COMIRB Protocol

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Protocol #: 20-2957
Project Title: Acute application of intrawound antibiotic powder in open extremity fracture wounds
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I. Hypotheses and Specific Aims:

We aim to investigate the effectiveness of preoperative intrawound antibiotic powder in preventing infection and reducing bacterial burden after open fracture. Our primary outcome is the incidence of surgical site infection after open fracture with and without antibiotic powder application in the preoperative setting. Secondary outcomes will be based on culture and PCR quantitative analysis. . We hypothesize that the subjects who receive preoperative intrawound antibiotic powder will have fewer superficial and deep surgical site infections compared to subjects who do not receive the antibiotic powder. We also anticipate that the application of the antibiotic powder to open fracture wounds preoperatively will decrease the bacterial burden.

Specific Aim 1: To compare the incidence of surgical site infection across the three study groups, standard of care, vancomycin antibiotic powder, and tobramycin antibiotic powder.
Hypothesis 1: There will be a significant decrease in the incidence of surgical site infection in the groups that receive the antibiotic powder relative to the standard of care group.

Specific Aim 2: To compare changes in open fracture wound bacterial burden between the pre and intra-operative timepoints.
Hypothesis 2: There will be larger decrease in bacterial load as measured by the Shannon index in the antibiotic groups relative to the control group.

Specific Aim 3: To compare changes in the presence of bacterial species commonly associated with open fracture surgical site infection between the pre and intra-operative time points.
Hypothesis: There will be a significant group*time interaction. Changes in the presence of bacterial species commonly associated with surgical site infection will significantly depend on group.

II. Background and Significance:

Current open fracture management involves expedited administration of systemic antibiotics which has been shown to reduce infection rate[1-3], yet infection remains the most common and significant complication after high-energy fractures, with rates ranging from 15% to 40%[4-7]. Rehospitalization rates for complications after severe open fractures have been reported as high as 57% and one of the

major drivers of hospital readmission is deep infection[5,8]. Deep infection is a common problem in combat casualties with open fractures as well[6]. Furthermore, surgical site infections are associated with poor long-term outcomes and is considered one of the most common potentially preventable post-operative complication[5,8,9].

The issue with systemic intravenous therapy alone is that the antibiotic concentration in critically injured tissues is often less than the desired therapeutic level[10]. Furthermore, the open fracture wound is exposed to the outside environment and even nosocomial bacteria before systemic therapy can be initiated. These factors offer great opportunity for bacteria to attach to soft tissues and bone, and initiate biofilm production, a recognized source of chronic infections[4,11]. Given this dilemma, multimodal antibiotic therapies are being increasingly used by surgeons to prevent biofilm development, with evidence in the literature supporting combined systemic and local administration[12,13].

Antibiotic powder has gained substantial interest in infection prevention in orthopedic surgery, with the majority of research in spine literature. It has been found to be safe and effective in decreasing the infection burden in a local manner, with low systemic risk in prior studies[14-23]. It is also inexpensive and easy to use. Although there is a growing interest and use of antibiotic powder in the trauma setting, supporting research is limited[24].

An additional past study conducted at Denver Health Medical Center reviewed the utilization of antibiotic powder applied in the operative setting. A sample of 614 independent surgeries in 514 patients were reviewed in which 152 (25%) patients received topical antibiotic powder based on the discretion of the attending surgeons. Patients received either Vancomycin alone (53 patients) with the median dose of 1 g (1 g to 2 g) or Vancomycin/Tobramycin (97 patients) with a median dose of 2 g (1 g to 2 g)/1.2 g (1.2 g to 1.2 g) respectively. This project supports a successful methodology of antibiotic powder application in the acute orthopedic trauma population and more importantly, supports that intrawound powdered antibiotics at the proposed dosages do not pose a greater risk than that of the standard of care[25].

Of the studies available on this topic, all of them investigate the effect of antibiotic powder in the intra-operative setting. To date, there is no known study investigating the effects of antibiotic powder in reducing infection risk when applied to an open fracture wound in the emergent setting, before the patient is taken to the operating room.

Orthopedic trauma surgeons continue to face a significant rate of infection related complications associated with open fractures. Patients who experience these complications often face multiple surgeries and poor outcomes resulting in a significant burden to both the patient and the health care system. By identifying ways to help decrease infection rates in open fractures, we can strive for improved patient care and outcomes.

III. Preliminary Studies/Progress Report

A preliminary project has been conducted at the University of Colorado Hospital in conjunction with National Jewish Health Genomics Core Laboratory to test the effectiveness and utility of the proposed culture collection and next-generation quantitative PCR analysis of bacteria from open wounds. Three subjects were enrolled with intrawound specimen swabs collected in the (1)

emergency department, (2) before prepping in OR, (3) after standard operative prepping, and (4) a tissue sample collected intraoperatively as well. A sterile, flocked swab was utilized to collect a sample from the wound bed and was then stored in a proprietary buffer solution for storage and analysis. This pilot project demonstrated feasibility in the collection process and the analysis using 16S metagenomics of both DNA and RNA. This protocol will support the proposed project and demonstrates validity of the methods that will be utilized to address the secondary outcome measure.

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IV. Research Methods

A. Outcome Measure(s):

- **Primary outcome**
  Incidence of surgical site infection during the post-operative follow-up period
- **Secondary outcome**
  - Shannon's index measure of bacterial diversity based on wound cultures (see Figure XX).
  - Simpson index measure of bacterial diversity based on the wound cultures.
  - Presence of bacterial species commonly attributed to surgical site infections based on would cultures including: Staph, enterococcus, acinetobacter, enterobacter, e. coli, klebsiella, and pseudomonas.

B. Description of Population to be Enrolled:

Inclusion Criteria for Subjects:

- Subject or proxy willing and able to provide written informed consent.
- Age between 18 years and 80 years (inclusive), with an upper or lower extremity Gustilo Type II, IIIA, or IIIB open fractures requiring debridement and internal fixation.
- Open extremity fractures
- Time from injury to study intervention 24 hours or less

Exclusion Criteria for Subjects:

- Individuals under the age of 18 years or over 80 years
- Type I or IIIC open fractures
- Over 24 hours from time of injury

- Subjects who have received acute operative care of the open fracture at an outside facility prior to presenting at the Emergency Department.
- Open fractures distal to the wrist and midfoot
- History of chronic infection in the extremity involved.
- Subjects who are currently pregnant
- Subjects who are Prisoners
- Subjects with a known allergy to vancomycin or tobramycin
- Subjects with a condition or social circumstances that would reduce adherence and follow-up.
- Subjects Participating in other clinical research involving investigational antimicrobial products within 30 days of randomization.

Subjects meeting the study criteria will be consented for debridement and fixation along with enrollment in the study if possible before proceeding to the operating room. Based on trauma registry data from both institutions, we estimate to have 105 enrolled participants in 18 months, which includes an anticipated ten percent failure to enroll. Each arm of the study will enroll approximately 35 subjects each, See Power and Sample Size

C. Study Design and Research Methods

A randomized clinical cohort study will be utilized to investigate the efficacy of pre-operative intrawound antibiotic powder to decrease bacterial burden and subsequent infection after open fractures. The study will be performed at two collaborative Level 1 Trauma Centers in a large, urban community (University of Colorado Hospital and Denver Health Medical Center). Both institutions have orthopedic trauma research teams with experience in the application of antibiotic powder and the collection of biologic samples from open wounds.

The intervention will have three treatment arms: standard open fracture wound care (no powder), standard of care along with 1.0 gram vancomycin powder application to the open wound in the emergency department, or standard or care along with 1.2 gram tobramycin powder application to the open wound in the emergency department. This will be a single-blind study as only the participant will be blinded to the treatment. The clinical and research teams will be aware of the treatment arm for each participant. Subjects will be randomized to the three arms using a blocked stratified randomization scheme. Randomization strata will be defined by the fracture severity (type II vs type III open fractures). Investigators will be blinded to block size to preserve integrity of the randomization strategy. Randomization assignments will be created using the proc plan procedure within SAS.

All subjects will receive standard open fracture care in the form of tetanus update (if needed), intravenous antibiotics, and splinting/traction as indicated. Gustilo Type II open fractures will receive intravenous first generation cephalosporin. Gustilo Type IIIA and IIIB open fractures will receive intravenous first generation cephalosporin and aminoglycoside. Intravenous penicillin will be added for anaerobic coverage as indicated. In the emergency department, prior to any antibiotic introduction, a swab of the wound bed will be collected and stored.

Arms of the study are as follows:

Arm 1 – Open fracture wounds are dressed with damp saline-soaked gauze and given standard open fracture care. This group will act as internal control.
Arm 2 – Same as Arm 1, with addition of 1 gram of vancomycin powder applied to the wound bed before the dressing is applied.
Arm 3– Same as Arm 1, with addition of 1.2 grams of tobramycin powder applied to the wound bed before the dressing is applied.

Before proceeding with surgical debridement in the operating room, swabs of the wound bed and tissue samples will be obtained. Samples will be collected as described in the METRC Bioburden study.[4] Additional samples may be obtained for microbiology analysis as part of the standard of care and results will be utilized as a comparison for the identification of bacteria present in the wound. All treating surgeons will be fellowship trained orthopedic traumatologists and will be trained in proper sample collection technique. Subjects will be prospectively followed through their electronic medical record to track infection occurrence and other adverse events for six months following final debridement and fixation.
Samples will be vortexed for 1 minute or agitated manually for 3 minutes immediately following specimen collection and submersion into the appropriate buffer solution. All manually agitated specimens will be vortexed for 1 minute prior to centrifuge. Samples will then be centrifuged at 1000rpm for 15 seconds and stored in a -80C freezer for temporary storage.

Samples will be analyzed at the National Jewish Health Genomics Core Laboratory using the 16S metagenomics sequencing on the Illumina platform. Both RNA and DNA extracts will be utilized to identify and quantify the bacteria load in the wound bed at the time immediately prior to surgical debridement. Total RNA and genomic DNA will be isolated using standard kits from Qiagen (Valencia, CA, USA). The isolated total RNA and genomic DNA will be processed for next-generation sequencing (NGS) library construction for analysis with a Life Technologies (Carlsbad, CA, USA) Ion Gene Studio S5 Prime NGS platform. An Ion Ampliseq Pan-Bacterial Research Panel (Carlsbad, CA, USA) will be used to target more than 400,000 bacterial 16s regions. These 16s regions can then be used to identify bacteria at the genus and species level. Library construction will start from isolation of total RNA and genomic DNA, followed by reverse transcription using random priming (RNA), target amplification, adaptor ligation, amplification, and bead templating. Once validated, the libraries will be sequenced as barcoded-pooled samples on a 550 chip, as routinely performed by the NJH Genomics Facility. Once sequencing is finished, the data will be analyzed through the Pan Bacterial Analysis Plug-In through the Torrent Suite software.

The Ion Ampliseq Pan-Bacterial Research pipeline will allow us to identify the bacterial species present in the wound cultures. The vegan R package will be used to calculate the Shannon index and the Simpson index of biodiversity. This information will allow us to determine whether changes in biodiversity differ across the study arms. The purpose of this proof of concept method will be to confirm that the bacterial powder causes quantifiable changes in the presence of bacterial species relative to the standard of care (no powder group). We acknowledge that diversity is an overly broad approach. Changes in diversity may not necessarily reflect changes in the bacterial species commonly associated with surgical site infection. Therefore, we will also test for differences in the presence of bacterial species commonly associated with surgical site infection before and after antibiotic powder treatment. Due to differences in antibiotic coverage between the two antibiotics, we expect changes in these species will depend on group (group*time interaction). Together the three aims will provide valuable information about the feasibility of the proposed study allowing us to more appropriately design a definitive RCT to test the efficacy of antibiotic powder treatment.

The investigational drug service pharmacy team covering these institutions will be working with the study team in dispensing the antibiotic powder. Both institutions participating in this study have pharmacy personnel in the emergency department for efficient access to antibiotic powder. All orthopedic trauma team members will be trained on a standard application of the antibiotic powder and sample collection to ensure consistent therapy and method of sample collection respectively.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

On presentation to the ER, the Subjects will undergo the standard of care as outlined above. The application of local antibiotic powder to the open fracture wound is very low risk as detailed in the significance and background section. Potential risks include allergic reaction, systemic absorption of antibiotic causing known side effects from both tobramycin and vancomycin such as acute kidney injury. However, these risks are very low and well detailed in the literature.

Five samples will be collected as follows:

P1_Swab_1: ED Pre Antibiotics Patient 1
P1_Swab_2: OR Pre Prep Patient 1
P1_Swab_3: OR Debridement Patient 1
P1_Swab_4: Closure Patient 1
P1_Tissue: Tissue at debridement

P2_S1: ED Pre Sterilization Patient 2
Etc: Follow Patient 1 order for Patient 2, 3….

When collecting samples, we will be using a cotton tipped swab which will not cause further injury to the soft tissues or bone surrounding the open fracture. Samples will be obtained intra-operatively as well by similar methods and the risk of further soft tissue damage is extremely low.

Steps for Collection: Swab

1. Swab patient.
2. Immediately submerge swab in respective tube.
3. Cut handle of swab low enough so it can fit in the tube, and the cap can be closed completely.
4. Begin vortexing tube immediately. Vortex tube for 1 minute. **If tube cannot be immediately vortexed, begin agitating by inversion for 3 minutes. Once a vortexer is available vortex for 1 minute.**
5. Spin down sample in centrifuge at 1000rpm for 15 seconds.
6. Place sample in -20C or -80C freezer as soon as possible. (Sample is stable at room temp for a significant amount of time; however, immediate freezing will guarantee the best results).
7. Keep sample frozen and deliver to NJH on dry ice.

Steps for Collection: Tissue

1. Cut tissue ~1 cubic centimeter.
2. Immediately submerge tissue in respective tube with colored dot.

3. Begin vortexing tube immediately. Vortex tube for 1 minute. **If tube cannot be immediately vortexed, begin agitating by inversion for 3 minutes. Once a vortexer is available vortex for 1 minute.**
4. Spin down sample in centrifuge at 1000rpm for 15 seconds. Be sure tissue is completely submerged in buffer before freezing.
5. Place sample in -20C or -80C freezer as soon as possible. (Sample is stable at room temp for a significant amount of time; however, immediate freezing will guarantee the best results).
6. Keep sample frozen and deliver to NJH on dry ice.

The procedures in the previous section are the most invasive portion of our study. The data collection and analysis performed by National Jewish has no direct risk to the patient's health or ability to heal their fracture. The risks associated with the data analysis by National Jewish are similar for other studies dealing with protected health information. All patient's information will be de-identified and placed into a secured database (REDCap) with a randomized study participant ID. Subjects will be consented by proxy if they do not have the ability to consent at the time of arrival to the emergency department. This will be indicated in instances where the subject is intubated or unconscious due to trauma.

Subjects or their proxy will be able to elect to remove themselves from the study at any time. They will be provided with the primary investigators phone number and may reach out at any time to have their data and specimens destroyed. At the time of notification, all patient data will be removed from the REDCap database. If patient swabs or culture tubes are still present, the respective laboratory will be notified to destroy and dispose of the specimen properly. This process will be described in the consent.

Planned Duration of the Entire Study

The study grant is for 24 months between January 1, 2021 and December 31, 2022. Enrollment and data collection will take place for about a year and six months. Final analysis will occur subsequently.

E. Potential Scientific Problems:

Potential problems with this study include the execution of the methodology when the patient comes into the emergency department. Potential issues including obtaining the antibiotic powder in a timely fashion once the injury is identified, standardization of obtaining samples in the ER, and consent of subjects involved in poly-traumas who are unable to provide consent for the study in the emergent setting.

To address the ability to obtain the antibiotic powder, there will be a specific order placed into EPIC to streamline the process for both orthopedic team member, ER team, and pharmacy. This order will be of the desired antibiotic powder with the correct dose with instruction to not re-constitute the powder and to directly provide the bottle to the orthopedic team member for administration.

In regard to timing, pre-made kits will be available in the ER for the orthopedic team member that will include all supplies for the protocol including swab sticks, test tube vials, 4x4s, chloraprep stick etc. and an instruction sheet that will include a QR code that is scannable by any mobile device with a camera. This QR code will allow the orthopedic team member to enter the patient's MRN into a box

which will populate into a secure redcap database and randomize the patient into the correct arm of the study which will then allow the provider to order the correct antibiotic powder.

For standardizing the protocol, there will be a formal training on how to perform the protocol in the ER to all orthopedic team members participating in the study. In addition, there will be detailed instructions within the pre-made kits. This will help to standardize the collection of samples and administration of antibiotics.

Given many patients have other associated injuries with open fractures, we will absolutely have subjects who are obtunded, altered, or unable to provide consent in the emergency department setting. Given the critical timing of managing open fractures and the procedures involved in managing these injuries, such as stabilization of the fracture in a splint and identifying other potentially life-threatening injuries, we will be requesting the use of consenting by proxy for specific circumstances at the time of consent for surgery.

F. Data Analysis Plan:

Samples obtained from the wound bed will be analyzed as detailed above by National Jewish. In addition, we will be sending cultures to the site's laboratories for standard aerobic, anaerobic, acid-fast bacilli, and fungal cultures.

The post-operative data observing the presence of surgical site infection will be collected and placed into the secure redcap database.

Sample Size and Power:

The anticipated sample size in the current study is fixed based on study constraints including funding availability, funding duration, and patients eligible for the study during the funding period. Based on typical clinical volume, we anticipate we will be able to enroll approximately 117 subjects into the study. Assuming attrition is approximately 10% (reasonable assumption given most of data is collected at surgery and/or within 90 days of surgery), we anticipate a total sample size of 105 available for analysis. We simulated data, 100,000 iterations, to evaluate the power available to test our primary aim, infection risk, assuming a sample size of 35 individuals per group. We evaluated several scenarios to explore power available in the current study. The parameters used in the simulation studies including the anticipated infection rate in the standard of care group, were based on a recent systematic review that reported the incidence of infection in lower extremity fractures that underwent operative treatment ranged between 5 and 40%. The incidence of infection was highest among open fractures, and thus we assumed the infection rate in the current study will be closer to the higher range of the infection estimates. As outlined below, assuming a common effect in the two antibiotic groups, 15% risk of infection, and a higher infection risk in the standard of care group, 40% risk of infection, the current study will have >80% power to reject the null hypothesis of no difference in infection risk between groups (antibiotic groups vs standard of care). As outlined in the table below, power decreases if there is heterogeneity in antibiotic effect and/or if the treatment effect is modest.

Study Power Available Under Different Scenarios

	Consistent Treatment Effect		**Heterogeneous Treatment Effect**	
	Infection Rate	Power: Antibiotics vs Standard of Care	Infection Rate	Power: Vancomycin vs Tobramycin vs Standard of Care

Large Treatment Effect				
Vancomycin	15%		15%	
Tobramycin	15%	81.8%	20%	61.7%
Standard of Care	40%		40%	
Moderate Treatment Effect				
Vancomycin	15%		15%	
Tobramycin	15%	45.8%	20%	26.1%
Standard of Care	30%		30%	

Statistical Methods:
Descriptive statistics will be used to summarize group characteristics in the two study groups. Due to the small sample size, standardized mean differences will be used to screen for imbalance in relevant covariates. Covariates unequally distributed across groups will be adjusted for in subsequent analyses using multivariable models. To address aim 1, *to test for differences in the incidence of post-operative infection risk,* Chi-Square tests will be used to test for differences in infection risk during the first 90 days post-operatively across the study arms. We will first test for differences between groups assuming a common effect among the two antibiotics groups. We will test the null hypothesis of no difference between antibiotics arms relative to the control group. In order to account for randomization strata (fracture severity type II vs type III), we will calculate the common risk difference and 95% confidence intervals using the Mantel-Haenszel method. Alternatively, if there is heterogeneity in the incidence of infections across the three groups, we will test for pairwise differences in infection risk between groups. To address aim 2, *changes in wound culture diversity indices,* linear mixed models will be used to compare changes in diversity indices between the pre- and intra-operative time points. A random intercept model will be used to account for clustering of multiple observations within subjects. We will test the null hypothesis that the difference between time points depends on group (group*time interaction). To address aim 3, *changes in presence of bacterial species commonly associated with surgical site infection*, generalized linear mixed models will be used to test for changes in presence of bacterial species, binary yes vs no, before and after application of the antibiotic powder. We will test whether changes in the bacterial species depends on group. We will account for multiple sources of clustering, multiple bacterial species (Staph, enterococcus, acinetobacter, enterobacter, e. coli, klebsiella, and pseudomonas) and time (pre-operative and intra-operative prior to wound debridement). In this analytic framework, we will test the average of the bacterial species-specific binary outcome variables. We will also consider testing whether the changes in time points between groups depends on species (group*time*species interaction).

Alternative Approaches: The primary analysis assumes that follow-up will be consistent across the three groups. Cox proportional hazards regression analyses will be considered if subjects are not routinely followed for 90 days.

G. Summarize Knowledge to be Gained:
Overall, this study will allow us to identify the utility of using antibiotic powder in open fracture wounds at the time of presentation. We will be able to either support or refute the use of these antibiotics and their ability to decrease surgical site infection in open fractures. Given the prevalence of surgical site infection in open fractures, this study may

change the management of open fractures within the orthopedic community in both civilian and military populations. This study if successful will lead to further multi-center national studies to be performed.

H. References:

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