Primary Statistical Analysis Plan

Version 1.0

Multi-Drug Resistant Organism Network (MDRO Network)

SNAP: Study Network of Acinetobacter baumannii as Carbapenem-Resistant Pathogen

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1 Introduction

1.1 Purpose

This Statistical Analysis Plan (SAP) describes the planned and ad hoc analyses for Study Network of *Acinetobacter baumannii* as Carbapenem-Resistant Pathogen (SNAP), part of the Multi-Drug Resistant Organism Network (MDRO), an observational study of Carbapenem-Resistant *Acinetobacter baumannii* (CRAB). This SAP outlines the general statistical approaches that will be used in the analysis of the study. It has been developed to facilitate discussion of the statistical analysis components among the study team, and to provide agreement between the study team and statisticians regarding the statistical analyses to be performed and presented in the statistical analysis report. Post hoc discussions are included in this plan.

Analyses for the Primary Analysis Report will be initiated once the CRF database has been frozen for analysis, central lab antibiotic susceptibility testing (C-AST) results have been received, and whole genome sequencing (WGS) results have been received.

1.2 Key updates to SAP

Version	Changes Made	Rationale	Effective Date
1.0	Original Version		

2 Protocol Overview

2.1 Study Design

This study is specifically designed to provide data which can help in the design of future randomized clinical trials on both therapeutics and diagnostics, and describe, on the basis of observational data, patients with CRAB infections in hospitalized patients. These data will include a detailed clinical and epidemiological description of patients including potential barriers to enrollment in future trials. In addition, data will be collected on species, strain type, and mechanism of drug resistance of the causative organism.

A cohort will be constructed for CRAB eligible patients by investigating hospitalized patients who have CRAB isolated in clinical cultures from any anatomical site. This study will be conducted under a waiver of informed consent to facilitate universal inclusion. All patients who are admitted to one of the participating hospitals and who have CRAB – as defined in the specific sub-study protocol (Appendix) – isolated in a clinical culture are targeted for inclusion.

2.2 *A priori* and *Post Hoc* Analyses

The following comparisons were defined *a priori*:

- Geographic region
- Geographic region, limited to infections
- Infection vs colonization (non-infection)
- Presence of a carbapenmase
- Presence of a carbapenmase, limited to infections
- Modeling

  - DOOR probability
  - Inverse probability weighted (IPW) DOOR probability

The following comparisons were *post-hoc*:

- Central Meropenem MIC levels
- Central Meropenem MIC levels, limited to infections
- Clonal Group 2 vs other clonal groups
- Clonal Group 2 vs other clonal groups, limited to infections
- Modeling
  - Logistic regression model with site as random effect

2.3 Hypotheses

CRAB infection is associated with significant morbidity and mortality, including in comparison with CRAB colonization (non-infection). Specific patient characteristics and clinical outcomes, and molecular and microbiological of the strains, will differ across regions, information which will aid in the design of randomized clinical trials on therapeutics and diagnostics for CRAB infections.

2.4 Study Objectives

The objective of this study is to provide observational data that will aid in the design of randomized clinical trials on therapeutics and diagnostics for CRAB infections. To this end, clinical and epidemiological data will be collected on patients who have CRAB isolated from clinically indicated cultures during hospitalization as well as descriptions of the outcomes of patients treated with various antimicrobial regimens. Molecular and microbiological characterization will also be performed on CRAB isolates.

2.4.1 Primary Objectives

- To determine 30 day all-cause mortality of CRAB infections

2.4.2 Secondary Objectives

- To determine 30-day Desirability of outcome Ranking (DOOR) of CRAB infections
- To determine 90-day all-cause mortality of CRAB infections
- To elucidate antimicrobial susceptibility and molecular epidemiology of the index CRAB strains

2.5 Inclusion criteria

Inclusion criteria for the MDRO protocol was all hospitalized patients, including pediatric patients, who have at least one MDRO (CRAB for this analysis) isolated from a clinical culture will be eligible for inclusion.

Additional inclusion criteria for this analysis:

- Meets study carbapenem resistance requirement (resistant to at least one carbapenem, other than ertapenem, per susceptibility testing at the local lab)
- Outcome information available for 30 days post index culture

Subjects will be excluded from the analysis for the following reasons:

- WGS sequencing result not useable
- WGS did not detect acinetobacter baumanii
- C-AST not resistant to meropenem

3 Statistical Methods

3.1 General considerations

- Global tests of statistically significant differences across any of the regions are provided. For pairwise comparisons between regions, USA will be the reference group.
- Tables are summarized by region, infection/colonization, meropenem MIC levels, presence of carbapenemase, and Clonal Group 2 vs other clonal groups.
- Categorical variables are tested using the chi-square test. Continuous variables and ordered categorical variables are compared using the Kruskal Wallis test.
- The Miettinen-Nurminen (score) method is used to calculate the confidence interval (CI) of the difference in proportions.
    - Please note that the only censoring is administrative censoring at 30 days. As this is an observational study with information collected from the medical record, unless subjects are known to have died, they are considered to be alive.
- Modeling will be limited to subjects with an infection.
- DOOR probabilities are calculated using Halperin CI.
- IPW adjustment for DOOR probability and difference in proportions will be utilized.
- Logistic regression is performed with study site as random effect.
- Analyses will be summarized for all subjects and repeated for the subset of subjects with an infection.
- Throughout this report we consider $p< 0.05$ with a two-sided test to be statistically significant.

3.2 Outcome measure definitions

Carbapenem susceptibility: a culture is included if it has a corresponding susceptibility form with documented resistance at the local lab to at least one carbapenem (meropenem, imipenem, doripenem).

Infection vs. colonization (non-infection): only cultures that meet the study surveillance definition of infected are counted as infected. For CRAB, respiratory isolates are considered to be infected if the respiratory diagnosis on the CRF was tracheobronchitis, pneumonia, no mechanical ventilation, ventilator-associated pneumonia, or an “other” diagnosis identified by 2 study investigators (Satlin and Doi). All other cultures, including cultures missing information needed for the assignment of infection/colonization (non-infection), are considered to be colonized (not infected).

30-day mortality: an admission is considered alive at 30 days if they are not recorded as dead. If an admission is discharged alive and there is no other information on the 90-day form or if they have a discharge date but no disposition, then they are assumed to be alive.

90-day mortality: an admission is considered alive at 90 days if they are not recorded as dead. If an admission is discharged alive and there is no other information on the 90-day form or if they have a discharge date but no disposition, then they are assumed to be alive.

DOOR: The DOOR variable reported in MDRO studies is an ordered categorical variable measuring status at 30 days following the culture collection date. The categories are:

- Clinical Response at 30 days, with no events
    - Symptomatic response, no anti-CRAB antibiotic, no relapse
- 1 event
- 2 or 3 events
- Death

The possible events are:

- No clinical response at 30 days
- "Bad" discharge within 30 days or readmitted within 30 days
- Renal failure post-culture or *C. difficile* infection.

4 Data sources

- CRF data, frozen on from the June 27, 2020 transfer.
- Meropenem AST data from the central lab transferred via Box on November 4, 2021, updated on May 13, 2022.
- WGS data from Meridian Hackensack transferred on December 2, 2021.
- AST panel from China was data 31 March 2022.
- AST panel from US central lab was dated 07 April 2022.

5 Report Components

- Inclusion/Exclusion reasons
- Enrollment by region
- Enrollment by region/site

The following tables will be summarized with the following sets of column variables:

- Geographic region
- Geographic region, limited to infections
- Infection vs colonization (non-infection)
- Presence of a carbapenmase
- Presence of a carbapenmase, limited to infections
- Central Meropenem MIC levels
- Central Meropenem MIC levels, limited to infections
- Clonal Group 2 vs other clonal groups
- Clonal Group 2 vs other clonal groups, limited to infections

5.1 Tables

Variables to be summarized:

- Baseline and Admission characteristics
    - Gender
    - Age (continuous and categorical)
    - Race
    - Ethnicity
    - Origin location (all origins + hove vs all others)

  - ICU admission prior to first positive culture
  - Charlson comorbidity index
  - Comorbidities
    - Diabetes
    - Coronary artery disease
    - Congestive heart failure
    - Heart disease
    - Peripheral vascular disease
    - Cerebrovascular disease
    - Dementia
    - Connective disuse disease
    - Peptic ulcer disease
    - Hemiplegia
    - Chronic kidney disease
    - Severe chronic kidney disease
    - Any chronic renal replacement therapy
    - Renal dysfunction
    - Liver disease
    - Cirrhosis
    - Cirrhosis and history of variceal bleeding
    - Cirrhosis and portal hypertension
    - Hepatitis B infection
    - Hepatitis C infection
    - Chronic obstructive pulmonary disease
    - History of malignancy
    - Immunocompromised
    - Solid organ transplant
    - Recent or current chemotherapy
    - Stem cell transplant
    - TNF-alpha blocker in the last month
    - HIV with AIDS diagnosis
    - Other monoclonal antibody in last month
    - Prednisone 10 mg/day or equivalent
- Culture information
  - WGS species
  - CRF species
  - Variables from CRFs
    - Infection/colonization
    - Culture anatomic source
    - Infection/colonization by source
    - Days from admission to culture (continuous)
    - ≥ 2 days from admission to culture
    - Healthcare associated/non-hospital acquired (CRACKLE II analysis definition)
    - Pitt score (continuous)
    - Pitt score < 4/≥ 4
    - Patient location at first positive culture
    - Did other pathogen from the same source grow? (polymicrobial)
  - Meropenem MIC via central lab

  - Molecular results
    - Carbapenemase present (yes/no)
    - Specific carbapenemase present
    - Clonal group
    - Capsular polysaccharide locus (KL)
    - Lipooligosaccharide outer core locus (OCL)

  - Central AST results (limited to those run by both labs China/US)
    - Amikacin
    - Cefepime
    - Ceftazidime
    - Ceftriaxone
    - Ciprofloxacin
    - Imipenem
    - Levofloxacin
    - Meropenem
    - Piperacillin tazobactam
    - Polymixin B/Colistin
  - CRF Susceptibility results (S/I/R and S/NS)
    - Amikacin
    - Ampicillin-sulbactam
    - Cefepime
    - Ceftazidime
    - Ceftriaxone
    - Ciprofloxacin
    - Colistin
    - Doripenem
    - Fosfomycin
    - Gentamicin
    - Imipenem
    - Levofloxacin
    - Meropenem
    - Piperacillin-tazobactam
    - Polymyxin B
    - Tobramycin
    - Trimethoprim-sulfamethoxazole
- Outcomes
  - Days from culture to discharge/death
  - Days from admission to discharge/death
  - Disposition after discharge
  - 30 day all-cause mortality
  - 90 day all-cause mortality
  - 30 day DOOR outcomes
  - DOOR outcome components
    - Unsuccessful discharge
    - No clinical response
    - Renal failure or *C. difficile*
  - 30 day clinical response

5.2 Outcome analyses

The difference in proportion in 30- and 90 day mortality will be summarized with confidence intervals. The IPW difference in proportion, with 95% CI, will also be summarized. Pairwise DOOR probabilities and 95% CI will be estimated using Halperin's method. Both an unadjusted and IPW adjusted DOOR probability with 95% CI will be estimated. IPW models for infection vs. non-infection comparisons included:

- Geographic region
- Immunocompromising conditions
- Preadmission location (home origin vs other)
- Age-adjusted Charlson comorbidity index

IPW models for regional comparisons (infections only) included:

- Anatomical source of infection
- Immunocompromising conditions
- Preadmission location (home origin vs other)
- Age-adjusted Charlson comorbidity index

Post hoc, a logistic regression model was used to explore risk factors for mortality. Variables included in the analysis were:

- Geographic region (USA as reference)
- Anatomical source of infection (blood as reference)
- Aged-adjusted Charlson comorbidity index
- Immunocompromising conditions
- Pre-admission location
- Monomicrobial vs polymicrobial culture
- Acquired carbapenemase gene
- Clonal group (CG2 as reference)
- Capsular polysaccharide locus (KL)
- Lipooligosaccharide outer core locus (OCL)
- Site (random effect)