Fecal Microbiota Transplantation Using RBX2660 for the Prevention of Recurrent Urinary Tract Infections Due to Multidrug Resistant Organisms

Clinical Protocol
August 22, 2018
Version #7
IND #16293; NCT03367910

Investigational Drug: Fecal microbiota transplantation (FMT)

Sponsor-Investigator:
Erik R. Dubberke, MD, MSPH
Washington University School of Medicine
660 S. Euclid Avenue, Campus Box 8051
St. Louis, MO 63110

Study Monitor:
Human Research Protection Office
Washington University School of Medicine
660 South Euclid Avenue
Campus Box 8089
St. Louis, MO 63110

Investigators:
Erik R. Dubberke, MD, MSPH
Washington University School of Medicine
660 S. Euclid Avenue, Campus Box 8051
St. Louis, MO 63110

Co-Investigators:
Jennie H. Kwon, DO, MSCI
Washington University School of Medicine
660 S. Euclid Avenue, Campus Box 8051
St. Louis, MO 63110

Clinical Laboratory:
Barnes-Jewish Hospital Clinical Microbiology Laboratory

CONFIDENTIAL

1.0 Introduction

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. Antimicrobial resistance in bacteria has now outpaced new antimicrobial development and is a public health crisis.[1] Highly prevalent multidrug resistant organisms (MDRO) include carbapenem-resistant *Enterobacteriaceae* (CRE), extended spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL), MDRO *Acinetobacter,* and MDRO *Pseudomonas aeruginosa*. Once acquired, these MDROs can cause difficult to treat infections in the host and are transmitted to other people in high risk healthcare settings and in the community. Not surprisingly, all of these MDRO are common inhabitants of the human colon.[2]

The most common type of infection caused by these MDRO is urinary tract infections (UTIs). As inhabitants of the colon, these MDRO contaminate the periurethral area and migrate to the bladder.[3] Patients with MDRO UTI frequently experience multiple relapses and hospitalizations, which both increase the individual's morbidity and mortality and leads to additional MDRO nosocomial spread.[4-8] There are few options available to prevent MDRO UTIs, and there are limited strategies to identify patients at risk for recurrent MDRO UTI and prevent or reverse MDRO colonization.[9,10] Changes in the fecal and urine microbiome, urine metabolome, and MDRO concentration in the stool may predict patients at risk for persistent MDRO colonization and recurrent UTI. Once patients at risk are identified, a potential novel method to reverse MDRO colonization and prevent recurrent UTI would be by repopulating the gut microbiome with “healthy” microbiota by fecal microbiota transplantation (FMT).

Rebiotix, Inc. has developed a standardized FMT product, RBX2660, for the treatment of recurrent *Clostridium difficile* infections (CDI) (IND #15439). Rebiotix has completed enrollment for three prospective trials (phase 2, expanded access, and phase 2b) is about to start a phase 3 trial. The phase 2b trial was a double blinded randomized controlled trial, and there were no differences in adverse events between those who received RBX2660 or placebo. Notably, a secondary analysis of the RBX2660 Phase 2 study found that 72.7% of patients colonized with vancomycin-resistant *Enterococcus* (VRE) prior to FMT converted to VRE negative status post-FMT.[11] It is possible that RBX2660 may be able to safely prevent MDRO UTI infections and/or reverse MDRO colonization by introducing “healthy” microbiota to the gut.

The purpose of this study is to determine the safety and impact of FMT with RBX2660 on the fecal and urine microbiome, urine metabolome, risk of recurrent UTI, and persistent MDRO colonization of patients with a history of recurrent MDRO UTIs. This is an open label phase 1-2 study.

1.1 Specific Aims:

Determine the safety and impact of FMT on the fecal and urine microbiome, urine metabolome, risk of recurrent UTI, and persistent MDRO colonization.

1.2 Hypothesis:

FMT will restore microbial communities that are protective against recurrent MDRO UTI, enhance metabolite production that inhibit growth of uropathogens, and suppress the MDRO.

1.3 FMT product:

150 mL of RBX2660 (microbiota suspension) will be used in this study. FMT will be delivered via enema. RBX2660 is being developed to be a commercially available FMT product made by Rebiotix, Inc. This

product completed Phase 2 and 2b clinical trials to determine the safety and efficacy of RBX2660 for treatment of recurrent *C. difficile* infections, and a phase 3 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.

1.4 Definitions:

Antimicrobials with rate-limiting toxicities: 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.

MDRO UTI: Urinary tract infection caused by a multidrug-resistant Gram negative bacteria.

- Eligible organisms will include, but are not limited to: *Acinetobacter* species, *Citrobacter* species, Enterobacteriaceae, *Enterobacter* species, *E. coli*, *Hafnia* species, *Klebsiella* species, *Morganella* species, *Pantoea* species, *Pseudomonas* species, *Plesiomonas* species, *Proteus* species, *Providencia* species, and *Serratia* species.

Multidrug resistant organism (MDRO): An MDRO causing UTI meeting inclusion criteria for this study will be defined as follows:

- *Pseudomonas aeruginosa* or *Acinetobacter* species resistant to any carbapenem (meropenem, imipenem, doripenem, ertapenem)
- *Pseudomonas aeruginosa* resistant to cefepime and ciprofloxacin
- *E. coli*, *Klebsiella* species, or *Proteus mirabilis* resistant to any third generation cephalosporin (ceftriaxone, cefpodoxime, cefotaxime, ceftazidime, cefixime, cefdinir)
- Enterobacteriaceae resistant to ciprofloxacin or levofloxacin
- Enterobacteriaceae species resistant to at least three of these classes of antimicrobials:
    - Carbapenems (meropenem, imipenem, doripenem, ertapenem)
    - Aminoglycosides (amikacin, tobramycin, gentamicin, streptomycin, kanamycin)
    - Fluoroquinolones (levofloxacin, ciprofloxacin, moxifloxacin)
    - Fourth generation cephalosporins (cefepime)
    - Piperacillin/tazobactam

  (Enterobacteriaceae resistant to ertapenem and susceptible to meropenem, imipenem, and/or doripenem will be eligible.)

- Enterobacteriaceae with any of the following resistance mechanisms identified:
    - Extended spectrum beta-lactamase (ESBL)

- Carbapenem-resistant Enterobacteriaceae (CRE)
- *Klebsiella pneumonia* Carbapenemase (KPC)
- New Delhi metallo-beta-lactamase 1 (NDM-1)
- OXA-48 Carbapenemase (OXA-48)
- IMP metallo-B-lactamase (IMP, IMP-1)
- VIM metallo-B-lactamase (VIM)

UTI: Patients must have a positive urine culture with an MDRO, as defined above, and one or more of the following symptoms: fever and/or chills with no other explanation; change in urinary frequency; urinary hesitancy, bladder not emptying, bloody urine, cloudy urine, pain and/or burning on urination, flank pain, or bladder/suprapubic pain.

2.0 Methods

2.1 Patient Population: Patients with eligible recurrent MDRO UTIs will be invited to participate.

Inclusion criteria will include:

- Age ≥18 years old.
- Outpatient status at time of FMT.
- History of at least three recurrent UTIs 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.
- 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:

- Age <18 years
- Inpatient status at time of FMT
- Ineligible UTI
- >1 organism in urine (other than minimal contaminants)
- Decline to participate
- Recurrent Clostridium difficile infection
- Presence of intra-abdominal devises
- Neutropenia (ANC <500 mm3)
- Intestinal mucosal disruption
- Unlikely to survive 6 months
- Pregnancy or unwillingness to use contraceptives
- Short gut syndrome
- Use of medications that affect intestinal motility
- Gastrointestinal motility disorder
- Inflammatory bowel disease
- Recent abdominal surgery
- Active typhlitis
- Active diverticulitis
- Current gastrointestinal graft versus host disease
- HIV with lack of antiretroviral therapy (ART)
- CD4 count <200 mm3

- Peritoneal dialysis
- Cirrhosis with ascites
- Active intra-abdominal malignancy
- Presence of chronic indwelling foley catheter, chronic suprapubic catheter, or ileal conduit
- Active hepatitis C
- Active hepatitis B
- Presence of ureteral stent
- Active kidney stone that is believed to be a persistent source of bacterial colonization
- Any condition where the investigator feels the risks of FMT outweigh the benefits

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 patient-by-patient basis. After clinical evaluation, patients 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.[12,13] 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,[14] 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, indicating the patient is well enough to not be hospitalized. Performing FMT only in outpatients ensures none of the patients are acutely ill at the time of FMT. If a patient 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.

Patients 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 patient with food allergies will be carefully assessed by the study investigator to determine if the patient is at risk for an allergic reaction and whether the benefit of FMT outweighs the risk. The potential patient will also be informed of the theoretical risk of an allergic reaction.

2.2 Study sites: This study will be performed at four sites:
- Washington University Medical Center, St. Louis, Missouri
- Rush University, Chicago, Illinois
- Duke University, Durham, North Carolina
- University of Pennsylvania, Philadelphia, Pennsylvania

2.3 IRB oversight: The Washington University Human Research Protection Office (HRPO) will serve as the single/central IRB for this study.

2.4 Study procedures:

See Appendix A for an overview of the entire study procedures for each patient.

Recruitment: The four participating sites for this study are currently conducting a prospective research study analyzing the natural history (risk factors, metabolomics, and metagenomics) of MDRO UTIs. As part of this study, patients with MDRO UTIs submit stool and urine specimens at designated time points up to six months post-UTI diagnosis, and patients are monitored for UTI recurrences. Patients enrolled in the natural history study who meet inclusion and exclusion criteria for this study will be eligible for enrollment.

In addition to patients already enrolled in the natural history of MDRO UTI study, additional FMT-eligible patients may be identified through referrals from colleagues at the participating sites, through routine clinical care, or during the natural history of MDRO UTI screening process. Patients identified in these manners will undergo the same screening process as patients already enrolled in the natural history of MDRO UTI study.

Enrollment: If a patient with an MDRO UTI who meets inclusion/exclusion criteria is identified, a site investigator will review the patient's symptoms, laboratory results, and medical history to determine if the patient is a potential candidate for FMT. If the patient appears to be eligible, study personnel will contact the patient and describe the FMT study. Study coordinators will perform a phone screen to assess eligibility, the results of which will be reviewed by a site investigator. If the patient appears to be eligible and is interested in FMT, he/she will provide **verbal consent** to participate over the phone and arrange to submit pre-FMT stool/urine specimens. He/she will also schedule an outpatient clinic visit with study investigators, including a site investigator.

Before the clinic visit, study personnel (including an infectious disease physician) will review the patient's medical chart to determine if required laboratory screening tests from within the previous 30 days are available (see below). If not, study personnel will arrange for these tests to be performed prior to the patient's clinic visit.

Patients will submit stool/urine specimens to study personnel at two time points before FMT: upon enrollment, and at the end of MDRO UTI antibiotic therapy (+/-48 hours for both).

FMT Clinic visit: The patient's FMT clinic visit will be scheduled to occur within 48 hours after the completion of the patient's MDRO UTI antibiotic treatment. During the clinic visit, a study investigator will discuss the risks and benefits of FMT with the patient. All patients will provide **written, informed consent** for FMT and stool/urine collection and storage at this time. Before administration of FMT, a site 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 30 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 prior to the FMT visit. The maximum window between screening tests and FMT will be 30 days; beyond 30 days, the screening tests will be repeated.

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

FMT administration: FMT administration will occur at an outpatient clinic at each participating site. 150mL of RBX2660 will be used for each enema. The day prior to FMT, study personnel will receive the RBX2660 from Rebiotix and thaw it in the refrigerator. The enema bag kit from Rebiotix will 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 patient's stool samples. The remaining FMT material will be administered to the patient 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 patient 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 patient's rectum. The patient will be instructed to tighten the anal sphincter to create a firm seal around the enema tubing. The patient 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 patient is ready, the FMT will be administered slowly with frequent checks to ensure the patient's comfort. If the patient 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 patient will be monitored for 30 minutes after the enema, with repeat measurement of blood pressure, heart rate, and respiratory rate immediately after the enema and every 15 minutes until the monitoring time is complete. Recipients will be assessed for hypersensitivity during the 30 minutes post-FMT.

The patient 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. The patients will also be instructed to call study personnel if there is concern for any adverse events between visits (see Risks to Human Patients section).

Post-FMT follow-up: Patients will have a follow-up visit with a study investigator 30 days post-FMT (+/- 1 week). The following will occur at the 30 day post-FMT visit:

- The clinical investigator will:
    - Perform a physical examination.
    - Review the patient'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.
- The patient will return his/her adverse event diary at this visit for review by study personnel.

Patients will provide post-FMT stool and urine specimens at the following time points:

- Day 3 post-FMT (+/- 48 hours)
- Day 7 post-FMT (+/- 48 hours)

- Day 14 post-FMT (+/- 72 hours)
- Day 30 post-FMT (+/- 1 week)
- Every 30 days up to 6 months post-FMT (+/- 1 week)

At each stool/urine specimen submission point, patients will also submit a 24-hour food log and a log detailing any recent changes to their medical history (medications, hospitalizations, new/changes in comorbidities, etc.), UTI symptoms, and bowel movement habits. Study personnel will review the medical history log at each submission point for potential adverse events. If the patient's form indicates an adverse event may have occurred, study personnel will contact the patient for more details and complete an adverse event form. All adverse event forms will be reviewed by site investigators for severity and relatedness to FMT.

Patients will have follow-up phone calls at 6 months and 12 months post-FMT (+/- 1 week). During these calls, study personnel will review the patient's recent medical history and potential adverse events. The 6-month follow-up call will occur regardless of whether the patient reported any potential adverse events on his/her medical history log submitted with the 6-month stool and urine specimens.

Stool and urine samples will be stored for future analysis of MDRO carriage and changes in the microbiome. Additionally, an aliquot of the FMT product will be stored for analyses.

Remuneration: Patients will receive $15 for each stool/urine specimen submission. Patients will receive $50 upon sending in their final, 6-month stool and urine specimens. Patients will be eligible for a total of $200. Patients who withdraw early will only receive compensation for the stools/urine they have already submitted.

2.5 Data Management: Demographic data, including age, gender, race/ethnicity, height, weight, comorbidities, and tobacco and alcohol history, will be obtained on enrollment. Patients will submit stool and food history logs at each stool/urine specimen submission time point. At the clinic visit, all medications the patient received in the 6 months prior to first stool collection will be recorded, and inclusion and exclusion criteria eligibility will be documented.

All data collection tools will be stored in locked cabinets in locked offices and will be transported in such a way that no PHI is visible. Electronic data files will be stored on secured servers and password-protected computers. A RedCap database will be create for this study, and only key study personnel will have access to this database.

2.9 Stool specimen processing and storage: Stool and urine specimens will be submitted to the study team via courier and processed according to standard operating procedures. Briefly, 2ml aliquots of specimen (with and without TSB/glycerol) will be made and stool specimens will stored at -80^{o} C .

3.0 Analysis: All adverse events will be recorded and assessed for relatedness to study drug or procedure, and incidence of recurrent UTI will be recorded. All donor stools will undergo shotgun sequencing. All FMT recipient urine and stool specimens will be cultured for the MDRO of interest and undergo 16S rRNA gene sequencing. Shotgun sequencing will be done on key stools and urines: baseline, post-antimicrobial, day 7, and when the MDRO of interest is no longer cultured from stool. The number, types, severity, and relation of adverse events to study procedures or product will be analyzed with descriptive statistics. Clinical, culturomic, metagenomics, and metabolic data before and after FMT will be analyzed. To identify donor and recipient factors responsible for favorable versus unfavorable clinical

outcomes, a Random Forest predictor will be used to classify factors as protective or permissive to UTI or MDRO colonization. The model will be iteratively trained and tested on reserved data points. Error will be estimated using N-fold cross validation; multiple resampling will be used to obtain mean and standard deviation classification error.

4.0 Risks to Human Patients

4.1 Human Patients Involvement, Characteristics, and Design:

This will be a prospective phase 1-2 study to determine the safety and impact of FMT with RBX2660 on the fecal and urine microbiome, urine metabolome, risk of recurrent UTI, and persistent MDRO colonization of patients with a history of recurrent MDRO UTIs. We anticipate enrolling 40 patients. Stool and urine samples will be obtained before FMT, at the end of MDRO UTI antibiotic therapy, and at 8 time points after FMT. Data on medication exposures, infections, and comorbidities will be collected.

Patients at the four study sites will be identified through referrals from clinical colleagues, identification through clinical care, or prior enrollment in the natural history of MDRO UTI study. All study procedures will take place at the outpatient medical clinics or inpatient facilities at each site. Inclusion and exclusion criteria were described above (section 2.1).

4.2 Sources of Materials:

Research material obtained from the study patients will include, but are not limited to, demographic data, history and symptoms of UTIs and MDRO infections, medication exposures, comorbidities, bowel movement history, and food history. Stool and urine will also be collected from study patients. The stools and urine will be de-identified and stored at -80^{o}C in a secured laboratory. Stool and urine specimens from non-WU participating sites will be shipped to WU in a de-identified manner for analyses. Only key study personnel will have access to study data and materials. All key personnel will have HIPAA and human patients research training.

Data from study patients will be collected through interviews. Protected health information (PHI) to be collected will include study patient name, contact information, current living address, date of birth, and dates of interviews/questionnaires/stool specimen collection. This PHI is necessary to ensure all data and specimens are linked within a study patient. Each study patient will receive a study number. All PHI will be destroyed as soon as possible after the data are analyzed.

Stool and urine specimens will be transported from patients to study sites 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.

Limited study data will be entered at each site into a RedCap database developed by WU. Stool and urine specimens will be stored in Dr. Dubberke's and Dr. Kwon'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 offices, and by using password protected and secured servers for computerized databases (including RedCap) 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 locked, secured research laboratories.

4.3 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 patients 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 patients 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 patients 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, and neither have been reported in the literature. 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 patient 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 patient developed the infection due to FMT, the stored specimens can be used to determine if the new MDRO came from the donor. In a double-blinded randomized controlled trial of RBX2660 vs placebo for recurrent *C. difficile* infection, there was no difference in number or type of adverse events reported between the study groups.[15]

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

4.4 Adequacy of Protection against Risks

Recruitment and Informed Consent: Study patients will be recruited to participate from referrals to study investigators, infectious disease consults, or through enrollment/screening in the natural history of MDRO UTI study. Potential study patients will discuss the study with the study investigator. If the potential patient is interested, the inclusion and exclusion criteria will be reviewed, and the patient will provide verbal consent via phone so that stool/urine specimens can be collected pre-FMT. At the clinic visit, patients will provide written informed consent. At both points, research personnel will review the consent documents, re-confirm inclusion and exclusion criteria are met, and answer any questions the study patient may have.

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 patients 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 WUSM. Study patients 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. Potential adverse events will also be recorded and monitored. Unanticipated Problems will be reported to the Washington University Human Research Protection Office according to WU HRPO Reporting Policies. All other adverse events possibly or definitely related to the study drug or procedures will be reported within 7 days to the WU site investigator. The study will be suspended and policies and procedures will be reviewed if a patient develops a serious adverse event.

5.0 Potential Benefits of the Proposed Research to Human Patients and Others

Patients with recurrent MDRO UTIs often have few treatment options available to them. If the FMT successfully reverses MDRO carriage, the patient's future risk of UTI due to MDRO 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 patient 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.

6.0 Importance of Knowledge to be Gained

The prevalence, costs, morbidity, and mortality of MDRO UTI continue to increase at a time when there are fewer antimicrobials being developed.[16,17] FMT may be a successful new measure to help combat the MDRO public health crisis and treat patients with recurrent MDRO UTIs. 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 in patients with MDRO UTIs.

7.0 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 patient seeks medical attention.

The dates of onset and resolution, actions taken as a result, and patient outcomes of adverse events will be recorded. Patients will be given a post-FMT diary (see Supplementary Materials) to record solicited adverse events in the 7 days post-FMT. Patients 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 30 days post-FMT. In addition, the patient will be assessed for adverse events, including serious, at the 30 day post-FMT clinic visit and all time points the patient will be contacted (see post-FMT follow-up above).

Safety reporting will be as per 21 CFR 312.32 and in accordance with the WU HRPO reporting policy 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 a site investigator 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 Unanticipated Problems will be reported to the WU site investigator and Washington University Human Research Protection Office according to WU HRPO Reporting Policies. All other adverse events definitely or probably related to study drug or procedure will be reported within 7 days to the WU site investigator. The FDA will be notified of serious adverse events definitely or probably related to study drug or procedure 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 patients 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. 10% of the planned number of enrolled subjects or more experience similar adverse events of a severe or greater grade related to study drug or procedure.
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 stools over baseline per 24-hour period OR IV fluid replacement indicated if not indicated at baseline	Life-threatening consequences, e.g., hypotensive shock
Abdominal cramping/pain	Discomfort/pain causing no or minimal interference with usual social and	Discomfort/pain causing greater than minimal interference with usual social and	Discomfort/pain causing inability to perform usual social and functional activities	Disabling pain causing inability to perform basic self-care OR inpatient

Parameter	Grade1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
	functional activities	functional activities		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
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	NA

Parameter	Grade1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
			medications / treatments	
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 oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of 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,

Parameter	Grade1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
				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.*

References

1. Spellberg B, Blaser M, Guidos RJ, et al. Combating antimicrobial resistance: policy recommendations to save lives. *Clin Infect Dis.* 2011;52 Suppl 5:S397-428.
2. Sommer MO, Dantas G. Antibiotics and the resistant microbiome. *Curr Opin Microbiol.* 2011;14(5):556-563.
3. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews Microbiology.* 2015;13(5):269-284.
4. Donskey CJ. The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. *Clin Infect Dis.* 2004;39(2):219-226.
5. Hill S. New antibiotics. *Practitioner.* 1990;234(1484):229-231.
6. Schechner V, Kotlovsky T, Tarabeia J, et al. Predictors of rectal carriage of carbapenem-resistant Enterobacteriaceae (CRE) among patients with known CRE carriage at their next hospital encounter. *Infect Control Hosp Epidemiol.* 2011;32(5):497-503.
7. Sethi AK, Al-Nassir WN, Nerandzic MM, Bobulsky GS, Donskey CJ. Persistence of skin contamination and environmental shedding of Clostridium difficile during and after treatment of C. difficile infection. *Infect Control Hosp Epidemiol.* 2010;31(1):21-27.
8. Zimmerman FS, Assous MV, Bdolah-Abram T, Lachish T, Yinnon AM, Wiener-Well Y. Duration of carriage of carbapenem-resistant Enterobacteriaceae following hospital discharge. *Am J Infect Control.* 2013;41(3):190-194.
9. Lucet JC, Koulenti D, Zahar JR. Persistence of colonisation with MDRO following discharge from the ICU. *Intensive Care Med.* 2014;40(4):603-605.
10. Sohn S, Climo M, Diekema D, et al. Varying rates of Clostridium difficile-associated diarrhea at prevention epicenter hospitals. *Infection Control and Hospital Epidemiology.* 2005;26(8):676-679.
11. Dubberke ER, Mullane KM, Gerding DN, et al. Clearance of Vancomycin-Resistant Enterococcus Concomitant With Administration of a Microbiota-Based Drug Targeted at Recurrent Clostridium difficile Infection. *Open Forum Infect Dis.* 2016;3(3):ofw133.
12. Kelly CR, Ihunnah C, Fischer M, et al. Fecal microbiota transplant for treatment of Clostridium difficile infection in immunocompromised patients. *Am J Gastroenterol.* 2014;109(7):1065-1071.
13. Di Bella S, Gouliouris T, Petrosillo N. Fecal microbiota transplantation (FMT) for Clostridium difficile infection: focus on immunocompromised patients. *J Infect Chemother.* 2015;21(4):230-237.
14. Hamilton MJ, Weingarden AR, Unno T, Khoruts A, Sadowsky MJ. High-throughput DNA sequence analysis reveals stable engraftment of gut microbiota following transplantation of previously frozen fecal bacteria. *Gut Microbes.* 2013;4(2):125-135.
15. Dubberke ER, Lee C, Orenstein R, Khanna S, Hecht G, Fraiz J. Efficacy and safety of RBX2660 for the prevention of recurrent Clostridium difficile infection: results of the PUNCH CD 2 trial. ID Week; October 26-30, 2016; New Orleans, LA.
16. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2013. 2014; http://www.cdc.gov/drugresistance/threat-report-2013/. Accessed 1/9/2016.
17. *Antimicrobial Resistance: Global Report on Surveillance.* Geneva, Switzerland: World Health Organization;2014.