Aztreonam for Pharyngeal Gonorrhea – A Demonstration Study

Study Protocol

Version 1.3

April 26, 2019

Executive Summary

The Centers for Disease Control and Prevention has identified antimicrobial-resistant (AMR) *Neisseria gonorrhoeae* (NG) as one of the nation's top three urgent AMR threats. Since the advent of antibiotics in the 1930s, NG has developed resistance to every first-line antibiotic. Parenteral third-generation cephalosporins are now the only class of drug with consistent efficacy against NG. New therapies are urgently needed. Although some novel antimicrobials are under development, reevaluating older drugs is another option for quickly identifying additional treatments for gonorrhea. We propose a demonstration study to test a single dose of aztreonam for the treatment of pharyngeal gonorrhea. We chose to focus on pharyngeal gonorrhea because these infections are common, play an important role in fostering gonococcal resistance, and are harder to eradicate than genital infections. Although aztreonam appears to be >98.6% efficacious for anogenital NG, its efficacy at the pharynx may be less. Only 8 cases of pharyngeal gonorrhea have been documented to be treated with aztreonam, but of those, only 5 (62.5%) were cured. The dose used in those studies was 1g of aztreonam. Most antibiotics have a lower efficacy at the pharynx than anogenital sites, which is likely due to drug pharmacokinetics, i.e. difficulty in penetrating pharyngeal tissue. Thus, in the proposed study, we plan to treat 50 subjects with untreated pharyngeal gonorrhea with 2g IM Aztreonam.

Objectives

The proposed study aims to evaluate the efficacy of a single 2g intramuscular (IM) dose of aztreonam in the treatment of pharyngeal gonorrhea. Secondary objectives include documenting the efficacy stratified by minimal inhibitory concentration (MIC) compared with the previously document area under the curve (AUC) in order to estimate a pharmacodynamic criterion. We will also attempt to determine whether aztreonam monotherapy induces antimicrobial resistance among treatment failures. Lastly, we will evaluate the tolerability of 2g of IM aztreonam. The specific aims are:

1) Determine the proportion of persons whose pharyngeal gonococcal infections are cured with a single dose of 2g aztreonam intramuscularly.
2) Determine the proportion of persons whose urethral gonococcal infection are cure with a single dose of 2g aztreonam intramuscularly
3) Evaluate the tolerability of 2g IM of aztreonam .
4) Estimate the best pharmacodynamics criterion (i.e. AUC/MIC ratio) for pharyngeal gonorrhea treated with aztreonam using previously published AUC for 2g aztreonam and NG isolate MIC.
5) Among treatment failures, conduct exploratory analyses comparing pre- and post-treatment MIC for evidence of induced resistance.

Study Design: Prospective cohort

Study Population & Inclusion Criteria:

Persons diagnosed with pharyngeal gonorrhea or gonococcal urethritis who are undergoing pharyngeal gonorrhea testing, who are not yet treated.

Exclusion criteria:

Age less than 16 years
Receipt of antibiotics in ≤30 days
Known allergy to aztreonam
History of renal disease (including diagnosis of solitary kidney, chronic renal insufficiency, renal cell carcinoma etc.)
Concurrent infection with syphilis or chlamydia
Pregnancy and/or nursing
Unable to return for a follow-up visit 4-7 days (+/- 1 day).
Study team's discretion

Intervention: 2g IM aztreonam x 1

Primary Outcome: Negative gonorrhea culture 4-7 days (+/- 1 day) after treatment

Sample Size: 50 persons

Statistical Analysis Plan:

The primary endpoint is the pharyngeal cure rate – that is, the proportion of subjects who return for TOC who clear their infections as evidenced by a negative culture 4-7 (+/-1) days after treatment. Persons who test negative for NG at their enrollment visit (i.e. infection cleared between initial clinical screening test and enrollment or had gonococcal urethritis in the absence of pharyngeal gonorrhea) will be excluded from analysis. Treatment failure will be defined as a positive culture 4-7 (+/-1) days after treatment. The cure rate will be calculated as the number of cured subjects divided by the total number of subjects who received treatment and returned for follow-up testing in a modified an intention to treat (mITT) analysis. We will also calculate the 95% CI for this proportion.

For our second objective, we will compare the MIC of cures to treatment failures using a stratified approach (Table 2), and we will correlate this data with known (i.e. previously published) AUC for 2g aztreonam to estimate the ideal pharmacodynamic (PD) criterion (Obj. 3). For persons who fail treatment we will also compare the pre- and post-treatment aztreonam MIC to estimate whether single dose aztreonam therapy can "induce" resistance (Obj. 4) as an exploratory analysis. We will consider an increase in the MIC by two doubling-dilutions (i.e. from MIC of 2 to 8 μg/mL, or 8 to 32 μg/mL) to represent induced resistance, and we will calculate the proportion of isolates among those that fail treatment that demonstrate induced resistance following receipt of aztreonam. Lastly, we will report the number and proportion of subjects who report side effects (Obj 2).

We will evaluate subject's tolerability (Obj. 2) using a standardized symptom questionnaire conducted at the enrollment visit (for questions related to injection) and the TOC visit. The TOC visit symptoms questioned will include, but not be limited to, rash, tinnitus, decreased hearing, nausea, vomiting, diarrhea, headaches, and decreased appetite. There will also be a section for free response.

Responsibilities

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Co-Investigator:	Matthew R. Golden, MD, MPH Department of Medicine, Division of Allergy and Infectious Diseases, University of Washington
Study Clinician:	Jennifer Morgan, ARNP STD Clinic, PHSKC
Study Coordinator:	Angela LeClair, CCRC STD Clinic, PHSKC
Medical Monitor:	Ann Collier, MD Professor of Medicine, Division of Allergy and Infectious Diseases University of Washington Director, UW AIDS Clinical Trials Unit Associate Director, Center for AIDS Research University of Washington and Fred Hutch
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Recruitment and Retention of Subjects

Patients who screen positive for pharyngeal gonorrhea, with or without concomitant GC at other anatomic sites, and have not yet received treatment for this infection or other antibiotics in the past 30 days are eligible for this study. We will also include men with GC urethritis (per gram stain) who will undergo pharyngeal GC screening the same day. We will not exclude based on gender or HIV status. Recruitment will primarily occur at PHSKC STD Clinic, however, we will also encourage high-diagnosing partner clinics to refer eligible patients as well. We plan to enroll 50 persons with untreated pharyngeal gonorrhea for this study.

Recruitment at PHSKC STD Clinic:

We will employ several strategies to recruit at PHSKC STD Clinic.

1) Patients who screen positive for pharyngeal gonorrhea and are called with their test results to return for treatment will be told on the phone that they may be eligible for a treatment study. (PHSKC STD Clinic providers (ie. ARNP, PA and/or RN) routinely call patients with positive tests results, thus these clinical staff will tell patients about their eligibility for the study, however, PHSKC STD Clinic providers will not conduct formal study eligibility screening nor will they elaborate about study procedures (see script, Appendix A).) If patients indicate interest, the PHSKC provider will make an appointment for the patient with the study coordinator (for more information about the study, eligibility screening and enrollment procedures) and study clinician (for testing and treatment procedures) at their earliest convenience, ideally the next day.
2) Male patients with gonococcal urethritis (as defined as urethral discharge and gram-negative diplococci on gram stain) who are to have pharyngeal testing that day, will also be invited to participate. PHSKC STD Clinic clinicians will be asked to refer these patients to study clinician.
3) Some patient may not receive a phone call with test results prior to coming in for treatment (i.e. they see results electronically on MyChart [patient portal] or they were not informed of the study via a test results phone call, or were referred by an outside clinic to the PHSKC STD Clinic for treatment as their provider does not stock the recommended therapy in their office). Patients who arrive at the PHSKC STD Clinic for treatment of pharyngeal gonorrhea and have not yet been informed about the study will be told by the triage RN or their clinician that they may be eligible and inquire about their interest. If they indicate any interest in the study, they will be referred to the study coordinator and/or study clinician at that time.

Recruitment outside of PHSKC STD Clinic:

We will advertise the Aztreonam Study with high diagnosing providers in the community. Through a large CDC surveillance grant, SURRG, we already have a relationship with many of these providers: Madison Clinic, Dr. Shalit's office, and Capitol Hill Medical

Group. We will inform providers in these clinics about the study and provide study info cards (see Appendix B). When they diagnose a patient with untreated pharyngeal gonorrhea, they will inform the patient that they may be eligible for this study, and if the patient is interested, either the provider or patient can call the study coordinator to set up an appointment.

Recruitment at Madison Clinic:

Madison Clinic patients diagnosed with pharyngeal gonorrhea, in the absence of chlamydia and syphilis, will be contacted about their test results by the Madison Clinic nurse. Patients will be informed about the need for treatment and their potential eligibility in a research study. The Madison clinic nurse will refer interested patients to the study coordinator to schedule an appointment.

Retention of subjects:

This study consists of two study visits. Once enrolled, subjects need to return 4-7 days (+/- 1day) for a test of cure (TOC) visit. At their enrollment visit, the subject will set up a TOC visit with either the study coordinator or study clinician, hand them an appointment reminder card (see Appendix C). Less than 24 hours prior to the appointment, the study coordinator will call the patient with an appointment reminder call. If the subject does not show up for their appointment, the study coordinator will call them that day to set up another TOC visit appointment.

Study Procedures

Screening & Enrollment

Pre-screening will occur by the PHSKC STD Clinic MA/RR who reviews all abnormal laboratories. Patients with untreated pharyngeal gonorrhea will be call the patient to inform them of their infection, let them know they need to come back for treatment and inform that they may be eligible for a study, and ask if they'd like to hear more. Interested potential study subjects will verbally consent to an eligibility screen over the phone and then will schedule and appointment with the study clinician. Alternatively, subjects who arrive at the clinic for treatment of pharyngeal gonorrhea or gonococcal urethritis will be identified by clinical staff as potentially eligible based on their chart. These patients will be referred to the study clinician who will offer the study in person and proceed with the other study procedures (eligibility screening, consent). We will use a standardized screening eligibility form (see Appendix D) to determine patient's eligibility. Patient will be excluded if they meet any of the following criteria:

- Age less than 16 years
- Receipt of antibiotics in ≤30 days
- Known allergy to aztreonam
- History of renal disease (including diagnosis of solitary kidney, chronic renal insufficiency, renal cell carcinoma etc),
- Known concurrent infection with syphilis or chlamydia
- Pregnancy and/or nursing
- Unable to return for a follow-up visit 4-7 days (+/- 1 day).
- Study team's discretion

Once it has been determined that the patient is eligible for and interested in the study, the study team member will go through the informed consent with the patient in detail. After the subject has signed the informed consent, formal study procedures will begin.

1. *Intake & Data Entry Form*:
   The study coordinator or clinician will fill out the first section of the data entry form which includes subject contact information, demographic information (include sex assigned at birth, current gender identity, sexual orientation, race/ethnicity), HIV status, anatomic sites of infection (or screening if presenting with gonococcal urethritis), height, weight, concurrent medications and medical history.

2. *Diagnostics and Baseline Clinical Data*:
   The study clinician will obtain specimens for culture from all anatomic sites that were screen positive for gonorrhea. This will include the pharynx (by inclusion criteria) and possibly the rectum, urethra and endocervix, per routine clinical care. A pharyngeal specimen is taken by gently touching a swab to the posterior aspect of the throat and moving it up and down, and over the tonsils for 5-10 seconds. Rectal swabs

involved inserting a small q-tip like swab in the rectum approximately 3 cm and twirling it around approximately five times. To obtain a urethral swabs, clinician use a smaller swab and insert it approximately 0.5 – 1 cm into the urethra and twirl 1-2 times. Women who are able to get pregnant will have a pregnancy test. If not pregnant, women who screen positive for vaginal gonorrhea will have endocervical specimen collected which requires a pelvic exam with speculum. Once the cervical os is visualized the clinician inserts the swab into the cervical os approximately 1 cm and spins it about 5 times for an adequate sample. All swabs for culture will be plated directly onto Thayer-Martin media and placed in a candle (CO2) jar. Plates will be transported to the Neisseria Reference Laboratory (NRL) for culture and antimicrobial susceptibility testing within < 24 hours. The procedures described above are all part of routine clinical care.

3. *Treatment*:
   Once all diagnostic procedures have been completed, the study clinician will administer 2g aztreonam IM. The aztreonam injections will be given as 2 injections of 4 mL each, one in each gluteus maximus. (Reconstitute Aztreonam with 6mL of sterile water)

4. *Counseling*:
   The study clinician will counsel the subject to avoid sexual activity, and most specifically performing oral sex, deep kissing, and oral-anal sex until they return for the TOC visit.

Test of Cure Visit

Subjects will return to the PHSKC STD Clinic to meet with the study clinician 4-7 days (+/- 1 day) after their enrollment visit. The study coordinator will call the subject the day prior to the visit as a reminder. At the TOC visit:

1. *Diagnostics*:
   The study clinician will obtain a pharyngeal culture for *N. gonorrhoeae.* If the culture is positive for *N. gonorrhoeae*, the subject will be called to return for standard of care treatment – Ceftriaxone 250mg IM plus Azithromycin 1g orally once.

2. *Behavior and Symptom screen*:
   The study clinician will administer a standardized symptom screening form to assess for aztreonam side effects and tolerability. The form will also ask about sexual activity, including kissing, in the time period between treatment and TOC.

Retention Plan

The study coordinator will attempt to schedule the TOC visit at 4 days after the enrollment visit so that if the subject fails to show to that visit, there are still opportunities to reschedule the TOC visit. Additionally, although the subject will be compensated for their time in the study, the will not receive compensation until they return for the TOC visit. This is an incentive for them to keep their follow-up visit.

Data Sources and Data Management

PHSKC STD Clinic Medical Record: We will use gonorrhea test results that occurred prior to enrollment visit from the STD clinic medical record for inclusion criteria. This data will be extracted by chart review during the enrollment visit using subject name and DOB as identifiers. The information will then be entered using a subject ID in the study database. We will also use demographic and sexual behavior data from the medical record.

Neisseria Reference Laboratory: The NRL will conduct and provide culture results and the antimicrobial susceptibility data for the *N. gonorrhoeae* isolates recovered at both enrollment and TOC visits. Dr. Soge will inform the study team of positive cultures via email using only the study ID, and will also provide an official paper result. Full antimicrobial susceptibility testing (AST) includes beta-lactamase, penicillin, tetracycline, ciprofloxacin, cefixime, ceftriaxone, azithromycin, gentamicin and aztreonam. Dr. Soge will provide AST Results for both enrollment and treatment failure isolates using an excel spreadsheet listing Study ID, date of collection, anatomic site, and whether the isolates was collected at enrollment or TOC visit.

Data Entry Form*:* The study coordinator and study clinician will record key data points on a paper data entry form which will be transcribed into electronic data entry form in RedCap. Data to be collected will come from both the STD Clinic medical record and patient interview. Data points will include: date of visit, gender identity, sex assigned at birth, age, race, height, weight, site of infections pre-enrollment and type of test used to diagnosis; results of treatment visit testing (culture results at each site); date of TOC visit, symptoms since treatment; sexual activity since treatment (including kissing, oral-anal, oral-penile, anal-penile, penile-vaginal etc.); anatomic sites tested by culture and results; repeat treatment needed etc.

Data Management

We will use RedCap to collect and store all study data. Data can be exported from RedCap as a Stata database, which will be used for analysis. Upon export from RedCap, we will remove identifiers except for subject ID and maintain a separate database to link subject identifiers/contact info to subject ID.

We will use Appointments Everywhere software for appointment scheduling and reminders, including text message reminders for TOC visit.

Study Confidentiality and Security

We will use study IDs to label data items that only pertain to the study – i.e. data entry form, and culture specimens. Specimens that are also clinical specimen, will be labeled with the patient's clinic label. Data will be obtained from the medical record and entered into the data entry form which will only be labeled with the Study ID. We will maintain a separate data base in RedCap that links the patient study ID and PHI. RedCap is 21 CFR part 11 compliant. We will destroy the link between the patient's identifying data and study ID after the study has been published or by December 31, 2021, whichever is sooner.

Laboratory Processing

N. gonorrhoeae *cultures and Antimicrobial Susceptibility Testing*

We will send specimens for culture and identification, antimicrobial susceptibility testing (AST) to the Neisseria Reference Laboratory. Gonorrhea isolates undergo AST according to the Clinical and Laboratory Standards Institute (CLSI) recommended agar dilution method for further surveillance.[13] AST testing includes: Penicillin (PEN), Tetracycline (TET), Cefixime (CFM), Ceftriaxone (CRO), Ciprofloxacin (CIP), Azithromycin (AZM), Gentamicin (GEN) and Aztreonam (AZT). We used CLSI [15] breakpoints to interpret MICs for penicillin (≥2.0μg/ml), tetracycline (≥2.0μg/ml), spectinomycin (≥128.0μg/ml), and ciprofloxacin (≥1.0μg/ml),[15] and used GISP alert values for azithromycin (≥2.0μg/ml), cefixime (≥0.25μg/ml), and ceftriaxone (≥0.125μg/ml) as CLSI has not established breakpoints for these antimicrobial agents.[12] Neither CLSI nor CDC have published breakpoints for aztreonam. Full AST results will be reported to the PI.

Data Safety and Monitoring Plan, Resolving & reporting Protocol Deviations, AEs and Unanticipated problems

Aztreonam Known Side Effects:

Aztreonam is usually a very well tolerated antibiotic in adults. Documented side effects (including, neutropenia, eosinophilia, thrombocytopenia, elevated liver function tests, skin rash, diarrhea, nausea, and vomiting) occur in <2% of cases. There may be pain related to the injection. Given that we are only dosing aztreonam once, we anticipate side effects to be rare. As with all drugs, there is a risk of allergic reaction. Type 1 allergic reaction (i.e. IgE mediated—anaphylaxis reactions) usually occur immediately. We will observe subjection for approximately 15 minutes following injection of aztreonam. Other types of allergic reaction occur later and are usually, but not always, less severe. A study clinician will evaluate the patient 4-7 days following administration of the drug and will ask about interim side effects.

DSMB OVERSIGHT RESPONSIBILITIES

Day-to-day oversight of the trial is provided by the Principal Investigator (PI), Dr. Barbee, along with Dr. Golden (co-I). Dr. Barbee assures that informed consent is obtained prior to performing any research procedures, that all subjects meet eligibility criteria, and that the study is conducted according to the IRB-approved research plan. Drs. Barbee and Golden review all study data and any adverse events (AEs) real-time, and report all SAEs to the Medical Monitor and IRB according to the approved DSMP.

Medical Monitor: Monitoring for the study will be provided by a volunteer Medical Monitor, Dr. Ann Collier, Professor of Medicine in the Division of Allergy and Infectious Diseases, who has over 25 years of experience with clinical trials, but is not directly related to the study team.

MONITORING PROCEDURES

The Medical Monitor, Dr. Collier, will review study conduct at the end of the study. The Medical Monitor will review serious adverse events (SAEs), and serious drug allergies in real-time. Study data are provided to the Medical Monitor prior to the interim and final analysis. Data reports are prepared by the PI, Dr. Barbee.

MONITORING REPORT

The Medical Monitor, Dr. Collier, will provide a written report to the study team with recommendations for study modification, study continuation/discontinuation as relevant. The study team is responsible for forwarding the report to the IRB.

COLLECTION AND REPORTING OF SAEs AND AEs

For this study, the following standard AE definitions are used:

Adverse event: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure.

Serious Adverse Event: Any AE that results in any of the following outcomes:

- Death
- Life-threatening
- Event requiring inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity

AEs are graded according to the following scale:

Mild: An experience that is transient, & requires no special treatment or intervention. The experience does not generally interfere with usual daily activities. This includes transient laboratory test alterations.

Moderate: An experience that is alleviated with simple therapeutic treatments. The experience impacts usual daily activities. Includes laboratory test alterations indicating injury, but without long-term risk.

Severe: An experience that requires therapeutic intervention. The experience interrupts usual daily activities. If hospitalization (or prolongation of hospitalization) is required for treatment it becomes an SAE.

The study uses the following AE attribution:

Not related: The AE is clearly not related to the study procedures (i.e., another cause of the event is most plausible and/or a clinically plausible temporal sequence is inconsistent with the onset of the event).

Possibly related: An event that follows a reasonable temporal sequence from the initiation of study procedures, but that could readily have been produced by a number of other factors.

Related: The AE is clearly related to the study procedures.

AEs are identified at the TOC visit using the symptom screen.

SAEs and specific procedure-associated AEs are reported to the Medical Monitor within 24 hours. In addition, all AEs are reported according to the University of Washington AE reporting guidelines.

MANAGEMENT OF RISKS TO SUBJECTS

Expected AEs

Expected AEs associated with aztreonam include:

- Pain at injection site
- Bruising at injection site

AE Management

- Warm compresses as needed
- Tylenol as needed

DATA ANALYSIS PLANS

The proposed study aims to evaluate the efficacy of a single intramuscular (IM) dose of 2g of aztreonam in the treatment of pharyngeal gonorrhea. Secondary objectives include evaluating the tolerability of these regimens, and estimating the ideal pharmacodynamic criterion. In exploratory analyses, we will attempt to determine whether aztreonam-monotherapy induces antimicrobial resistance among treatment failures. The specific aims are:

1) Determine the proportion of persons whose pharyngeal gonococcal infections are cured with a single dose of 2g aztreonam intramuscularly alone,
2) Determine the proportion of persons whose urethral gonococcal infections are cured with a single dose of 2g aztreonam intramuscularly alone
3) Evaluate the tolerability of 2g IM of aztreonam.
4) Estimate the best pharmacodynamics criterion (i.e. AUC/MIC ratio) for pharyngeal gonorrhea treated with aztreonam using previously documented AUC for 2g aztreonam and NG isolate MIC.
5) Among treatment failures, conduct exploratory analyses comparing pre- and post-treatment MIC for evidence of induced resistance.

Analyses

The primary endpoint is the pharyngeal cure rate – that is, the proportion of subjects who return for TOC who clear their infections as evidenced by a negative culture 4-7 (+/-1) days after treatment. The cure rate will be calculated as the number of cured subjects divided by the total number of subjects who received treatment and returned for follow-up testing in a modified an intention to treat (mITT) analysis. We will also calculate the 95% CI for this proportion.

$$\textit{Cure rate} = \frac{\textit{number of subjects with negative pharyngeal culture at TOC}}{\textit{number of subjects with + throat test at enrollment \& received aztreonam}}$$

Persons who test negative for pharyngeal NG at their enrollment visit (i.e. infection cleared between initial clinical screening test and enrollment or were negative) will be excluded from the primary analysis. Treatment failure will be defined as a positive culture 4-7 (+/-1) days after treatment.

For our secondary objectives, we will evaluate subject's tolerability (Obj. 2) using a standardized symptom questionnaire (see Appendix F) conducted at both the enrollment and the TOC visit. At enrollment, questions will focus on tolerability of the injection. At TOC, the symptoms questioned will include, but not be limited to, rash, tinnitus, decreased hearing, nausea, vomiting, diarrhea, headaches, and decreased appetite. There will also be a section for free response.

For persons who fail treatment we will also compare the pre- and post-treatment aztreonam MIC to estimate whether single dose aztreonam therapy can “induce” resistance (Obj. 5) in an exploratory analysis. We will consider an increase in the MIC by two doubling-dilutions (i.e. from MIC of 2 to 8 µg/mL, or 8 to 32 µg/mL) to represent induced resistance, and we will calculate the proportion of isolates among those that fail treatment that demonstrate induced resistance following receipt of aztreonam. Should we see a signal for induced resistance, we plan to apply for additional funding to conduct whole genome sequencing to look for genetic determinants of resistance.

Table 2: Proposed Description of Primary Study Findings

Aztreonam MIC (µg/mL)	Cure (N=XX)	Treatment Failures (N=XX)
0.03		
0.06		
0.12		
0.25		
0.5		
1.0		
2		
>2		