Project title
Beta-lactam continuous versus intermittent infusion and associated bacterial resistance and therapy outcomes in critically ill patients with severe pneumonia

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Abstract

Infections caused by resistant microorganisms are affecting millions of people and causing thousands of deaths every year in the United States. This has created a problem along with the slow development of new antimicrobials which limited the treatment options for infected patients. In the intensive care unit (ICU), pneumonia is the most commonly encountered bacterial infection, which is associated with severe outcomes including longer ICU stay, mechanical ventilation, and high mortality rate. Such infection is usually treated with beta-lactams using standard doses which may be inadequate to treat serious infections in ICU patients. To date, no published clinical trials evaluated the impact of beta-lactam continuous vs. intermittent infusion on the bacterial resistance in patients with severe pneumonia.

We plan to randomize a total of 240 patients infected with Gram-negative bacterial pneumonia to receive beta-lactam (meropenem, cefepime, or piperacillin/tazobactam) continuous or intermittent infusion and collect baseline and regular follow-up respiratory cultures to assess the development of new resistance. In addition, we will measure beta-lactam concentration to assess the impact of drug exposure on the bacterial resistance. The primary outcome in this trial will be the incidence of Gram-negative bacterial resistance, while the secondary outcomes will be the microbiologic eradication, clinical cure, length of stay, mortality, and incidence of adverse events. Given the sample size, the admission rate, and the multidisciplinary team involved, we expect to adequately describe the microbiologic and clinical outcomes associated with the beta-lactam continuous vs. intermittent infusion.

Background

Being a worldwide problem, antimicrobial resistance is putting millions of people's lives at risk (WHO, 2015). Resistant bacteria and fungi cause 3 million infections and >35,000 deaths every year in the United States. In their most recent Antibiotic Resistance threats report, the Centers for Disease Control and Prevention specified certain bacteria to be associated with great threat to people due to their resistance pattern and high mortality rates. These bacteria include carbapenem-resistant and extended-spectrum beta-lactamase-producing Enterobacteriaceae and multidrug-resistant *Pseudomonas aeruginosa* (CDC, 2019). With the slow development of new antibiotics, the resistance problem has created a barrier to treating patients and limited the available options.

Sepsis is considered a major problem in the intensive care unit (ICU). Globally, the sepsis-associated mortality is 25% (Kaukonen 2014, McPherson 2013, Fleischmann 2016). One of the most common bacterial infections in the ICUs is pneumonia, which has been consistently associated with prolonged mechanical ventilation, ICU stay, and high mortality rate (Papazian 2020). Early antibiotic therapy is important in these infections and resistance may develop in this population if there is suboptimal antimicrobial exposure that is insufficient to eradicate the invading bacteria (Roberts 2008, Tam 2005). Beta-lactams are frequently prescribed to treat these infections in the ICUs. The bacterial killing of beta-lactams is driven by the amount of time the free antibiotic concentration is above the minimum inhibitory concentration (fT>MIC). Currently suggested doses in the package inserts may be inadequate to treat the serious infections encountered in the critically ill patients, including pneumonia (Lipman 1999, Crandon 2010, Koomanachai 2010, Al-Shaer 2020).

To date, no published clinical trial evaluated the impact of beta-lactam continuous infusion on the emergence bacterial resistance in patients with severe pneumonia, which will be addressed in this project.

Statement of Work

Scope

This randomized, controlled clinical trial aims to identify the benefits and risks of beta-lactams continuous vs. intermittent infusion in critically ill patients with Gram-negative bacterial severe pneumonia, who will be admitted to the medical ICU at UF Health Shands from 2021 to 2023. We will compare the microbiologic, clinical, and safety outcomes between the two groups.

Objectives

Primary objective

- Compare the incidence of Gram-negative bacterial resistance between patients treated with continuous and intermittent infusion beta-lactam regimens.

Secondary objectives

Compare the following outcomes and variables between the continuous and intermittent beta-lactam infusion groups:

- Superinfection
- Microbiologic eradication
- Clinical cure
- PK/PD target attainment (calculated fT>MIC and fT>4×MIC)
- Length of stay
- Mortality rate
- Incidence of adverse events

Definitions

Bacterial resistance will be defined as new numeric increases (≥2-fold) in the bacterial MIC during the follow-up period compared to the baseline when starting beta-lactam therapy. Superinfection will be defined as the growth of resistant Gram-negative bacteria during the follow-up period which was not isolated in baseline culture. Microbiologic eradication is the absence of bacterial growth during the follow-up period with no subsequent positive culture from any site. Clinical cure is the resolution of infection-related symptoms at the end of therapy, including normalization of body temperature and WBC count and taking the patient off mechanical ventilation or vasopressors, and non-initiation of a new antibiotic within 48 hours of stopping the original one. Adverse events include drop in platelets ($<125\times10^3$ cells/mm^3) or WBC (<2,500 cells/mm^3), increase in serum creatinine (≥1.3 times of baseline) (HHS/NIH/NIAID/DAIDS 2017), *Clostridioides difficile* colitis, and neurotoxicity. Beta-lactam neurotoxicity is defined as meeting ≥2 of the National Cancer Institute (NCI) criteria for neurological toxicity including symptoms such as presence of new onset confusion, delirium, and drowsiness (NCI CTCAE v5.0), electroencephalogram (EEG) findings consistent with beta-lactam toxicity as per the neurologist, and improvement of signs and symptoms of neurotoxicity after beta-lactam discontinuation. The EEG findings will be categorized based on the 2012 American Clinical Neurophysiology Society standardized EEG critical care terminology (Hirsch 2013). Patients who will develop neurotoxicity will be extensively reviewed by a neurologist to assess the cause of this event (beta-lactam therapy vs. other factors). Patients with serious adverse events will be assessed for stopping or changing therapy as per clinician discretion.

Specific aims and tasks

Aim 1. To describe the incidence of Gram-negative bacterial resistance in the continuous and intermittent beta-lactam infusion groups

Study design

A randomized, open-label study of patients admitted to UF Health Shands Hospital in Gainesville, FL.

Inclusion criteria

- Admission to the medical ICU with severe pneumonia defined as per IDSA/ATS 2019 criteria on mechanical ventilation: presence of signs, symptoms and confirmatory chest imaging consistent with pneumonia (e.g. fever, cough and pulmonary infiltrate by chest radiograph) requiring mechanical ventilation due to respiratory failure
- Age ≥18 years
- Positive rapid identification test and respiratory culture for Gram-negative bacteria including, but not limited to, *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *S. marcescens*, *H. influenzae*, Enterobacter spp., *M. catarrhalis*, *A. baumannii*, Achromobacter spp., *P. mirabilis*, and/or *B. cepacia*
- Received within the last 48 hours or will receive meropenem, cefepime, or piperacillin/tazobactam therapy

Exclusion criteria

- Pregnancy
- Prisoners
- Allergy to the beta-lactams to be administered in this study
- On renal replacement therapy at the time of randomization
- Gram-negative bacteria identified by rapid testing only with negative respiratory culture at baseline
- COVID patients enrolled in other trials

Patients who will develop renal impairment requiring renal replacement therapy after randomization will be included in the analysis and, in case they were on continuous infusion, will be switched to intermittent infusion (see *intention to treat (ITT)* and *per protocol (PP) analyses* under *Statistical Analysis* section below).

Procedures and Intervention arms

Patients will be identified from the medical ICU admission list by the study team. Once the patient is intubated, a bronchoalveolar lavage or endotracheal aspirate will be

sent for culture and susceptibility testing as per standard of care. As part of the screening process, the same specimen will be used for quick semi-quantitative pathogen identification using the BioFire® FilmArray® pneumonia panel (BioFire Diagnostics Salt Lake City, Utah, USA) that detects 15 common potential agents of pneumonia in intubated patients within 1 hour in the laboratory. If Gram-negative bacteria were identified, patient or their representative will be approached and provided with informed consent form, and if they agree to participate the patient will be enrolled in the study.

Alternate Plan: The BioFire® FilmArray® pneumonia panel is expected to be available at UF Health starting Summer 2021. The availability and quantity offered may vary based on the supply. In case the panel will not be available for this study, the research team will use the positive respiratory cultures at baseline, using growth on MacConkey agar in the first 24 hours as a proxy for presumptive growth and identification of appropriate culture results for inclusion into the study.

The beta-lactam agent and duration of therapy will be specified by the treating physician. A 1:1 randomization scheme based on the infusion duration (continuous over 24 hours or intermittent over 30 minutes) with stratification based on the beta-lactam prescribed (cefepime, meropenem, or piperacillin/tazobactam) will be developed before starting the trial using a random number generator. Only the PI will have access to the randomization list. On recruitment, the study coordinator will contact the PI, who will assign a unique study identifier in sequential order and provide the team with the randomization arm. These identifiers correspond with entries in the randomization list, which indicates either starting/switching the patient to 24-hour continuous infusion beta-lactam therapy (intervention group) or 30-minute intermittent infusion beta-lactam therapy (control group) as shown in **Table 1**. For continuous infusion regimens, piperacillin and cefepime will be provided by the pharmacy as one 24-hour-dose bag, whereas meropenem provided as two 12-hour-dose bags based on previous experience with its stability (Venugopalan 2018). In case the patient was randomized to an infusion group then the treating physician decided to switch the beta-lactam to another one in this study before completing therapy, the patient will remain in the study and their new beta-lactam will be infused based on the original randomization group the patient was assigned to. If the patient was switched to another agent not included in this study within the first 72 hours, the patient will be withdrawn from this study. If the switch to a different agent was after 72 hours of beta-lactam therapy, the patient will remain in the study and their respiratory samples will be collected for up to 4 weeks to assess the emergence of resistance. Patients in the ICU will have triple lumen IV line so they have a dedicated line for the beta-lactam infusion. Once the patient is transferred out of the ICU and still needs to continue the beta-lactam therapy, a dedicated peripheral line will be inserted if needed.

Table 1: Initial beta-lactam doses per infusion group and CrCl value

Creatinine clearance	Intermittent infusion arm	Continuous infusion arm
Cefepime		
>60	2 g IV every 8 hr	6 g IV daily
30-60	2g IV every 12 hr	4 g IV daily
10-30	2g IV every 24 hr	2 g IV daily
<10	1g IV every 24 hr	1 g IV daily
Meropenem		
>50	2 g IV every 8 hr	6 g IV daily
25-50	2g IV every 12 hr	4 g IV daily
10-25	1g IV every 12 hr	2 g IV daily
<10	1g IV every 24 hr	1 g IV daily
Piperacillin/tazobactam		
>40	4.5 g IV every 6 hr	18 g IV daily
20-40	3.375 g IV every 6 hr	13.5 g IV daily
<20	2.25 g IV every 6 hr	9 g IV daily

Tracheal aspirate (intubated patients) or sputum samples (extubated patients) will be collected twice weekly and sent for culture and susceptibility testing for a maximum of 4 weeks from the initiation of beta-lactam therapy. More cultures may be drawn as per standard of care and clinicians discretion which will be included in the analysis. The MICs for the bacteria will be determined in the onsite UF Health Shands microbiology laboratory. Bacteria will be identified by standard microbiologic methods using VITEK® Mass Spectrometry and Vitek® II (AST GP 72, AST GP 78, AST GN 73, and AST XN06) (bioMérieux, Inc., Durham, NC, USA). MICs will be done by E-test.

As per standard of care, patients will have their beta-lactam plasma drug concentration measured in the first days of therapy, and repeated as needed. The ID/ICU pharmacist or physician will order a peak (1 hour after the end of the infusion) and a trough (before the start of next dose) sample for patients on intermittent infusion. We recommend two random samples, while the patient is on the same infusion bag, to be ordered for those on continuous infusion. A minimum of one sample will be considered acceptable for continuous infusion patients. Beta-lactam plasma concentration will be quantified at the IDPL using validated LCMS-MS assays. The IDPL is a CLIA-licensed clinical laboratory. All assays will be performed using standard operating procedures (SOPs) that have been reviewed and certified by the College of American Pathologists (CAP). The standard curve (calibrators) consists of 6 concentrations of samples. A minimum of three quality control samples are assayed along with the standard curve samples. The calibration curves for meropenem, cefepime, and piperacillin are linear and range from 2 to 100 mcg/mL ($R^2 \geq 0.99$). The

ranges of the coefficient of variation for the plasma meropenem, cefepime, and piperacillin quality control samples are 3.7%–8.6%, 3.3%–9.8%, and 4.6%–6.3% for inter-day precision and the ranges are 1.4%–12.6%, 3.7%–11.0%, and 4.1%–7.3% for intra-day precision, respectively. Meropenem-d_6, cefepime-d_3, and piperacillin-d_5 are used as internal standards. A Thermo Scientific Altis triple quadrupole mass spectrometer (Thermo Fisher, San Jose, CA), coupled with a Vanquish autosampler and pump (Thermo Fisher) will be used to analyze the samples. The fT>MIC will be calculated for each patient using BestDose™ software (LAPKB, University of Southern California) and therapy adjusted based on the clinician discretion (Table 2), while each patient remaining in their randomized infusion group.

Table 2: Suggested therapy adjustment for patients above or below the target

Therapy group	**<100% $fT_{>MIC}$**	**>100% $fT_{>5\times MIC}$**
Intermittent infusion	25-50% increase in frequency of administration	50% decrease in dose or 25-50% decrease in frequency
Continuous infusion	25-50% increase in dose	50% decrease in dose

Data will be collected include patients' age, sex, comorbidities, APACHE II and SOFA scores, vital signs, cultures and susceptibilities, bronchoalveolar lavage culture data and cell count, lab results including complete blood count, basic metabolic panel, C-reactive protein, erythrocyte sedimentation rate, and procalcitonin, antimicrobial regimens, vasopressors requirement, ventilation settings and requirements, and EEG findings if available.

Outcomes

Primary outcome, incidence of Gram-negative bacterial resistance, will be reported under this aim. Definitions were mentioned previously (see *Definitions* under *Objectives*).

Statistical analysis

Descriptive statistics of median and interquartile range or mean and standard deviation will be reported for the continuous variables, whereas counts and percentages will be reported for categorical variables. The primary outcome under this aim will be assessed using ITT analysis, and PP analysis including only those who finished their therapy on the randomized beta-lactam infusion. Multiple regression models will be built

to assess the incidence of resistance while controlling for beta-lactam infusion time, drug exposure (fT>MIC and trough/MIC), APACHE and SOFA scores, mono- vs. polymicrobial infections, administration of other antibacterial agents (fluoroquinolones, aminoglycosides, polymixins), and source of infection. Time-to-event analysis and log-rank test will be used to compare the time to developing new resistance between groups. Data will be analyzed both individually for each beta-lactam and pooled since target exposure for resistance suppression in pre-clinical studies was similar for most beta-lactams (i.e. trough/MIC ≥4) (Sumi 2019). The study team will do their best to avoid missing any datapoint. In case there is missing data in certain outcomes or variables for a patient, that patient will be dropped from that specific analysis and the number of patients included in each analysis will be reported. Inability to provide sputum sample for culture for a patient who is improving clinically with no subsequent positive cultures will be considered as no new resistance developed. JMP Pro v15.0 (SAS) will be used for the statistical analysis.

Assuming the incidence of new resistance will be 20% in the intermittent vs. 5% in the continuous infusion groups, with 80% power and 20% dropout rate, a sample size of 76 (one-sided test) or 96 (two-sided test) per group will be needed. We will aim to enroll a total of 240 patients (120 per group) based on the current rate of admissions and positive respiratory cultures in the medical ICU.

Aim 2. To compare clinical outcomes, including microbiologic eradication, clinical cure, mortality rate, and length of stay, between the two infusion groups

The protocol mentioned previously in Aim 1 will be followed for participant enrollment, treatments, and follow-up evaluations.

Outcomes

Secondary outcomes, including microbiologic eradication, clinical cure, mortality rate, and length of stay, will be reported under this aim. Definitions were mentioned previously (see *Definitions* under *Objectives*).

Statistical analysis

Descriptive statistics of median and interquartile range or mean and standard deviation will be reported for the continuous variables, whereas counts and percentages will be reported for categorical variables. Outcomes under this aim will be assessed using ITT analysis, and PP analysis including only those who finished their therapy on the randomized beta-lactam infusion. Multiple regression models will be built to assess the microbiologic eradication, clinical cure, mortality, length of stay, days on mechanical ventilation, and days on vasopressors while controlling for beta-lactam infusion time, drug exposure (fT>MIC and trough/MIC), APACHE and SOFA scores, mono- vs. polymicrobial infections, administration of other antibacterial agents (fluoroquinolones, aminoglycosides, polymixins), and source of infection. Time-to-event analysis and log-

rank test will be used to compare the time to microbiologic eradication and transfer out of ICU and time on mechanical ventilation and vasopressors between groups. Data will be analyzed both individually for each beta-lactam and pooled while controlling for achieving the optimal PK/PD target for clinical efficacy (i.e. fT>MIC meropenem >40%, cefepime >70%, and piperacillin >50%) (Heffernan 2018). The study team will do their best to avoid missing any datapoint. In case there is missing data in certain outcomes or variables for a patient, that patient will be dropped from that specific analysis and the number of patients included in each analysis will be reported. Inability to provide sputum sample for culture for a patient who is improving clinically with no subsequent positive cultures will be considered as presumed microbiologic eradication. JMP Pro v15.0 (SAS) will be used for the statistical analysis.

Assuming the absolute difference in clinical cure will be 15% between the intermittent and the continuous infusion groups (Dulhunty 2013), with 80% power and 5% level of significance, a total sample size of 60 (two-sided test) will be needed. Aiming to enroll a total of 240 patients (120 per group) for our Aim 1 (detection of new resistance), our study will have enough power to detect a difference in clinical cure.

Aim 3. To compare safety of both infusion strategies

The protocol mentioned previously in Aim 1 will be followed for participant enrollment, treatments, and follow-up evaluations.

Data collection for this aim includes platelets and WBC count, serum creatinine, *Clostridioides difficile* colitis, and EEG reports for neurotoxicity. Neurotoxicity has been defined previously. Patients who will develop neurotoxicity will be extensively reviewed by a neurologist to assess the cause of this event (beta-lactam therapy vs. other factors). Patients with serious adverse events will be assessed regularly for stopping or changing therapy as per clinician discretion and the Safety Officer will review all safety data on a regular basis (see *Data Safety Monitoring Plan*).

Outcomes

Secondary outcome, incidence of adverse events, will be reported under this aim. Definitions were mentioned previously (see *Definitions* under *Objectives*).

Statistical analysis

Descriptive statistics of median and interquartile range or mean and standard deviation will be reported for the continuous variables, whereas counts and percentages will be reported for categorical variables. Outcomes under this aim will be assessed using ITT analysis, and PP analysis including only those who finished their therapy on the randomized beta-lactam infusion. Multiple regression models will be built to assess the incidence of adverse events while controlling for beta-lactam infusion time, drug exposure (fT>MIC, area under the concentration-time curve, trough/MIC), APACHE and

SOFA scores. Time to event analysis and log-rank test will be used to compare the time to developing the adverse event between groups. Data will be analyzed for each beta-lactam individually. The study team will do their best to avoid missing any datapoint. In case there is missing data in certain outcomes or variables for a patient, that patient will be dropped from that specific analysis and the number of patients included in each analysis will be reported. JMP Pro v15.0 (SAS) will be used for the statistical analysis.

Given that no previous clear data on the toxicity associated with different infusions of beta-lactams, the analysis in this aim will be exploratory and hypothesis generating.

Tasks

We are dividing our deliverables into pilot, proof-of-concept, and final groups of patients. As a result, the tasks and subtasks will be the same for all aims as we will be assessing the three aims above (Aim 1 – 3) for every group of patients.

- **Task 1** (Base period): Pilot group (first 60 patients) (timeframe: months 0 to 6):
  - **Subtask 1.** Screening 100 patients using BioFire pneumonia panel:
    - Study coordinator will be available on a daily basis for reviewing admission list and identifying the eligible patients. Dr. Trillo will oversee the admission to the medical ICU and help identify the patients who may be eligible at the time of intubation. If a patient fulfills the inclusion criteria, Dr Trillo or his clinical team (including Drs. Kaseer and Jutba) will ask the patient or their representative if they are interested to enroll in this study. If yes, Dr. Trillo's clinical team will inform the study coordinator who will approach the eligible patient's family or representative for consenting. If informed consent was obtained from an authorized representative, it will be obtained from the patient again once they are able to provide one and still enrolled in the study. Dr. Rand will oversee the respiratory samples coming to the microbiology lab for pneumonia panel screening and assess the quality of the results. The results will be available for the study team and clinicians through the secured EMR.
  - **Subtask 2.** Randomizing 60 patients to continuous or intermittent infusion beta-lactam therapy after confirming the result of the pneumonia panel:
    - The study coordinator will be in touch with the microbiology lab for the final results of the pneumonia panel. Dr. Alshaer will provide the study coordinator with the randomization code and oversee the randomization process. Dr. Venugopalan, along with ID and ICU pharmacists, will oversee the adherence to the randomization protocol in terms of beta-lactam infusion time and order beta-lactam plasma samples to quantify the drug exposure. Dr. Peloquin will oversee the samples received at the IDPL, assess the quality of the

results generated by the assay, and make sure the results are released in a timely manner to EMR. Drs. Trillo, Kwara, and Venugopalan will evaluate the clinical status of the patients on a regular basis and determine the clinical cure and incidence of adverse events with the help of the assigned nurses.

- **Subtask 3.** Obtaining and sending respiratory cultures for randomized patients twice weekly for a total of 4 weeks:
  - Dr. Trillo or designate and Dr. Kwara will order regular respiratory cultures (twice per week) for up to 4 weeks from randomization. Respiratory therapist will obtain the samples and the study coordinator will take them to the microbiology lab. Dr. Rand will oversee the respiratory samples coming to the microbiology lab for Gram-negative bacteria identification and MIC testing, and assess the quality of the results. The results will be available for the study team through the secured EMR.
- **Subtask 4.** Calculating beta-lactam target attainment and drug exposure for randomized patients using plasma concentration and BestDose™:
  - As the beta-lactam plasma concentrations become available, Dr. Alshaer will use BestDose™ to calculate the PK and PK/PD parameters for each patient, using their plasma concentration and dosing info, to be included later in the analysis. BestDose™ will use the entire discrete joint distribution of PK parameters (i.e. support points) generated in a PK model to identify each patient's total drug exposure using Multiple Model Bayesian adaptive control, in which the most probable support point, the one with the best fit, for each patient will be used to generate the full PK exposure. Dr. Alshaer will assess the model fit to the patient's data and evaluate the bias and imprecision of the predictions.
- **Deliverables:** Number of patients screened, distribution of Gram-negative bacteria identified using pneumonia panel, number of patients randomized to each arm and their initial beta-lactams regimens, distribution of Gram-negative bacteria identified using respiratory cultures and their MICs, number of patients for each objective (new resistance [primary], microbiologic eradication, clinical cure, days on mechanical ventilation and vasopressors, days in ICU and hospital, mortality rate, and PK/PD target attainment [secondary]) along with statistical analysis to compare the two infusion groups.

- **Task 2** (Option 1): Proof-of-concept group (second 60 patients) (timeframe: months 6 to 12):
  - **Subtask 1.** Screening the next 100 patients using BioFire pneumonia panel: details above.
  - **Subtask 2.** Consenting and randomizing the next 60 patients to continuous or intermittent infusion beta-lactam therapy after confirming the result of the pneumonia panel: details above.

- **Subtask 3.** Obtaining and sending respiratory cultures for randomized patients twice weekly for a total of 4 weeks: details above.
- **Subtask 4.** Calculating beta-lactam target attainment and drug exposure for randomized patients using plasma concentration and BestDose™: details above.
- **Deliverables:** Number of patients screened from month 6 to 12, distribution of Gram-negative bacteria identified using pneumonia panel, number of patients randomized to each arm and their initial beta-lactams regimens, distribution of Gram-negative bacteria identified using respiratory cultures and their MICs, number of patients for each objective (new resistance [primary], microbiologic eradication, clinical cure, days on mechanical ventilation and vasopressors, days in ICU and hospital, mortality rate, and PK/PD target attainment [secondary]) along with statistical analysis, updated analysis for all patients randomized in the first year of the study (target 120 patients).

- **Task 3** (Option 2): Final group (last 120 patients) (timeframe: months 12 to 24):
  - **Subtask 1.** Screening the next 200 patients using BioFire pneumonia panel: details above.
  - **Subtask 2.** Consenting and randomizing the last 120 patients to continuous or intermittent infusion beta-lactam therapy after confirming the result of the pneumonia panel: details above.
  - **Subtask 3.** Obtaining and sending respiratory cultures for randomized patients twice weekly for a total of 4 weeks: details above.
  - **Subtask 4.** Calculating beta-lactam target attainment and drug exposure for randomized patients using plasma concentration and BestDose™: details above.
  - **Deliverables:** Number of patients screened from month 12 to 24, distribution of Gram-negative bacteria identified using pneumonia panel, number of patients randomized to each arm and their initial beta-lactams regimens, distribution of Gram-negative bacteria identified using respiratory cultures and their MICs, number of patients for each objective (new resistance [primary], microbiologic eradication, clinical cure, days on mechanical ventilation and vasopressors, days in ICU and hospital, mortality rate, and PK/PD target attainment [secondary]) along with statistical analysis, updated analysis for all patients randomized in the whole study period (target 240 patients).

Protection of Human Subjects

This study is considered an ‘Applicable Clinical Trial.’ At University of Florida, all clinical studies will need to be reviewed by the Institutional Review Board (IRB) once funded and before enrollment of participants. As part of the IRB review, the study will be directed to specialized staff for registration at ClinicalTrials.gov registry.

This study will enroll both men and women equally as well as elderly patients fulfilling the inclusion criteria. Given the current practice in the adult medical ICU, use of beta-lactams of interest (meropenem, cefepime, and piperacillin), and the study scope (isolated Gram-negative bacteria from respiratory specimens), pediatrics will not be included in this study.

1. Risk to the subjects

a. Human subject involvement and characteristics

The Statement of Work provides a detailed description of the participation of human subjects in this project, including participant characteristics, numbers, age and inclusion/exclusion criteria. To summarize, this study will enroll 240 patients hospitalized at UF Health Shands in Gainesville, Florida with severe pneumonia who are 18 years or older and on (or will be on) meropenem, cefepime, or piperacillin. Potential subjects will be excluded if they are pregnant, allergic to beta-lactam therapy in the study, on renal replacement therapy, and/or COVID patients enrolled in other trials. The IRB of University of Florida will review the protocol and informed consent, and approve the study before enrollment begins. All subjects or surrogate will provide signed written informed consent. The informed consent will describe the purpose of the study, the procedures to be followed, the risk and benefits of participation. A copy of the consent forms will be given to the subject. Dr. Alshaer is responsible for overseeing the conduct of the clinical trial.

b. Sources of Materials

Potential participants will be screened in the medical ICU to ensure that they meet the inclusion criteria. As part of the screening process, eligible patients will have their respiratory samples sent to the microbiology lab for bacterial cultures and MIC determination at the time of intubation as per standard of care. To limit the study-related invasive procedures, the same sample will be used for rapid identification of bacteria. Screening evaluation will be recorded on electronic sheet and will be stored on the secured server of the University of Florida, which is only accessible to staff involved in this study.

Once the participant is enrolled and the study has started, regular respiratory samples (from regular suctioning in intubated patients [usual care practice] or sputum samples in extubated patients) will be obtained for research purposes. Respiratory samples will be handled like other clinical samples within the closed hospital system. Blood samples for drug concentrations will be drawn as per standard of care and sent to the IDPL for quantification within 24 hours on weekdays.

Routinely collected antibiotic side effects data will be extracted and entered into the research database for the purposes of research analysis. Drug concentration data will be made available to the clinicians for routine care. All patients' data including laboratory specimens, evaluation forms, reports and other records extracted from EPIC will be assigned a coded number to maintain subject confidentiality; only research staff will be able to identify the subject from the code. All records will be kept in a secured server at University of Florida.

c. Potential Risks

All the study medications are approved for clinical use and prescribed by the treating physicians of participants. Both the continuous and intermittent beta-lactam infusion regimens are administered on daily basis to the medical ICU patients. As a result, both the clinicians and study team have long experience with these regimens. Antibiotic dose adjustments will be based on recommendations by ID and ICU pharmacists and physicians as per usual care. Close clinical and laboratory monitoring is routinely done for hospitalized patients. The patient-to-nurse ratio is 1:1 to 2:1 in the medical ICU, and 4:1 to 5:1 in the floors. The patients will be under continuous observation by their clinical nurse during the study period. In the event of a serious medical emergency related to the study, treatment will be provided. Adverse events related to the beta-lactam infusion or respiratory sampling for research are reported to the appropriate University of Florida IRB according to existing regulations.

2. Adequacy of protection against risks

a. Recruitment and Informed Consent

All protocols, consent forms, and recruitment materials and procedures will be reviewed and approved by University of Florida IRB. Since participants will be intubated and sedated, a patient's representative will receive a complete description of the study procedures, including risks and benefits, from one of the study investigators or a designate. Informed consent also includes alternatives and principal investigator's phone numbers. When the explanation is completed and subjects' representatives indicate that all of their questions have been answered, they will sign the consent document, with an understanding that participation is voluntary. There are no waivers or modifications of the consent process. Subjects (or their representatives) will receive a copy of the consent form, and details of medical records or other patient data will be kept confidential except as required by law. If informed consent was provided initially by an authorized representative, the study coordinator will

obtain another informed consent from the patient once they are able to provide one and still enrolled in the study.

b. Protection Against Risk

The treating physicians will take responsibility for medical coverage, and for assuring the safety of study participants. All medications are given with ongoing monitoring and supervision by nurses and treating physicians. Medical coverage, including resuscitation coverage, is available at all times, and a physician is responsible for overseeing patient care. Should participants or representative have study related questions or problems that arise during the course of the study, it will be addressed by the research team in person or by phone. The numbers are listed on each consent form, a copy of which is provided to each subject.

Every effort is made to protect the confidentiality of study-related data. All paper documents are stored under lock and key in a secure area, while electronic data kept on a secured server only accessible to the study team. All patients data are coded, with only the PI and designated research associates having access to the code.

3. Potential benefits of the proposed research to the subjects and others

There are no immediate direct benefits to individual subjects. However, the risks are considered acceptable based on the known adverse event profile associated with respiratory samples through suctioning or sputum samples. Also, patients in both groups will receive the same beta-lactam total daily dose based on their renal function initially and will have their beta-lactam drug concentration measured to make sure they are not having very high or toxic concentration. On the other hand, there may be substantial benefits to the society in general in further optimizing beta-lactam regimens to prevent the emergence of resistance in Gram-negative bacterial pneumonia.

4. Importance of the knowledge to be gained from this work

Gram-negative bacterial resistance represents a major worldwide problem. Robust clinical trials are needed to prove the efficacy of different beta-lactam dosing strategies against the emergence of resistance. Meropenem, cefepime, and piperacillin are used very often in the ICUs but there is currently limited data to support the suppression of new resistance with certain infusion strategy. This study helps answering this question in patients with severe pneumonia, the most common infection in the ICUs, coupled with measuring the drug exposure. Thus, the proposed work would provide applicable data that could inform the future recommendation for beta-lactams dosing.

5. Data and Safety Monitoring Plan

Typically, a data safety monitoring board would regularly review the safety of study and the data generated. Given that it is a relatively small, single-center, 2-year study of fairly safe approved antibiotics at clinically used dosages, no experimental dosages or drugs administered, and the only research component is the administration modality (i.e. continuous versus intermittent), we propose an independent Safety Officer instead. The independent Safety Officer will be from a different clinical team, as free of conflict of interest as possible, and have experience with clinical research and clinical expertise relevant to this study (Dr. Bethany Shoulders, Clinical Assistant Professor, UF and ICU Pharmacy Specialist, Surgical ICU, UF Health). A Safety Officer charter will be developed to define the role of the Safety Officer, describe the reviews and meetings, define the data to be reviewed, and outline the content of the Safety Officer reports. The study investigators will meet with the Safety Officer through conference calls and present progress, interim analyses, and safety data to them every 6 months and as needed to ensure that the study continues to be safe for participants. The interim analyses will be performed after enrolling the first 60 patients (6 months from starting the study), second 60 patients (12 months from starting the study), and last 120 patients (24 months from starting the study). Should there be any unusual adverse outcomes or breaches in confidentiality, this will be reported immediately to the responsible IRB and the Safety Officer for the necessary corrective actions. Given that the amount of risk in this study might be minimal, there is no specific safety stopping criteria for this study and the Safety Officer will evaluate the data as they become available. The Safety Officer will check the data for any adverse events trends along with the statistical analysis for comparison. The same procedure will be followed for efficacy endpoint and the Safety Officer will review the data with the statistical analysis.

Conflict of Interest

The investigators have no conflict of interest to disclose.