Omada microbiome

IRB00081259

NCT05515562

IRB Approval Date: 24APR2023

Protocol Synopsis

Title: Effects of IV Omadacycline on Gut Microbiome

Version: 2.0

Protocol Number: 001
Version Date: 24APR2023

Principal investigator: John C. Williamson, PharmD

Accrual Target: 8 participants

Population: Healthy participants between ages 18 and 40 years.

Regimen: Participants will receive a 10-day course of omadacycline with 5 days of intravenous therapy followed by 5 days of oral therapy.

Study Duration:

Length of study: Approximately 12 months from First Participant First Visit to Last Participant Last Visit.

Length of participation of study participants: Up to 36 days.

Locations:

University of Houston College of Pharmacy
Laboratory and Data Analysis Center
Jinhee Jo, Chenlin Hu, Anne J Gonzales-Luna, Khurshida Begum, M Jahangir Alam, and Kevin W. Garey

Wake Forest University Health Sciences at Atrium Health Wake Forest Baptist
Data and Sample Collection Center; IRB of record
John Williamson, PharmD

High Point University Fred Wilson School of Pharmacy
Recruitment Center
Travis Carlson, PharmD

1.0 STUDY OBJECTIVES

1.1.1 *Primary Objective:*

To characterize the impact of 5 days of IV omadacycline followed by 5 days of oral omadacycline on the gut microbiome of healthy volunteers**.**

2.0 BACKGROUND AND SCIENTIFIC RATIONALE

Clostridioides difficile infection is a major health threat in the United States that is most often an outcome of perturbation of gut microbiota following antibiotic use [1]. Even the limited number of antibiotic regimens effective in the treatment of *C. difficile* infection have the ability to impact gut microbiome in a negative way and may increase the risk of relapse due to such perturbations [2,3,4]. Thus, there is an increasing need to identify candidate therapies for *C. difficile* that target this pathogen but have minimal effect on the native gut microbiome.

Omadacycline is an aminomethylcycline tetracycline antibiotic that is approved by the US Food and Drug Administration (FDA) for the treatment of community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSSI) [5]. A recent study demonstrated *in vitro* activity of omadacycline against *C. difficile* [6].

Given the clinical need to improve upon current antibiotic regimens for the treatment of *C. difficile* infection with a particular focus on the impact of therapies on gut microbiome, we propose this study to characterize the impact of IV omadacycline on gut microbiome of healthy volunteers. We plan to enroll healthy volunteers between the ages of 18 and 40 years old and without history of cardiovascular, gastrointestinal, hepatic, or renal disease to receive 5 days of intravenous omadacycline followed by 5 days of oral omadacycline.

2.1 Reproductive and Developmental Toxicity

Based on animal data, there is reasonable suspicion that omadacycline has the potential to cause fetal harm [5,7]. We will only enroll females of childbearing potential who are not pregnant at baseline and who agree to use a reliable method of birth control or remain abstinent. Additionally, females of childbearing potential who use hormone-based oral contraceptives (OCs) will be required to use a second form of birth control (e.g. condom) because of a possible drug interaction with omadacycline which may lower the effectiveness of OCs.

3.0 PATIENT SELECTION

3.1 Selection Criteria

#	Yes	No	Inclusion Criteria
1	☐	☐	Is aged 18-40 years.
2	☐	☐	Is willing and able to comply with all study procedures including providing informed consent, and visiting the ID Clinical Trials Center at Wake Forest Baptist Medical Center at the specified times to receive study medication and/or to deliver samples (must have reliable transportation).
3	☐	☐	Is considered healthy, without history of cardiovascular, gastrointestinal, hepatic, or renal disease.
4	☐	☐	Meets one of the three categories below: ☐ **1. Is female and of child bearing potential** and agrees to use highly effective contraception during the study and for at least 7 days after the last dose of study product ☐ **2. Is female and not of child bearing potential** due to *(One must be checked):* ☐ surgical sterility OR ☐ congenital sterility OR ☐ post-menopausal state OR ☐ pre-menarchal state ☐ **3. Is male**

#	Yes	No	Exclusion Criteria
1	☐	☐	Has consumed probiotics within 30 days prior to enrollment.*
2	☐	☐	Has consumed antibiotics within 90 days prior to enrollment.*
3	☐	☐	Has a known hypersensitivity to omadacycline or tetracycline-class antibacterial drugs.
4	☐	☐	Is pregnant or breastfeeding.
5	☐	☐	In the opinion of the investigator, is experiencing signs or symptoms of acute illness that increase the risk of adverse effects from participating in the study.
6	☐	☐	Has previously participated in the study.

*Potential study volunteers who are excluded due to probiotic or antibiotic consumption may be re-screened at a later date when outside the exclusionary window(s).

Investigator Signature	
Date of Signature (DDMMMYYYY)	

3.2 Sample Size
The study will enroll 8 participants.

4.0 INVESTIGATIONAL PLAN

4.1 Screening

After signing informed consent, participants will undergo a brief interview about medications taken within the last 90 days with a particular focus on probiotic and antibiotic use. Volunteers who have received probiotics in the past 30 days and volunteers who have received antibiotics within the past 90 days will not be considered for participation. Participants will also undergo a limited review of their medical history to capture cardiovascular, gastrointestinal, renal, and hepatic diagnoses or conditions. Once the investigator confirms that the participant meets all patient selection criteria, the investigator and study team will schedule the patient's first dose to occur on study Day 1. If dosing is to occur immediately, the participant will provide a stool sample directly following informed consent but before omadacycline dosing. If dosing is to occur on a subsequent day, the participant will bring a stool sample (obtained the morning of Day 1) to the clinic prior to dosing or will provide the sample in clinic just prior to Day 1 dosing. The screening period will begin no more than 5 days before study drug administration on study Day 1.

4.2 Omadacycline dosing:

Study Day	Dose	Route of administration and schedule
Day 1	200 mg	Intravenous, infused over 60 minutes
Days 2 – 5	100 mg	Intravenous, infused over 30 minutes
Days 6 – 10	300 mg	Oral, two tablets once daily (dispensed as ten 150 mg tablets)

Omadacycline powder will be provided by the sponsor, reconstituted with Sterile Water, and then further diluted for Injection under aseptic conditions as follows: 2 vials (200 mg) to 2 mg/mL, and 1 vial (100 mg) to 1 mg/mL. Each subject will receive 2 vials on day 1 followed by 1 vial on days 2-5 (4 additional vials). Subjects will then receive 2 oral tablets (300 mg) daily on study days 6 through 10 (5 days).

4.3 Monitoring subjects during omadacycline infusions

During the first infusion of omadacycline, subjects will be monitored by either a research nurse or investigator. At a minimum, the monitor will be present with the subject during the first 10 minutes of the infusion and will return every 5-10 minutes or sooner thereafter for the remainder of the 60-minute infusion. The monitoring study team member will,

- Observe the general appearance of the subject
- Examine skin color for possible infusion reaction

- Examine the site of the infusion for possible redness or swelling
- Ask the subject if they are experiencing discomfort at the infusion site
- Ask the subject if they are experiencing any of the following symptoms: nausea, headache, dizziness, lightheadedness, fever, chills, shortness of breath, flushing, itching, or chest discomfort
- Report any symptoms to the investigator immediately, if investigator is not already present
- Along with investigator, assess the severity of any reported symptoms

The research nurse will perform and record vital signs for any clinically significant symptoms. Depending on the severity of any symptoms reported, the investigator will determine whether the infusion should be held or discontinued. If needed, the research nurse or investigator will activate the institution's code response system for "sudden illness" or "cardiac-pulmonary arrest".

If the subject tolerates the first infusion, subsequent infusions will be administered with less frequent monitoring. For these infusions, the monitoring research team member will be present with the subject for the first 5 minutes of the infusion and return every 10 minutes or sooner for the remainder of the 30-minute infusion. The same approach to monitoring with be undertaken (see methods above).

4.4 Laboratory Samples

Female participants of childbearing potential must produce a negative urine pregnancy test on Day 1 prior to dosing. Results from pregnancy tests performed within 3 days prior to Day 1 will be accepted for eligibility. Due to the teratogenic potential of omadacycline, a urine pregnancy test must be repeated and confirmed negative on Day 5 prior to infusion. Omadacycline dosing will be discontinued if the Day 5 pregnancy test is positive.

Participants will provide a total of 13 stool samples and 12 saliva samples during study participation. Two stool samples (one before and one after dosing) and one saliva sample will be provided on Day 1. On Days 2-10, Day 13 (+1), and Day 31 (±1) participants will provide the first stool sample of the day and one saliva sample per study day. Participants should deliver any home-collected stool samples to the clinic by 4:30pm on the day of collection and should store the sample in the refrigerator if not immediately bringing to the clinic. If the first stool sample of the day is collected after 4:30pm, it may be stored in the refrigerator overnight and delivered to the clinic the next morning.

4.5 Schedule of Events

	Screening (-5 or 1)[I]	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 13 (+1)	Day 31 (±1)
Informed Consent	X												
Eligibility	X												
Enrollment	X												
Limited Medical History[A]	X												
Limited Medication History[B]	X												
Investigational Product													
Omadacycline IV 200 mg[C]		X											
Omadacycline IV 100 mg[D]			X	X	X	X							
Omadacycline Oral 300 mg							X	X	X	X	X		
Laboratory Assessments													
Stool Sample[E,F]	X	X	X	X	X	X	X	X	X	X	X	X	X
Saliva Sample		X[G]	X	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test	X[J]					X[J]							
Safety Assessments													
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications[H]		X	X	X	X	X	X	X	X	X	X	X	X

A History of cardiovascular, gastrointestinal, hepatic, or renal disease is exclusionary

B No probiotic consumption within 30 days of enrollment. No antibiotic consumption within 90 days prior to enrollment.

C Infused over 60 minutes

D Infused over 30 minutes

E All stool samples should be delivered to the clinic less than 24 hours after collection. Delivery will typically occur the same day as collection, but could be the following morning if the sample is collected after 4:30pm

F On all study days after Day 1, the first stool of every day should be the one collected

G The saliva sample on Day 1 should be provided before omadacycline dosing

H Assessment of antibiotic and probiotic use to occur at each study visit

I Re-screening is allowable

J Results from a recent pregnancy test (within 3 days prior to Day 1) will be accepted for eligibility determination. Pregnancy tests should be performed and confirmed negative prior to dosing on Days 1 and 5.

4.6 Setting

4.6.1 *Recruitment and advertising*

Advertisements will be posted on the campus of High Point University (HPU) in High Point, NC and Atrium Health Wake Forest Baptist (AHWFB) in Winston-Salem, NC. Study team members at HPU and AHWFB will manage recruitment.

4.6.2 *Study procedures*

Study procedures including informed consent, eligibility confirmation, medical and medication history review, investigational product dispensing and administration, data collection, safety monitoring, and sample collection, temporary storage, and shipment will take place in the Wake Forest University Health Sciences Infectious Diseases Clinical Trials Center (IDCTC) at the Winston-Salem, NC campus of Atrium Health Wake Forest Baptist.

4.6.3 *Laboratory and Data Analysis*

The Garey Lab at University of Houston College of Pharmacy in Houston, TX will perform laboratory and data analysis.

5.0 SUBJECT RECRUITMENT METHODS

Study team members at HPU and AHWFB will post advertisements, receive initial contact from interested study candidates, and perform pre-screening. The contact information of candidates who appear eligible based on pre-screening will be transferred to the staff of the IDCTC team for management of consent and consent process documentation, final sign-off on eligibility, and study enrollment.

6.0 ANALYTICAL PLAN

6.1 University of Houston Microbiome analysis:

DNA extraction:

Prior to DNA extraction, the OMNIgene tubes will be vortexed vigorously for at least 10 seconds. DNA will be extracted from the tube by aliquoting 300 µL of the tube sample using the AnaPrep system DNA extractor (AnaPrep 2, BioChain Instruments, Newark CA) and the following protocol. Approximately 400 uL of lysed bacteria will be mixed and loaded into the AnaPrep system and gDNA will be extracted using 400 microliter input volume and 100 uL elution volume. DNA will be eluted in 100µL of buffer and concentration measured using a Nanodrop 2000 (Fisher Scientific, Waltham, MA). If the DNA concentration is less than 10 µg/mL, the extraction will be repeated. A 50 µL portion of the extracted DNA will be used for qPCR analysis and the remainder used for 16S rDNA amplicon generation.

High-throughput metagenomics sequencing:

Microbiome characterization will be performed by sequencing the V3-4 region of the 16S rRNA gene followed by extensive bioinformatics analysis related to microbial composition, diversity, and community structure. Microbial DNA extraction, 16S rRNA gene-based next-generation sequencing (using the Illumina MiSeq), quality filtering, and microbiome-based analysis will be performed [8]. Each sample will be amplified using a

barcoded primer, which yields a unique sequence identifier tagged onto each individual sample library. Illumina-based sequencing will yield > 15,000 reads per sample. Operational taxonomic units (OTUs) will be defined as ≥99% identity. To analyze differences across timepoints, the OTU table will be normalized to the lowest number of sequences per sample, then consolidated by summing to species level and to each successively higher taxonomic level. The alpha diversity parameters Chao and Shannon (based on number of OTUs) and Phylogentic Diversity (PD) (based on phylogenetic relationships), will be generated in QIIME or R; alpha diversity is widely regarded as an indication of microbiota health. For beta diversity, which describes the relationships between samples, weighted Unifrac distance matrices will be generated in QIIME.

6.2 Statistical analyses

In general, the healthy volunteer analyses will assess changes in total and species-specific microbiota over time as well as changes in microbial diversity. Significance differences over time will be calculated using Mann-Whitney U test. Analyses will be performed using SAS (SAS Institute, Cary NC) or R on log-transformed data. The LEfSe (Linear Effect Size) algorithm [9] will be used to identify significant differences in microbiota composition between baseline and each study time point, followed by Wilcoxon Signed Rank tests on identified taxa. The Benjamini-Hochberg procedure will be used to control the false discovery rate at 0.10 [10]. The MaAsLin algorithm will be used to find associations of taxa with treatment. For beta diversity, principal co-ordinates analyses on weighted Unifrac distance matrices generated in QIIME will be performed in the vegan package in the statistical program R. Finally, using the methods described by Burdet at al, Shannon diversity (a measure of alpha diversity) will be compared at each time point for subjects that receive moxifloxacin or comparators[11].

6.3 Omadacycline pharmacokinetics

All subject's stool samples will be aliquoted for omadacycline concentrations.

7.0 CONFIDENTIALITY AND PRIVACY

Confidentiality will be protected by collecting only information needed to assess study eligibility and outcomes, minimizing to the fullest extent possible the collection of any information that could directly identify subjects, and maintaining all study information in a secure manner. To help ensure subject privacy and confidentiality, only a unique study identifier will appear on the data collection form. With the exception of full names and signatures on the informed consent document and consent process documentation form, any patient information corresponding to the unique study identifier will be maintained and stored separately from the patient's study chart in WFUHS's Clinical Trials Management Software, OnCore or "Wake Integrated Solution for Enterprise Research (WISER)". WISER access is limited to designated study personnel. Following data collection, subject identifying information will be destroyed 6 years after study closure, consistent with data validation and study design, producing an anonymous analytical data set. Data access will be limited to study staff. Data and records will be kept locked and secured, with any computer data password protected. No reference to any individual participant will appear in reports, presentations, or publications that may arise from the study.

8.0 DATA AND SAFETY MONITORING

8.1 Stopping Criteria for the Study

The study will be stopped if any of the following events occur or, if in the judgment of the investigator, participant safety is compromised:

8.1.1 Unexpected death of a participant.
8.1.2 Occurrence of a life-threatening hypersensitivity reaction.
8.1.3 A trend of unexpected SAEs related to the study product.
8.1.4 A pattern of clinical or laboratory events considered to be associated with the study product that may be considered minor in terms of individual events but collectively represent a serious potential concern.
8.1.5 Any other event(s) that is/are considered to be SAE(s) in the clinical judgment of the responsible investigator.

8.2 Safety

The principal investigator will be responsible for the overall monitoring of the data and safety of study participants. The principal investigator and appropriately trained appointees will be responsible for reporting unanticipated problems (UAP), adverse events (AE), serious adverse events (SAE), and protocol deviations (PD) in compliance with Wake Forest University Health Sciences Institutional Review Board (WFUHS IRB) policies. All events will be logged on either a PD log or AE log as appropriate, and the log will be retained in the regulatory binder. A current version of the staff signature log and delegation of duties will be retained in the regulatory binder.

In addition to ongoing reporting as UAPs, PDs, AEs, and SAEs arise, self-monitoring will take place at the time of IRB Continuing Review to ensure protection of rights and well-being of study participants and data integrity. Self-monitoring will be performed by the principal investigator or an appropriately trained investigator appointee with protocol familiarity, such as a study coordinator or project manager of the Infectious Diseases Clinical Trials Unit (IDCTU). Self-monitoring will include the following:

8.2.1 Review study file to ensure compliance with local policies.
8.2.2 Verify written informed consent is on file for each participant.
8.2.3 Review the data collection forms for completion.
8.2.4 Note any safety issues including AEs, SAEs, UAPs, and PDs and verify appropriate reporting took place in accordance with local requirements.

9.0 REPORTING OF UNANTICIPATED PROBLEMS, ADVERSE EVENTS, OR DEVIATIONS

9.1 Definitions

9.1.1 *Adverse Event Definition:*

An AE is any untoward medical occurrence in humans, whether or not considered drug-related, which occurs during the conduct of a clinical trial. An AE can therefore be any change in clinical status, routine labs, x-rays, physical

examinations, etc., that is considered clinically significant by the study investigator.

9.1.2 *SAE Definition:*

An SAE or serious suspected adverse reaction or serious adverse reaction as determined by the investigator, or the sponsor is an AE that results in any of the following serious outcomes:

- Death
- Life Threatening
- Requiring hospitalization
- Permanently disabling
- Causing cancer
- Congenital anomaly/ birth defect
- Important medical event that may not result in one of the above outcomes, but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event

9.1.3 *UAP Definition:*

An unanticipated problem is any event, incident, experience, or outcome (including, but not limited to, adverse events and serious adverse events) which occurs in a research study and meets ALL of the following criteria:

- Unexpected in nature, frequency, or severity (not articulated in the study protocol, informed consent or Investigator's Brochure or not expected as a consequence of the natural history of a disease under study)
- Related or possibly related to participation in the research (there is a reasonable possibility the that the event, incident, experience, or outcome may have been caused by the drug/device, procedures or interventions involved in the research)
- Places subjects or others at a greater risk of harm than was previously known or recognized (causes physical, psychological, economic, or social harm to a human subject; increases the risk of harm of any kind; or otherwise compromises subject's safety, rights, welfare, or privacy).

9.2 Assessing Severity and Causality of AEs, SAEs, UAPs

9.2.1 *Criteria for Assessing Severity*

The Investigator will evaluate comments of the subject and any signs or symptoms associated with the event occurring in response to the antibiotic in order to judge the nature and severity of the adverse event. Severity refers to the intensity of discomfort/impairment of health and will be assessed according to the following criteria:

Mild: Awareness of sign, symptom, or event, but easily tolerated

Moderate: Discomfort enough to interfere with usual activity and may warrant intervention

Severe: Incapacitating with inability to do usual activities or significantly affects clinical status and warrants intervention

To clarify the difference between the terms "serious" and "severe", which are not synonymous, the following note is provided:

> The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is *not* the same as "serious", which is based on patient/event *outcome or action* criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

9.2.2 *Criteria for Assessing Causality*

The question of the relationship of an adverse event to study drug will be determined by the Investigator after thorough consideration of all facts that are available. Assessment of causality is based on considering associative connections (time or place), pharmacological explanations, previous knowledge of the drug, presence of characteristic clinical or pathological phenomena, exclusion of other causes, and/or absence of alternative explanations. The causal relationship of an adverse event to study drug will be assessed according to the following criteria: The Investigator will be responsible for determining the relationship between the administration of study drug and the occurrence of an AE as not suspected or suspected.

Not suspected: The temporal relationship of the adverse event to study drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions, or underlying conditions provides a sufficient explanation for the observed event.

Not related: Temporal relationship to study drug administration is missing or implausible, or there is evidence of another cause.

Unlikely related: Temporal relationship to study drug administration makes a causal relationship improbable; and other drugs, chemicals, or underlying disease provide plausible explanations.

Suspected: The temporal relationship of the adverse event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.

Possibly related: Reasonable time sequence to administration of study drug, but the event could also be explained by concurrent disease of other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

Definitely related: Plausible time relationship to study drug administration; event cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible.

9.3 Reporting and Monitoring of AEs or SAEs

9.3.1 *Reporting of Adverse Events*

All adverse events from the time of enrollment will be recorded in the Adverse Events section of the case report form. All adverse events will be described in terms of duration, severity, possible relation to study medications and treatment.

All adverse events will be followed until resolution.

Pregnancies that occur while on-study will not be considered an AE, but will be documented. Pregnancies will be followed to outcome if outcome occurs beyond the participant's Final Study Visit. Any pregnancy-associated SAE will be reported to the extent that information can be collected.

If the adverse event meets any of the criteria for Serious Adverse Events (see Section 9.1), the event will also be documented on a separate SAE Form.

9.3.2 *Reporting of Serious Adverse Events*

In the event of a Serious Adverse Event, information will be collected and recorded on the Serious Adverse Event section of the case report form. Serious Adverse Events will be reported from the time informed consent is obtained through the last protocol-specified study visit; whichever occurs later.

9.3.3 *Serious Adverse Event (SAE) Reporting to IRB and Study Sponsor:*

The Principal Investigator will be responsible for reporting any SAE thought to be directly attributable to the study medication to the IRB and Study Sponsor.

9.4 Deviations

Protocol deviations not meeting the WFUHS IRB Major Protocol Deviation definition will be tabulated and submitted to the IRB at the time of continuing review. Deviations meeting the criteria of a Major Protocol Deviation as defined by the WFUHS IRB will be reported per WFUHS IRB Policy.

10.0 REFERENCES

1. Duan J, Meng X, Liu S, Zhou P, Zeng C, Fu C, et al. Gut microbiota composition associated with *Clostridium difficile*-positive diarrhea and *C. difficile* type in ICU patients. Front Cell Infect Microbiol, 11 May 2020. doi.org/10.3389/fcimb.2020.00190.

2. Jacques Pépin, Marie-Eve Alary, Louis Valiquette, Evelyne Raiche, Joannie Ruel, Fulop, K., Dominique Godin, & Claude Bourassa. (2005). Increasing Risk of Relapse after Treatment of Clostridium difficile Colitis in Quebec, Canada. *Clinical Infectious Diseases*, *40*(11), 1591–1597. http://www.jstor.org/stable/4484249

3. Petrella LA, Sambol SP, Cheknis A, Nagaro K, Kean Y, Sears PS, Babakhani F, Johnson S, Gerding DN. Decreased cure and increased recurrence rates for Clostridium difficile infection caused by the epidemic C. difficile BI strain. Clin Infect Dis. 2012 Aug;55(3):351-7. doi: 10.1093/cid/cis430. Epub 2012 Apr 20. PMID: 22523271; PMCID: PMC3491778.

4. Singh T, Bedi P, Bumrah K, Singh J, Rai M, Seelam S. Updates in Treatment of Recurrent Clostridium difficile Infection. J Clin Med Res. 2019 Jul;11(7):465-471. doi: 10.14740/jocmr3854. Epub 2019 Jun 11. PMID: 31236163; PMCID: PMC6575119.

5. Omadacycline [package insert]. Boston, MA: Paratek Pharmaceuticals, Inc.; 2018.

6. Begum, Khurshida & Bassères, Eugénie & Miranda, Julie & Lancaster, Chris & Gonzales-Luna, Anne & Carlson, Travis & Rashid, Tasnuva & Eyre, David & Wilcox, Mark & Alam, PhD, M. Jahangir & Garey, Kevin. (2020). In vitro Activity of Omadacycline, a New Tetracycline Analog, and Comparators Against Clostridioides difficile. Antimicrobial Agents and Chemotherapy. 64. 10.1128/AAC.00522-20.

7. Tetracycline [package insert]. Sellersville, PA: Teva Pharmaceuticals USA; 2013.

8. Fadrosh DW, Ma B, Gajer P, et al. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome **2014**; 2(1): 6.

9. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol **2011**; 12(6): R60.

10. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological) 1995; 57(1):289±300.

11. Burdet C, Nguyen TT, Duval X, et al. Impact of Antibiotic Gut Exposure on the Temporal Changes in Microbiome Diversity. Antimicrob Agents Chemother **2019**; 63(10).