for microbiome sequence analysis and ARB testing

C.9.14.3. Study Visit 8: Day 28 (+/- 5 Day) - Intermediate Safety Assessment

- Clinical assessment by study nurse/physician, specifically enquiring about adverse events related to the FMT
- Data collection at visit:
    - Interim medical history (See C.19.3)
    - Concomitant medication
    - Significant changes in diet
    - Changes in stool consistency (Bristol Stool Scale) and frequency
    - Clinical assessment findings
    - Vital signs: Temperature, heart rate, blood pressure, weight

    - General health status
    - Adverse events (NIH criteria)
- Stool samples and rectal swabs will be collected for 16S sequencing and ARB testing. If a stool sample is not available (for example due to constipation), rectal swabs should be collected, as per SOP, from the participant for microbiome sequence analysis and ARB testing

C.9.14.4. Study Visit 9: Month 6 (+/- 14 Day) - Long Term Safety Assessment

- Telephonic interview by study nurse/doctor, specifically enquiring about adverse events related to the FMT
- Data collection at visit:
    - Interim medical history (See C.19.3)
    - Concomitant medication
    - Significant changes in diet
    - Changes in stool consistency (Bristol Stool Scale) and frequency
    - Clinical assessment findings
    - Vital signs: Temperature, heart rate, blood pressure, weight
    - General health status
    - Adverse events (NIH criteria)
- Stool samples and rectal swabs will be collected for 16S sequencing and ARB testing. If a stool sample is not available (for example due to constipation), rectal swabs should be collected, as per SOP, from the participant for microbiome sequence analysis and ARB testing

C.9.15. Early Termination Visit

If the participant terminates the study early, if feasible the study staff should perform an early termination study visit. Ideally, this will include an in-person clinical assessment, but if not feasible (i.e. participant leaves facility), the remaining data should be obtained from the medical record if available.

Study staff will complete an 'Early Termination CRF' and the following clinical assessments:

- Clinical assessment by study nurse/physician, specifically enquiring about adverse events related to the FMT
- Data collection at visit:
    - Reason/s for withdrawing from study
    - Interim medical history (See C.19.3)
    - Concomitant medication
    - Significant changes in diet
    - Changes in stool consistency and frequency
    - Clinical assessment findings

    - Vitals: Heart rate, blood pressure, temperature, weight
    - General health status
    - Adverse events (NIH criteria v 5.0)
- If permitted by the participant, stool biobank stool samples and/or rectal swabs will be collected for 16s sequencing and ARB testing. If a stool sample is not available (for example due to constipation), rectal swabs should be collected, as per SOP, from the participant for microbiome sequence analysis and ARB testing

C.9.16. Unscheduled Study Visit

In the event that a participant develops an ARB associated infection or if believed to be in the participant's best interest by the site PI, study staff will complete an 'Unscheduled Study Visit CRF' and the following clinical assessments:

- Data collection at visit (if applicable):
    - Onset of infectious episode
    - Method of confirmation of ARB infection
    - Site from which organism was isolated
    - Prescribed antibiotics
    - Interim medical history (C.19.3)
    - Concomitant medication
    - Changes in stool consistency and frequency
    - Clinical assessment findings
    - Vitals: Heart rate, blood pressure, temperature, weight
    - Adverse events (NIH criteria)
- Stool and/or rectal swab collection for 16S sequencing and ARB testing

C.10. Study Procedures / Evaluations

C.10.1. Clinical Evaluation at Enrollment

Enrollment: A comprehensive past medical history will be obtained from all participants. Study physician/nurse practitioner will conduct a clinical examination on all participants at enrollment and at FMT study visit (D0).

C.10.2. Evaluation at Follow-Up Study Visits

The study physician/nurse practitioner will conduct the clinical assessment at all follow-up study visits.

Vital signs that will be recorded at each study visit include temperature, blood pressure and pulse rate measurement. Height and weight will be measured at enrollment if feasible for the patient; otherwise this will be abstracted from the medical record.

Study physician/nurse practitioner will enquire about adverse events at each study visit following the FMT (*i.e.* starting at visit 6). All adverse events including solicited and unsolicited, minor and severe adverse events will be recorded at each study visit beginning with study visit number 6. All adverse events will be recorded on the study's adverse event CRF and all serious adverse events will be recorded on the study's serious adverse event form and must be reported to the sponsor within 24 hours of knowledge of the event.

C.10.3. Evaluation of Safety Outcome

All adverse events both unrelated and related to the FMT procedure will be recorded during the course of this study. The primary outcome of this study is to evaluate the safety (short-term) of FMT in this study population at day 7 that is defined as the absence of NIH Grade ≥2 adverse events. This primary outcome will be evaluated using the study adverse event CRFs.

C.10.4. Laboratory Evaluations

C.10.4.1. Enrollment

Stool samples and rectal swabs should be collected from all participants.

C.10.4.2. Screening Only Samples

Samples greater than 10g but less than 40g will be screened for CRE, VRE , ESBL, *C. difficile* and common enteric pathogens (*Salmonella* spp., *Shigella* spp., *Campylobacter* spp.), but will NOT be processed into enema.

C.10.4.3. Samples Processed into Enemas
Stool samples meeting a minimum enema processing weight threshold of 40 grams will be tested for CRE, VRE, ESBL, *C. difficile* and common enteric pathogens (*Salmonella* spp., *Shigella* spp., *Campylobacter* spp.), using either a PCR test or culture-based assay. These stool samples will then be processed into an auto-FMP enema. The participants who test positive for ARB at this stage will be excluded from the randomization portion of the study. A portion of stool from all participants will be aliquoted into 4 equal aliquots and stored at -80°C for future DNA extraction and 16S rRNA sequencing. Stool will be homogenized [redacted] and filtered [redacted].

C.10.4.4. Study Visit Stool Collection
Stool from each study visit will be collected for biobanking, ARB testing and planned future calculation of the microbial disruption index (MDI).

C.10.4.5. Rectal Swabs
Rectal swabs should be collected pre-FMT and at all post-FMT study visits (visits 4 through 9) for 16S rRNA sequencing and ARB testing. All swabs will be processed according to the sample collection SOP.

a. MRDO testing: Swabs that are collected for MDRO testing will be sent [redacted] at room temperature within 2 days of collection.
b. CDI testing: Swabs that are collected for CDI (PCR) testing will be sent [redacted] on dry ice.
c. 16S rRNA sequencing: Swabs for 16s rRNA sequencing will be stored in in -80˚C freezer for biobanking purposes.

C.10.5. Specimen Handling and Shipping
All patients will be assigned a unique barcode number/participant ID. Each floor in a long-term care facility has a dedicated nurse manager and team of certified nurse assistants (CNA), who will be informed about the study and the participants who are enrolled into the study. Study staff will collect the stool and rectal swabs at the bedside and label the specimens. All specimens and swabs will be treated under strict infection control procedures, which include gloves to be worn at all times when handling samples and strict hand sanitizing before and after handling specimens. The labeled stool and rectal swab specimens will then be packaged and transported.

Courier services will be utilized to transport specimens on the day of collection. Specimens will be closely tracked during transportation using the labeling and log books. Formal hand-overs with recording of specimen labels in tracking

logbooks will be required during the transport of specimens (i.e. study staff handover to courier service, courier service handover to laboratory staff).

Stool to be used for the autologous FMT will be processed under aseptic conditions in the laboratory into an auto-FMP enema and stored in a -80°C freezer. Once a participant has been identified as requiring a course of antibiotics for an infection, their auto-FMP enema will be dispatched to the study site. Specific laboratory software will be used to track location of all collected specimen at all times.

Once an FMT date has been scheduled, the enema treatment for that patient will be dispensed by the study staff. A 'double check system' will be employed when dispensing auto-FMP product from freezer storage and before administration of auto-FMP product to recipients. Two staff members (duplicate) are required to check that the specimen barcode matches the donor study identification.

C.11. Assessment of Safety

Safety will be assessed by the frequency and severity of adverse events (AE). For additional information, see Section C18 'Safety Reporting and Definitions'.

C.11.1. Definition of Adverse Events (AE)

Adverse events (AEs) will be recorded at each regular scheduled study visit starting with the FMT visit (visit number 5) 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 who is 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 (including an abnormal laboratory finding), 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 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)

If there is any doubt as to whether a clinical observation is an AE, the event should be reported. AEs must be graded for severity and relationship to study product.

C.11.2. NIH Grading of Severity of the Event

AEs will be assessed by the clinician using the NIH Common Terminology Criteria for Adverse Events (CTCAE) defined grading system version 5.0 (see C.18.2). 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

C.11.3. Relatedness

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, 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.

C.11.4. Solicited Adverse Events After Auto-FMP

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 FMT through to 6 months after FMT):

Symptoms clinically more severe than at 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	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated;	Life-threatening consequences; urgent intervention indicated	Death

	output compared to baseline	output compared to baseline; limiting instrumental ADL	severe increase in ostomy output compared to baseline; limiting self-care ADL		
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 living	Severe pain; limiting self-care activities of daily living	n/a	n/a
Constipation	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas; limited instrumental activities of daily living	Obstipation with manual evacuation indicated; limiting self-care activities of daily living	Life-threatening consequences; urgent intervention indicated	Death
Bloating	Mild symptoms; intervention not indicated	Moderate; persistent; psychosocial sequelae	n/a	n/a	n/a

C.11.5. Serious Adverse Events

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

- Death
- Life-threatening adverse event*
- Inpatient hospitalization or prolongation of existing hospitalization
- 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 serious when,

based upon appropriate medical judgment, 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 PIs, it 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 (regardless of relatedness):

- Reported to the sponsor within 24 hours of knowledge of the event
- Recorded on the appropriate SAE CRF. In the event the eCRF system is unavailable, the site will notify the sponsor using an alternative means of encrypted communication and record the information in the CRFs once the system is back online
- Followed through resolution by a study clinician
- Reviewed and evaluated by a study clinician

C.11.6. Unsolicited Adverse Events

Upon enrollment in the study, the study patients will be instructed to contact the site PI if an AE occurs. These unsolicited, unrelated non-serious adverse events occurring from the time of FMT until 6 months following FMT.

C.11.7. Reporting of Adverse Events

Study participants will be instructed to contact the study nurse or physician if any serious or unexpected adverse event occurs. Study staff will enquire about AEs at each study visit. Reported AE's will be recorded in detail in an AE CRF.

AE information to be collected in the AE CRF:

- Nature of the event (diagnosis)
- Date of onset
- Concomitant treatment: product (generic name), indication, dosage, dosage interval, presentation, mode of administration, administration regimen – this information is captured in a separate concomitant medication log that is linked to the AE
- Duration of the AE
- Severity
- Seriousness
- Causality or relatedness
- Action taken with study drug
- Outcome

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

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

Any SAE (including death, irrespective of the cause) occurring during the study will be immediately reviewed by the site PI, i.e. within 24 hours and reported to the Sponsor.

A specific SAE CRF will be provided similar to C.18.2. 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, or until the end of the 6-month follow-up period, whichever comes first (unless additional follow-up is specifically requested by the medical safety monitor or the DSMB).

If determined to be a SUSAR (suspected unexpected serious adverse reaction – SUSAR; C.18.2 and C.18.3) by the site PI, study medical monitor or lead PI, the event will be reviewed by the DSMB. Regulatory filing to the FDA or local IRB as per standard practices will occur.

The DSMB will review all SAEs every 6 months or *ad hoc* depending on the clinical case at the discretion of the site PI, study medical monitor and lead PI.

For non-serious adverse reactions (related or not related) the site PI will complete a report and submit it to the medical monitor and lead PI. All non-serious adverse reactions will be reviewed by the DSMB every 6 months or *ad hoc* depending on the clinical case at the discretion of the site PI, study medical monitor and lead PI.

In accordance with safety requirements, the PIs will inform the IRB of the study at the Boston University-Boston Medical Center 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 site PI, be withdrawn from further treatment/placebo, and symptomatic treatment must be administered. The participant may opt to voluntarily provide sample for duration of study
- The measures taken must be recorded on the CRF

- In accordance with local legislation, the site PI will submit copies of the final SAE-report to their local IRB, if necessary
- The site PI will submit a copy of the final SAE-report to the Sponsor

C.11.8. Follow-up of Participants After Adverse Events

AEs will be followed until resolution or until the 6-month follow-up visit, whichever occurs first. 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 CRF.

C.11.9. Halting Rules

C.11.9.1. 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.
- 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.

CBER will be notified of any study halt that occurs as a result of any of the above halting criteria.

C.11.9.2. Individual's Halting Rules

Participants who meet any of the following criteria must be assessed by the PIs 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

C.11.10. Safety Oversight

C.11.10.1 Data and Safety Monitoring Board (DSMB)

Safety oversight will be under the direction of a DSMB. The DSMB is an independent group of experts who will advise the study PIs. The primary responsibilities of the DSMB are to 1) periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and when appropriate, efficacy, and 2) make recommendations to the continuation, modification or termination of the trial. The DSMB will be composed of at least 3 voting members. The membership will include a chairperson who has prior DSMB experience. One member will be an experienced gastroenterologist and one member will be an experienced geriatric specialist. All DSMB members will be separate and independent of study personnel participating in this trial and should not have scientific, financial or other conflict of interest related to the trial. Procedures for DSMB data reviews will be defined in the DSMB Charter that will include DSMB membership, responsibilities, and the scope and frequency of data reviews. The DSMB will have access to unblinded treatment assignments during the closed session of their meetings. The study should be reviewed by the DSMB at least bi-annually. The DSMB may conduct a safety interim analysis after 50% of patients have enrolled in the treatment arm.

C.12. Clinical Monitoring

C.12.1. Site Monitoring Plan

Site monitoring is conducted to ensure that the human participant protection, study and laboratory procedures, study intervention administration, and data collection processes are of high quality. The lead PI (or delegate) will conduct a site-monitoring visit(s) as detailed in a monitoring plan. The PIs will permit authorized representatives [redacted], regulatory agency and/or an auditing body to inspect facilities and records relevant to this study, if needed.

Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with site PIs to discuss any problems and actions to be taken and document visit findings and discussions.

C.13. Statistical Considerations

This is a pilot, randomized double blinded placebo controlled clinical trial to determine the safety and efficacy of autologous FMT on the intestinal microbiome and the prevention of ABR colonization and infection.

C.13.1. Study Objectives

To investigate the safety and the role of autologous fecal microbiota transplantation (FMT) for the prevention of antibiotic resistant infections through microbiome restoration.

C.13.2. Primary Endpoint of Short-Term Safety

Proportion of participants with AEs (NIH Grade ≥2) at Day 7 following autologous FMT.

C.13.3. Secondary Endpoints

C.13.3.1. Clearance of ARB among Patients Co-colonized at Day 3, Day 7, Day 28 and Month 6

Proportion of co-colonized participants who clear the ARB following autologous FMT.

C.13.3.2. Proportion of Participants Who Develop ARB Associated Infections at Day 3, Day 7, Day 28, and Month 6

Proportion of participants who develop ARB associated infections following autologous FMT.

C.13.3.3. Proportion of Participants Who Develop AE's by Day 28

Proportion of participants who develop AE's by Day 28 following autologous FMT.

C.13.4. Possible Exploratory Endpoint

C.13.4.1. Microbiome Disruption Indices (16S rRNA sequencing)

Description of severity of microbial community disruption following antibiotic administration. Microbiome disruption indices (MDI) will be developed by Finch Therapeutics, using 16S rRNA sequence data from samples collected pre- and post-FMT. We will assess the suitability of two measures of disruption, applied to pre- and post-intervention communities in comparison to baseline and reference communities, and determine which of these metrics best correlates with clinical outcomes. These measures will comprise: 1) MDI-community which assesses community-level divergence, as measured by the Jensen-Shannon divergence from a reference; and 2) MDI-species, which assesses species-level divergence, as measured by a simple loss measure that quantifies the fraction of

the baseline (or reference) community lost due to disruption (*i.e*. the percent of the reference community that is represented by OTUs missing in the disrupted community). The reference data set will consist of a metacommunity that we will build using healthy OpenBiome donors. MDI will be assessed as the deviation from the healthy metacommunity.

C.13.5. Sample Size

As this is a pilot proof-of-concept study, no formal power calculation is possible and a sample size of convenience was utilized (n=20). In order to yield the target sample size, a total of 180 participants is predicted to be required assuming:

1. 40% exclusion for positive ARB in stool per Trick and colleagues study[3]
2. 35% of residents will develop an infection requiring antibiotics annually based on direct historical observations at these sites (C.19.2)
3. Adjusted for 7.5-month treatment window to meet target study period (C.19.3)
4. An anticipated drop-out rate of 15%

Given that the 4 nursing homes participating in the study house a total of 866 residents, the total enrollment of 180 participants can be achieved with a 20.8% enrollment rate. The sites feel this is realistic given that most participants will only need to undergo informed consent and provide a stool sample for processing and testing. See C.19.2 for a schematic of this process.

C.13.6. Final Analysis 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.

For the primary outcome of AEs at day 7, the proportion of AEs occurring in the intervention group will be compared to the proportion of AEs occurring in the control group at day 7, using the Z-score test for two populations with 95% confidence intervals. Given the limited sample size, the confidence intervals will likely be quite wide. The p-value will be two tailed with a significance level of 0.05.

For the secondary outcome of ARB colonization at days 3, 7, and 28, the proportion of ARB colonizations occurring in the intervention group will be compared to the proportion of ARB infections occurring in the control group at each time point, using the Z-score test for two populations with 95% confidence intervals. Given the limited sample size, the confidence intervals will likely be quite wide. The p-value will be two tailed with a significance level of 0.05.

C.14. Source Documents and Access to Source Data/Documents

Each participating site 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.

C.15. Quality Control and Quality Assurance

The site PI or delegate is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site 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. The site PI will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The site PI will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements.

C.15.1. Procedural Quality Control

Data coordination center chair in conjunction with site PIs will implement quality control procedures including a 'double-check' procedures when collecting/labeling stool and rectal swab specimens and administering FMTs at study sites.

Study personnel will be required to verify that specimens have been labelled appropriately at collection. Similar, stringent tracking and labelling processes will be implemented while stool specimens are being processed. Two study personnel will be required to verify identification of the participant during the administration of fecal microbiota to recipients, thereby ensuring that the recipient receives their own donated stool during the FMT.

C.15.2. Database Quality Control

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 communicated to the site(s) for clarification and resolution.

C.16. Ethics/Protection of Human Participants

C.16.1. Ethical Standard

The PIs will ensure that this study is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable.

C.16.2. Institutional Review Board

Each participating institution will provide for the review and approval of this protocol and the associated informed consent documents, by an appropriate ethics review committee or IRB. Any amendments to the protocol or consent materials must also be approved before they are placed into use unless change is for the safety of the participant. Only those IRB members who are independent of the PIs should provide an opinion on study related matters. Verification of IRB approval of the protocol and the written informed consent will be transmitted by the PIs or designee prior to the shipment of clinical trial material. No deviations from or changes to the protocol will be initiated without prior approval of an appropriate amendment unless change is for the safety of the participant. Each participating institution is responsible for ensuring Continuing Review at least once a year and for keeping the IRB apprised of the progress of the study and any changes to the protocol.

C.16.3. Informed Consent Process

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 should be implemented 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 should be presented both orally and in written form. A site PI or designee will describe the protocol to potential participants and/or substitute decision maker/proxy guardian face-to-face, over the phone, and/or by email. The Participant Information and Consent Form may be read to the participants, but in any event, the site PI 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 and/or substitute decision maker/proxy 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 (including

a statement that the particular treatment or procedure may involve risks to the participant or to the embryo or fetus, if the participant is or may fathers a child, that are currently unforeseeable), the expected duration of the participant's participation in the trial, the procedures of the research study, including all invasive procedures, and the probability for random assignment to treatment groups. Participants and/or substitute decision maker/proxy will be informed that 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 site PI nor the trial staff should 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 must be in a language fully comprehensible to the prospective participants. Informed consent shall 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 PIs 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.

C.16.4. Exclusion of Women, Minorities, and Children (Special Populations)
Children and pregnant women are excluded for safety reasons.

C.16.5. Participant Confidentiality
Participant confidentiality is held strictly in trust by the site PIs and their designees. 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 Sponsor.

The study monitor or other authorized representatives of the PIs and FDA may inspect all documents and records required to be maintained by the site PI, 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.

C.16.6. Study Discontinuation
The Sponsor has the right to terminate this study or an individual site's participation at any time. Reasons for terminating the study may include, but are not limited to, the following:

- Incidence or severity of adverse events indicates a potential health hazard
- Data recording is inaccurate or incomplete

- Site PI does not adhere to the protocol or applicable regulatory guidelines in conducting the study

C.16.7. Future Use of Stored Specimens

Any leftover specimens will be stored and may be used for future research. 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 de-identified barcoded specimens are stored for future use. For participants who choose not to allow storage of their samples for future use, their samples will be destroyed at the end of the study.

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.

Samples may be shared with other investigators at other institutions with the consent of the lead PI. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect participant's confidentiality.

Research using stored specimens may be conducted by other institutions. Any specimens and data provided to the receiving-institution will be coded. Unequivocally, neither individual personal identifiers nor the key linking coded data to individuals will be released to the receiving-institution. The use of any of these specimens for any future studies will only be performed after the lead PI has authorized the use of these specimens.

C.16.8. Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse Events must be graded, assessed for severity and causality, and reviewed by the site PI or designee and reported to the sponsor.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the site PI must maintain complete and accurate documentation for the study.

C.16.9. Data Capture Methods

Clinical data (including AEs, concomitant medications, and solicited events data) and clinical laboratory data will be entered into a 21CFR11-compliant Internet Data Entry System, REDCap. 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.

C.16.10. Types of Data

Data for this study will include clinical, safety and microbiological outcome measures.

C.16.11. Timing/Reports

Interim reports for the DSMB will be prepared when approximately 50% of treating participants complete enrollment and every 6 months. Interim statistical reports may be generated as deemed necessary and appropriate by the lead PI. Other safety summary reports may be generated for the DSMB. A final report will be prepared following the availability of all the clinical, safety and efficacy data.

C.16.12. Study 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 and 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, if applicable. It is the responsibility of the lead PI to inform the site PI when these documents no longer need to be retained. Consent forms for future use will be maintained as long as the sample exists.

C.16.13. 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 site PI, or the study site 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 site PI 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 lead 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. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.
All protocol deviations must also be reported to the sponsor. A report of all deviations should be sent via email to the sponsor on a monthly basis. Any deviation that per site guidelines is considered a major deviation should be reported to the sponsor within 24 hours of knowledge of the deviation.

C.17. References

1. CDC. *Antibiotic Resistance Threats in the United States, 2013*. Atlanta, GA: US Department of Health and Human Services, CDC; 2013.

2. Lim CJ, Kong DC, Stuart RL. Reducing inappropriate antibiotic prescribing in the residential care setting: Current perspectives. *Clin Interv Aging*. 2014;9:165-177. doi:10.2147/CIA.S46058.

3. Trick WE, Weinstein RA, DeMarais PL, et al. Colonization of skilled-care facility residents with antimicrobial-resistant pathogens. *J Am Geriatr Soc*. 2001;49(3):270-276.

4. Loeb MB, Craven S, McGeer AJ, et al. Risk factors for resistance to antimicrobial agents among nursing home residents. *Am J Epidemiol*. 2003;157(1):40-47. doi:10.1093/aje/kwf173.

5. CDC. *Core Elements of Antibiotic Stewardship for Nursing Homes*. Atlanta, GA: US Department of Health and Human Services, CDC; 2015.

6. Yates M, Horan MA, Clague JE, Gonsalkorale M, Chadwick PR, Pendleton N. A study of infection in elderly nursing/residential home and community-based residents. *J Hosp Infect*. 1999;43(2):123-129. doi:10.1053/jhin.1999.0739.

7. Relman DA. The human microbiome: Ecosystem resilience and health. *Nutr Rev*. 2012;70(Suppl 1):S2-S9. doi:10.1111/j.1753-4887.2012.00489.x.

8. Becattini S, Taur Y, Pamer EG. Antibiotic-induced changes in the intestinal microbiota and disease. *Trends Mol Med*. 2016;22(6):458-478. doi:10.1016/j.molmed.2016.04.003.

9. Hentges DJ, Freter R. In vivo and in vitro antagonism of intestinal bacteria against Shigella flexneri: I. Correlation between various tests. *J Infect Dis*. 1962;110(1):30-37. doi:10.1093/infdis/110.1.30.

10. Freter R. In vivo and in vitro antagonism of Intestinal bacteria against Shigella flexneri: II. The inhibitory mechanism. *J Infect Dis*. 1962;110(1):38-46.

11. Bohnhoff M, Miller CP. Enhanced susceptibility to Salmonella infection in Streptomycin-treated mice. *J Infect Dis*. 1962;111(2):117-127.

12. Drekonja D, Reich J, Gezahegn S, et al. Fecal microbiota transplantation

for Clostridium difficile infection: A systematic review. *Ann Intern Med*. 2015;162(9):630-638. doi:10.7326/M14-2693.

13. Agrawal M, Aroniadis OC, Brandt LJ, et al. The long-term efficacy and safety of fecal microbiota yransplant for recurrent, severe, and complicated Clostridium difficile infection in 146 elderly individuals. *J Clin Gastroenterol*. 2016;50(5):403-407. doi:10.1097/MCG.0000000000000410.

14. Pamer EG. Resurrecting the intestinal microbiota to combat antibiotic-resistant pathogens. *Science (80-)*. 2016;352(6285):535-538. doi:10.1126/science.aad9382.

15. Halpin AL, McDonald LC. The Dawning of Microbiome Remediation for Addressing Antibiotic Resistance. *Clin Infect Dis*. March 2016:ciw187. doi:10.1093/cid/ciw187.

16. Donskey CJ, Chowdhry TK, Hecker MT, et al. Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. *N Engl J Med*. 2000;343(26):1925-1932. doi:10.1056/NEJM200012283432604.

17. Johnsen PJ, Simonsen GS, Olsvik O, Midtvedt T, Sundsfjord A. Stability, persistence, and evolution of plasmid-encoded VanA glycopeptide resistance in enterococci in the absence of antibiotic selection in vitro and in gnotobiotic mice. *Microb Drug Resist*. 2002;8(3):161-170. doi:10.1089/107662902760326869.

18. Foucault M-L, Depardieu F, Courvalin P, Grillot-Courvalin C. Inducible expression eliminates the fitness cost of vancomycin resistance in enterococci. *Proc Natl Acad Sci U S A*. 2010;107(39):16964-16969. doi:10.1073/pnas.1006855107.

19. Ubeda C, Bucci V, Caballero S, et al. Intestinal microbiota containing Barnesiella species cures vancomycin-resistant Enterococcus faecium colonization. *Infect Immun*. 2013;81(3):965-973. doi:10.1128/IAI.01197-12.

20. Crum-Cianflone NF, Sullivan E, Ballon-Landa G. Fecal microbiota transplantation and successful resolution of multidrug-resistant-organism colonization. *J Clin Microbiol*. 2015;53(6):1986-1989. doi:10.1128/JCM.00820-15.

21. Freedman A. Use of stool transplant to clear fecal colonization with carbapenem-resistant enterobacteraciae (CRE): Proof of concept. In:

IDSA. ; 2014.

22. Singh R, van Nood E, Nieuwdorp M, et al. Donor feces infusion for eradication of Extended Spectrum beta-Lactamase producing Escherichia coli in a patient with end stage renal disease. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2014;20(11):O977-978. doi:10.1111/1469-0691.12683.

23. Wei Y, Gong J, Zhu W, et al. Fecal microbiota transplantation restores dysbiosis in patients with methicillin resistant Staphylococcus aureus enterocolitis. *BMC Infect Dis*. 2015;15:265. doi:10.1186/s12879-015-0973-1.

24. Lombardo M-J. Vancomycin-resistant enterococcal (VRE) titers diminish among patients with recurrent Clostridium difficile infection after administration of SER-109, a novel microbiome agent. In: IDSA; 2015.

25. Dubois N, Ling K, Osman M, et al. Prospective assessment of donor eligibility for Fecal Microbiota Transplantation at a public stool bank: results from the evaluation of 1,387 candidate donors. In: San Diego: IDSA; 2015.

26. Kassam Z, Lee CH, Yuan Y, Hunt RH. Navigating long-term safety in fecal microbiota transplantation. *Am J Gastroenterol*. 2013;108(9):1538. doi:10.1038/ajg.2013.214.

27. Taur Y, Pamer EG. Autologous Fecal Microbiota Transplantation (Auto-FMT) for prophylaxis of Clostridium difficile infection in recipients of allogeneic hematopoietic stem cell transplantation. Clinical Trials Registry (NCT02269150).

28. Fischer M, Allegretti JR, Smith M, et al. A multi-center, cluster randomized dose-finding study of fecal microbiota transplantation capsules for recurrent Clostridium difficile infection. In: ; 2016.

29. Zipursky JS, Sidorsky TI, Freedman CA, Sidorsky MN, Kirkland KB. Patient attitudes toward the use of fecal microbiota transplantation in the treatment of recurrent Clostridium difficile infection. *Clin Infect Dis*. 2012;55(12):1652-1658. doi:10.1093/cid/cis809.

30. Hochberg NS, Rekhtman S, Burns J, et al. The complexity of diagnosing latent tuberculosis infection in older adults in long-term care facilities. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis*. 2016;44:37-43. doi:10.1016/j.ijid.2016.01.007.

31. Crobach MJT, Planche T, Eckert C, et al. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for Clostridium difficile infection. *Clin Microbiol Infect*. 2016;20 Suppl 2(Cdi):1-26. doi:10.1016/j.cmi.2016.03.010.

32. Claesson MJ, Jeffery IB, Conde S, et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature*. 2012;488(7410):178-184. doi:10.1038/nature11319.

33. Mariat D, Firmesse O, Levenez F, et al. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol*. 2009;9:123. doi:10.1186/1471-2180-9-123.

34. Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. *Am J Gastroenterol*. 2012;107(5):761-767. doi:10.1038/ajg.2011.482.

35. Youngster I, Mahabamunuge J, Systrom HK, et al. Oral, frozen fecal microbiota transplant (FMT) capsules for recurrent Clostridium difficile infection. *BMC Med*. 2016;14(1):134. doi:10.1186/s12916-016-0680-9.

C.18. Safety Reporting and Definitions

C.18.1. Investigational New Drug Safety Reporting Definitions

21 CFR §312.32 IND safety reporting.

(a) *Definitions.* The following definitions of terms apply to this section:

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

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

Serious adverse event or *serious suspected adverse reaction.* An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or *unexpected suspected adverse reaction.* An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue

of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

C.18.2. Sample Adverse Event Reporting Form with NIH Severity Grading Scale (version 5.0)

Adverse Events Recording Form

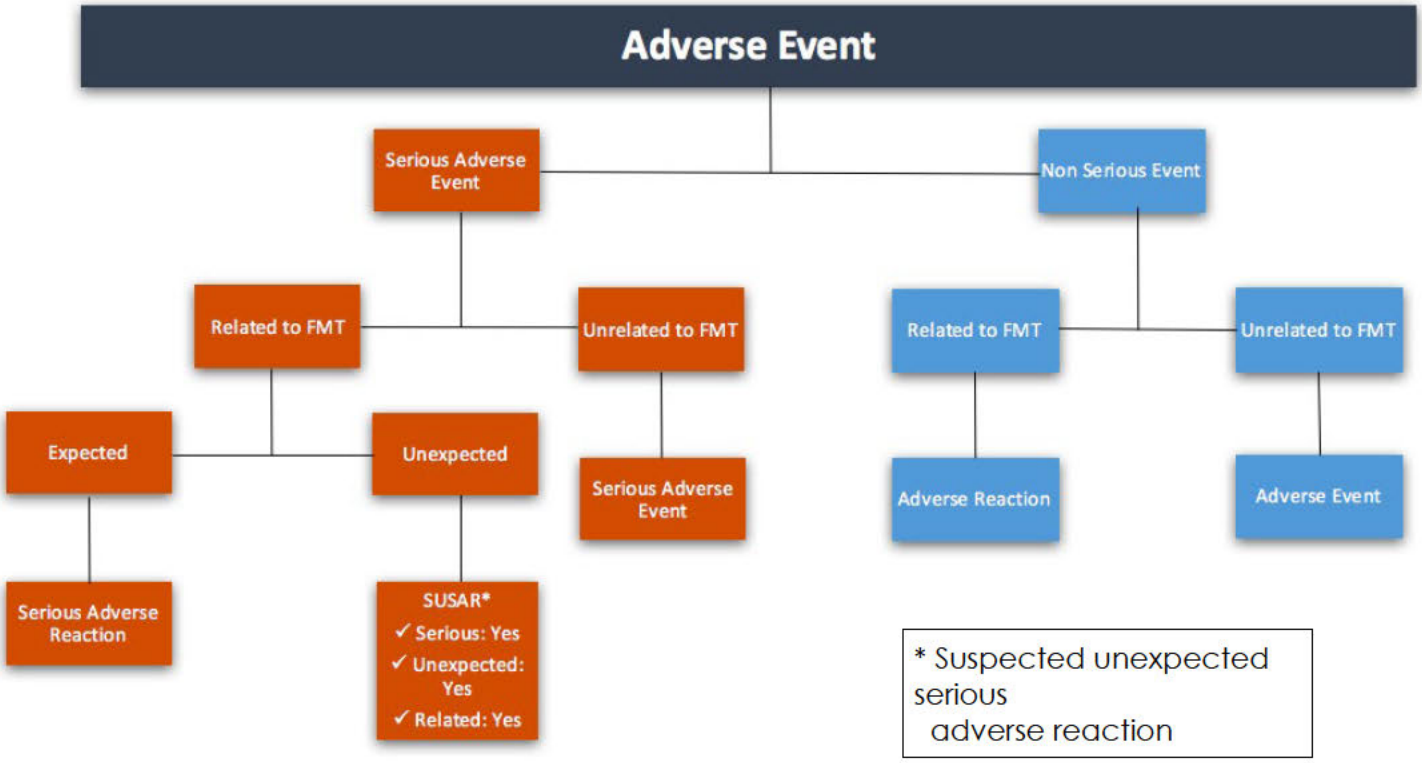
Record of adverse events:				
System:	**Present**	**Grade**	**Attribute**	**Describe reaction (refer to appendix 9)**
Systemic				
Infection				
Injection site reaction				
Skin/dermatologic				
Cardiovascular				
Gastrointestinal				
Neurologic				
Respiratory				
Musculoskeletal				
Genitourinary				
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

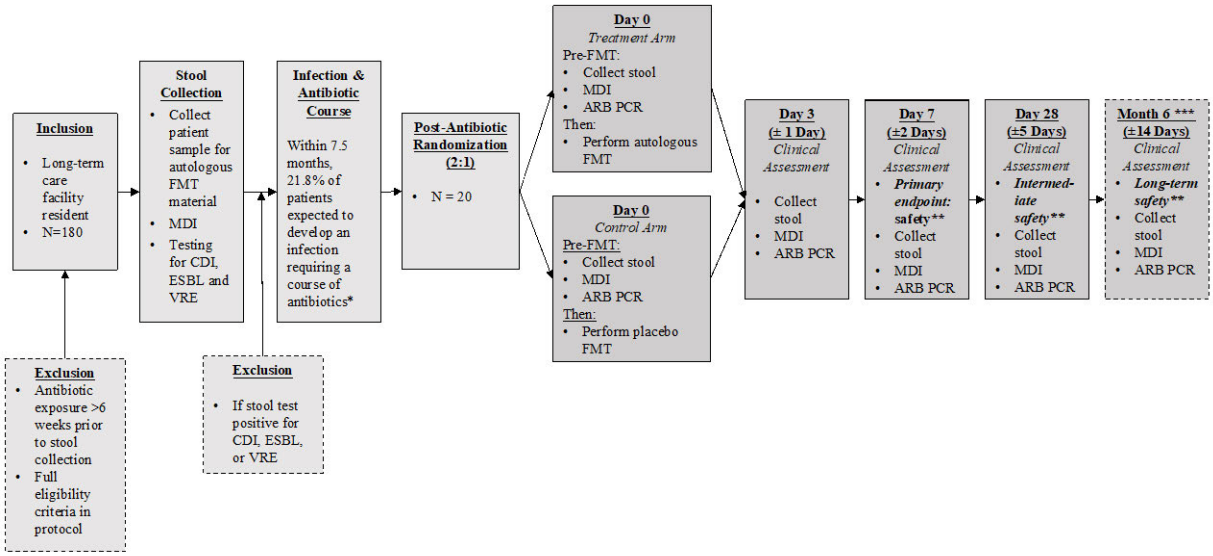
C.18.3. Adverse Event Assessment Flowchart



C.19. Study Design

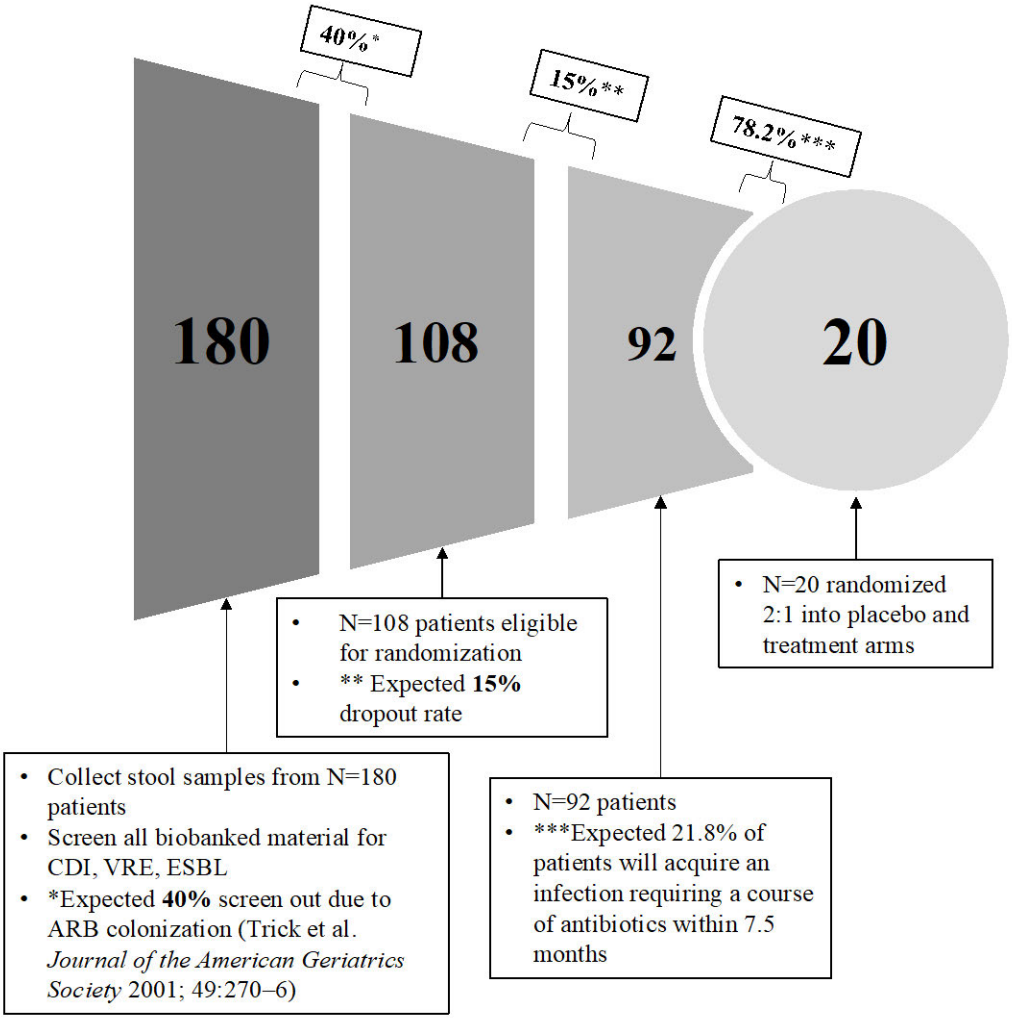
C.19.1. Study Design Schematic

Study Schematic– RACE: Randomized controlled trial of Autologous microbiome reconstitution to prevent Colonization by antibiotic rEsistant bacteria



* Based on clinical site infection rates
**Safety: As defined by NIH Severity Grade ≥2 and IND safety reporting criteria defined in 21 CFR § 312.32
***The Month 6 safety FDA-mandated follow-up will not be part of the grant timeline; however, it will be completed along with MDI analysis as a follow-up study at no additional funding.
Expected 15% dropout rate

C.19.2. Patient Enrollment Pipeline



	Screening Visit and Patient Information (Visit 1)	Collection & Processing (Visit 2)	Infectious Episode (Visit 3)	Post-Antibiotic (Visit 4)	FMT Study Visit* (Visit 5)	Day 3 (Visit 6)	Day 7 (Visit 7)	Day 28 (Visit 8)	Month 6 (Visit 9)
Informed consent	X	-	-	-	-	-	-	-	-
Medical History	X	-	X	-	X	X	X	X	X
Concomitant Medication	X	-	X	-	X	X	X	X	X
Adverse Events	-	-	-	-	-	X	X	X	X
Physical Exam	X	-	-	-	X	X	X	X	X
Enema	-	-	-	-	X*	-	-	-	-
Stool/swab sample	-	X**	-	X	X	X	X	X	X
CRE, VRE, ESBL 16s and CDI testing*	-	X	-	X	X	X	X	X	X
Processing of stool into auto-FMT	-	X	-	-	-	-	-	-	-

C.19.3. Study Schedule

*Participants who become severely immunocompromised, as defined by the investigator/treating physician, will be excluded prior to receiving intervention.

** Participants with a history of gastrointestinal (GI) illness within the past 30 days prior to enrollment stool collection, that at the discretion of the site investigator could reasonably be caused by one of the following pathogens 1) *Vibrio* spp. 2) Norovirus 3) Rotavirus 4) Adenovirus 5) Shiga toxin, will be excluded from stool collection.

C.20. Common Conditions in the Geriatric Population

Anemia
Angina or chest pain
Asthma
Atrial fibrillation
Bipolar
Bleeding disorder
Blood clots / venous thromboembolism
Bypass surgery
Cancer
Chronic Obstructive Pulmonary Disease/emphysema
Cirrhosis
Congestive heart failure
Connective tissue disorder (lupus, scleroderma)
Coronary artery disease
Crohns Disease
Dementia
Depression
Dialysis
Dysphagia (trouble swallowing)
Gout
Heart attack/Myocardial Infarct
Heart valve replacement
Hepatitis
Hiatial hernia
High blood pressure
High cholesterol
Hip fracture
HIV/AIDS
Irritable bowel
Kidney failure
Liver disease
Memory problems
Neuropathy
Palpitations
Parkinsons
Poor circulation/claudication
Rheumatoid Arthritis
Schizophrenia
Seizures
Spinal surgery
Stomach ulcers
Stroke
Transient Ischaemic Attack (TIA)
Type 2 Diabetes Mellitus
Ulcerative colitis

INFORMED CONSENT STATEMENT

RACE: Randomized controlled trial of Autologous microbiome reconstitution to prevent Colonization by antibiotic rEsistant bacteria

IRB #: H-35722
Sponsor: OpenBiome (full name: Microbiome Health Research Institute Inc.)

Principle Investigators: Zain Kassam, MD, MPH, FRCPC [redacted]

Christine Liu, MD, MSc [redacted]

Study-Related Phone Numbers: Regular business hours: [redacted]
24 hours: [redacted]

BACKGROUND

It is normal for friendly bacteria to live in a person's large intestine (or colon). These friendly bacteria help the body to stay well and keep normal function. They help in a number of ways: making certain vitamins; fighting off disease; and digesting food. When the number of bacteria gets low, a person can become ill. A common illness is diarrhea. Recently doctors have started treating one type of diarrhea (*C. difficile)* by transplanting friendly bacteria from the colons of healthy people into people who have the diarrhea. This process is called a fecal bacteria transplantation and will be referred to as the "bacteria transplant" throughout this form.

Treatment with antibiotics is known to disrupt the health of friendly bacteria in the colon. Antibiotics can lead to a colon infection with bad bacteria that resist the effects of antibiotics. The purpose of this research is to learn if a friendly bacteria transplant can stop infection with bad bacteria that resist antibiotics. We want to try this study in a nursing home. People who live in nursing homes are more subject to be infected with bad bacteria.

The stool donation would be taken from your own colon. The transplant dose will be made from friendly bacteria extracted from your own stool (bowel movement). After you have a bowel movement, we would then take the stool, clean the stool, and then save the bacteria to use in the future. This is similar to donating your own blood before you have surgery. If you get treated with antibiotics, you would get the bacteria transplant made of your own friendly bacteria. The transplant will given via an enema.

Not everyone in the study will receive the actual bacteria transplant. Half the people in the study will have an enema that contains no fecal material (placebo). Comparing two groups will help the researchers learn if the bacteria transplant makes a difference in the development of antibiotic resistant bacteria infection.

Taking part in this research study is voluntary. You can decide to stop taking part in this research study at any time for any reason. If you stop being in this research study, it will not affect how you are treated at the nursing home or your relations with your medical care team.

Your doctor may also be an investigator in this research study. Being an investigator means your doctor is interested in both you and the study. You may want to get a second opinion about being in the study. You can do so now or at any time during the study. Another doctor who is not an investigator can give you a second opinion about being in the study. You do not have to agree to be in this study even though it is offered by your doctor.

PURPOSE

This is not a treatment study. The researchers first want to see if this type of bacteria transplant is safe and if it is possible to perform. In addition, there will be examinations to see if the bacteria transplant made a difference in the type of bacteria in your colon and to check the type of antibiotic resistant bacteria in your colon.

This study would use your own friendly bacteria from your stool, which OpenBiome and their research collaborators will collect and store for use if you are prescribed antibiotics.

WHAT WILL HAPPEN IN THIS RESEARCH STUDY

If you agree to be in the study, you will provide us with a stool sample. If you are prescribed antibiotics, you will be assigned to receive a treatment of your own stool or a placebo. A computer will randomly choose which of the two study groups you will be assigned. The choice will neither be based on your decision nor the researcher's decision. The placebo looks exactly like the bacteria transplant treatment, but does not contain stool. Placebos are used in research studies to see if the results are due to the study treatment or other reasons. Neither you nor the study staff will know which one you receive. Study staff will follow up with you for at least 6 months after the treatment.

If you agree to participate, you will be one of 180 subjects who will be participating in this research.

Before You Begin the Study:

The study staff will briefly review your health records and information to ensure that you are a good candidate for the study. They will discuss with you the risks and benefits of being in the study, and answer any questions you have regarding the study.

After You Begin the Study:

Study staff will examine your medical chart to determine your demographics, past medical history and allergies, current medication list, and dietary, social, travel and family history. A short physical exam including measuring weight and height will be performed. You will be asked to provide a stool sample that will be collected by the study staff. At the same time, we will also swab your rectum with a small swab the size of a Q-tip. The stool will then be cleaned and then processed to into the bacteria transplant and saved. We may need to collect several stool samples until there is one that is of adequate size to make into a transplant. After you provide a stool sample to process into an enema, the study team will monitor your medical records to see if you will be treated with antibiotics.

Your Bacteria Transplant Procedure:

If you develop an infection needing antibiotic treatment, the study team will offer you treatment from the study. If you decide undergo the treatment, the study team will first collect another stool sample and rectal swabs from you. If a stool sample is not available (for example due to constipation), only rectal swabs will be collected. A brief clinical examination will also be performed to collect vitals.

Then you will receive the bacteria transplant via enema. This will happen within eight weeks of you finishing your antibiotics. You will receive either your own colon bacteria or a placebo. Neither you nor your physician will know which study group you are in. Your other treatments will remain unchanged. The bacteria transplant is the administration of prepared stool and water mixture from your previously provided stool sample.

For the bacteria transplant, you will be asked to lie on your left hand side and a study doctor or nurse will administer the enema. You will be asked to try and hold the enema fluid this for 30 minutes if you can, continuing to rest during this time. While you are getting the enema, a study doctor or nurse will be with you the entire time.

After Your Bacteria Transplant Procedure:

Study staff will meet you four times after you get the bacteria transplant:

- 2-4 days after your bacteria transplant
- 5-9 days after your bacteria transplant
- 23-33 days after your bacteria transplant
- 5 ½ to 6 ½ months after your bacteria transplant

During each visit, the study team will perform a brief physical examination to evaluate and identify any changes in your health. We will monitor your medical chart to determine any changes to your medical history and any changes to your medications. We will also collect a stool sample and rectal swabs at each of these follow-up visits to allow us to learn the type of bacteria in your stool. If you are unable to provide a stool sample, we will only collect rectal swabs.

Sending Study Information to Research Collaborators:

In addition to OpenBiome and Boston Medical Center researchers having your study information, we will send some of your study information and/or samples to researchers working with us.

These researchers may use your information and/or samples along with other information that they may later on develop for internal and external programs such as publication, future research trial design, or commercial purposes. You will not get any money from the programs.

We will label all your study materials with a code instead of your name. The key to the code connects your name to your study information and samples. The key to the code will be kept at OpenBiome and will not be shared with our research collaborators.

Unanticipated Findings:

During the screening process, we might find out that you have problems with your memory or have a certain kind of bacteria in your stool. If we find these problems, we would like to share this information with your regular doctor, as it may affect future medical treatment. However, we will only share this information with your permission. We will suggest to your regular doctor that our findings be verified, as the test we used may differ from the tests they prefer. Your doctor may or may not want to change your medicines or current treatments depending on what they think of the test results. We are happy to discuss what these possible changes may be, but it is ultimately up to your regular doctor to make the final recommendation as they know you best.

Biorepository Bank:

As part of your participation in this study, you will give us stool for testing. After testing for infectious disease and filtering the friendly bacteria for transplant, part of your samples may be left over. These samples would normally be thrown away. We are asking you to allow us to collect and store this leftover stool in a research tissue bank. The collection of stool is being done as part of your participation in this research study and would be done whether or not you give the study staff permission for your samples to be stored in the tissue bank. There will be no extra procedures or time commitment involved for allowing us to store your leftover samples.

If you agree, the leftover samples will be frozen and sent to the bank. We are also asking for your permission to store some of your health information with your samples so that your samples can be more useful for research. We plan to continue to review your medical record to update your health information in the tissue bank computer database. We will ask that you contact us if you are required to take antibiotics in the future for any reason.

Your samples and information will be used mainly to understand the general effects of fecal transplantation and future antibiotic use of patients who have gone through this procedure. The long-term goals of the research are to learn how to better understand, prevent, diagnose or treat frequent c diff infections. It is not possible to list every research project. Also, we cannot predict all of the research questions that will be important over the next years. As we learn more, there are new research questions and new types of research related to *C. difficile* and the microbiome.

Your samples and information may also be used for research on other conditions (e.g. comparisons to other diseases). This could include a wide variety of conditions such as diabetes, cancer or other chronic illnesses.

We may plan to do genetic research on the DNA in your tissue sample. DNA is the material that makes up your genes. All living things are made of cells. Genes are part of cells with instructions that tell our bodies how to work, and determine physical characteristics such as hair and eye color. Genes are passed from parent to child.

There is no limit on how long OpenBiome may store your samples and information for research. Samples will only be released after a project is approved by a committee of experts who will review each request to make sure the proposed research is ethical, useful, and based on good science. The code linking your samples to your medical record may be kept indefinitely so that your samples and updated health information may be used for research in the future.

You have a right to withdraw your permission at any time. If you withdraw, your samples and information will be destroyed. However, it will not be possible to destroy samples and information that have already been given to researchers. If you decide to withdraw, you should contact the tissue bank's staff by phone: [redacted].

You will not directly benefit from research conducted on your samples stored in the research tissue bank. We hope that research using the samples and information will help us understand, prevent, treat or cure the illnesses and conditions studied.

RISKS & DISCOMFORTS

Most persons enrolled in the study will only donate stool and likely not have to take the bacteria transplant. However, if you do need to have a bacteria transplant, here are some important considerations of possible events:

- Symptoms that last only for a short while and usually get better on their own:
  - Diarrhea (70%)
  - Abdominal cramps/discomfort (20%)
  - Nausea and/or vomiting (<5%)
  - Constipation (20%)
  - Excess flatulence (25%)
  - Related to enema:
    - Irritation of the perianal area, including exacerbation of any potential pressure ulcers in the perianal area from enema
    - Abdominal or rectal discomfort during enema administration
- More serious symptoms:
  - Infection: Although this material has been screened for bacteria, viruses, fungi and parasites there is a risk of transmission of known and unknown infectious organisms. This will be characterized by fever, chills and possibly low blood pressure. There have been two cases of a bacterial infection in the blood after the transplant but it is unclear if this was from the bacteria transplant, and the patient did well on antibiotics.
  - Receiving non-autologous bacteria transplant: there is always a risk, as in any procedure that administers any biological product based on bodily fluids, that the person receives the incorrect product. In this case, this would be a bacteria transplant of stool from another person.
  - Inflammatory bowel disease (IBD) flare in those with underlying IBD
  - Bowel perforation due to the pressure required to administer the enema
  - Hemorrhoid bleeding from the enema may occur. We will undertake precautions to prevent bleeding from your hemorrhoids.

The long-term side effects of the bacteria transplant are **not** currently well known. During the study, you should report anything that you feel is relevant or causing you concern. There may be unknown risks or discomforts involved.

POTENTIAL BENEFITS

There may be a direct benefit to you if the therapy works as it is intended. The bacteria transplant may prevent a serious infection after taking antibiotics. If the study therapy does not work or you receive the placebo, you may not receive any benefit. However, you being in the study may help investigators learn how to improve available treatment in the future.

ALTERNATIVES

If you decide not to participate in this study and still want to prevent antibiotic resistant bacteria, you can try probiotics such as Culturelle capsules, which are available at local drug stores. However, the ability of probiotics to prevent antibiotic resistant bacteria has not been definitely proven.

COSTS

There are no costs to you for being in this research study.

PAYMENT

You will be compensated for your participation. Once you have completed the consent process, you will receive a $30 gift card. If you undergo a study intervention (treatment or placebo), you will receive a $70 gift card after the procedure.

CONFIDENTIALITY

As this study involves the use of your identifiable, personal information, there is a chance of a loss of confidentiality. The researchers have procedures in place to lessen the possibility of this happening. The research study will be conducted according to detailed standard operating procedures that protect patient confidentiality. We will do our best to keep your information safe. However, we cannot guarantee confidentiality.

Federal and state agencies, if they are required by law or are involved in research oversight, may access information about you from this study including your health information. Such agencies may include the U.S. Department of Health and Human Services, the Food and Drug Administration, the National Institutes of Health, and the Massachusetts Department of Public Health.

The results of this research study may be published in a medical book or journal, or used to teach others. However, your name or other identifying information will **not** be used for these purposes without your specific permission.

A description of this clinical trial will be available on **ClinicalTrials.gov**, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

The repository has standard operating procedures to protect your confidentiality. Staff at the bank will assign your sample a code number and store it in a freezer. They will not keep your name or other information that could identify you with your sample. They will use the code number to connect your sample to your health information that is stored in a computer database. The computer database is password protected. Only staff at the bank will know the password.

USE AND DISCLOSURE OF YOUR HEALTH INFORMATION

Boston Medical Center and the study staff want to use and share your health information as part of this research study. The law requires Boston Medical Center to get your permission to do so.

Health information that might be used or given out during this research includes:

- Information from your hospital or office health records or elsewhere. This applies to information that is reasonably related to the aims, conduct and oversight of the research study. If health information is needed from your doctors or hospitals, we will ask you for permission for these records to be sent to the researcher.
- New health information from tests, procedures, visits, interviews, or forms filled out as part of this research study

The reasons that your health information might be used or given out to others are:

- To do the research described here
- To make sure we do the research according to certain standards set by ethics, law, and quality groups or otherwise as required by law

The people and groups that may use or give out your health information are:

- Researchers involved in this research study from Boston Medical Center
- Researchers from other institutions or organizations that are involved in this research study
- Other people at Boston Medical Center who may need to access your health information to do their jobs (e.g. treatment, research administration, payment, billing, or health care operations)

- People or groups who the researchers use to help conduct the study or provide oversight for the study
- The Institutional Review Board that oversees the research and other people or groups who are part of the Human Research Protection Program that oversees the research
- Research monitors, reviewers, or accreditation agencies and other people or groups that oversee research information and the safety of the study
- The sponsor of the research study, listed on the first page, and people or groups hired to help them do the research

Some people or groups who get your health information may not be obligated to follow the same privacy laws we follow. We ask anyone who gets your health information from us to protect the privacy of your information. However, after your information has been shared with others, we cannot promise that it will be kept private.

The time period for using or giving out your health information:

- Because research is an ongoing process, we cannot give you an exact date when we will either destroy or stop using or sharing your health information

Your privacy rights are:

- You have the right not to sign this form that allows us to use and give out your health information for research. If you do not sign this form, you cannot be in the research. This is because we need to use the health information to do the research. Your decision not to sign the form will not affect any treatment, health care, enrollment in health plans, or eligibility for benefits.
- You have the right to withdraw your permission to use or share your health information in this research study. If you want to withdraw your permission, you must write a letter to the Principal Investigator at the address listed on the first page of this form. If you withdraw your permission, you will not be able to take back information that has already been used or shared with others. This includes information used or shared to do the research study or to be sure the research is safe and of high quality. If you withdraw your permission, you cannot continue to be in the study.
- You have the right to see and get a copy of your health information from the Principal Investigator that is used or shared for research. However, you may only get this copy after the research is finished.
- The results of this research study may be published in a medical book or journal, or used to teach others. However, your name or other identifying information will not be used for these purposes without your specific permission.

COMPENSATION FOR INJURY

If you think that you have been injured by being in this study, please let the investigator know right away. Use the phone number on the first page of this form. You can get treatment for the injury at Boston Medical Center or at any healthcare facility you choose. There is no program to provide compensation for the cost of care for research related injury or for other expenses. Other expenses may include lost wages, disability, pain or discomfort. You or your insurance will be billed for the medical care you receive for a research injury. You are not giving up any of your legal rights by signing this form.

In the event of physical injury resulting from your participation in this research, necessary medical treatment will be provided to you and billed as part of your medical expenses. Costs not covered by your health care insurer will be your responsibility. Also, it is your responsibility to determine the extent of your health care coverage. There is no program in place for other monetary compensation for such injuries. However, you are not giving up any legal rights or benefits to which you are otherwise entitled. If you are participating in research that is not conducted at a medical facility, you will be responsible for seeking medical care and for the expenses associated with any care received.

SUBJECT'S RIGHTS

By consenting to be in this study, you do not waive any of your legal rights. Consenting means that you have been given information about this study and that you agree to participate in the study. You will be given a copy of this form to keep.

If you do not agree to be in this study or if you withdraw from this study at any time, you will not suffer any penalty or lose any benefits you are entitled. Your participation is completely up to you. Your decision will not affect your ability to get health care or payment for your health care. It will not affect your enrollment in any health plan or benefits you can get.

It is important to tell the study doctor if you are thinking about stopping so any risks from the bacteria transplant can be evaluated by your doctor. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and testing could be most helpful for you.

During this study, we may find out something that might make you not want to stay in the study. If this happens, we will tell you as soon as possible.

We may decide to have you stop being in the study even if you want to stay. Your participation may be terminated by the investigator without regard to your consent in the following circumstances:

- If he/she believes it is in your best interest or your health
- If you experience any side effects or if you become ill during the research
- If it is part of the research plan that people who develop certain conditions may not continue to participate
- If you do not follow the study rules
- If the study is stopped

QUESTIONS

The investigator or a member of the research team will try to answer all of your questions. If you have questions or concerns at any time, contact Christine Liu, MD, MSc [redacted]. You may also call if you need to report an injury while being in this research or if you are calling after normal business hours.

You may also call [redacted] or email [redacted]. You will be talking to someone at the Boston Medical Center and Boston University Medical Campus IRB. The IRB is a group that helps monitor research. You should call or email the IRB if you want to find out about your rights as a research subject. You should also call or email if you want to talk to someone not part of the study about your questions, concerns or problems.

BIOREPOSITORY BANK

We would like to ask your permission to collect and store leftover stool for a research tissue bank. Please initial your choice below:

____Yes ____No You may collect and store leftover stool for a research tissue bank.

RE-CONTACT

We would like to ask your permission to contact you again in the future. This contact would be after the study has ended. Please initial your choice below:

____Yes ____No You may contact me again to ask for additional information related to this study.

____Yes ____No You may contact me again to ask for additional biological samples related to this study.

SIGNATURES

Subject: _____
Printed Name of Subject

By signing this consent form, you are indicating:

- You have read this form (or it has been read to you)
- Your questions have been answered to your satisfaction
- You voluntarily agree to participate in this research study
- You permit the use and release of information that may identify you as described, including your health information

To be completed by subject if personally signing

_____ _____
Signature of Subject Date

To be completed by LAR if subject does not personally sign
I am providing consent on behalf of the subject.

_____ _____
Printed Name of Legally Authorized Representative (LAR) Relationship to Subject

_____ _____
Signature of LAR Date

Researcher: _____
Printed Name of Person Conducting Consent Discussion

To be completed by researcher if subject personally signs

I have personally explained the research to the above-named subject and answered all questions. I believe that the subject understands what is involved in the study and freely agrees to participate.

_____ _____
Signature of Person Conducting Consent Discussion Date

To be completed by researcher if subject does not personally sign

I have personally explained the research to the above-named subject's Legally Authorized Representative (LAR) and answered all questions. I believe that the LAR understands what is involved in the study and freely agrees to have the subject participate.

I consider that the above-named subject who is being enrolled in the study (check one):

- ☐ is capable of understanding what is involved in the study and freely agrees to participate.
- ☐ is not capable of understanding what is involved in the study.

_____ _____
Signature of Person Conducting Consent Discussion Date

To be completed by witness if researcher reads this form to the subject/LAR

_____
Printed Name of Witness (a person not otherwise associated with this study)

_____ _____
Signature of Person Conducting Consent Discussion Date