PROCALBAN

STATISTICAL ANALYSIS PLAN

Use of procalcitonin point-of-care test to guide de-escalation of empiric antibiotic therapy in adult patients with sepsis in a tertiary hospital in Bangladesh (PROCALBAN): a randomised, controlled, open-label trial

Use of procalcitonin, a blood test to guide antibiotic therapy for sepsis in adults

ACRONYM: PROCALBAN study

Version 1.0
Date 15th October 2024

WRITTEN BY:

FORHAD UDDIN HASAN CHOWDHURY

DATE: 15 OCTOBER 2024

PRINT NAME

DPHIL STUDENT/PROJECT COORDINATOR/CO-PRINCIPAL INVESTIGATOR

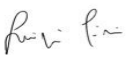
SUPERVISED AND APPROVED BY:

MAVUTO MUKAKA

DATE: 15 OCTOBER 2024

PRINT NAME

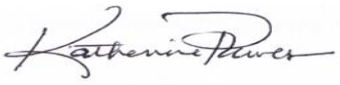
TRIAL STATISTICIAN

LUIGI PASANI 

DATE: 15 OCTOBER 2024

PRINT NAME

INVESTIGATOR

KATHERINE PLEWES 

DATE: 15 OCTOBER 2024

PRINT NAME

INVESTIGATOR

ARJEN DONDORP 

DATE: 15 OCTOBER 2024

PRINT NAME

PRINCIPAL INVESTIGATOR

TABLE OF CONTENTS

1. Analysis Considerations

1.1 General Analysis Approach

The main analysis strategy for the primary outcome will be the intention-to-treat (ITT) principle. In this analysis, patients will be analysed according to the arm of randomisation, irrespective of the treatment that was actually given. These ITT analyses will be followed by the per protocol (PP) analysis. In this analysis, only those that adhered to the protocol with respect to the primary outcome will be included for analysis of the primary outcome. All participants will be included in the Intention to Treat (ITT) analysis to maintain the balance achieved through randomisation. For the Per Protocol Analysis (PPA), patients who meet exclusion criteria before hospital discharge or who receive an alternative non-infectious diagnosis during the trial will be exlcuded.

Key secondary endpoints such as Days of Therapy (DOT), safety and tolerability data will be analysed similar to the ITT approach for the main primary outcome. In this analysis, patients will be analysed according to the arm of randomisation irrespective of the treatment that was actually given, and all patients will be included in the analyses irrespective of their follow-up status, provided data collection is complete. Withdrawals and losses to follow up will not affect the analyses of this data as long as the relevant data needed for these analyses is available prior to withdrawal or loss to follow up.

Data analysis will be performed using Stata 18 or higher, StataCorp, 4905 Lakeway Drive College Station, Texas 77845 USA or in R software.

1.2 Data cleaning and verification

All data will be cleaned and verified prior to statistical analysis. The study site is visited by the Study Monitor periodically at time points agreed on with the Investigators. At the time of each monitoring visit, the Study Monitor will review the completed CRFs to ascertain that all items have been completed and that the data provided are accurate and obtained in the manner specified in the protocol. The Study Monitor will also check that the data in the CRF are consistent with the clinical records (Source Data Verification [SDV]) and that study results are recorded completely and correctly. The Data Manager will ensure that clean data is submitted to the statistician for analysis. The statistician will cross-check that the available data for analysis is clean. Any data cleaning queries will need to be resolved before statistical analyses.

1.3 Locking the dataset

After data cleaning and responding to all data queries, the clean data will be locked in the database that was used for data capturing. The data may also be locked and stored in other user-friendly formats such as MS Excel and Stata. The locked data will be stored at an identifiable secure place and should be available to the relevant researchers upon request following proper request procedures.

1.4 Data format and Analysis logs

Prior to dispensing data to the Trial Statistician, the head of Data Management will make sure that the data to be sent to the Trial Statistician is clean. This will help the statistician to provide the analysis results in a timely manner as there will be a reduced number of queries if clean data is provided. Data will be given to the Trial Statistician by the head of Data Management (or designated person) in a format that is compatible with statistical software reading. Statistical analyses will be performed in Stata, version 18 or higher or R software. Statistical programs and output logs will be kept for all analysis and made available upon request.

1.5 Interim analyses

Two interim analyses on clinical /laboratory data are conducted to assess the safety of procalcitonin guided de-escalation of antibiotic therapy. The interim reports will be reviewed by the DSMB. The interim analyses are performed after the first 40 patients, first 250 patients and at additional time-points before the planned interim analyses, as indicated by the DSMB after their review, if deemed necessary. After this, safety analysis will be performed based on recommendations by the DSMB. No stopping rules, either statistical or clinical will be specified. The need to stop the trial will be based on the evaluation of the accumulating data by the DSMB. DSMB evaluation mainly focuses on patient safety data, but efficacy data will also be considered.

The trial Statistician in collaboration with Trial Coordinator produces the report for the DSMB. Only relevant data included in a specific interim report are made available to the DSMB members at the time of sending the report. During a DSMB meeting, the report is presented to the DSMB members by the Trial Coordinator in line with the meeting agenda. In preparation of the scheduled DSMB meetings, the interim report is sent out to the members at least a week before the meeting.

2. Introduction and study summary

Antimicrobial resistance (AMR) is a global problem, resulting in increased morbidity and mortality from multidrug resistant bacterial infections that are challenging to treat. Low- and middle-income countries (LMICs), like Bangladesh, are disproportionally affected by AMR. Excessive use of antibiotics both inside and outside the hospital is thought to be an important driver of AMR in Bangladesh and other LIMCs. Several studies have shown that sequential assessment of serum procalcitonin (PCT) concentrations is an effective and safe strategy to shorten the duration of antibiotic therapy in critically ill patients. In this strategy, a decrease of procalcitonin below a certain threshold prompts the clinician to stop or reduce antibiotic treatment. However, these studies were conducted in high resource settings with standard laboratory PCT testing at considerable costs. We propose to evaluate the use of sequential serum PCT measurement to guide de-escalation of antibiotic therapy in a randomised clinical trial in patients with sepsis in a large tertiary referral hospital in Chattogram, Bangladesh, using low-cost point-of-care quantitative procalcitonin measurements. Main endpoint of the trial will be the difference in antibiotic therapy duration between the study groups. If this study shows that PCT guided de-escalation of antibiotics results in a safe reduction in antibiotic usage, this will be an important strategy to reduce the antibiotic burden in hospitals in Bangladesh and comparable resource-limited settings, as well as other consequences of prolonged antibiotic therapy, including increased costs, prolonged duration of intravenous lines and duration of stay, and microbiota changes.

2.1 Study objectives and endpoints

2.1.1 Primary objective

To assess the **efficacy** of antibiotic de-escalation rules based on daily procalcitonin measurements to reduce the length of antibiotic treatment in hospitalized patients with sepsis in a low-resourced setting.

2.1.2 Primary endpoint

Length of antibiotic treatment: number of days of antibiotic treatment during the study period (till 28 days after hospital discharge). The primary outcome data for participants with recurrent infections will be censored at the time of recurrent infection diagnosis.

2.1.3 Secondary objectives and endpoints

- To assess if procalcitonin guided de-escalation reduces antibiotic **consumption** and days of therapy with antibiotics.
    - Consumption of antibiotics expressed as the Defined Daily Dosage (DDD)

      Definition of DDD: The basic definition for a defined daily dose is the assumed average maintenance dose per day for a drug used in its main indication in adults. Antibiotic use using the WHO DDD definition (https://www.whocc.no/atc_ddd_index/) will be determined for the most frequently prescribed Drugs in Bangladesh

    - Days of therapy with antibiotics (DOT)

      Definition of DOT: One DOT represents the administration of a single antibiotic on a given day regardless of the number of doses administered or dosage strength. If a patient receives more than one antibiotic it will be added on with DOT of the first antibiotic. (Dalton et al. 2015)

    - Number of days of parenteral antibiotic during hospitalisation period
    - Number of days of antibiotic treatment during the hospitalisation period.

- To assess the safety of procalcitonin guided de-escalation of treatment
    - Overall mortality, mortality-associated with recurrent infection, and non-lethal recurrent infections.

- To assess the effect of procalcitonin guided de-escalation on length of hospital stay
    - Duration of hospital stay (ICU/ general ward)

- To determine the spectrum of antimicrobial resistance among bacteria identified in clinical specimens from Bangladeshi patients hospitalized with sepsis

    - Bacterial isolates from blood cultures will be identified, tested for antibiotic susceptibility. In a subset of antibiotic resistant isolates, genetic correlates of resistance will be assessed (A comprehensive microbiological analysis plan is provided in section 3.6).

- To compare the patient care costs between study arms
    - Direct medical costs of prescribed antibiotics, the PCT assay and hospital stay costs.

2.2 Study design

Brief Description

Trial design

Randomised controlled, non-blinded, two-arm, parallel, clinical trial comparing Standard practice to procalcitonin guided de-escalation of antibiotic therapy.

Trial settings

Trial site is Chattogram Medical College Hospital (CMCH), Bangladesh. CMCH is a tertiary care hospital with undergraduate and postgraduate teaching facilities. This tertiary hospital receives referrals from urban and rural areas of southern Bangladesh and has basic facilities for intensive care and haemodialysis.

Randomisation and blinding

Consecutive patients with suspected sepsis admitted at CHMCH are eligible for enrolment, provided informed consent is obtained. After eligibility is confirmed, the patient is randomised to either the experimental (procalcitonin) arm or control (standard therapy) arm. Randomisation and treatment allocation is determined by a central computerized binary sequence number generator in a 1:1 ratio with pre-determined block sizes by the study statistician. The study statistician designs the randomisation schedule. The subject's randomisation number is on the sealed opaque envelope as well as on and insert inside the envelope. The trial staff opens the envelopes sequentially for enrolled trial participants and allocates the patient to the treatment arm that is denoted on the insert of that envelope. The allocated arm is noted on the eCRF and other applicable documents. The medical staff and patients are not blinded to intervention randomisation.

2.3 Determination of sample size

The sample size calculation is based on the expected difference in mean length of antibiotic treatment between the experimental and control arm. Based on retrospectively collected data from CMCH, the mean length of therapy (LOT) in sepsis patients was 5.2 days with a standard deviation (SD) of 8 days. The SD of the duration of antibiotic prescription in population level was derived by dividing the difference between the maximum and minimum value of LOT that was obtained from the retrospective data by four as one of the rules of thumb. The SD is assumed to be the same in the experimental and the control arms. We hypothesize to have a higher mean number of days of LOT in the control group as compared to the experimental group – i.e 5.2 days in the control group vs. 3.2 days in the experimental group, for an absolute reduction of mean LOT of 2 days. The level of significance was set at 5% (i.e., alpha=0.05). Based on these assumptions and estimating with 80% power, a total of 506 participants are required (i.e., 253 in the experimental arm and 253 in the control arm) to verify the study hypothesis. We anticipate a 5% loss to follow-up and to compensate for this, a total of 532 participants will be enrolled (266 in the experimental arm and 266 in the control arm). The sample size calculations have been performed in R software (version 4.2.1).

3. Data Analysis

3.1 Trial Profile

The number of patients screened, reasons for non-enrolment, number of patients randomised, number of patients lost to follow-up and the number of patients assessed for final endpoint are summarised in a CONSORT flow diagram, as shown in Figure 1.

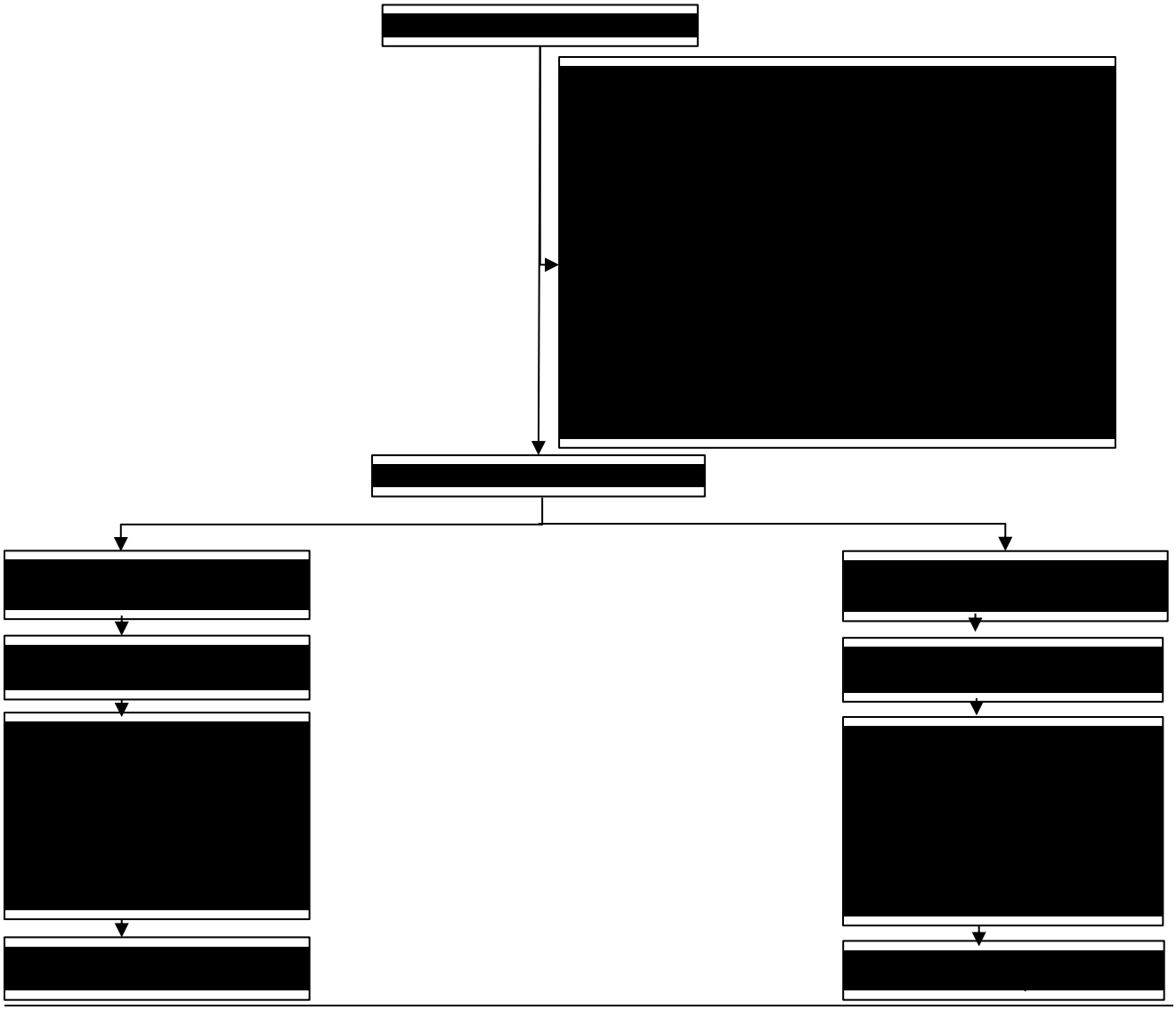


Figure 1: Consort Trial Profile by Arms

3.2 Demographics and other baseline characteristics

The following baseline characteristics are described, according to study arm as shown in Table 1. Continuous variables will be summarised using mean and standard deviation or median (IQR) as appropriate. Categorical variables such as sex, marital status, education, employment status, residences and infection status at baseline will be summarised using frequencies and percentages.

Table 1. Baseline characteristics for the participants by study group

Characteristics	Procalcitonin (N=XX)	Control (N=XX)	Total (N=XX)
Age (years), median (IQR)	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)
Female, n (%)	XX (XX)	XX (XX)	XX (XX)
Weight (Kg), median (IQR)	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)
Height (cm), median (IQR)	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)
BMI (kg/m^2), median (IQR)	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)
Marital Status, n (%)			
Married	XX (XX)	XX (XX)	XX (XX)
Single	XX (XX)	XX (XX)	XX (XX)
Widowed	XX (XX)	XX (XX)	XX (XX)
Divorced	XX (XX)	XX (XX)	XX (XX)
Highest education, n (%)			
No formal education	XX (XX)	XX (XX)	XX (XX)
Primary education	XX (XX)	XX (XX)	XX (XX)
High School	XX (XX)	XX (XX)	XX (XX)
College education	XX (XX)	XX (XX)	XX (XX)
Graduate or post-graduate	XX (XX)	XX (XX)	XX (XX)
Employment status, n (%)			
Government service	XX (XX)	XX (XX)	XX (XX)
Private sector	XX (XX)	XX (XX)	XX (XX)
Casual worker (pay by the day)	XX (XX)	XX (XX)	XX (XX)
Unemployed	XX (XX)	XX (XX)	XX (XX)
Residences, n (%)			
Urban	XX (XX)	XX (XX)	XX (XX)
Rural	XX (XX)	XX (XX)	XX (XX)
Temperature (ºC), median (IQR)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)
Respiratory rate (breaths/min), median (IQR)	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)
Heart rate (beats/min), median (IQR)	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)
Systolic blood pressure (mmHg), median (IQR)	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)
Diastolic blood pressure (mmHg), median (IQR)	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)
SPO2	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)
WBC (Total/Cmm), median (IQR)	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)

Neutrophil (%), median (IQR)	XX (XX - XX)	XX (XX - XX)	XX (XX - XX)
Infection status at enrolment, n (%)			
Suspected bacterial infection	XX (XX)	XX (XX)	XX (XX)
XXX	XX (XX)	XX (XX)	XX (XX)

3.3 Analysis of primary outcome (length of antibiotic therapy), number of mortalities, pattern of major organ dysfunction, and recurrent infections

Summary of intention to treat (ITT) and per protocol analysis (PP) Inclusion and Exclusion Criteria

A. Included in ITT but Excluded in PP

a. Participants who meet exclusion criteria/alternative non-infection diagnosis during the study:

- Outcome will be censored after the duration of empiric antibiotic therapy until the date patient receives a definitive diagnosis.

b. Patients transferred to other hospitals:

- Last observed outcome before transfer to the other hospital will be used

c. Patients who left against medical advice:

- Last observed outcome before leaving against medical advice will be used

d. Patients without complete primary outcome data:

- Patients that do not have complete outcome data are included in the table with baseline data.

e. Patients who die early (before the intervention started):

f. Patients without 28-day follow-up data:

- Outcome data will be censored at the last day of follow-up

g. Patients who received prophylactic antibiotics:

- Outcome data will be censored at the day of start of prophylactic antibiotics

B. Included in Both ITT and PP

a. Patients for whom the non-binding advice to stop antibiotics was not followed:

- Provided the patient does not meet criteria for an alternative diagnosis

b. Patients presenting with recurrent infections:

- If it is a recurrent infection the outcome will be censored at the time of recurrent infection. The amount of antibiotic a patient receives after enrollment in the study, up until the occurrence of a recurrent infection episode, will be included in the analysis

c. Patients discharged before procalcitonin reached the predifined target value for antibiotic de-escalation

d. Patients who restarted antibiotics without having a recurrent infection:

- Included in the analysis, except if antibiotics were started after discharge from CMCH for other reasons than a recurrent infection.

C. Pre-defined Subgroups:

1. **By source of infection:**
   There will be two subgroup analyses:

- The first will be based on the initial diagnosis made by the study team.
- The second will be based on the updated final diagnosis, with the same categorisation structure as used in the initial diagnosis for the source of infection.

Categories of diagnostic subgroups:

Urinary Tract Infection (UTI):
This category will encompass all infections involving the urinary tract, including pyelonephritis, and other related infections.

Skin and Soft Tissue Infection:
Any infection primarily affecting the skin, subcutaneous tissues, or muscles will be classified under this category. This includes cellulitis, abscesses, and other similar conditions.

Undifferentiated Sepsis:
In cases where sepsis is present without a clearly identifiable source, the diagnosis will be labeled as "Undifferentiated Sepsis."

Enteric Fever:
Both suspected and microbiologically confirmed cases of enteric fever will be grouped under this diagnosis. This ensures uniformity whether or not laboratory confirmation has been obtained.

Lower Respiratory Tract Infection (LRTI):
All infections involving the lower respiratory tract, including pneumonia, exacerbations of chronic obstructive pulmonary disease (COPD), bronchiectasis, and interstitial lung disease, will be categorized as LRTI.

Upper Respiratory Tract Infection (URTI):
Infections of the upper respiratory tract, such as acute tonsillitis, and similar conditions, will fall under this label.

Meningoencephalitis:
This diagnosis will be used for infections involving the central nervous system, specifically those affecting the meninges and/or the brain, such as meningitis and encephalitis.

Intra-abdominal Infection:
This category includes infections within the abdominal cavity, such as acute gastroenteritis, cholangitis, and other intra-abdominal infectious processes.

Other diagnostic subgroups (viral infections, cancer, tuberculosis, connective tissue disease)

2. **By presence of bacterial culture positivity:**
    - Culture-positive sepsis
    - Culture-negative sepsis
    - **Note:** in this subgroup analysis we will exclude infections that require standardised prolonged therapy (e.g., Burkholderia).

3. **By antibiotic resistance:**
    - Patients with antibiotic-resistant infections (based on antimicrobial susceptibility testing results of the admission specimen)
    - Patients with antibiotic-susceptible infections (based on antimicrobial susceptibility testing of initial specimen)

4. **By admission severity:**
    - Subgroup based on severity at enrolment, using either:
        - Based on NEWS score: Aggregate NEWS score 5–6 (medium clinical risk and ≥7 (high clinical risk)
        - Based on SOFA score: **0-5**: Low risk, mild organ dysfunction, **6-9**: Moderate risk, moderate organ dysfunction, **≥10**: High risk, severe organ dysfunction.

5. **By presence of co-morbidities:**
    - Participants with co-morbidities such as Diabetes Mellitus (DM), Chronic Kidney Diseases and other chronic diseases)
    - Participants without co-morbidities

Data will be represented as summarised in **Table 2**.

We will summarise LOT, DOT, DDD using mean (SD) or median IQR depending on the distribution. If it is skewed the range may also be reported in addition to the IQR.

Table 2. Summary of length of antibiotic therapy, length of hospital stay, number of mortalities, pattern of major organ dysfunction, and recurrent infections

Characteristics	Procalcitonin (N=XX)	Control (N=XX)	Total (N=XX)	P value
Length of antibiotic therapy (LOT) days, median (IQR)	XX (XX-XX)	XX (XX-XX)	XX (XX-XX)	X.XXX
days, mean (95% CI)	XX (XX-XX)	XX (XX-XX)	XX (XX-XX)	X.XXX
Day of therapy (DOT) days, median (IQR)	XX (XX-XX)	XX (XX-XX)	XX (XX-XX)	X.XXX
days, mean (95% CI)	XX (XX-XX)	XX (XX-XX)	XX (XX-XX)	X.XXX
Defined Daily Dose (DDD) days, median (IQR)	XX (XX-XX)	XX (XX-XX)	XX (XX-XX)	X.XXX
days, mean (95% CI)	XX (XX-XX)	XX (XX-XX)	XX (XX-XX)	X.XXX

Length of hospital stay days, median (IQR)	XX (XX-XX)	XX (XX-XX)	XX (XX-XX)	X.XXX
days, mean (95% CI)	XX (XX-XX)	XX (XX-XX)	XX (XX-XX)	X.XXX
Mortality, n (%)	XX (XX)	XX (XX)	XX (XX)	X.XXX
Pattern of major organ dysfunction, n (%)				
Convulsions	XX (XX)	XX (XX)	XX (XX)	X.XXX
Pulmonary oedema (Clinical)	XX (XX)	XX (XX)	XX (XX)	X.XXX
ICU admission	XX (XX)	XX (XX)	XX (XX)	X.XXX
ICU referral	XX (XX)	XX (XX)	XX (XX)	X.XXX
Invasive mechanical ventilation	XX (XX)	XX (XX)	XX (XX)	X.XXX
Non-invasive ventilation or high Flow nasal oxygenation	XX (XX)	XX (XX)	XX (XX)	X.XXX
Shock	XX (XX)	XX (XX)	XX (XX)	X.XXX
Inotrope/vasopressors	XX (XX)	XX (XX)	XX (XX)	X.XXX
Cardiovascular dysfunctions	XX (XX)	XX (XX)	XX (XX)	X.XXX
Fluid bolus required	XX (XX)	XX (XX)	XX (XX)	X.XXX
Spontaneous bleeding	XX (XX)	XX (XX)	XX (XX)	X.XXX
Blood transfusion	XX (XX)	XX (XX)	XX (XX)	X.XXX
Renal failure	XX (XX)	XX (XX)	XX (XX)	X.XXX
Haemodialysis / Peritoneal dialysis	XX (XX)	XX (XX)	XX (XX)	X.XXX
Recurrent infection confimed or suspected after de-escalation of antibiotic	XX (XX)	XX (XX)	XX (XX)	X.XXX
XXX	XX (XX)	XX (XX)	XX (XX)	X.XXX

Handling of missing data

Data validation rules will include range checks on numeric variables, skip-logic for conditional questions and consistency checks between related variables. Stringent checks will be performed for datapoints that are critical for the primary and secondary study outcome measures.

3.4. Safety and tolerability of procalcitonin-guided de-escalation of antimicrobials

The safety and tolerability outcome measures are assessed based on the occurrence of adverse events (AEs) and serious adverse events (SAEs) in the study arms. AEs will be summarised as shown in **Table 3 and Table 5**. Adverse events (AEs) defined in the study protocol and occurring among participants during

the study will be graded according to CTCAE Version 5 and presented for comparison across both study arms.

Safety analyses will be performed on all enrolled patients who received anbiotics. Safety and tolerability of the procalcitonin arm versus control arm will be assessed by comparing the frequency (as %) of AEs and SAEs, using the Fisher's exact test or Chi-square test, as appropriate. Safety data will be presented in tabular and/or graphical format and summarised descriptively. Patients safety data will be analysed following the intention to treat analysis. All adverse event summaries will refer to adverse events related to antibiotic treatment, i.e. adverse events that developed or increased in intensity after antibiotic administration, such as hospital acquired fungal infections. AEs will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The relevant CTCAE table is appended to this document. The safety and tolerability summaries will be presented as shown in **Table 3 and Table 5**.

The 95% confidence intervals will be reported for proportions. The proportions will be compared between intervention and control groups. The analyses will be summarised in **Table 3 and Table 5**.

Table 3. Summary of adverse events by study group

Adverse events	**Procalcitonin**		**Control**		**Total**
Number of participants	XXXX		XXXX		XXXX
Total number of events	XXXX		XXXX		XXXX
Grading of adverse events	1-2	3-4	1-2	3-4	
Symptoms, n/N (%)					
Septic thrombophlebitis	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Catheter-associated urinary tract infection	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Skin reaction	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Diarrhea	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Allergic reaction	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Others (e.g. hospital acquired infections)					

3.5. Relationship of AEs to procalcitonin arm versus control arm

Table 4. AE relationship to study group

Adverse events	**Procalcitonin**		**Placebo**		**Total**
Number of related AEs (Yes)	XXXX		XXXX		XXXX
Total number of events	XXXX		XXXX		XXXX
Symptoms, n/N (%)					
Septic thrombophlebitis	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)

Catheter-associated urinary tract infection	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Skin reaction	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Diarrhea	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Allergic reaction	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
XXX	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Others (e.g. hospital acquired infections)					

Table 5. Summary of severe adverse events by study group

Severe adverse events	**Procalcitonin**		**Control**		**Total**
Number of participants	XXXX		XXXX		XXXX
Total number of events	XXXX		XXXX		XXXX
Grading of adverse events					
Events, n/N (%)					
Deaths related to the study intervention such as death dierectly related to the early stopping of antibiotics or complications of prolong use of antibiotics	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)

Table 6. SAE relationship to study group

Severe adverse events	**Procalcitonin**		**Control**		**Total**
Number of participants	XXXX		XXXX		XXXX
Total number of events	XXXX		XXXX		XXXX
Grading of adverse events					
Events, n/N (%)					
Deaths (related to intervention)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)

3.6 Microbiological analysis

Phenotypic and genotypic data analsyis of the bacterial isoates

3.6.1 Antimicrobial Susceptibility Testing (AST) Analysis Plan

Descriptive Statistics

Antimicrobial susceptibility testing (AST) results will be categorised as susceptible (S), intermediate (I), or resistant (R) based on the breakpoints provided by CLSI guidelines. Any missing data will be handled appropriately and noted in the analysis.

Resistance patterns for each antibiotic will be summarised, including multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) bacteria. Frequencies and percentages of susceptible, intermediate, and resistant isolates will be reported for each antibiotic and bacterial species.

A summary table will be presented with the resistance rates for each antibiotic tested.

Comparative Analysis

Comparative analysis will be conducted between drug-resistant and drug-susceptible bacteria. Chi-square or Fisher's exact tests will be used, depending on the data distribution. A p-value of <0.05 will be considered statistically significant.

Multivariate Analysis

Multivariate analysis, such as logistic regression, will be conducted to identify risk factors associated with antimicrobial resistance. Potential confounders and covariates will be adjusted for in the models, and results will be presented as adjusted odds ratios with 95% confidence intervals.

Reporting Guidelines

The results will be presented in visual formats such as bar charts, heatmaps, and antibiograms to clearly convey resistance profiles. Data will be summarised visually to aid in understanding the susceptibility patterns.

Table 7. AST Analysis Plan

Step	Description
1. Descriptive Statistics	Categorise AST results as susceptible, intermediate, or resistant. Handle missing data appropriately. Summarise resistance patterns for each antibiotic, including MDR, XDR, and PDR bacteria. Present frequencies and percentages in a summary table.
2. Comparative Analysis	Conduct comparative analysis between drug-resistant and drug-susceptible bacteria using Chi-square or Fisher's exact tests. A p-value of <0.05 will be considered statistically significant.
3. Multivariate Analysis	Perform logistic regression or other multivariate analyses to identify risk factors for antimicrobial resistance. Adjust for confounders and present adjusted odds ratios (AORs) with 95% confidence intervals.
4. Reporting Guidelines	Present results visually using bar charts, heatmaps to clearly convey resistance profiles.

Table 8. Summary of culture positivity by culture type

There are XX number of culture positive. Of XX, there were XX in Procalcitonin and XX were in Control.

Culture type	Out of total	Of culture positive
Blood, n/N (%)	X/X (X.XX)	X/X (X.XX)
Urine, n/N (%)	X/X (X.XX)	X/X (X.XX)
Sputum, n/N (%)	X/X (X.XX)	X/X (X.XX)
Pus, n/N (%)	X/X (X.XX)	X/X (X.XX)
Blister Fluid, n/N (%)	X/X (X.XX)	X/X (X.XX)
XXX	X/X (X.XX)	X/X (X.XX)

Table 9. Summary of Species name

Species name	Of culture positive
Acinetobacter spp. , n/N (%)	X/X (X.XX)
Burkh. pseudomallei, n/N (%)	X/X (X.XX)
Burkhodelria cepacia, n/N (%)	X/X (X.XX)
Enterococ. faecalis, n/N (%)	X/X (X.XX)
Esch coli, n/N (%)	X/X (X.XX)
Klebsiella pneumoniae, n/N (%)	X/X (X.XX)
Pseudomonas aeruginosa, n/N (%)	X/X (X.XX)
Pseudomonas spp. , n/N (%)	X/X (X.XX)
Salmonella typhi, n/N (%)	X/X (X.XX)
Staphylococcus aureus, n/N (%)	X/X (X.XX)
Staphylococcus epidermidis, n/N (%)	X/X (X.XX)
Strept. pneumoniae, n/N (%)	X/X (X.XX)
Strept. pyogenes, n/N (%)	X/X (X.XX)
XXXX	X/X (X.XX)

Table 10. Summary of drug sensitivity only in ESC

Drug name	S	R	I	Total
Amoxicillin (AMO), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Amikacin (AMI), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Ampicillin (AML), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Azithromycin (AZM), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Aztreonam (ATM), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)

Cefepime (FEP), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Cefixime (CFM), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Ceftriaxone (CRO), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Ciprofloxacin (CIP), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Colistin (CT), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Gentamycin 10 (CN), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Gentamycin 120 (CN), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Levofloxacin (LEV), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Ceftazidime (CAZ), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Ceftazidimeavibactum, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Cotrimoxazole(SXT) , n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Meropenem (MEM) , n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Nitrofurantoin(F) , n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Piperacillin/Tazobactam(TZP) , n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Polymyxin B (PB) , n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Tetracycline (TE) , n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Tigecycline (TGC) , n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Tobramycin (TOB), n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
XXXX	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
XXXX	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)

Table 11. Summary of drug sensitivity grouping only in ESC

Drug name	S	R	I	Total
Aminoglycoside, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Penicillin, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Macrolids, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Monobactams, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Quinolones, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Carbapenem, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Cephalosporin+Beta lactam inhibitor, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Cephalosporins, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Nitrofurans, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Penicillin+Beta lactam inhibitor, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Polymyxins, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Sulfonamides, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)

Tetracyclines, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
XXXX	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
XXXX	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)

Definition of MDR Bacteria

For Multi-Drug Resistant (MDR) bacteria, all of them are MDR as resistant (R) to two or more classes of antibiotics (Table 12).

Table 12. Summary of MDR Bacteria

Species name	MDR number of antibiotics
Acinetobacter spp.	XX
Burkh. pseudomallei	XX
Burkholderia cepacia	XX
Enterococ. faecalis	XX
Esch coli	XX
Klebsiella pneumoniae	XX
Pseudomonas aeruginosa	XX
Pseudomonas spp.	XX
Salmonella typhi	XX
Staphylococcus aureus	XX
Staphylococcus epidermidis	XX
Strept. pneumoniae	XX
Strept. pyogenes	XX
XX	XX

Table 13. Summary of bacterial species in different culture positive

Species name	Blood	Urine	Sputum	Pus	Blister Fluid	Total
Acinetobacter spp., n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Burkh. pseudomallei, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Burkhodelria cepacia, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Enterococ. faecalis, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Esch coli, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Klebsiella pneumoniae, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Pseudomonas aeruginosa, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Pseudomonas spp., n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Salmonella typhi, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Staphylococcus aureus, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)

Staphylococcus epidermidis, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Strept. pneumoniae, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Strept. pyogenes, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)

Table 14. Summary of bacterial species in CRO

Species name	S	R	I	Total
Acinetobacter spp., n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Enterococ. faecalis, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Esch coli, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Klebsiella pneumoniae, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Pseudomonas aeruginosa, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Salmonella typhi, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Staphylococcus aureus, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Strept. pneumoniae, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Strept. pyogenes, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)

Table 15. Summary of bacterial species in CXM

Species name	S	R	I	Total
Burkh. pseudomallei, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Esch coli, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Klebsiella pneumoniae, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)
Staphylococcus aureus, n (%)	X(X.XX)	X(X.XX)	X(X.XX)	X(X.XX)

Table 16. Summary of Initial Diagnosis

Initial Diagnosis	Frequency n (%)
Enteric Fever	X(X.XX)
Intra-abdominal Infection	X(X.XX)
Lower Resp Tract Infection	X(X.XX)
Meningoencephalitis	X(X.XX)
Skin and Soft Tissue Infection	X(X.XX)
Undifferentiated Sepsis	X(X.XX)
Upper Resp Tract Infection	X(X.XX)
Urinary Tract Infection	X(X.XX)

3.6.2 Analysis plan of the whole genome sequencing data

Whole Genome Sequencing
Bacterial isolates will undergo whole genome sequencing using MicrobesNG's Short Read service. The sequencing will be performed using Illumina technology, providing 2 × 250 bp paired-end reads with a minimum coverage of 30X. Genomic DNA will be extracted from the bacterial isolates using standardised protocols, and sequencing libraries will be prepared according to the manufacturer's instructions. If required, MicrobesNG will also handle the DNA extraction at no extra cost.

Bioinformatics Analysis
Raw sequencing data will be processed using bioinformatics tools and pipelines to ensure high-quality genome assemblies. The analysis will include the following steps:

1. Quality Control: Raw reads will be assessed for quality using tools like FastQC. Low-quality reads and adapter sequences will be trimmed using software such as Trimmomatic.

2. Genome Assembly: High-quality reads will be assembled using de novo assemblers like SPAdes or Velvet. The quality of assemblies will be evaluated using metrics such as N50 and total assembly length.

3. Annotation: Assembled genomes will be annotated to identify genes, coding sequences, and other genomic features using tools like Prokka or Bakta.

4. Comparative Genomics: Resistance genes and mutations conferring antibiotic resistance will be identified using NCBI AMR finder+. Plasmid sequences will be identified to examine any common plasmids or other mobile genetic elements carrying AMR genes.

5. Phylogenetic Analysis: Phylogenetic trees will be constructed to examine the evolutionary relationships among the bacterial isolates. This will help in understanding the transmission dynamics and evolutionary history of MDR bacteria in the study population.

3.7 Economic Analysis

Objective
The economic analysis aims to compare the direct medical costs associated with antibiotic therapy and hospital stay between the control and intervention groups in the trial. The goal is to evaluate whether procalcitonin (PCT)-guided de-escalation of antibiotic use reduces the overall economic burden of treatment. A detailed analysis plan on the economic evaluation will be provided separately. Initial components are listed below:

3.7.1 Cost Calculation

Antibiotic Therapy Costs

Intervention Group:

- Let $C_{antibiotic_{ij}}$ be the cost of antibiotic therapy for patient i using antibiotic j in the intervention group. The standard maximum retail prices set by the producer will be used in this case.
- Let C_{PCT} be the cost of the procalcitonin test per patient. The average PCT testing charge set by the diagnostic centers will be used in this calculation.
- Total cost for antibiotic therapy for the intervention group $C_{antibiotic,\ intervention}$ is:

$$C_{antibiotic\ plus\ PCT,\ \ intervention} = \sum_{i}^{266}\sum_{j}^{n} C_{antibiotic_{ij}} + 266\ X\ \ C_{PCT}$$

- Per patient cost for antibiotic therapy in the intervention group $\bar{C}_{antibiotic,\ \ intervention}$ is

$$\bar{C}_{antibiotic\ plus\ PCT,\ \ intervention} = \frac{C_{antibiotic\ plus\ PCT,\ \ intervention}}{266}$$

Control group:

- Let $C_{antibiotic_{ij}}$ be the cost of antibiotic therapy for patient i using antibiotic j in the intervention group.
- Total cost for antibiotic therapy for the control group $C_{antibiotic,\ \ control}$ is:

$$C_{antibiotic,\ \ control} = \sum_{i}^{266}\sum_{j}^{n} C_{antibiotic_{ij}}$$

- Per patient cost for antibiotic therapy in the control group $\bar{C}_{antibiotic,\ \ control}$ is

$$\bar{C}_{antibiotic,\ \ control} = \frac{C_{antibiotic,\ \ control}}{266}$$

Hospital Stay Costs

- Let LOS_i be the length of stay for patient i.
- Let $C_{inpatient\_day}$ be the average inpatient cost per day for septic patients. The analysis will use an estimated cost from previous research.

Intervention Group:

- Total hospital stay cost for the intervention group $C_{hospital,\ \ intervention}$ is

$$C_{hospital,\ \ intervention} = \sum_{i=1}^{266} LOS_i\ X\ C_{inpatient\_day}$$

- Per patient hospital stay cost in the intervention group $\bar{C}_{hospitl,\ \ intervention}$ is

$$\bar{C}_{hospital,\ \ intervention} = \frac{C_{hospital,\ \ intervention}}{266}$$

Control Group:

- Total hospital stay cost for the control group $C_{hospital,\ \ control}$ is

$$C_{hospital,\ \ control} = \sum_{i=1}^{266} LOS_i\ X\ C_{inpatient\_day}$$

- Per patient hospital stay cost in the control group $\bar{C}_{hospital,\ control}$ is

$$\bar{C}_{hospital,\ control} = \frac{C_{hospital,\ control}}{266}$$

3.7.2 Cost Comparison

Total costs

Intervention Group:

- Total per patient cost in the intervention group $\bar{C}_{total,\ intervention}$ is:

$$\bar{C}_{total,\ intervention} = \bar{C}_{antibiotic\ plus\ PCT,\ intervention} + \bar{C}_{hospital,\ intervention}$$

Control Group:

- Total per patient cost in the intervention group $\bar{C}_{total,\ control}$ is:

$$\bar{C}_{total,\ control} = \bar{C}_{antibiotic,\ control} + \bar{C}_{hospital,\ control}$$

3.7.3 Costs Analysis

- Difference in average total cost per patient $\Delta\ \bar{C}_{total}$ is:

$$\Delta\ \bar{C}_{total} = \bar{C}_{total,\ control} - \bar{C}_{total,\ intervention}$$

- Perform t-test to determine if $\Delta\ \bar{C}_{total}$ is statistically significant.

3.7.4 Cost Savings

- Calculate absolute cost savings per patient $Savings_{absolute}$:

$$Savings_{absolute} = \Delta\ \bar{C}_{total}$$

- Calculate percentage cost savings $Savings_{percentage}$:

$$Savings_{percentage} = \left(\frac{\Delta\ \bar{C}_{total}}{\bar{C}_{total,\ control}}\right) X\ 100$$

4. Appendix

In the systematic review titled, "Metrics for quantifying antibiotic use in the hospital setting: results from a systematic review and international multidisciplinary consensus procedure," the panel recommended that antibiotic use should be expressed using at least two metrics simultaneously (1). For our analysis of antibiotic use, we will apply three metrics: Length of Therapy (LOT), Days of Therapy (DOT), and Defined Daily Dose (DDD)

4.1 Guide to the calculation of the Length of Therapy (LOT), Days of Therapy (DOT) and the Defined Daily Dose (DDD)

Length of therapy (LOT) calculation.

LOT is defined as the number of days of antibiotic treatment during the study period. Length of treatment will be measured from the day of enrolment to completion of the study follow up (28 days after hospital discharge). The primary outcome data for participants with recurrent infections will be censored at the time of recurrent infection diagnosis, i.e. the reccurent date will be used as the stop date in the calculation of LOT. If the reccurent infection occurs after the patient already stopped antibiotics for the initial presenting infection, the actual stop date of the last dose of antibiotics administered for the initial infection will be used in the LOT calculations and not the recurrent infection date.

The stop date for each antibiotic will be when the treating team discontinues it. In special circumstances, such as patients who left the hospital against medical advice or were transferred to another facility, the stop date will be recorded as the date they left or were transferred, as it was not feasible for the study team to track antibiotic data consistently in both arms beyond these points. If a patient developed an alternative diagnosis during the study that required an extended or modified antibiotic regimen, each case was reviewed individually by an investigator panel to determine the maximum antibiotic duration that could be included in the analysis.

Scenario 1: As an example, suppose patient A was admitted to the hospital on 02 February 24 at 10:00 AM due to a urinary tract infection. At 4:00 PM the same day, this patient was enrolled into the PROCALBAN study and was discharged from the hospital on 08 February 24 at 10:00 AM. This patient received intravenous ceftriaxone, 1 gram administered every 12 hourly from the date of enrolment and then upon discharge, this patient was prescribed oral cefuroxime, 500mg every 8 hours, for a duration of 7 days i.e. the actual stop date time for this oral cefuroxime will be subsequently recorded in the database by the end of follow-up for this patient. The calculation of LOT (the primary outcome) will proceed as follows:

1. Hospital stay duration during enrolment will be calculated as follows:
   - Enrollment: February 2nd, 4:00 PM
   - Discharge: February 8th, 10:00 AM
   - Hospital stay duration:
     - February 2nd, 4:00 PM to February 3rd, 4:00 PM = 24 hours
     - February 3rd, 4:00 PM to February 4th, 4:00 PM = 24 hours
     - February 4th, 4:00 PM to February 5th, 4:00 PM = 24 hours
     - February 5th, 4:00 PM to February 6th, 4:00 PM = 24 hours
     - February 6th, 4:00 PM to February 7th, 4:00 PM = 24 hours
     - February 7th, 4:00 PM to February 8th, 10:00 AM = 18 hours

- Total hospital stay duration = 24 + 24 + 24 + 24 + 24 + 18 = 138 hours (approximately 5 days and 18 hours).

Definition of 'hosptial stay duration':

Hospital stay duration = discharge date time – enrolment date time.

If only date (without time) is available for one or both date fields used in these calculations, the dates will be used for calculating the length of therapy.

2. Post-discharge oral antibiotic therapy duration:
   - Prescribed for 7 days
   - Total duration for oral cefuroxime therapy: 7 days * 24 hours/day = 168 hours

3. Total duration up to discharge:
   - Total therapy duration = Hospital stay duration + oral cefuroxime therapy duration
   - Total therapy duration = 138 hours (hospital stay) + 168 hours (oral cefuroxime therapy) = 306 hours (approximately 12 days and 18 hours)

In total, the total length of therapy from enrolment up to discharge for Patient A is approximately 12 days and 18 hours, or 306 hours. For analysis, this will be expressed in days.
In short LOT=stop date time (for last dose) – start date time (for first dose).

Similarly, if only date (without time) is available for one or both date fields used in these calculations, the dates will be used for calculating the length of therapy.

Scenario 2 : A patient is prescribed ceftriaxone IV and oral clarithromycin to treat an infection. Both antibiotics are started on 03 September 2024 and discontinued on 07 September 2024.

- **LOT Calculation**: Since LOT is based on the total duration of exposure to any antibiotic, regardless of how many different antibiotics are administered, we calculate it by counting the calendar days from the start date to the stop date.
  - **Start Date**: 03 September 2024
  - **Stop Date**: 07 September 2024
  - **Total LOT**: 5 calendar days

Result: The LOT for this course of therapy is **5 days**, representing the patient's exposure to antibiotic treatment over this period, irrespective of the number of antibiotics given.

Definition of DOT (Days of therapy)

One DOT represents the administration of a single antibiotic on a given day regardless of the number of doses administered or dosage strength. If a patient receives more than one antibiotic, the DOTs for all the given antibiotic therapies will be added together to come up withe the total DOT for this patient. DOT will be greater than or equal to LOT because each antibiotic administered counts as a separate DOT.
For calculating DOT, the start time for any antibiotic initiated prior to enrolment will be recorded as the time of enrolment, while for antibiotics started post-enrolment, the actual start time will be used.

Scenario: As an example, suppose patient B was admitted to the hospital on May 5, 2024 with pneumonia and was discharged on May, 9, 2024. During the hospital stay, Patient B received Intravenous

ceftriaxone every 12 hours, denoted by "1 dot" for each day. Upon discharge, Patient B was prescribed oral amoxicillin 500mg every 8 hours to be taken at home for 7 days, denoted by "1 DOT " for each day.

Calculation:
1. Hospital stay duration: 4 days
2. Intravenous ceftriaxone therapy: Given every 12 hours for 4 days (total of 2 doses per day, counted as "1 DOT " for each day)
3. Oral amoxicillin therapy: Prescribed for 7 days, taken every 8 hours (total of 3 doses per day, counted as "1 DOT " for each day)
4. Overall Therapy Duration: 4 days (hospital stay) + 7 days (oral antibiotic therapy) = 11 DOTs

The actual DOT for each antibiotic drug that was given will be recorded by the end of the patient's follow-up and will be used for the calculations. The actual date (or date time if available) will be used to calculate the DOT for each antibiotic drug and then these DOTs will be summed up.

In calculating DOT, if an antibiotic is administered every 48 hours, it will not be counted as every alternate day. For example, if a patient receives meropenem every 48 hours over a span of 7 days, it will be counted as 7 DOT, not 4 DOT. This approach considers the overall exposure to the antibiotic.

Number of days of parenteral antibiotic during hospitalisation period: will be measured from the day of enrolment to the day of stopping of parenteral antibiotic during hospitalisation period

Number of days of antibiotic treatment during the hospitalisation period: Length of treatment will be measured from the days of enrolment to stopping of antibiotic during hospitalisation period.

DDD (Defined Daily Dose)

According to WHO "The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adult"
In the absence of electronic medication management systems, the Defined Daily Dose (DDD) is a valuable metric for assessing drug consumption. The Anatomical Therapeutic Chemical (ATC)/DDD methodology, endorsed by the World Health Organization (WHO), is an internationally recognised standard for evaluating drug usage. Guidelines for calculating DDD can be found on the WHO website here. In this study, we will apply the WHO ATC/DDD methodology to determine DDD, with the aim of producing high-quality, practical, and comparable drug utilisation statistics (2).

Drug utilisation data expressed in DDDs offers an approximate estimate of consumption but does not precisely reflect actual usage. DDDs serve as a standardised unit of measurement, unaffected by factors such as price, currency, packaging size, or strength, allowing researchers to examine trends in drug utilisation and compare usage across different population groups.

To calculate the Defined Daily Dose (DDD) for each antibiotic, use the following steps:

1. **Identify the Route of Administration**: Confirm the administration route (e.g., IV, oral) for each antibiotic dose, as the DDD value varies with the route.
2. **Total Dose Administered**: Sum the total dose of each antibiotic administered per patient, as recorded in the dataset.

3. **Apply DDD Standard Values**: Divide the total dose administered by the standard DDD value for each antibiotic (specific to its route of administration) provided by the WHO ATC/DDD Index.

Example of a DDD calculation

Scenario

A 57-year-old male patient was admitted to the hospital with a severe respiratory infection and was prescribed intravenous amoxicillin as part of his treatment. To manage the infection, the medical team ordered a 1-gram dose of IV amoxicillin administered every 8 hours.

Treatment Summary

- **Dosage per administration**: 1 gram of IV amoxicillin
- **Frequency**: Every 8 hours (3 times per day)
- **Total doses administered over the course of treatment**: 13 doses

DDD Calculation

To assess antibiotic use, the team calculates the Defined Daily Doses (DDDs) based on the WHO DDD standard for IV amoxicillin, which is 3 grams per day.

1. **Total dose administered**:
    - Total dose = 1 gram × 13 doses = 13 grams
2. **Total DDDs**:
    - Total DDDs = Total dose administered / WHO DDD for IV amoxicillin
    - Total DDDs = 13 grams / 3 grams = approximately 4.33 DDDs

DDD Patient Days

Drug utilisation figures should ideally be presented using a relevant denominator for the health context. In the hospital setting, this would be the number of patients or number of bed days in the setting and period monitored. Description and calculation of the most common indicators such as DDD/patients and DDD/100 bed days, DDD/1000 population/day gives an approximate measure of how many people are taking an antibiotic on any given day. Alternatives include:

- DDD per 1000 inhabitants per day
- DDD per 100 bed-days

Definition of DDD per 1,000 Patient Days:

- DDD per 1,000 patient days is a standardized measure that compares the total amount of antibiotics used (in DDDs) to the number of patient care days, adjusted to a standard of 1,000 patient days. This helps compare antibiotic consumption across different settings, regardless of patient volume.

- Formula for DDD per 1,000 Patient Days:
    - The formula is:

$$\text{DDD per 1,000 patient days} = \frac{\text{Total DDD}}{\text{Total patient days}} \times 1,000$$

Example calculation of DDD per 1,000 Patient Days:

- Suppose we have the same three patients with the following hospital stays:
    - Patient 1: 5 days

  - Patient 2: 3 days
  - Patient 3: 7 days
- Total patient days = 5 + 3 + 7 = 15 patient days.
- Suppose the total DDD (Defined Daily Dose) for all patients combined is 20.

Applying the Formula:

$$\text{DDD per 1,000 patient days} = \frac{20\,\text{DDDs}}{15\,\text{patient days}} \times 1,000$$

Breaking Down the Calculation:

First, divide the total DDD by the total patient days: 20/15=1.3333
Then, multiply by 1,000 to standardize:1.3333×1,000=1,333.33

The DDD per 1,000 patient days is 1,333.33.

4.2 Considerations for timepoints in calculating Length of Therapy (LOT), Days of Therapy (DOT), and Defined Daily Dose (DDD)

Length of Therapy (LOT)	**Days of Therapy (DOT)**	**Defined Daily Dose (DDD)**
Starting Point: Enrolment date and time.	Starting Point: Enrolment date and time.	Starting Point: Enrolment date and time.
Stopping Point: Actual antibiotic stopping time as recorded by the treating team.	Stopping Point: Antibiotic stopping time as per treating team's decision.	Stopping Point: Antibiotic stopping time as per treating team's decision.
Exceptions: Recurrent Infections: Censor the LOT during recurrent infection episodes. Left Against Medical Advice (LAMA): Consider the date the patient left as the stopping point. Transfer to Another Hospital: Use the transfer date as the stopping point. Atypical Cases: Determine a reasonable stopping date through team discussion.	Exceptions: Same as those applied in the LOT calculation, using specific stopping points for recurrent infections, LAMA, transfers, and atypical cases.	Exceptions: Same as those applied in the LOT and DOT calculation, using specific stopping points for recurrent infections, LAMA, transfers, and atypical cases.

4.2 Atypical Cases in PROCALBAN:

1. **Antibiotic Management**: For cases where an alternative diagnosis is confirmed, the stopping date for empirical antibiotic therapy will be recorded as the actual stop date.
2. **Alternative Diagnosis**: If antibiotic therapy is continued despite the confirmation of an alternative diagnosis, such as Melioidosis (which requires a prolonged course of antibiotics), the date on which the treating team identified this alternative diagnosis will be considered as the stopping date for the initial antibiotic treatment.
3. **Additional Diagnoses**: For cases where an additional diagnosis accompanies the initially suspected infection and antibiotic therapy is continued, the full duration of the antibiotic course will be included in the outcome analysis (e.g., Enteric Fever combined with Viral Infection).
4. **Sepsis Mimickers**: In cases where sepsis-like symptoms are attributed to other conditions, such as cancer, connective tissue diseases, or suspected/confirmed tuberculosis, the duration of empiric antibiotic treatment given before an alternative diagnosis is confirmed through clinical assessment, laboratory findings, or radiological evidence will be used for outcome analysis.
5. **Discrepancies in Diagnosis**: If there is a disagreement among investigators regarding the final diagnosis, a third investigator, who has not previously reviewed the case, will assess it to determine the most appropriate antibiotic duration based on the case's clinical details.

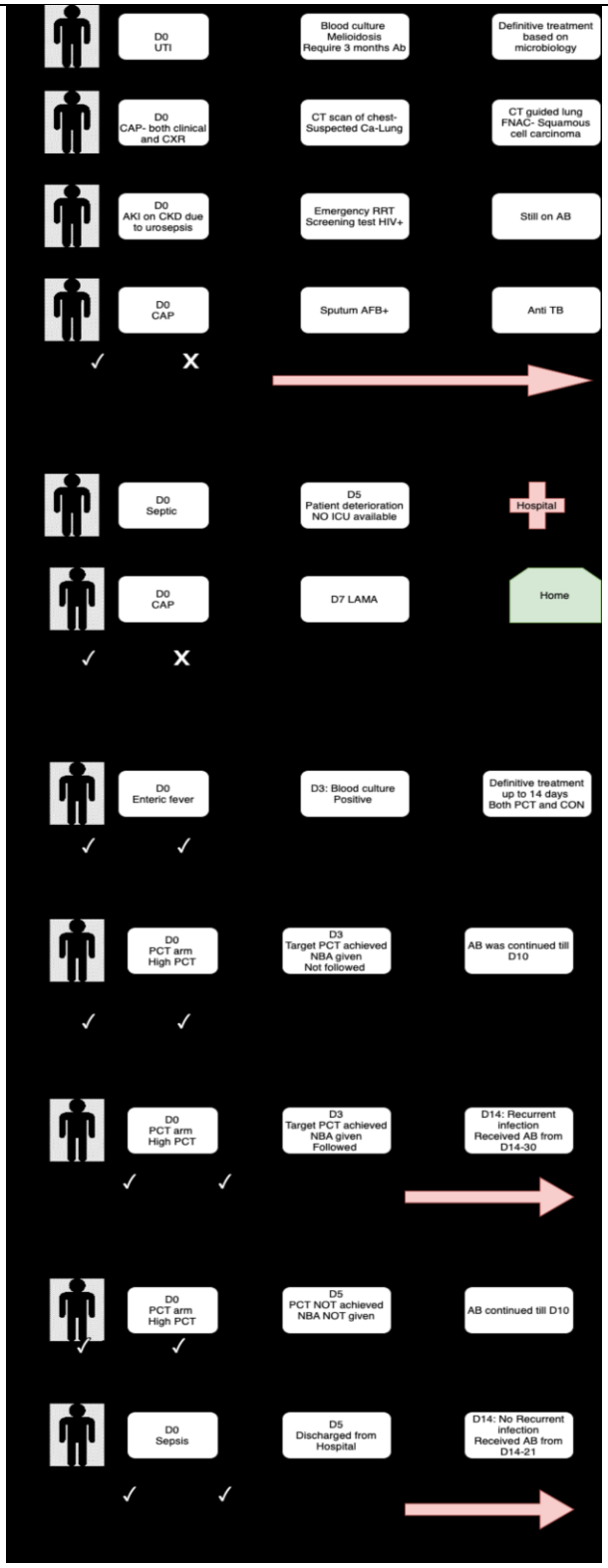


Figure 2: Pictorial Representation of ITT and PP Analysis Plan for Participants

5. References:

1. Stanić Benić M, Milanič R, Monnier AA, Gyssens IC, Adriaenssens N, Versporten A, et al. Metrics for quantifying antibiotic use in the hospital setting: results from a systematic review and international multidisciplinary consensus procedure. Journal of Antimicrobial Chemotherapy [Internet]. 2018 Jun 1 [cited 2024 Nov 1];73(suppl_6):vi50–8. Available from: https://academic.oup.com/jac/article/73/suppl_6/vi50/5033637

2. WHO. ATC/DDD Index 2024 [Internet]. 2024 [cited 2024 Jun 1]. Available from: https://atcddd.fhi.no/atc_ddd_index/?code=J&showdescription=no