# Use of Fecal Microbiota Transplantation (FMT) to Reverse Multi-Drug Resistant Organism Carriage

**Clinical Protocol v4**

**Date 9.1.15.**

**NCT #02312986**

## A. Introductory Statement and General Investigational Plan

### 1.0 Introductory Statement

This proposed protocol involves the use of the fecal microbiota transplantation (FMT) to suppress or reverse colonization with multidrug resistant organisms (MDRO) in subjects with recurrent MDRO infections due to organisms of likely enteric origin. The purpose of the proposed FMT is to prevent relapse of the MDRO infection.

FMT will be performed on adult subjects with a history of at least three recurrent infections due to MDRO; at least two recurrent, severe infections due to MDRO requiring hospitalization; or at least two recurrent infections due to MDRO for which only antimicrobials with rate limiting toxicities are available.

The objective of this protocol is to determine if fecal microbiota transplantation (FMT) will be able to prevent additional recurrences of infections due to MDRO by suppressing or reversing enteric colonization with MDRO.

### 2.0 General Investigational Plan

The discovery of antimicrobials revolutionized medicine in the twentieth century. However, almost immediately after antimicrobials were introduced into clinical practice, resistance to these life-saving medications was identified. Bacterial resistance has now outpaced new antimicrobial development, and there are now some bacteria resistant to most, if not all, of our current antimicrobials with few new antimicrobials being developed.[1] Highly prevalent resistant bacteria include *Acinetobacter*, *Pseudomonas aeruginosa,* vancomycin resistant enterococci (VRE), extended spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL), and carbapenem-resistant *Enterobacteriaceae* (CRE). A major concern is many of these multidrug resistant organisms (MDRO) are related to bacteria that normally colonize the colon.[2] Once colonization with an MDRO is established, patients can remain colonized with these MDROs for prolonged periods of time, serving as a reservoir for infection in the colonized patient. Often, the only antibiotic treatment options available to these patients have significant disadvantages: they can only be administered intravenously, or are associated with high toxicity.

A major risk factor for acquiring, shedding, and developing MDRO infections is exposure to antimicrobials.[3-13] A healthy fecal microbiome provides "colonization resistance" against potentially pathogenic bacteria and MDROs. Even limited exposure to antimicrobials can disrupt the microbiota enough to allow for colonization with MDROs, and this increased susceptibility to become colonized with resistant bacteria can persist for months after the antimicrobial exposure ends.[2] Continued and repeated antimicrobial exposure is associated with both increased duration of MDRO colonization and concentration of resistant bacteria in stool. Unfortunately, this results in a cycle where patients develop infections due to MDROs and are treated with antibiotics, which in turn further disrupts the microbiota and allows the MDRO colonization to persist, leading to future infections. Eventually over time, if the patient does not receive additional antibiotics, the MDRO typically no longer is detectable. Presumably this is because the intestinal microbiome "normalizes" and suppresses the MDRO after the selective pressure of antibiotics is removed.[6]

FMT is a potential novel method to rapidly suppress or reverse MDRO enteric colonization and the cycle of MDRO colonization-infection-antibiotics-colonization-reinfection. FMT could repopulate the intestines with normal, healthy microbiota that would compete with MDRO and reduce MDRO colonization. This, in turn, would prevent future MDRO infections. FMT has been demonstrated to be effective against recurrent *C. difficile* infections, with a high clinical cure rate and very few adverse events.[14;15] A recent case report presented at the 2014 ID Week meeting demonstrated FMT was successful in aborting the infection-antibiotics-infection cycle in a girl with recurrent disseminated CRE infections.[16]

Although all of the risks and benefits of FMT are not known at this time, existing data indicate there are no known acute, serious adverse events associated with FMT. There are reports tallying hundreds of patients in the literature who have undergone FMT without a signal for any FMT-associated adverse events.[17-19] FMT also appears safe in immunocompromised patients, possibly by repopulating the gut with less pathogenic bacteria than those that are present pre-FMT.[20;21] The highest quality data on potential adverse events associated with FMT come from a phase 2 study of RBX2660, an FMT product being developed by Rebiotix, Inc, Roseville, MN, for recurrent CDI. 34 study subjects received RBX2660 under this protocol (NCT01925417), adverse events were predominantly mild to moderate and included flatulence, belching, constipation, and occasional bouts of diarrhea. There were 9 serious adverse events, none of which were felt to be related to study product or it administration.

This is a prospective pilot study of fecal microbiota transplantation in patients with a history of recurrent MDRO infections. RBX2660 will be provided free of charge and administered by enema. Subjects who meet inclusion/exclusion criteria and provide written, informed consent will provide a pre-FMT stool sample. The FMT will be administered by enema in the outpatient setting by trained personnel. Patients will provide stool and urine samples 30 days, 6 months, and 12 months post-FMT. The subjects will also be monitored for potential adverse events, recurrence of MDRO infections, infections that may be related to FMT, and worsening of existing comorbidities or development of new comorbidities. As this is an unfunded protocol intended to provide desperate patients with limited options a potential therapeutic with theoretical benefit and low risk, the stool and urine specimens will be collected and stored in hopes that funding will be available in the future to determine the impact FMT had on MDRO colonization.

**B. Clinical Protocol**
**Use of Fecal Microbiota Transplantation (FMT) to Reverse Multi-Drug Resistant Organism Carriage (v4. Date 9.1.15)**

**Investigational Drug:** Fecal microbiota transplantation (FMT)

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## 1.0 Methods

**1.1 Hypothesis:** Fecal microbiota transplantation (FMT) will be able to rapidly and safely reverse carriage of multi-drug resistant organisms (MDRO) in patients with recurrent infections due to MDRO.

**1.2 Specific Aims:**

1. To provide FMT as a treatment option to prevent infection recurrence for patients with recurrent MDRO infections of likely enteric origin.
2. To determine the safety of FMT when administered to prevent recurrent MDRO infections.

**1.3 Definitions:**

- Antimicrobials with rate-limiting toxicities will include aminoglycosides, colistin/polymixin, or any antimicrobial the patient is unable to tolerate due to a severe allergic reaction not amenable to desensitization (e.g. Stevens-Johnson Syndrome) or ≥grade 2 adverse events according to the severity grading scale included in the data safety and monitoring section.
- An MDRO will be defined as an infection caused by a Gram negative bacteria resistant to at least three of the following antibiotics: ciprofloxacin, 4$^{th}$ generation cephalosporins, aminoglycosides, carbapenems, piperacillin / tazobactam; or enterococci resistant to ampicillin and vancomycin.
- Organism of likely enteric origin will be defined as a bacteria recognized to be a common colonizer of the human intestinal tract.
- Eligible infections will include but are not limited to urinary tract infections, bloodstream infections, and metastatic infections.

**1.4 Patient Population:** This study will be conducted through the Washington University School of Medicine (WUSM) Infectious Diseases Clinic. We estimated that approximately 5 patients per year will be eligible to participate. The maximum number of subjects enrolled will be 20.

Inclusion criteria will include:

- Age ≥18 years old.
- Outpatient status at time of FMT.
- History of at least three recurrent infections due to an MDRO; at least two recurrent, severe infections due to MDRO requiring hospitalization; or at least two recurrent infections due to MDRO for which only antimicrobials with rate limiting toxicities (see above) are available AND the MDRO is likely of enteric origin. Only MDROs of likely enteric origin will be included.
- Be without active infection due to the MDRO at the time of FMT.
- Not be receiving antimicrobials (therapeutic or suppressive) within 48 hours of FMT.

Exclusion criteria will include:

- Subjects <18 years old.

- Subjects unable to be seen as an outpatient.
- Use of enteral or systemic antimicrobials at time of FMT.
- Planned use of enteral or systemic antimicrobials up to 6 months post-FMT.
- Pregnancy or inability/unwillingness to use contraceptives.
- Recent intra-abdominal surgery
- Short gut syndrome
- Gastrointestinal motility disorders
- Use of medications that affect intestinal motility an inability to cease using those medications at the time of FMT.
- Post-allogeneic hematopoietic stem cell transplant recipients with previous or current gastrointestinal graft versus host disease.
- ANC $<500/mm^3$
- HIV+ and not well controlled on antiretroviral therapy, or CD4+ $<200/ mm^3$
- At increased risk for peritonitis: presence of intra-abdominal devices, receiving peritoneal dialysis, or ascites.
- Any acute illness
- Recurrent *C. difficile* infection.
- Unlikely to survive for 3 months.
- Investigator feels the risks of FMT outweigh potential benefit, including other conditions or medications that the investigator determines puts the subject at greater risk from FMT.

Other than these, there will be no absolute contraindications for inclusion. The risk of recurrent infection and of additional antimicrobial therapy will be weighed against the risks of the study drug and procedure on a subject-by-subject basis. After clinical evaluation, subjects will be excluded if the study investigator feels the risk of FMT outweighs the benefits for that patient.

Immunocompromised patients are at highest risk for MDRO infections and will not be automatically excluded. Immunocompromised patients are both at increased risk for infections due to MDRO and also at increased risk for morbidity due to infection. FMT has been given to immunocompromised patients, including solid organ transplant recipients, hematopoietic stem cell transplant recipients, and patients with refractory inflammatory bowel disease without any reports of harm due to FMT.[21;22] Infections due to enteric organisms in immunocompromised patients tend to be due to Proteobacteria and enterococci. FMT has been shown to reduce the prevalence of Proteobacteria and enterococci in stool,[23] and therefore may be associated with a decreased risk of infection compared to no FMT administration. Furthermore, neutropenic or immunocompromised patients routinely are able to receive suppositories and have bowel movements safely. Administration of FMT and stool and urine collection should pose no increased risk to patients beyond those routine events. In addition, FMT will be done only in the outpatient setting. The immunocompromised population at greatest risk for infection due to translocation of enteric bacteria are patients who undergo intensive chemotherapy who develop mucositis and a neutrophil count <100. It is standard practice at Barnes-Jewish Hospital / Washington University that these patients remain hospitalized until neutrophil recovery; therefore this population will be excluded by default. Also, risk of translocation of intestinal bacteria is greatest during acute illness. Performing FMT only in outpatients ensures none of the subjects are acutely ill at the time of FMT. If a subject does appear acutely ill on the day of the

FMT, FMT will be deferred and the patient's primary physician will be contacted to arrange admission to the hospital.

A history of recurrent MDRO infections (as described above) will be used as the primary inclusion criteria instead of MDRO colonization status because enrolled subjects will have recently completed treatment for MDRO infection and therefore MDRO levels present in stool may be below the level of detection. In addition, the subject's MDRO infection may relapse while waiting for culture results. However, stool and urine samples will be obtained from enrolled subjects just prior to the infusion of the study drug and stored for future testing.

Subjects with food allergies will not be excluded from the study. There have been no reports of allergic reactions in stool transplant recipients, either from RBX2660 or other FMT products. A potential subject with food allergies will be carefully assessed by the study investigator to determine if the subject is at risk for an allergic reaction and whether the benefit of FMT outweighs the risk. The potential subject will also be informed of the theoretical risk of an allergic reaction.

**1.5 Study procedures:** Potential subjects will be identified through referrals to the WUSM Infectious Diseases Clinic or infectious disease consults at Barnes-Jewish Hospital. All study procedures will take place at the outpatient Infectious Diseases Clinic.

If during the course of routine clinical care for management of an MDRO it is determined a patient might be eligible, a study investigator will discuss the risks and benefits of FMT with the subject. **All subjects will provide written, informed consent** for FMT and stool collection and storage**.** Patients will then schedule an outpatient visit at the WU Infectious Disease Clinic for FMT administration. Study specific procedures will include acquisition of the FMT product, administration of the FMT, collection of stool and urine specimens, and collection of adverse events (solicited adverse events immediately following FMT). All other activities will be part of patient management/routine clinical care and would occur regardless of enrollment in this study protocol (e.g. clinic visits, physical exams, labs, etc), but records of these events and results will be maintained as part of the study protocol.

- The investigator will:
    - Perform a physical examination.
    - Interview the patient about his/her medical history.
    - Review the patient's history of MDRO infections.
    - Review the results of screening tests collected within the previous 14 days (CMP, CBC, PT/INR, PTT, pregnancy test if of childbearing potential). If these tests were not performed during routine care, the study investigator will order them. The maximum window between screening tests and FMT will be 30 days; beyond 30 days, the screening tests will be repeated.

- The patient will provide a pre-FMT stool and urine specimen.

If all criteria are met and the patient provides consent, an FMT date will be scheduled. If the patient is currently taking antimicrobials for the MDRO infection, they will be stopped 48 hours prior to FMT.

Subjects will return to the WU Infectious Disease Clinic for FMT administration. Women of childbearing potential will perform a urine pregnancy test before FMT administration. For the FMT, subjects will receive 150 mL of RBX2660 (Rebiotix, Inc.), an FMT product being developed, via enema. Further details of the FMT procedure are described below (section 1.8).

The subject will be provided with a diary to record solicited adverse events for the seven days following FMT (see Data and Safety Monitoring Plan below). This diary will be collected at the day 30 visit.

Subjects will have follow-up clinic visits with a study investigator 30 days, 6 months, and 12 months post-FMT (+/- 1 week for all). The following will occur at these visits:

- The clinical investigator will:
    - Perform a physical examination.
    - Review the subject's recent medical history, including potential adverse events (solicited and unsolicited; see Table 1 below), recurrence of MDRO infections, infections that may be related to FMT, concomitant medications, worsening of existing comorbidities, or development of new comorbidities. All unsolicited adverse events will be recorded at the post-FMT visits, regardless of relatedness to FMT.
- Patients will provide post-FMT stool and urine specimens at each visit. Stool and urine samples will be stored for future analysis of MDRO carriage and changes in the microbiome. The subjects will also be instructed to call if there is concern for any adverse events between visits.

**1.6 Data Management:** Demographic data, including age, gender, race/ethnicity, height, weight, comorbidities, and tobacco and alcohol history, will be obtained on enrollment. At the screening visit, all medications the subject received in the 6 months prior to first stool collection will be recorded. The subjects will be instructed to call if he/she believes an adverse event is being experienced (see details below under Risks to Human Subjects).

**1.7 Stool specimens**: Stool specimens will stored at -80$^{o}$ C for future research.

**1.8 FMT:** 150 mL of RBX2660 (microbiota suspension) will be used. RBX2660 is being developed to be a commercially available FMT product made by Rebiotix, Inc. This product completed a Phase 2 clinical trial to determine the safety of RBX2660 for treatment of recurrent *C. difficile* infections, and a phase 2b study for recurrent CDI is underway. Each 150mL bag of RBX2660 product consists of a suspension of at least $10^7$ live organisms in polyethylene glycol 3350/0.9% sodium chloride irrigation, USP, solution. RBX2660 stool donors have undergone rigorous testing for stool and blood pathogens, including: *C. difficile,* Norovirus, Rotavirus, Adenovirus, enteric pathogens (*Shigella, Salmonella, Campylobacter,* sorbitol-negative *E. coli, Aeromonas, Plesiomonas, Yersinia*, shiga toxins, *Giardia, Cryptosporidium, Cyclospora, Isospora*, other ova and parasites, VRE, MRSA, Vibrio, Listeria, HIV, Hepatitis A, B, and C,

and Treponema. RBX2660 is not screened for all MDROs (such as CRE/ESBL); however, RBX2660 stool donors are at very low risk for these MDROs. All donors are healthy people who reside in the U.S. and have not received antimicrobials. RBX2660 will be shipped to the study investigators to arrive frozen the day before the scheduled FMT date and thawed in the refrigerator prior to use. The Rebiotix Kit containing RBX2660 will also include enema supplies: rectal tube assembly, instructions for use, and a biohazard disposal bag.

A 5mL aliquot of RBX2660 will be reserved for microbiological comparison with the subject's stool samples if funding is obtained to process the specimens. The remaining FMT material will be administered to the subject by enema. Blood pressure, heart rate, and respiratory rate will be measured. If abnormal, the results will be reviewed with the study investigator. All healthcare personnel in the room will don non-sterile gloves, gowns, and wear protective eyewear. The subject will then be placed into the left lateral decubitus position. The enema tubing will be attached to the RBX2660 bag, and the tip will be lightly lubricated with water soluble lubricant. The enema tubing will then be gently inserted ~6 cm into the subject's rectum. The subject will be instructed to tighten the anal sphincter to create a firm seal around the enema tubing. The subject will be told the infusion will start, and to let it be known if he/she has the urge to have a bowel movement or cramping. Once the subject is ready, the FMT will be administered slowly with frequent checks to ensure the patient's comfort. If the subject reports the urge to have a bowel movement or cramping, the FMT administration will be held until this passes. After the FMT is administered the enema tubing should be pinched and tilted upward to prevent backflow of the FMT. Administration of the FMT should take a total of 5 to 10 minutes. After the FMT has been administered the enema tubing will be removed. The recipient will then be placed in the prone position for up to 15 minutes, and then the right lateral decubitus position for 5 minutes. The patient will be instructed to retain the FMT for as long as possible, up to 24 hours. The subject will be monitored for 60 minutes after the enema, with repeat measurement of blood pressure, heart rate, and respiratory rate immediately after the enema and every 10 minutes until the monitoring time is complete. Recipients will be assessed for hypersensitivity during the 60 minutes post-FMT. Because the FMT procedure will take place in the WUSM Infectious Diseases Clinic, appropriate medications and healthcare staff will be on hand to treat a reaction should one occur.

**1.9 Analysis:** Descriptive analysis of the safety and outcomes data will be conducted. Primary safety endpoints will include incidence, severity, and relatedness of solicited, unsolicited, and serious adverse events occurring within 12 months of FMT. Secondary endpoints to be analyzed will include the proportion of subjects free of recurrent MDRO infections 30 days, 6 months, and 12 months post-FMT.

**2.0 Risks to Human Subjects**

2.1 Human Subjects Involvement, Characteristics, and Design: This will be a prospective pilot study to examine whether fecal microbiota transplantation (FMT) is able to suppress or reverse carriage with MDRO in patients with recurrent infections in order to prevent future recurrences. We anticipate enrolling approximately 5 patients per year. Stool and urine samples will be obtained before FMT and at four time points after FMT (30 days, 6 months, and 12 months). Data on medication exposures, infections, and comorbidities will be collected. This protocol is

intended to provide desperate patients with limited options a potential therapeutic with theoretical benefit and low risk.

Subjects will be identified through referrals to the WUSM Infectious Diseases Clinic or infectious disease consults at Barnes-Jewish Hospital. All study procedures will take place at the outpatient Infectious Diseases Clinic. Eligibility criteria were described above (briefly, adult outpatients with a history of at least three recurrent infections due to an MDRO; at least two recurrent, severe infections due to MDRO requiring hospitalization; or at least two recurrent infections dues to MDRO for which only antimicrobials with rate limiting toxicities are available AND the MDRO is likely of enteric origin, outpatient status, and not on antimicrobials within 48 hours of FMT). Patients will be excluded if the study investigator feels the risk of FMT outweighs the benefits for that patient or if the patient is unlikely to survive 3 months, if the patient is <18 years old, has any other medical contraindication (as described above), or has recurrent *C. difficile* infection. Eligible MDRO will include but are not limited to those caused by: vancomycin-resistant *Enterococcus* (VRE), extended-spectrum betalactamase-producing organisms (ESBL), carbapenem-resistant *Enterobacteriacaea* (CRE), *Acinetobacter*, or *Pseudomonas* (see above).

2.2 Sources of Materials: Research material obtained from the study subjects will include demographic data, medication exposures, and comorbidities. Stool and urine will also be collected from study subjects. The stools and urine will be de-identified and stored at -80ºC in a secured laboratory. Only key study personnel will have access to study data and materials. All key personnel will have HIPAA and human subjects research training.

Data from study subjects will be collected through interviews. Protected health information (PHI) to be collected will include study subject name, contact information, current living address, date of birth, and dates of interviews/questionnaires/stool specimen collection. This PHI is necessary to ensure data are linked within a study subject at all time points the data and specimens are collected. Each study subject will receive a study number. All PHI will be destroyed as soon as possible after the data are analyzed. Stool specimens will be transported to WUSM by a courier service. There are other research protocols at WU that involve stool pick-up and delivery by a courier service. There have been no breeches of PHI with use of the courier service for his project. This project will adhere to the same protocols to ensure PHI is protected.

Study subject data will be stored at the WU Division of Infectious Diseases and stool specimens will be stored in Dr. Dubberke's laboratory. We will protect against the risk of breaches in confidentiality by de-identifying our data, using locked file cabinets to store all paper records in a secured, locked office, and by using password protected and secured servers for computerized databases to which only the Principal Investigator, co-investigators, and other key personnel identified by the Principal Investigator will have access. Stool and urine specimens will be stored in a locked, secured research laboratory. These methods have proven very effective at maintaining confidentiality at our institution.

2.3 Potential Risks: Potential risks include breach of confidentiality, side effects of an enema, or side effects from FMT. As with any research, there is a potential risk of loss of privacy and inadvertent release of protected health information, and this can lead to psychological, social, or

legal distress. As data will be de-identified and all paper data will be stored in locked file cabinets in a secured, locked office, all electronic data will be password protected on secured servers to which only the Principal Investigator, co-investigators, and other key personnel identified by the Principal Investigator will have access, and all stool specimens will be stored in a secured area without patient identifiers attached to the specimen, the risk of loss of privacy is minimal.

There are potential risks to an enema, including anal leakage, a vagal reaction, and mucosal tears. The volume to be administered by enema is small (approximately 150 ml); this should ensure anal leakage is rare and minimize its occurrence. Study subjects will be lying down at the time of the enema administration, monitored closely for 60 minutes afterwards, and instructed to stand-up slowly after the enema. Study subjects will be asked if they have had symptoms consistent with a vagal reaction in the past in response to sensations of pressure in the bladder or bowel, or with needle sticks. These subjects will be monitored more closely. These measures should minimize the occurrence of a vagal reaction. There is a possibility of mucosal tears. The likelihood of a mucosa tear is very low. It is not uncommon for people to self-administer enemas at home. The enemas will be administered by trained study personnel to minimize the risk of mucosal tears.

There are potential risks to FMT, including non-specific febrile reactions and bloodstream infections. FMT involves the administration of a large volume of bacteria into the colon. This may result in a non-specific febrile reaction from bacterial components or a bloodstream infection. The risk of these occurring is rare. Bloodstream infections secondary to FMT have not been reported in the literature. Dr. Dubberke is the director of the FMT program at Washington University, and he has yet to observe a mucosal vagal reaction, mucosal tear, febrile reaction, or bloodstream infection. There is also a potential risk of infection or colonization with a pathogen due to FMT. All RBX2660 stool donors are screened for most known pathogens and are healthy and at low risk for MDRO colonization, which minimizes the risk. Stool from study subject will be collected at various time points, and an aliquot of donor FMT will be saved. If any new MDRO occurs, and there are concerns the study subject developed the infection due to FMT, the stored specimens can be used to determine if the new MDRO came from the donor.

As a pilot study, there are no alternative treatments or procedures. All study subjects will be informed they can withdraw from the study at any time.

**3.0 Adequacy of Protection against Risks**

3.1 Recruitment and Informed Consent: Study subjects will be recruited to participate from referrals to the WUSM Infectious Diseases Clinic or infectious disease consults at BJH. Potential study subjects will discuss the study with the study investigator. If the potential subject is interested, the inclusion and exclusion criteria will be reviewed and a consent form will be signed if the subject meets inclusion and exclusion criteria. The research personnel will review the consent document, re-confirm inclusion and exclusion criteria are met, and answer any questions the study subject may have.

3.2 Protection Against Risk: We will protect against the risk of breaches in confidentiality by de-identifying the data, using locked file cabinets to store all paper records in a secured, locked

office, and by using password protected and secured servers for computerized databases to which only the Principal Investigator, co-investigators, and other key personnel identified by the Principal Investigator will have access. Stool specimens will be stored in secured laboratories to which only the Principal Investigator, co-investigators, and other key personnel identified by the Principal Investigator will have access. These methods have proven very effective at maintaining confidentiality at our institution.

Study subjects will be monitored for adverse events due to enema and FMT. The risk of adverse events due to these procedures is low. As described above, measures will be taken to minimize the risk, and these measures have proven effective at our institution. Study subjects will be informed of signs and symptoms to be aware of, and instructed to contact study personnel if they occur. Study personnel are available 24 hours a day. Dr. Dubberke, the study investigator, is board certified in internal medicine and infectious diseases. He, or a pre-identified physician in the Infectious Diseases Division at Washington University, will be available to evaluate the study subject in case there is a potential study-related adverse event. Dr. Jennie Kwon will be a sub-investigator for this protocol. However, over the course of routine clinical care, one of Dr. Dubberke's or Dr. Kwon's ID colleagues at Washington University School of Medicine may be contacted regarding a study subject. Potential adverse events will also be recorded and monitored. All serious adverse events will be reported to the Washington University Human Research Protection Office within 24 hours of its occurrence. The study will be suspended and policies and procedures will be reviewed if a subject develops a serious adverse event.

**4.0 Potential Benefits of the Proposed Research to Human Subjects and Others**

Patients with recurrent MDRO infections often have few treatment options available to them. If the FMT successfully reverses MDRO carriage, the patient's future risk of infection due to MDRO (urinary tract infection, bloodstream infection, etc.) will be decreased. The procedures involved in this study, enema and FMT, are common, and are in general well tolerated with a very low risk for severe adverse events. The study subject will have the opportunity to withdraw from the study at any time. This research will benefit others in the future by providing a new method to eliminate multidrug resistant organism (MDRO) colonization of the colon plus protect those patients who have not yet acquired MDRO. Study participants may be protected from future infections with MDRO. The knowledge that will be gained more than justifies the minimal risks associated with the study.

**5.0 Importance of Knowledge to be Gained**

The prevalence, costs, morbidity, and mortality of MDRO continue to increase at a time when there are fewer antimicrobials being developed. FMT may be a successful new measure to help combat the MDRO public health crisis and treat patients with recurrent MDRO infections. FMT has the potential to eliminate MDRO colonization of the colon plus protect those patients who have not yet acquired MDROs. This will be the first study to examine whether FMT can reverse MDRO carriage.

**6.0 Data and Safety Monitoring Plan**

An adverse event will be defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with a study procedure, whether or not considered related to the study procedure. Adverse events will be classified as indicated

below (Table 1). The study investigator will assess all adverse events and classify them as definitely/probably/possibly/not related to FMT/FMT administration/underlying condition. Relatedness will be defined as follows:

**Definitely related:** It is clear that the event was caused by the study treatment (FMT). There is a strong temporal relationship between the event and study treatment and an alternative cause is unlikely.

**Probably related:** There is a reasonable possibility that the event is likely to have been caused by the study treatment. There is a temporal relationship between the event and study treatment, but a potential alternative cause may be present.

**Possibly related:** There is a reasonably possibility that the event might have been caused by study treatment, but an alternative cause seems more likely or a possible relationship to study treatment cannot be reasonably ruled out.

**Not related:** The cause of the event is known and the event is not related to study treatment. If there is any uncertainty regarding causality of the event, then the event must be assessed as possibly related.

Solicited adverse events will include: gas, abdominal distension or bloating, rectal irritation or pain, chills, abdominal pain or cramping, diarrhea, constipation, rectal bleeding, nausea, vomiting, fever, and any new illness or injury.

A serious adverse event will be defined as an adverse event grade 4 (see Table 1); one that is fatal, life-threatening, results in hospitalization or prolongation of hospitalization, results in persistent or significant disability, congenital abnormality, or any event deemed to be serious by the study investigators. A medically attended adverse event will be defined as an event for which the subject seeks medical attention.

The dates of onset and resolution, actions taken as a result, and subject outcomes of adverse events will be recorded. Subjects will be given a post-FMT diary (see Supplementary Materials) to record solicited adverse events in the 7 days post-FMT. Subjects will return this diary at their 30-day post-FMT clinic visit. All participants will be instructed to contact study investigators if they experience any of the adverse events listed in Table 1 in the 28 days post-FMT. In addition, the study investigator will assess for all adverse events, including serious, at each clinic visit (day 30, 6 months, 12 months).

Safety reporting will be as per 21 CFR 312.32. Safety information, including serious adverse events, will be included in annual reports and the final clinical study report to the FDA. All adverse events will be reviewed by Dr. Dubberke (the PI) to determine if the adverse event is felt to be related to a study procedure, FMT, or underlying condition (not related, unlikely related, possibly related, definitely related) and whether it is a serious adverse event. All serious adverse events will be reported to the Washington University Human Research Protection Office within 24 hours, and all other adverse events will be reported within 7 days. The FDA will be notified of serious adverse events within 15 days, or within 7 calendar days of initial receipt of an

unexpected fatal or life-threatening suspected adverse event. In the event of a serious adverse event, no additional study subjects will be enrolled until the adverse event is reviewed and a decision is made as to whether it is safe to proceed with the study protocol. Study halting criteria will include:

1. Transmission of any infection assessed as possibly, probably, or definitely related to the FMT.
2. Any 2 or more subjects experience similar adverse events of a severe or greater grade.
3. Any serious adverse event at least possibly related to FMT.

The FDA will be notified immediately in the event that the study halting criteria are triggered.

Table 1. Classification of Adverse Events

| Parameter | Grade1: Mild | Grade 2: Moderate | Grade 3: Severe | Grade 4: Potentially Life-threatening |
|---|---|---|---|---|
| Flatulence | Symptoms causing no or minimal interference with usual social and functional activities | Symptoms causing greater than minimal interference with usual social and functional activities | NA | **NA** |
| Belching (burping) | Symptoms causing no or minimal interference with usual social and functional activities | Symptoms causing greater than minimal interference with usual social and functional activities | NA | **NA** |
| Abdominal distension or bloating | Symptoms causing no or minimal interference with usual social and functional activities | Symptoms causing greater than minimal interference with usual social and functional activities | Symptoms causing inability to perform usual social and functional activities | **NA** |
| Diarrhea | Increase of ≤ 3 stools over baseline per 24-hour period | Increase of 4 – 6 stools over baseline per 24-hour period | Bloody diarrhea if not present at baseline OR increase of ≥ 7 | Life-threatening consequences, e.g., |

| Parameter | Grade1: Mild | Grade 2: Moderate | Grade 3: Severe | Grade 4: Potentially Life-threatening |
|---|---|---|---|---|
| | | | stools over baseline per 24-hour period OR IV fluid replacement indicated if not indicated at baseline | hypotensive shock |
| Abdominal cramping/pain | Discomfort/pain causing no or minimal interference with usual social and functional activities | Discomfort/pain causing greater than minimal interference with usual social and functional activities | Discomfort/pain causing inability to perform usual social and functional activities | Disabling pain causing inability to perform basic self-care OR inpatient hospitalization ≥ 24 hours |
| Constipation | Occasional or intermittent symptoms, occasional use of stool softeners, laxatives, dietary modifications or enema | Persistent symptoms with regular use of laxatives or enemas indicated | Symptoms causing inability to perform usual social and/or functional activities | Life-threatening consequences, e.g., obstruction, toxic megacolon |
| Colitis | No symptoms, regardless of pathologic or radiographic evidence of inflammation | Abdominal pain, mucus or blood in the stool | Abdominal pain, fever, change in bowel habits with ileus; peritoneal signs | Life-threatening consequences, e.g., perforation, bleeding, ischemia, necrosis, toxic megacolon |
| Fever | 37.7 – 38.2°C (99.9 – 100.8° F) | 38.3 – 38.7°C (100.9 – 101.7° F | 38.8 – 39.5°C (101.8 – 103.1° F) | > 39.5°C (103.1° F) |
| Fatigue/malaise | Symptoms causing no or minimal interference with usual social and functional activities | Symptoms causing greater than minimal interference with usual social and functional activities | Symptoms causing inability to perform usual social and functional activities | Incapacitating fatigue/malaise symptoms causing inability to perform basic self-care functions |

| Parameter | Grade1: Mild | Grade 2: Moderate | Grade 3: Severe | Grade 4: Potentially Life-threatening |
|---|---|---|---|---|
| Chills | Symptoms causing no or minimal interference with usual social and functional activities | Symptoms causing greater than minimal interference with usual social and functional activities | Symptoms causing inability to perform usual social and functional activities | NA |
| Rectal discomfort or irritation | No symptoms or symptoms not requiring medical intervention | Symptomatic with medical intervention (topical medications / treatments) indicated | Symptoms causing inability to perform usual social and functional activities or requiring medical intervention other than topical medications / treatments | NA |
| Rectal bleeding | Mild or intermittent without transfusion | Persistent without transfusion | Requires transfusion | Life-threatening consequences |
| Nausea | Transient (≤ 24 hours) or intermittent nausea with nor or minimal interference with oral intake | Persistent nausea resulting in decreased intake for 24-48 hours | Persistent nausea resulting in decreased intake > 48 hours OR aggressive rehydration indicated, e.g., IV fluids | Life-threatening consequence, e.g., hypotensive shock |
| Vomiting | Transient (≤ 24 hours) or intermittent vomiting with nor or minimal interference with oral intake | Frequent episodes of vomiting with no or mild dehydration | Persistent vomiting resulting in orthostatic hypotension OR aggressive rehydration indicated, e.g., IV fluids | Life-threatening consequence, e.g., hypotensive shock |
| Hypotension | NA | Symptomatic, corrected with | Symptomatic, IV fluids indicated | Shock requiring use of |

| Parameter | Grade1: Mild | Grade 2: Moderate | Grade 3: Severe | Grade 4: Potentially Life-threatening |
|---|---|---|---|---|
| | | oral fluid replacement | | vasopressors or mechanical assistance to maintain blood pressure |
| Adverse event not identified elsewhere in this table | Symptoms causing no or minimal interference with usual social and functional activities | Symptoms causing greater than minimal interference with usual social and functional activities | Symptoms causing inability to perform usual social and functional activities | Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death |

*****Adapted from *Division of AIDS Table for Grading of Severity of Adult and Pediatric Adverse Events* and *Addendum 3: Rectal Grading Table for Use in Microbicide Studies; May 2012.*

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