Prospective Study Characterizing Fecal Microbiome Disruptions During and After Receipt of Antimicrobials

Clinical Protocol

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Introduction

Study Purpose:
The purpose of this study is to evaluate the impact of antimicrobial exposures on the microbiome in healthy adults, specifically during and after usual courses of the antimicrobials used to treat community acquired pneumonia (CAP).

Background:
In the United States, approximately 2 million people develop infections due to multidrug-resistant organisms (MDRO), and approximately 23,000 die as a result.[1] The major risk factor to acquire MDRO colonization and develop subsequent MDRO infections is exposure to antimicrobials.[2,3] A healthy fecal microbiome provides "colonization resistance" against MDROs and antimicrobial-exposure induced disruptions facilitate MDRO selection and colonization.[4,5] The use of antimicrobials has also been associated with an altered and often less diverse composition of the fecal microbiome, and expansion of the resistome (i.e. the compendium of resistance genes present in the microbiome).[6-8]
Controlling MDRO threats is a multifaceted task, requiring the prevention of healthcare-associated infections (HAI), preventing the spread of MDROs within and between healthcare facilities, and by improving antimicrobial stewardship.[9] With regards to antimicrobial stewardship, what is not definitively known is whether certain antimicrobials lead to greater or lesser degrees and/or durations of microbiome alterations.[10,11] A better understanding of exactly how antimicrobials impact the microbiome is necessary to optimally guide MDRO prevention efforts and antimicrobial stewardship.[11]

To address this knowledge gap, we propose a prospective study of the impact of antimicrobial exposures on the microbiome in healthy adults. The goal of this study is to characterize microbiome disruptions observed during and after usual courses of antimicrobials used to treat community acquired pneumonia (CAP). Healthy volunteers will be recruited and then be randomized to receive one of four standard antimicrobial courses for CAP. Fecal, salivary, skin, and urine specimens will be collected before, during, and after receipt of antimicrobials and analyzed to determine the impact of the antimicrobials on the microbiome. These data will allows us to develop microbiome disruption indices (MDI), which can be used to characterize the MDRO risk associated with specific antimicrobials.

A better understanding of how courses of antimicrobials alter the microbiome can be used to improve antimicrobial prescribing practices, allowing for selection of antimicrobials that minimize MDRO colonization risk. Through this study, we will develop standardized protocols for subject recruitment, antibiotic administration, specimen collection, culture, and metagenomic specimen analysis. These standardized protocols can then be used to compare the impact a variety of antimicrobial treatment regimens on the microbiome. These data will lead the development of MDIs, which have a multitude of potential uses in the fields of antimicrobial development, antimicrobial stewardship, infection control, and identification of patients who require microbiome remediation.[12] With the tremendous burden of MDRO infections and a paucity of safe and effective therapies against MDROs, data to develop MDIs are necessary to improve our antimicrobial stewardship and infection control measures.

Specific aims and hypotheses:
Specific aim 1: Determine the effects of antimicrobials used to treat community acquired pneumonia (CAP) on the fecal microbiota architecture and resistome (i.e. compendium of antibiotic resistance genes within the microbiota) in healthy adults.
Hypothesis 1: We hypothesize that, compared to the pre-antimicrobial state, antimicrobials will cause a decrease in microbial diversity, increase the concentration of antimicrobial resistance genes and MDROs in the fecal microbiome, and the microbiome alterations will vary between antimicrobials.

Specific aim 2: Determine the time to restoration of the fecal microbiome after exposure to antimicrobials used to treat CAP.
Hypothesis 2: We hypothesize that the degree and time to stabilization of the fecal microbiome will vary between antimicrobials.

Specific aim 3: Create microbiome disruption indices (MDIs) based on 1) the degree of fecal microbiota disruption, 2)

duration of microbiota disruption, and 3) resistome expansion.
Hypothesis 3: We hypothesize that the impact of antimicrobials will vary in regards to the degree of microbiome disruption, duration of microbiome disruption, and potential for resistome expansion.

Methods

Study design: Prospective cohort study.

Study population: 20 healthy adults. Subjects will be randomized to receive a five day course of azithromycin, levofloxacin, cefpodoxime, or cefpodoxime with azithromycin, which are recommended antimicrobials for the treatment of CAP.[13]

Inclusion criteria: Healthy adults age 21-60 who provide written, informed consent.

Exclusion criteria:
History of allergic reaction to study antimicrobial(s)
Contraindication(s) to study antimicrobial(s)
Inability to provide regular stool samples
Any non-topical antimicrobial exposure in previous 6 months
Tube feeds as primary source of nutrition in previous 6 months
Pregnant or risk of becoming pregnant during study period
Breastfeeding during study period
Gastroenteritis in last 3 months
Any non-elective hospitalization in the previous 12 months
Incontinent of stool
Known colonization with an MDRO
Anticipated change in diet or medications during study period
Elective surgery during study period
History of an intestinal disorder
Inability to provide written, informed consent

Recruitment: The following recruitment strategies will be used:

1. Word of mouth
2. Volunteer for Health website posting
3. Volunteer for Health Facebook post with study flyer
4. Post flyers around the WUSM campus
5. Email blast to WUSM employees.

Because the number of subjects needed is small, study investigators may not need to use all recruitment strategies.

Study Procedures:
Institutional Board Review (IRB) approval through the WUSM Human Research Protection Office (HPRO) will be obtained prior to study implementation. Subjects interested in participation will contact study personnel via phone. Study personnel will describe the study and screen potential subjects by asking whether they have any allergies to antibiotics and whether they are able to provide regular stool samples. If the subject is still interested in participation, a clinic visit (WUSM Infectious Diseases Clinic) will be scheduled. At this visit, the study investigator will review the informed consent document and answer any questions the subject may have. If the subject agrees to participate, he/she will then provide written, informed consent. Upon enrollment, subjects will undergo a clinical evaluation and interview. Women of child-bearing age will take a pregnancy test. Demographic data, dietary preferences, medical/surgical history will be obtained. Each subject will be provided with a specimen collection kit with appropriate materials and instructions for stool, saliva,

skin, and urine collection. The subject will collect specimens at the designated time points (Table 1) and complete a questionnaire on bowel movement consistency, frequency, diet, medications, and any changes in medical history at each time point. A total of 15 specimen sets will be collected per subject, and the subject will be provided all materials for specimen collection. Prior to each specimen collection date, study personnel will contact the subject via phone and remind him/her to collect specimens and fill out a food log, stool log, and medical update form.

Table 1. Specimen Collection and Processing Overview

Specimen collection	Culture & shotgun sequencing (Stool only)	Storage for future analyses
Enrollment/Day 14 prior to ABX	X	X
Day 10 prior to ABX		X
Day 7 prior to ABX		X
Day 1 prior to ABX	X	X
Day 3 during ABX	X	X
Day 1 after ABX		X
Day 3 after ABX	X	X
Day 7 after ABX		X
Day 10 after ABX		X
Day 14 after ABX	X	X
Day 30 after ABX	X	X
Day 60 after ABX	X	X
Day 90 after ABX		X
Day 120 after ABX		X
Day 180 after ABX		X

ABX = Antimicrobials

Once the specimens are ready, the subject will contact a pre-identified courier (JS Logistics, St. Louis, MO) for specimen pick up, and then the specimens will then be promptly delivered to the WUSM lab. After the pre-antimicrobial stools are collected, subjects will be provided with a 5 day course of azithromycin, levofloxacin, cefpodoxime, or cefpodoxime with azithromycin. Explicit directions on how to take the antimicrobial will be provided, as well as memory aids and instructions to send the pill bottle back to confirm all doses were taken.

The specimen on days -14, -10, and -7 days before antimicrobials can be submitted +/- 48 hours from the scheduled date. The -1 day specimens may be submitted -48 hours from the scheduled date but not after the subject has begun antimicrobial therapy. The day 3 during antimicrobials specimen may be submitted +/- 48 hours from the scheduled date. The specimens on day +1, +3, +7, and +10 post-antimicrobials may be submitted +/- 48 hours from the scheduled date. The specimens on day +14 may be submitted +/- 72 hours from the scheduled date. The specimens on days +30-+180 may be submitted +/- 1 week from the scheduled date.

Study activities will be complete after the subject submits the 180-day specimens.

Subject Remuneration

Subjects will be provided with $30 per specimen set for the first 14 specimen sets and $80 for the final (day 180) specimen set. Total remuneration for subjects who submit all specimens will be $500. No compensation will be provided if a subject fails to submit a specimen set. Subjects who drop out of the study early will receive compensation only for the specimens already submitted.

Data management

Specimen storage and transport:

Specimens will be shipped to WUSM by study subjects via courier. All specimens will be de-identified and labeled with a study ID number and will be stored without PHI. All salivary, urine, skin and all remaining stool specimens will be frozen at -80°C for future analyses. Prior to freezing, specimens will be divided into aliquots and labeled in formats and containers appropriate for future metabolomic, metagenomics, and culture-based analyses. Specimens will be stored in locked laboratories.

Electronic data:

All electronic records will be stored on password-protected computers on secure network servers. Portable data storage, such as laptops, jump drives, etc., will not be used. Data on biological specimens will be stored without PHI and separately from the primary enrollment database.

Paper records:
All paper/hard copy records, such as signed consent forms, interview and exam findings, stool and food logs, and medical history updates, will be stored in locked files in locked offices. When being transported via courier, they will be in sealed packages without any visible identifying information. Only key study personnel will have access to identified data.

Data analysis

Specimen processing:
A subset of 7 stool specimens per subject will be analyzed by culture and shotgun sequencing (Table 1). All saliva, skin, urine, and remaining stool specimens will be stored at -80°C for future analyses. All study investigators will remain blinded to study group assignment until after the culture and metagenomic data are analyzed.

Culture methods:
Semi-quantitative cultures will be performed to assess the relative abundance of aerobic and anaerobic bacteria in the fecal specimens. A series of 10, 10-fold dilutions will be made of the fecal specimen then plated and incubated at 35°C. A "sweep" of the growth on each blood agar plate will be frozen in TSB with glycerol for additional analysis. Overall burden of MDRO in the fecal specimens will also be assessed by culture.

Shotgun sequencing:
Metagenomic DNA will be extracted from the fecal material using a standardized phenol chloroform method as we and others have previously published.[14-18] Each metagenomic sample will be tagged with a unique 7 bp nucleotide barcode. We will use the Illumina NextSeq platform to generate at least 400 million reads per lane. Total reads will be demultiplexed by barcode into individual sample sequence bins. The adaptors and barcodes will be removed by Trimmomatic and simultaneously filtered for quality. The Dantas lab has extensive experience in the Illumina library preparation pipeline for metagenomic sequencing.[19-21] Further details of metagenomic methods are delineated in SOW section, task 6: shotgun sequencing.

Metagenomic data analysis:
We will use methods previously published to conduct metagenomic analyses, including quantitative statistical techniques to compare microbial community composition and structure.[19,22] Antibiotic resistance genes composition and abundance will be identified directly from shotgun data using ShortBRED.[23] Once annotated, resistomes will be compared between samples by adapting a species-oriented metric, such as Bray-Curtis, to resistance gene class abundances.[24,25] For aim 1, we will compare microbial diversity, antimicrobial resistance gene abundance, and MDRO concentration in the pre-antimicrobial specimen to the post-antimicrobial specimens within a person, between persons, and to previously published data from healthy controls. For aim 2, we will define the duration of antimicrobial disruption that occurs until return to the pre-antimicrobial state within a person (if this achieved), between persons, and to previously published data. Alternatively, we will assay for return to an alternative stable microbiota state post-antimicrobials, within and between the aforementioned comparisons. For aim 3, we will characterize the degree and disruption that occurs with each antimicrobial, and work with the Centers for Disease Control and Prevention (CDC) to develop and test a quantitative MDI metric.

Risk to Subjects and Protection Against Risks

The primary risk to subjects is loss of confidentiality. To minimize this risk, the data management practices described above will be utilized. Study personnel will maintain strict adherence to HIPAA guidelines throughout the conduct of the study.

There are also uncommon risks associated with the study antimicrobials. These will be detailed on the informed consent document and discussed with study subjects. They include: diarrhea, nausea, vomiting, abdominal pain or bloating, vaginal fungal infection, skin rash, and headache.

There are rare risks associated with the study antimicrobials, including: allergic reaction, throat/tongue swelling, jaundice, abnormal heart rhythms/arrhythmias, death, liver or kidney failure or abnormal function, heart palpitations, C. difficile infection, chest pain, kidney infection, dizziness, sleepiness/fatigue, rash/itchy skin/hives, light sensitivity, indigestion/gas/belching, stroke, swelling, decrease appetite, insomnia, constipation, heart attack, tendon swelling or rupture, fungal infection, anemia, bleeding, loss of white blood cells, tremor, convulsions, nerve damage or changes in nerve sensation, anxiety/agitation/confusion, depression, hallucination, nightmares or other sleep disorder, muscle tightness or spasms, difficulty concentrating, or achiness.

To minimize the risk of side effects from the study antimicrobials, study personnel will screen and exclude any participants with a history of allergic reaction to antimicrobials or any contraindications. Study investigators will perform a physical exam and interview subjects regarding their medical history; any subject with a history that might increase his/her risk of an adverse event will be excluded from participation.

All participants will be provided with the contact information for the study team as well as the 24-hour number for the nurse line at the ID Clinic and instructed to call with any questions or concerns about adverse events. Dr. Kwon and/or Dr. Dubberke will supervise all enrollment and review data collection tools to identify adverse events or toxicities in enrolled subjects. They will document and report all adverse events in study participants. All adverse events will be reported to the WUSM Human Research Protection Office within 7 days of notification.

Future Benefits

There are no direct benefits to subjects for participating in this study; however, there are future potential benefits to society. The prevalence, costs, morbidity, and mortality of MDRO infections continue to increase at a time when there are few antimicrobials being developed. Methods to proactively prevent MDRO colonization, rather than reliance on reactive approaches to this problem, are urgently needed. Antimicrobial stewardship is a key component of MDRO prevention efforts; however, there is no method to determine which antimicrobials cause the greatest degree of microbiome disruption. A better understanding of exactly how antimicrobials alter the microbiome is necessary to optimally guide future MDRO prevention efforts and antimicrobial stewardship. This study would elucidate the impact of antimicrobials on the fecal microbiome and the duration of this effect.

The results of this study will improve our current body of knowledge by characterizing microbiome disruptions (from baseline) observed during and after usual courses of antimicrobials commonly used in medicine. This information can then be used to create microbial disruption indices (MDIs) for antimicrobial comparisons and future antimicrobial development. These indices, in turn, may be used to help physicians select treatments for their patients that will minimally disrupt their microbiomes.

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