PROTOCOL TITLE:

Antibiotic concentration after delivery to middle ear for chronic suppurative otitis media

STUDY IDENTIFIERS:

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PRINCIPAL INVESTIGATOR:

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VERSION NUMBER/DATE:

Version 2 (updated as of August 14, 2021)

*Dartmouth-Hitchcock (D-HH HRPP) IRB approval for this protocol is effective from July 7, 2021, until July 21, 2022.

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1	July 2, 2021	Responding to reviewer comments	AL
2	August 14, 2021	Follow-up date changed to 3-10 days	AL

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1.0 Study Summary

Study Title	Antibiotic concentration after delivery to middle ear for chronic suppurative otitis media
Study Design	Prospective observational study
Primary Objective	Investigate the reliability of ciprofloxacin concentration measurements from aspirates of middle ear fluid after self-administration of otic drops in patients with chronic otorrhea and non-intact tympanic membrane
Secondary Objective(s)	Elucidate the role of antibiotic concentration in varying clinical outcomes in patients with chronic middle ear infections by using antibiotic concentration analysis of aspirates.
Research Intervention(s)/ Investigational Agent(s)	Patients diagnosed with chronic suppurative otitis media prescribed with antibiotic drops (0.3% ciprofloxacin, 0.1% dexamethasone suspension) will apply drops 1-hour prior to visit. During visit, the ear will be cleaned of any debris and a microscopic aspirate of approximately 100 microliters will be collected with a modified Juhn Tym-Tap device.
IND/IDE #	N/A
Study Population	Adults in the community with diagnosed chronic middle ear infection
Sample Size	N=10
Study Duration for individual participants	An individual participant will be followed on a weekly basis until reaching either criteria of complete symptom resolution, new treatment prescription, or maximum duration of 3 months
Study Specific Abbreviations/ Definitions	Chronic suppurative otitis media (CSOM)

2.0 Objectives*

The purpose of this study is to develop methodology to better understand the potential effect of ototopical concentration on clinical outcomes in patients with chronic middle ear infections. The investigators will do so by measuring antibiotic concentration in aspirates from the middle ear of selected subjects with otorrhea due to Chronic Suppurative Otitis Media (CSOM) who are prescribed and instructed to self-administer ototopical ciprofloxacin.

The primary specific aim of the study team is to investigate the reliability of measuring the concentration of ciprofloxacin from aspirates of middle ear fluid after self-administration of otic drops in subjects with chronic otorrhea. A secondary aim is to correlate these concentrations with subject factors such as the extent of otorrhea and the anatomy of the ear.

The investigators hypothesize that patient self-administration of ciprofloxacin drops results in variable concentrations delivered to the tissue level that are significantly below the in vitro concentration of the prescribed solution and that these concentrations are below the bactericidal concentration of ciprofloxacin-resistant bacteria as determine by our previous work. Additionally, the investigators anticipate that the concentration levels of ciprofloxacin at the level of the tympanic membrane will correlate with specific patient factors and clinical outcomes.

3.0 Background*

Chronic suppurative otitis media (CSOM) is characterized by chronic inflammation of the middle ear with persistent discharge from a non-intact tympanic membrane. CSOM is notably associated with a significant burden of disease worldwide (1). The most common pathogens are staphylococcus spp., klebsiella spp., pseudomonas aeruginosa, corynebacterium spp, and proteus spp. (2). Topical fluoroquinolones are first line therapy for CSOM and are advantageous as compared to oral or intravenous therapy in that these antibiotics avoid systemic side effects and have the potential to deliver high antibiotic concentrations directly to the middle ear mucosa (3-5). However, because of their widespread use, fluoroquinolone resistance has steadily risen (6-8), making management of CSOM challenging.

A prior study by the study team confirmed 141 patients with ciprofloxacin-resistant otitis media had poor outcomes when using ciprofloxacin drops compared to alternative therapy options (9). Similarly, Jang et al. recovered ciprofloxacin-resistant P. aeruginosa from all patients (n=88) with unremitting CSOM in the context of topical ciprofloxacin therapy in a prospective population of 231 outpatients. Of these 88, over 40% required intravenous antibiotics (10). These findings contradict the previously accepted thought by many practicing otolaryngologists that the high concentrations (3000 mcg/ml) obtained with topical fluoroquinolone antibiotic preparations (3000 mcg/ml) would sufficiently

overcome all bacterial resistance to fluoroquinolones. This belief is based on the fact that minimum inhibitory concentration (MIC) levels are derived from available plasma concentrations. (4,11).

To further investigate these relationships, the study team examined the actual MIC and minimal bactericidal concentrations (MBC). In this study, Trinh et al. found that the actual MIC and MBC of ciprofloxacin-resistant ear pathogens are much higher than the plasma-derived MIC levels used to assign resistance, but generally still below the concentration of the ototopical solution (12). This study suggests elevated MIC or MBC levels alone do not explain the observed poor clinical outcomes in patients with ciprofloxacin-resistant infections. These findings have led to the hypothesis that poor clinical outcomes in fluoroquinolone resistance are due to multiple effects, including elevated MICs and poor drug delivery with failure to reach the necessary concentration at the tissue level (tympanic membrane or middle ear).

The potential role of ineffective ototopical delivery of fluoroquinolones and antibiotic resistance in patient outcomes has yet to be elucidated. As such, it is becoming increasingly clear that topical antibiotic delivery is more complex than previously considered. Understanding the complexities of drug delivery is critical to improving patient management and clinical outcomes. Only one study has reported on drug concentration of ototopical solutions at the level of the middle ear. Ohyama et al. studied clinician-administered ototopical fluoroquinolone after ear cleaning using high performance liquid chromatography to measure intratympanic ciprofloxacin concentration. This study demonstrated that commercially available preparations of ofloxacin (3000 mcg/ml) resulted in highly variable concentrations (1.2 to 602 mcg/g) in the middle ear mucosa in 11 patients, with undetectable levels in another five patients (13). These results allude to the variety of patient factors that can influence drug penetration and the availability in the middle ear, including volume of otorrhea, the narrowness of the ear canal, differences in surface tension affecting fluid dynamics, and size of the tympanic membrane. These patient related factors are ill defined within the literature and the magnitude of such individual effects is largely unknown. Furthermore, the delivery method employed in this prior study was not consistent with standard medical practice of patient self-administration. As such, the investigators are pressed to further examine the fundamentals of ototopical antibiotic delivery.

One such avenue of examination is understanding what is happening at the tissue level. Many authors have advocated for a more complex assessment of fluoroquinolone resistance that includes both the relative resistance of the organism, the available concentration at the tissue level and duration of drug exposure to those tissues (14, 15). Matta et al. demonstrated the ability to measure concentrations of ciprofloxacin in the plasma, urine, kidney and bladder of mice using a high throughput liquid chromatography-tandem mass spectrometric (LC-MS/MS) method (16). The team hopes to use this as the basis for the development of our assay in order to measure the concentration of ciprofloxacin at the level of the tympanic membrane. In doing so, the

investigators can begin to better define the variables that contribute to drug penetration and effectiveness of ototopical antibiotics in the context of CSOM.

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4.0 Study Endpoints*

The specific aim is to investigate the reliability of accurately measuring the concentration of ciprofloxacin from aspirates of middle ear fluid after self-administration of otic drops in subjects with chronic otorrhea. A secondary aim is to correlate these concentrations with patient factors such as the extent of otorrhea and the anatomy of the ear.

Objective	**Study Endpoint**	**Safety Endpoint**
Investigate the reliability of measuring concentration of ciprofloxacin from middle ear aspirates	Precise sampling of a known aspirate volume (100 microliters) and performance of LC-MS/MS ciprofloxacin assay development for middle ear aspirate analysis	
Correlate the concentrations with patient factors	Correlate ciprofloxacin concentration of middle ear aspirate to symptom resolution time, patient demographics, patient anatomy and clinical outcomes (infection resolution).	

5.0 Study Intervention/Investigational Agent

The standard of care for CSOM involves ototopical quinolone therapy such as ciprofloxacin or ofloxacin. The affected ear is routinely cleaned of any debris, and a Juhn Tym-Tap® device will be used to aspirate fluid for culture samples. The patient will be provided the standard course of treatment, which is a prescription

for antibiotic drops of ciprofloxacin 0.3% and dexamethasone 0.1% suspension with specific written instructions. The frequency of treatment is three times daily for ten days.

For this study, patients who meet inclusion-exclusion criteria will be offered an opportunity to participate by the prescribing provider. Informed consent will be obtained. Enrolled subjects will be asked to return 3 to 10 days after the initial visit. Additionally, the subject will be asked to keep a log of their medication use and to administer the ototopical medication one hour prior to their appointment. During the follow-up appointment, the ear will be cleaned of any wax or purulent debris. A microscopic aspirate (~100 μL) will then be collected of fluid at the level of the non-intact tympanic membrane will be collected using with a modified Juhn Tym-Tap®. This type of sampling of middle ear aspirates is commonly performed in the ENT clinic (performed more than 120 times in the Dartmouth-Hitchcock ENT clinic in the past year). After sampling, the volume of the aspirate will then be precisely measured with a device developed specifically for this study. The color and consistency of the sample will be noted. The sample will then be diluted with a known volume of water to an acceptable volume for the LC-MS/MS assay. Furthermore, other data beyond ciprofloxacin concentration such as otologic diagnosis, bacteria culture results, presenting symptoms, specific site of infection, external ear canal volume estimates (based upon tympanometry and physical exam), configuration of external auditory canal, prescribed treatment, treatment outcome, and duration of infection will be noted for each subject.

6.0 Procedures Involved*

Specimen collection: This will be a prospective observational study of adult subjects being diagnosed with chronic otorrhea at a tertiary care hospital, Dartmouth-Hitchcock Medical Center. To test our hypothesis, we will identify subjects with chronic otorrhea in clinic. Informed consent will be obtained. Subjects will be given a prescription for antibiotic drops containing ciprofloxacin 0.3% and dexamethasone 0.1% suspension from a pharmacy of their choice with specific written instructions for self-administration. A compliance card will be given to record adherence to three-time daily regiment for ten days. Drops will be dosed three times daily for ten days, and no other non-prescribed ototopical antibiotic therapy nor additional over the counter treatments should be used during this period. Subjects will return for a follow up visit between 3 to 10 days after their initial visit and will be asked to administer the drops in the affected ear one hour prior to their appointment. At that time, the ear will once again be cleaned of any debris and a microscopic aspirate (~100 μL) of fluid at the level of the tympanic membrane or middle ear will be collected using an aseptic technique with a Juhn Tym-Tap specimen trap to collect micro aspirates. This specimen will be analyzed, precisely measured for

volume, diluted, and sent for analysis using mass spectrometry (description below). Data collected will include patient demographics, otologic diagnosis, bacteria culture results, presenting symptoms, amount and type of drainage, specific site of infection, external ear canal volume estimates, configuration of the external auditory canal based on physical exam, prescribed treatment, treatment outcome, and duration of infection.

Ciprofloxacin concentration measurements: A reverse-phase liquid chromatography with tandem mass spectrometry (LC-MS/MS) assay will be developed to measure concentrations of ciprofloxacin in the auricular aspirate. The assay is based on that published by Matta et al (16). In brief, a C_{18} stationary phase column will be used with a gradient elution of 5mM ammonium formate in 0.1% formic acid buffer and acetonitrile for the mobile phase. External standards using a ciprofloxacin stock solution at low, medium, and high concentrations will be used to create a calibration curve of instrument response to known concentrations. For sample analysis, a 30-µL aliquot of the auricular aspirate sample will be combined with a 60-µL water and 100-µL acetonitrile, which would be spiked with a single standardized amount of the internal standard, deuterated 8 ciprofloxacin. By using this internal standard method of calibration, a precise quantification of the ciprofloxacin concentration in the auricular aspirate will be determined. This assay will be developed and validated by the staff of the Clinical Pharmacology Shared Resource directed by Dr. Lionel D. Lewis.

7.0 Data and Specimen Banking*

The electronic data will be managed in an excel file of a local computer drive with frequent electronic backups in a securely encrypted cloud storage service (ShareFile). The stored data will include date of birth, medical record number, assigned sex at birth, BMI, otologic diagnosis, degree of anatomic involvement, bacteria culture results, presenting symptoms, amount and type of drainage, specific site of infection, external ear canal volume estimates, configuration of the external auditory canal, prescribed treatment, treatment outcome, and duration of infection.

All specimens will not be stored longer than necessary to complete analysis and will be discarded through proper DHMC lab protocols.

8.0 Sharing of Results with Subjects*

The subjects will be asked whether they would like to receive the study results, which would be released in the form of a manuscript or journal article over email at the conclusion of the study. Furthermore, a form to share (or not share) any incidental findings and investigational diagnostic tests such as bacterial culture results will be provided as an option to the subject.

9.0 Study Timelines*

Proposal Timeline	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR
Funding Acquisition & IRB Process	X	X	X					
Assay Development		X	X	X	X			
Patient Recruitment & Sample Collection					X	X	X	
Statistical Data Analysis						X	X	X
Concluding Analysis, Article Submission							X	X

Subject enrollment is anticipated from December 2021 to January 2022 with subsequent data collection and analysis happening immediately afterwards. The subjects will be followed up with weekly until infection is resolved (for a maximum of 3 months). The study is anticipated to end in December 2021.

10.0 Inclusion and Exclusion Criteria*

Inclusion criteria include:

- Adult patients greater than or equal to 18 years of age
- Diagnosis of CSOM

Exclusion criteria include:

- Atypical presentation of CSOM or atypical anatomy of the ear
- Presence of additional ear pathophysiology beyond CSOM
- Adults unable to consent
- Individuals who are not yet adults (infants, children, teenagers)
- Pregnant women
- Prisoners

- Non-English speaking

11.0 Vulnerable Populations*

This is not applicable to this study.

12.0 Local Number of Subjects

Ten subjects will be accrued locally in the ENT clinic on 4F at DHMC in Lebanon, NH.

13.0 Recruitment Methods

The potential subjects will be recruited in the ENT clinic from July to September 2021 during otology clinic hours. Provider will screen using inclusion-exclusion criteria described above in conjunction with clinical presentation aligned with CSOM diagnosis.

14.0 Withdrawal of Subjects*

Circumstances for subject withdrawal from research may include non-compliance with prescribed antibiotic regiment or any additional necessary medical intervention beyond that prescribed for CSOM. The study team does not plan to collect survival data from subjects that withdraw from the study. The subject always has the option to voluntarily withdraw from the study.

15.0 Risks to Subjects*

Participation for the subject may cause:

- Mild physical discomfort with an additional study-related sample collection using a Juhn Tym-Tap device
- Psychological stress via social pressure to comply to medication regiment and research protocol
- Time and potential indirect financial expense, especially for subjects who may have higher reliance on hourly wage

16.0 Potential Benefits to Subjects*

The subject may benefit from the additional examination and cleaning 3 to 10 days after the follow up appointment as well as higher social pressure to comply to prescribed medication regiment.

17.0 Data Management* and Confidentiality

Given the small sample size (n=10) and expected variability of results, a Fisher's exact test will be used to describe and compare the measured concentration of ciprofloxacin at the tympanic membrane to subject demographics as well as extent of perforation, amount of aural drainage and treatment outcomes.

The data will be managed on a locally encrypted device in an Excel file while stored on the encrypted cloud storage service called ShareFile. Access will be provided to those on the research team on a need-to-know basis with particular caution to ensure separation of identifiers and data. Any communication in a non-encrypted platform will not contain any protected health information.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects*

This is not applicable to our study as our study does not involve more than minimal risk to the subject.

19.0 Provisions to Protect the Privacy Interests of Subjects

The subjects' privacy interests will be conserved in this study as the subject will primarily see the physician and will only be exposed to one more interaction should they give informed consent.

At every encounter between the subject and research team, the subjects will be offered a time to ask questions as well as an email and cell-phone number to call for any forgotten questions.

The research team will have access to the subjects' electronic medical records as well as the master data file stored onto the encrypted cloud storage. The excel data file will only be shared with people directly involved in the data entry and analysis.

20.0 Compensation for Research-Related Injury

This research does not involve more than minimal risk to subjects.

21.0 Economic Burden to Subjects

The subject will have direct time cost by participating in this research including the travel and clinic time associated with the additional ear sampling needed to obtain the study-related middle aspirate. This may have an indirect financial cost with time possibly taken off from work. Furthermore, the subjects will be expected to complete a medication compliance / symptoms log. To offset this economic burden, subjects will be compensated $50 for their participation.

22.0 Consent Process

Subjects who meet inclusion-exclusion criteria will be given the option to enroll into our study during their first visit for CSOM to the ENT clinic at DHMC in Lebanon, NH. The subjects will have the opportunity to read through the consent form in the clinic room without any medical staff present to remove possibility of coercion or undue influence.

After five to ten minutes, a member of the research team will talk to the subject as per HRP-090. Non-English speaking, cognitively impaired, pregnant, prisoners, and pediatric subjects are not included in this study.

The "SOP: Informed Consent Process for Research (HRP-090)" will be followed.

23.0 Process to Document Consent in Writing

The "SOP: Written Documentation of Consent (HRP-091)" will be followed.

24.0 Setting

The setting of this research project is at Dartmouth-Hitchcock Medical Center (DHMC) in Lebanon, NH. The potential subjects will be recruited from the ENT clinic on 4F. Laboratory analyses will be completed in the labs at DHMC.

25.0 Resources Available

The feasibility of recruiting ten suitable subjects is high given the prevalence of CSOM. I will be dedicating full-time commitment during the summer months of July and August with additional part-time commitment until the conclusion of this project. This research will be conducted in the ENT clinic at DHMC with the support of Dr. Galit Almosnino and Dr. James Saunders. Furthermore, Dr. Lionel Lewis of the Section of Clinical Pharmacology and his lab have agreed to develop and operate the ciprofloxacin assays.

Prior to the formal initiation of the study, all members of the research team will be presented with the protocol as well as a specific delineation of each member's role.

26.0 Multi-Site Research*

This is not currently applicable to this study.