

## **Study Protocol**

### **Restoration of the Fecal Microbiome after Antimicrobial Exposure with Autologous Fecal Flora Restoration Therapy**

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**ClinicalTrials.gov ID: 201312022**

## Methods

**Hypothesis:** Autologous fecal microbiota therapy (auto FMT) will be able to rapidly, and safely, restore a patient's fecal microbiome after antimicrobial exposure.

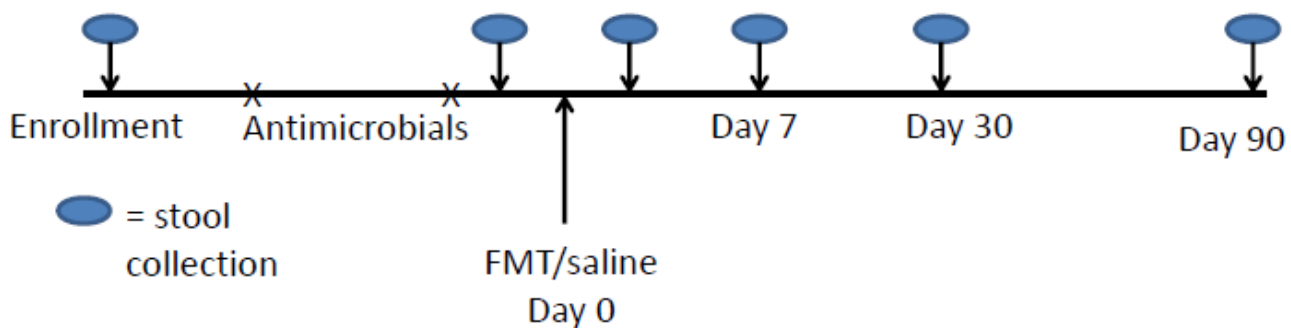
### Specific Aims:

1. Determine the effects five days of amoxicillin/clavulanic acid on the fecal and urinary microbiomes of healthy volunteers.
2. Determine whether an infusion of fecal filtrate collected prior to the antimicrobial exposure is capable of rapid restoration of a healthy fecal microbiome when infused after a course of antimicrobials.

**Patient Population:** Ten healthy volunteers will be recruited to participate through the Washington University Research Participant Registry (see Resources). Inclusion criteria will be healthy adults 21 to 70 years of age. Exclusion criteria will include a history of allergic reaction to beta-lactam antimicrobials; any non-topical antimicrobial exposure or tube feeds as a primary source of nutrition in the past six months; pregnant or risk of becoming pregnant during the study period; gastroenteritis in the last 3 months; any non-elective hospitalization in the previous 12 months incontinent of stool; prior resection or alteration of the stomach; small bowel, or colon; unwillingness to receive an enema/FMT; known colonization with an MDRO; anticipated change in diet or medications, or elective surgery, during the study period; positive HIV test; evidence of chronic medical conditions seen during physical exam; or a history of an intestinal disorder.

**Study Procedure** After written informed consent is obtained, a full physical exam including vital signs,  $\beta$ HCG pregnancy test in women of childbearing age, and an HIV 1/2 antibody test will be performed. The subject will be provided with a sealable stool collection device. The subject will be instructed to bring back stool and urine specimens at their convenience within the next week, preferably within two hours after the bowel movement/urination. If the bowel movement has been at room temperature for greater than 6 hours, the subject will be asked to bring back another stool specimen. In case the subject has a bowel movement after hours or is unable to deliver the specimen within two hours, the subject will be instructed to contact a pre-identified courier to pick up the urine and stool specimens. Please see below on how stool and urine will be processed for microbiome interrogation (stool and urine specimens) and FMT (autologous FMT). After the first stool specimen is collected, the subject will be provided with a five day supply of amoxicillin/clavulanic acid.

**Figure Study procedure overview.** 10 patients will be enrolled. Half will be randomized to FMT and the other half will receive saline enemas. Stool will be collected at enrollment, after the course of antimicrobials, the first bowel movement after FMT/saline, and days 7, 30, and 90 after FMT. The stool specimens and FMT product will be interrogated with DNA-based microbiome methods and semi-quantitative culture



Explicit directions on how to take amoxicillin/clavulanic acid will be provided, as well as instructions to bring the pill bottle back to confirm all doses were taken. A return visit will be scheduled one or two days after amoxicillin/clavulanic acid is to be completed. The subject will be instructed to provide stool specimens after

amoxicillin/clavulanic acid has been completed but before FMT/saline. The subject will be able to bring the specimens with them to the FMT/saline visit if they are less than two hours old, or drop off/courier specimens prior to the appointment. The FMT/saline will be administered by enema in the outpatient setting by trained research personnel. The subject will be instructed to collect the first bowel movement after FMT/saline (immediate release of enema material will be excluded), as well as urine at that time, and also bowel movements at day  $7 \pm 2$ , day  $30 \pm 2$ , and day  $90 \pm 2$ . The subject will be supplied with all necessary materials for stool collection and drop-off at each stool collection time point.

**Data Management:** Demographic data, including age, gender, race/ethnicity, height, weight, comorbidities, and tobacco and alcohol history, will be obtained on enrollment. All medications the subject received in the 6 months prior to first stool/urine collection will be recorded. The subject will be provided a questionnaire on bowel movement consistency (per Bristol stool chart) and frequency, diet in past week, and medications taken to be completed at time of enrollment, FMT/saline, and all subsequent stool/urine collection time points.

**Stool specimens:** Stool specimens will be collected as cited above. Fresh stool specimens will be cultured on to Brucella blood, Laked blood kanamycin vancomycin (LKV), PEA, Bacteroides Bile Esculin (BBE), MacConkey, Hectoen Enteric, Campylobacter blood, blood, Colistin-Naladixic, and cycloserine cefoxitin fructose (CCFA) agars semiquantitatively to assess viability of aerobic and anaerobic gram positive and gram negative organisms. DNA will be extracted prior to freezing, or processing of the pre-antimicrobial specimen, of residual stool using the MOBIO PowerSoil kit, as performed for the NIH Human Microbiome Project at Washington University (60;62). Parallel sequence datasets will be generated for each of the samples at the Genome Institute at Washington University as previously described for the HMP (58-60). This will involve 1) PCR amplification of bacterial rRNA genes using degenerate primers to capture rRNA genes from a broad diversity of bacteria, followed by sequencing of two separate segments of the 16S rRNA gene encoding either the V1 through V3 (V1–V3) or V3 through V5 (V3–V5) hypervariable regions on the 454 sequencing platform. Of note, the 454 sequencing primers used in this current study will be identical to the primers employed for characterizing the microbial communities in healthy individuals at different body sites, including the gastrointestinal tract by the Human Microbiome Project. All sequences will be processed as in the HMP to remove artifactual chimeric sequences and regions of low sequence quality, and then only sequences greater than a certain length (usually 200 bases) will be used for further analysis. Analyses will be performed by the methods used for previous studies at the Genome Institute (58;60;61) and will include quantitative statistical techniques to compare microbial community composition (taxa that are present) and structure (abundance of taxa). Where differences are found, it is usually possible to specify which species are most at variance between samples, and thus pinpoint potentially technical problems to be addressed in the preparation and re-establishment of fecal microbiomes. We also note that this methodology will allow us to initially classify the subjects as to which enterotype (63;64) their microbiome belongs to, in case this is a variable that would require stratification in application of the method.

We will also perform shotgun sequencing on a subset of the samples (the pre-antimicrobial sample, the enefusate, and the final 90 day sample). Shotgun sequencing allows for detection of non-bacterial entities such as viruses (50) and eukaryotic microbes, as well as detection of bacterial virulence factors, antibiotic resistance genes, and other relevant elements. Analyses will be as performed in the references cited above for the HMP in order to determine if these factors pose problems for the process.

**Autologous FMT:** The pre-antimicrobial stool specimen will be processed for FMT within two hours of collection. Approximately 150 gm of stool will be homogenized with 250ml of non-bacteriostatic saline. The slurry will be passed through stainless steel strainers to remove particulate matter. Sterile pharmaceutical grade glycerol will be added to a final concentration of 10%, and the specimen will be frozen at  $-80^{\circ}\text{C}$  (43). After the antimicrobials have been discontinued and the patient has provided a post-antimicrobial bowel movement, the enema material will be thawed over 2 to 4 hours in an ice bath for the patient undergoing autologous fecal flora restoration therapy. A 10 ml aliquot of the material will be saved for viability count

cultures and microbiome interrogation per above. The remaining material will be administered to the patient by enema, and the patient will be instructed to retain the material for as long as possible. A 10 ml aliquot of the non-bacteriostatic saline enema given to patients undergoing the sham procedure will also be collected for viability count cultures and microbiome interrogation as well.

**Analysis:** As mentioned above, analyses will be performed by the methods used for previous studies at the Genome Institute (58;60) and will include quantitative statistical techniques to compare microbial community composition (taxa that are present) and structure (abundance of taxa). Most methods are performed using functions in the R package, and include calculation of proximity measures such as Bray-Curtis index, comparison of communities using hierarchical clustering, multidimensional scaling, and/or Principal Component/Coordinate Analysis. The Genome Institute has recently developed additional metrics for community comparison using a tree-based approach (65). It is usually possible to specify which species are most at variance between samples, and thus pinpoint potentially technical problems to be addressed in the preparation and re-establishment of fecal microbiomes. Enterotype (63;64) analysis will also be performed on the initial subjects to determine if there is any correlation between this parameter and outcome.