BLING III

The **B**eta-**L**actam **I**nfusio**N** **G**roup

BLING III Study PK-PD Substudy Protocol

A phase III randomised controlled trial of continuous beta-lactam infusion compared with intermittent beta-lactam dosing in critically ill patients

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The University Of Queensland AUSTRALIA

UNSW SYDNEY

The George Institute for Global Health

ANZICS Clinical Trials Group

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1. Protocol synopsis

Title	Continuous vs intermittent infusion of β-lactam antibacterial drugs: impact on resistance and outcomes in sepsis
Short title	BLING III PK-PD sub-study
Design	This is a prospective, observational sub-study of the BLING III study.
Main Objectives	1. To compare target attainment in culture positive patients by randomisation status according to the following definitions: a) 'Equivalent PK-PD target' for beta-lactam antibiotic exposure, defined as 100%$fT_{>MIC}$ for both treatment arms b) 'Variable PK-PD target' for beta-lactam antibiotic exposure, defined as $fC_{ss\geq 4xMIC}$ for the continuous infusion arm and 100%$fT_{>MIC}$ for the intermittent infusion arm c) 'Alternative variable PK-PD target' for beta-lactam antibiotic exposure, defined as $fC_{ss\geq 4xMIC}$ for the continuous infusion arm and 50%$fT_{>MIC}$ for the intermittent infusion arm. 2. To explore the association in culture-positive patients between beta-lactam antibiotic exposure and the following outcomes by randomisation status: a) All-cause patient mortality at Day 90 post randomisation (primary outcome in the BLING III RCT) b) Patient clinical cure at Day 14 post randomisation (secondary outcome in the BLING III RCT) c) New acquisition, colonisation or infection with a multi-resistant organism or *Clostridium difficile* associated diarrhoea up to 14 days post randomisation (secondary outcome in the BLING III RCT)
Study procedures	Pharmacokinetic component Three blood samples collected to determine unbound plasma antibiotic concentrations (termed 'antibiotic exposure'). Samples collected between days 2 and 5 of beta-lactam antibiotic therapy. Pharmacokinetic analyses will be undertaken to describe beta-lactam antibiotic exposure in terms of: - unbound beta-lactam antibiotic concentration above the MIC for the entire dosing interval (i.e. $fT_{>MIC}$) - 24-hour area under the concentration-time curve of unbound beta-lactam antibiotic to MIC ratio (i.e. $fAUC_{0-24}$/MIC) Pharmacodynamic component For culture positive patients: Causative organism MIC will be preferentially determined via the broth microdilution and/or epsilometer test (Etest®) methods. Whole-genome sequencing will be performed on isolates of causative organisms to determine sequence types and characterise beta-lactamase genes (if applicable).

	For culture negative patients: see Section 5.2 for further detail.
Sample size	300 culture-positive patients. Due to study design, culture negative patients will also be included in additional analyses.
Patient inclusion criteria	Patients enrolled into the BLING III study from sites participating in the PK-PD sub-study and for whom there is written consent to collect and use blood samples can be enrolled in the substudy if the following inclusion criteria are met: • Patient has either an arterial line or central venous catheter from which to sample blood from • Patient is able to be sampled between days 2 and 5 post commencement of beta-lactam antibiotic therapy.
Site requirements	Sites participating in the BLING III study meeting the following criteria will be invited to participate in the sub-study: 1) Capacity to collect and process three timed blood samples 2) Capability of microbiology laboratory to collect and transfer clinical culture isolate samples 3) Provision to store aliquoted plasma samples and cultured isolates at -70^{o}C to -80^{o}C.

2. Administration information

The Beta-Lactam Infusion Group (BLING) III pharmacokinetic-pharmacodynamic (PK-PD) sub-study forms part of the BLING III randomised controlled trial (RCT). The BLING III Management Committee has overall responsibility for conduct of the BLING III trial and the PK-PD sub-study. A sub-study steering committee will provide expert advice on sub-study management.

The BLING III PK-PD sub-study has received endorsement from the Australian and New Zealand Intensive Care Society (ANZICS) Clinical Trials Group, the Australasian Society for Infectious Diseases Clinical Research Network, the European Society of Clinical Microbiology and Infectious Diseases and the European Society for Intensive Care Medicine.

2.1. Sub-study Steering Committee

The BLING III PK-PD Sub-study Steering Committee will comprise the following Investigators with additional representatives invited as required (Chair followed by alphabetical order):

- Professor Jason Roberts (CHAIR), Pharmacist Consultant, Royal Brisbane and Women's Hospital, and Professor of Medicine, Faculty of Medicine, UQ Centre of Clinical Research, The University of Queensland, QLD, Australia
- Dr Mohd Hafiz Abdul Aziz, Research Fellow, Faculty of Medicine, UQ Centre of Clinical Research, The University of Queensland, QLD, Australia
- A/Prof Mark Chatfield, Senior Biostatistican, Faculty of Medicine, UQ Centre of Clinical Research, The University of Queensland, QLD, Australia
- Dr Menino Osbert Cotta, Research Fellow, Faculty of Medicine, UQ Centre of Clinical Research, The University of Queensland, QLD, Australia
- Professor Joshua Davis, Infectious Diseases Physician, John Hunter Hospital, NSW, Australia
- Professor Jan De Waele, Surgical Intensivist, Department of Critical Care Medicine, Ghent University Hospital, Belgium
- Dr Joel Dulhunty, Research Fellow, Department of Intensive Care Medicine, Royal Brisbane and Women's Hospital, QLD, Australia
- Dr Andrew Henderson, Infectious Diseases Physician & Clinical Microbiologist, Princess Alexandra Hospital, QLD, Australia
- Professor Jeffrey Lipman, Director, Department of Intensive Care Medicine, Royal Brisbane and Women's Hospital, QLD, Australia
- Professor David Paterson, Infectious Diseases Physician, Royal Brisbane and Women's Hospital, QLD, Australia
- Ms Dorrilyn Rajbhandari (BLING III study PROJECT MANAGER), Critical Care & Trauma Division, George Institute for Global Health, NSW, Australia
- Dr Steven Wallis, Laboratory Manager and Senior Scientist, Faculty of Medicine, UQ Centre of Clinical Research, The University of Queensland, QLD, Australia

2.2. Coordinating Centre and Central Bioanalysis Laboratory

Antimicrobial Optimisation Group
Faculty of Medicine
Level 8, UQ Centre for Clinical Research
The University of Queensland
Royal Brisbane and Women's Hospital, Herston QLD 4029

2.3. Funding

The BLING III PK-PD sub-study has received part funding from the Royal Brisbane and Women's Hospital Foundation. Etests® for minimum inhibitory concentration (MIC) are donated by bioMérieux (Etoile, France). The sub-study has been awarded a United States Food and Drug Administration (FDA) Grant/Contract (Contract No. 75F40121C00126) commencing November 2021. Australian National Health and Medical Research Council (NHMRC) Centre of Research Excellence RESPOND (APP2007007) and a NHMRC Investigator Grant (APP2009736) to Professor Jason Roberts provide further funding to support the study.

2.4. Role of funding bodies

The BLING III PK-PD sub-study will be designed and conducted, and the results analysed, presented and published by the investigators independent of any funding agencies.

3. **Background and rationale**

Bacterial killing for beta-lactam antibiotics is related to the duration of time that bacteria are exposed to unbound antibiotic concentrations, or more accurately termed antibiotic exposure, that exceed the minimum inhibitory concentration (MIC) of the pathogen.[1] Both *in vitro* and *in vivo* mathematical PK-PD models have provided a means to accurately describe beta-lactam antibiotic exposure targets that are associated with maximum effect.[2] For the most part, these described PK-PD exposure targets have been supported by results reported in clinical studies.[3] However, there is still some discordance in the literature on what constitutes optimal beta-lactam antibiotic plasma concentrations from the point of view of clinical cure and microbiological eradication.

In some clinical studies, targets for beta-lactam antibiotic exposure associated with microbiogical eradication have been shown to correlate with unbound plasma concentrations maintained four to six times above the MIC throughout the entire dosing interval (i.e. 100% $fT_{>4\text{-}6xMIC}$).[4-6] Other pre-clinical data have shown maximal bactericidal effect occurs for only a proportion of, rather than the entire dosing interval. Based on this, PK-PD exposure targets for unbound concentrations remaining four times above the MIC (4xMIC) for 50%, 70% and 40% for the penicillins, cephalosporins and carbapenems, respectively, have been stipulated.[7] Hence, a suggested target for beta-lactam antibiotic exposure of unbound plasma concentrations above the MIC for the entire dosing interval (i.e. 100% $fT_{>1xMIC}$) has been advocated to ensure that 40-70% $fT_{>4xMIC}$ is achieved.[8]

However, whether 100% $fT_{>1xMIC}$ ensures 40-70% $fT_{>4xMIC}$ has recently been challenged, with a recent study using existing patient data to simulate first doses suggesting that only a small number of patients will achieve 40-70% $fT_{>4xMIC}$ (for beta-lactam antibiotics such as piperacillin, ceftazidime and cefepime) with dosing to a therapeutic PK-PD target of 100% $fT_{>1xMIC}$.[9] These investigators strongly suggest an alternative exposure target of unbound plasma concentrations maintained four times above the MIC throughout the entire dosing interval (i.e. 100% $fT_{>4xMIC}$) for intermittent infusion, and a steady-state concentration of the unbound antibiotic is at least four times greater than the MIC (i.e. $fC_{ss\geq4xMIC}$) for continuous infusion. Based on existing data, they propose that this target will ensure maximal bacterial killing, prevent bacterial regrowth and ensure positive clinical outcome.

The 2014 DALI (Defining Antibiotic Levels in Intensive care unit patients) study showed that clinical outcomes were better when 100% $fT_{>1xMIC}$ was achieved as compared to lower concentrations.[10] However, as this and other studies have not investigated outcomes associated with alternative PK-PD targets, such as 100% $fT_{>4xMIC}$, it is currently unknown if higher PK-PD targets lead to incrementally better patient outcomes in the critically ill population.

The large-scale recruitment during the BLING III study provides a unique opportunity for the collection of the largest and most comprehensive dataset of beta-lactam antibiotic PK-PD data. Serial blood samples and, if available, microbiological cultures will be collected. With this rich PK-PD dataset available, the BLING III PK-PD sub-study will be the best and perhaps, the only opportunity to describe the antibiotic exposure-clinical response relationship in the critically ill population. These data will define what method of administration (i.e., continuous infusion or intermittent infusion) and also, exposure (or concentration) of the beta-lactam antibiotic provide maximal therapeutic effect (patient survival) and also minimises the development of resistance in critically ill patients with sepsis. Additionally, by being conducted within the BLING III study, which is a pragmatic global trial, it will cost-effectively maximise generalisability across a range of infections, antibiotic resistance profiles and geographic regions/countries with different health care systems.

4. Study design

4.1. Aim

The primary aim of the BLING III PK-PD sub-study is to determine the relationship between beta-lactam antibiotic exposure and patient-related outcomes observed in a subset of patients enrolled into the BLING III RCT.

4.2. Main Objectives

1. To compare target attainment in culture-positive patients by randomisation status according to the following definitions:
   a) 'Equivalent PK-PD target' for beta-lactam antibiotic exposure, defined as 100%$fT_{>1xMIC}$[1] for both treatment arms[2]
   b) 'Variable PK-PD target' for beta-lactam antibiotic exposure, defined as $fC_{ss\geq4xMIC}$[3] for the continuous infusion arm and 100%$fT_{>1xMIC}$[1] for the intermittent infusion arm[2]
   c) 'Alternative variable PK-PD target' for beta-lactam antibiotic exposure, defined as $fC_{ss\geq4xMIC}$[3] for the continuous infusion arm and 50%$fT_{>1xMIC}$[4] for the intermittent infusion arm[5]
2. To explore the association in culture-positive patients between beta-lactam antibiotic exposure and the following outcomes by randomisation status:
   a) All-cause patient mortality at Day 90 post randomisation (primary outcome in the BLING III RCT)
   b) Patient clinical cure at Day 14 post randomisation[6] (secondary outcome in the BLING III RCT)

c) New acquisition, colonisation or infection with a multi-resistant organism or *Clostridium difficile* associated diarrhoea up to 14 days post randomisation (secondary outcome in the RCT) [expected outcome prevalence << 15%].

APACHE = Acute Physiology and Chronic Health Evaluation. BLING = Beta-lactam Infusion Group. MIC = minimum inhibitory concentration. PK-PD = pharmacokinetic-pharmacodynamic. RCT = randomised controlled trial.

1 - 100%$fT_{>1xMIC}$ = unbound beta-lactam antibiotic concentration above the MIC for the entire dosing interval.

2 - For patients randomised to the continuous infusion arm, the mean value of the 3 steady-state concentrations will be used; for patients randomised to the intermittent infusion arm, the measured 'trough' concentration will be used.

3 - $fC_{ss\geq4xMIC}$ = unbound beta-lactam antibiotic steady-state concentration four or more times above the MIC.

4 - 50%$fT_{>1xMIC}$= unbound beta-lactam antibiotic concentration above the MIC half-way (i.e. 'mid') through the dosing interval.

5 - For patients randomised to the continuous infusion arm, the mean value of the 3 steady-state concentrations will be used; for patients randomised to the intermittent infusion arm, the measured 'mid' concentration will be used.

6 - Clinical cure is defined as the completion of the beta-lactam antibiotic treatment course (on or before Day 14) without recommencement of antibiotic therapy within 48 hours of cessation for the same infectious episode. For the purposes of evaluating clinical cure, change of antibiotic therapy (i.e. either escalation or de-escalation) for the same indication for which the beta-lactam antibiotic was commenced is considered part of the antibiotic treatment course

4.3. Hypotheses

For Objective 1: It is hypothesised that 1) attainment of the 'Equivalent PK-PD target' will be higher in patients randomised to the continuous infusion arm, 2) attainment for 'Variable PK-PD target' will be equivalent between the two treatment arms, and 3) attainment for 'Alternative Variable PK-PD target' will be higher in patients randomised to the intermittent infusion arm.

For Objective 2: It is hypothesised that the beta-lactam antibiotic exposure vs outcome curves will be similar for both continuous and intermittent infusion randomisation groups. Increasing beta-lactam antibiotic exposure will be associated with improved patient outcomes (clinical cure up to Day 14 and survival up to Day 90) up to a point, beyond which there may be a worsening in patient outcomes due to a potential association between very high beta-lactam exposure and increased sickness severity. The effect of confounding will be minimised by inclusion of relevant factors in regression models. It is hypothesised that increasing beta-lactam antibiotic exposure will also be associated with a decrease in the incidence of new acquisition, colonisation or infection with a multi-resistant organism or *Clostridium difficile* associated diarrhoea up to Day 14

4.4 Study Design

This is a prospective, observational PK-PD sub-study of the BLING III study.

BLING III is a prospective, multi-centre, open, phase III, randomised controlled trial comparing continuous infusion of a beta-lactam antibiotic (piperacillin-tazobactam or meropenem) with intermittent infusion of the beta-lactam antibiotic over 30 minutes in critically ill patients with infection.

4.5 Patient inclusion criteria

All patients enrolled into BLING III at sites participating in the BLING III PK-PD sub-study are eligible for inclusion into this substudy if the following inclusion criteria are met:

- Patient has either an arterial line or central venous catheter from which to sample blood from
- Patient is able to be sampled between days 2 and 5 post commencement of beta-lactam antibiotic therapy.

4.6 Participation requirements for sites

Sites participating in the BLING III study meeting the following eligibility criteria will be invited to participate in the PK-PD sub-study:

1) Capacity to collect and process three timed blood samples
2) Capability of microbiology laboratory to collect and transfer clinical culture isolate samples
3) Provision to store aliquoted plasma samples and culture isolates at -70^{o}C to -80^{o}C.

5. **Study procedure**

5.1. Pharmacokinetic component

Sample collection

Patients successfully enrolled into the sub-study will have three blood samples collected to determine unbound plasma antibiotic concentrations. Sampling will occur between days 2 and 5 of beta-lactam antibiotic therapy.

Blood collection tubes with heparin are required to be used for all blood sample collections. Once collected, the blood samples need to be placed in an ice-bath until centrifugation can be performed. Centrifugation needs to occur within 6 hours of blood sample collection. Plasma from centrifuged samples will be pipetted into a clean polypropylene cryovial, labelled with sub-study identifiers and stored at -70°C to -80°C.

<u>Patients randomised to the continuous infusion arm of the BLING III study:</u>

Blood samples will be collected 4 hours apart during the sampling window period between days 2 and 5 of antibiotic therapy (**Figure 1**). The continuous infusion dose must be running at a constant rate during the sampling period.

Sample 1: Time 0 (e.g. 0800 hrs)

Sample 2: Time 4 hrs (e.g. 1200hrs)

Sample 3: Time 8 hrs (e.g. 1600hrs)

<u>Patients randomised to the intermittent infusion arm of the BLING III study</u>:

Blood sampling will occur during one dosing interval (**Figure 1**).

Sample 1: 30 minutes after the start of the intermittent infusion dose [‘0.5hr’]

Sample 2: Half-way through the dosing interval (i.e. if dosing interval is 8 hours, then sample will be taken at 4 hours) [‘Mid’]

Sample 3: Prior to the next scheduled intermittent infusion dose (must be within 30 minutes) ['Trough']

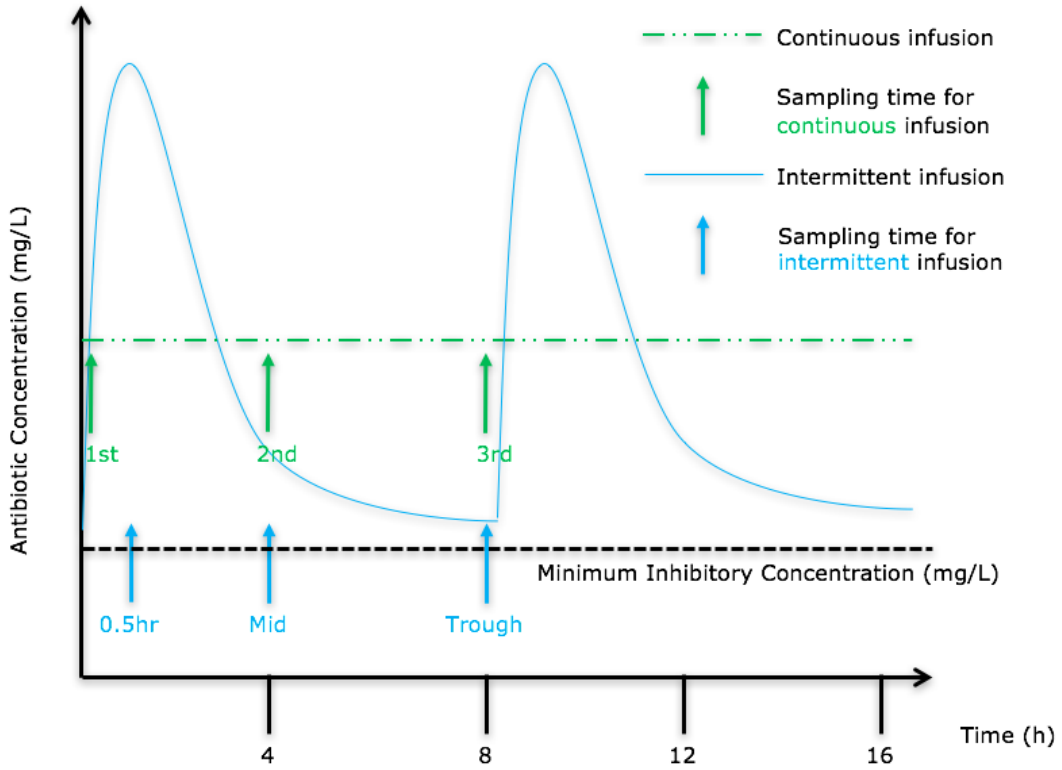

Figure 1. Sampling times between days 2 and 5 of antibiotic therapy for continuous infusion and an intermittent infusion administered at a 8-hour dosing interval

Sample analysis

Beta-lactam antibiotic assays of blood samples will be conducted by the Central Bioanalysis Laboratory at UQ Centre of Clinical Research (UQCCR) at The University of Queensland to determine unbound antibiotic concentrations. Only samples where written consent has been obtained will be transferred for analysis. Any part of a sample remaining after analysis will be destroyed. Samples where no written consent is obtained will be destroyed at study sites.

5.2. Pharmacodynamic component

Microbiological cultures

As part of routine clinical care at each participating site, biological samples will be sent to the clinical laboratory for microbiological culture. Viruses, fungi, parasites, rickettsiae and some fastidious bacteria (e.g., Legionella species, Brucella species and Leptospira species) are not considered relevant causative organisms in this sub-study.

"A causative organism" is defined as "a bacterium that is able to be grown by the participating site's clinical laboratory" and must meet one of the following criteria:

- **Sterile site isolates** (e.g., blood, bone, cerebrospinal fluid, deep fluid or pus, synovial fluid, and deep tissue): an organism which the treating team identifies as responsible for the current episode of sepsis. These should not include organisms generally considered to be contaminants, unless the organism is grown two or more times within a 72-hour period with identical antibiograms and the treating team identifies it as responsible for the current episode of sepsis
- **Sputum or endotracheal aspirates:** a pure or predominant organism which is a known pathogen in a purulent sample with moderate or heavy growth, and is identified by the treating team as responsible for the current episode of sepsis
- **Urine samples:** pure or predominant growth of at least 10^5 colony forming units per mL, and at least 10 white blood cells per high power field, and identified by the treating team as responsible for the current episode of sepsis
- **Other samples:** an organism grown from another non-sterile site may be included if the treating team identifies it as responsible for the current episode of sepsis.

For new acquisition, colonisation or infection with an MRO or *Clostridium difficile* associated diarrhoea up to 14 days post randomisation: a patient will be considered to have a relevant MRO based on defined clinical criteria and an identified organism (only bacterium) if cultured from samples taken **between Day 1 and Day 14 post-randomisation (inclusive).**

MIC determination

Isolates from causative organisms will be sent to UQCCR for formal susceptibility testing (by a Microbiological Research Scientist). Integrity of the isolate will be maintained by transfer of a 10 µL loop of culture into a labelled vial containing glycerol (one organism per vial) which will be stored at –70°C to –80°C before transfer to UQCCR. A commercial courier company specialising in transport of clinical samples on dry ice will collect the microbiological isolates from each site and deliver to Central Bioanalysis Laboratory at UQCCR (Brisbane, Australia) for microbiological analysis. Once received, the MIC of the organism will be determined by broth microdilution (BMD); as the gold standard and to provide maximum accuracy for antibacterial susceptibility testing and E-test® (to provide generalisability for an alternative susceptibility testing method which is more commonly used in clinical laboratories). Our approach will provide maximum accuracy for defining the β-lactam antibacterial concentration to MIC ratio from which achievement of the PK-PD exposure can be preferentially determined, both for BMD and E-test® methods of antibacterial susceptibility testing. When more than one causative organism has been identified, MICs will be determined for each causative organism. The highest MIC will be imputed into beta-lactam antibiotic exposure calculations. Where an additional antibiotic is prescribed for a pathogen which is non-susceptible to piperacillin-tazobactam or meropenem, the highest MIC of the other pathogen/s will be used (e.g. vancomycin co-prescribed with meropenem for a patient with cultures of both methicillin-resistant *S. aureus* and *P. aeruginosa*, the *P. aeruginosa* MIC would be used for defining PK-PD exposure for meropenem).

For culture negative patients: median MIC values for piperacillin-tazobactam and meropenem based on culture-positive patient data will be used (see 'Additional analyses' in

Appendix 1 for further detail). Additionally, analyses will be undertaken whereby MIC values in culture-negative patients will be based on MIC clinical breakpoints based on *Pseudomonas aeruginosa* susceptibility (i.e. 16 mg/L for piperacillin-tazobactam and 2 mg/L for meropenem).

Identification of beta-lactam antibiotic resistance genes

Whole-genome sequencing will be performed on isolates of causative organisms to determine sequence types and characterise beta-lactamase genes. Genomic DNA will be extracted using UltraClean DNA isolation kits (MoBio, Australia) and QIAmp DNA mini kits (Qiagen, Australia) and quantified by spectrophotometry (NanoDrop, ThermoFisher) and fluorometry (Qubit, ThermoFisher). Paired-end DNA libraries will be prepared using Nextera kits (Illumina, Australia). Whole-genome sequencing will be performed at the Central Bioanalysis Laboratory at UQCCR (Brisbane, Australia) by a Microbiological Research Scientist, using Illumina HiSeq (100 bp paired end) and MiSeq (300 bp paired end), as well as Illumina NextSeq (150 bp paired end) at the Forensic and Scientific Services Laboratory. Strains are also checked for contamination using Kraken (v1.1). Antibacterial resistance genes will be detected using assemblies and Abricate (v0.8) against the ResFinder and ARG-ANNOT databases. Resistance genes are determined as being present only when 100% coverage of the gene sequence is present.

Individual genes will be reviewed and characterised by their corresponding beta-lactamase enzymes and distinctive substrates using the Bush-Jacoby-Medeiros classification scheme. Isolates will be screened for: (a) beta-lactamase genes grouped as ESBL, plasmid-mediated ampC, and narrow-spectrum OXA; (b) Group 2b enzymes such as TEM-1 and SHV-1; and (c) carbapenemase genes.

6. Withdrawal from the PK-PD sub-study

The patient or their substitute decision maker may withdraw consent for participation in the BLING III study and/or the BLING III PK-PD sub-study at any time. If consent for use of previously collected blood samples is withdrawn or refused, then the samples will be destroyed and no anlysis performed.

7. Safety considerations

No additional risks are anticipated with participation in the BLING III PK-PD sub-study. Participation in the BLING III PK-PD sub-study will involve three blood samples (3-5 mL of blood per sample) taken either during one dosing interval (intermittent infusion) or over an 8-hour period (continuous infusion). Samples will be in addition to, and may occur more frequently than as per standard clinical care. However, samples will be collected via existing arterial catheters placed as part of standard clinical care. There should be no additional discomfort for patients participating in the PK-PD sub-study.

8. Data analysis methods

8.1. Sample size

This is an opportunistic exploratory study with an aim to recruit 300 culture-positive sepsis patients in the BLING III PK-PD sub-study. Due to study design, culture negative patients will also be included in additional analyses.

8.2. Statistical analysis plan

See Appendix 1 (below).

9. Data collection

The PK-PD sub-study will require collection of data pertaining to the timing details of blood sample collection and causative organism MIC, if available. It will not require any extra data demographic, or outcome collection above that already specified in the BLING III Study. Patient demographics, randomisation allocation and outcome measures required for data analysis will be obtained from the routinely collected BLING III data. Plasma antibiotic concentration data will be supplied by the Central Bioanalysis Laboratory at UQCCR using a password-protected data file.

10. Quality control and quality assurance

All centrifuged plasma samples will be stored at -70°C to -80°C until transferred to the Antimicrobial Optimisation Group, UQ Centre of Clinical Research at The University of Queensland for assay of unbound antibiotic concentrations. Unbound concentrations of piperacillin and meropenem in the biological samples will be determined by chromatographic methods (HPLC and LC-MS/MS) that are validated and conducted in accordance with the Food and Drug Administration's guidance for industry on bioanalysis.

11. Ethical considerations

The BLING III PK-PD sub-study will be conducted in accordance with ethical principles outlined as per the main BLING III study protocol (refer to section 10. 'Ethics and dissemination').

The protocol, as a sub-study of the BLING III Study, will be reviewed by the relevant Human Research Ethics Committee/Instiutional Review Board for each participating site, and where possible, under a single ethical review of multicentre trial. Approval from local research governance will be sought as required. Documentation of approval of the protocol and consent documents will be provided to the coordinating centre before the study can begin at any site. Consent will be obtained for all patients participating in the BLING III PK-PD sub-study and a tick box on the BLING III consent form will indicate the patients willingness or not to participate in the PK-PD sub-study.

12. Publication policy

The BLING III PK-PD sub-study will be conducted under the name 'BLING III PK-PD Sub-study'. Central project coordination and data management will be provided by the Antimicrobial Optimisation Group, UQCCR at the University of Queensland.

Authorship of publications arising from the sub-study will be consistent with current ANZICS Clinical Trials Group policies with full credit assigned to all collaborating investigators, research

coordinators and institutions. Responsibility for the content of manuscripts will rest with the BLING III PK-PD Sub-study Steering Committee.

It is expected that findings will be disseminated via publication in high-quality peer reviewed journals in the medical literature. Study findings will also be presented at regional, national, and international intensive care conferences.

Funding bodies will be acknowledged in all publications.

13. Proposed project timeline

The BLING III PK-PD sub-study will be conducted over a two- to three-year period with project milestones aligned with the main BLING III study.

14. References

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15. Appendix 1

Statistical Analysis Plan

The Beta-lactam Infusion Group (BLING) III Pharmacokinetic-Pharmacodynamic (PK-PD) sub-study is an embedded observational sub-study of the BLING III randomised controlled trial (RCT) conducted in patients with sepsis. This document describes the pre-specified statistical analysis plan finalised by the BLING III PK-PD sub-study statistician (MC) and lead investigators (JAR, MOC, JD and JL) and approved by the BLING III PK-PD sub-study Steering Committee before completion of patient enrolment and database lock.

Data analysis

The following data of PK-PD sub-study participants will be presented by treatment arm: baseline characteristics (**Table 1**), APACHE III diagnosis (**Table 2**), primary site of infection (**Table 3**), infective organisms identified in culture-positive patients (**Table 4**) and associations between beta-lactam antibiotic exposure and outcomes (**Tables 5** and **6**).

Analysis for Objective 1

According to randomisation status, a stacked bar chart showing various antibiotic exposure ranges will be presented for each treatment arm separately for definitions a), b) and c) (**Figure 2**).

Analysis for Objectives 2a) and 2b)

Multivariable fractional polynomial logistic regression models will be used. Each model will include main effects for: beta-lactam antibiotic (meropenem vs. piperacillin-tazobactam, severity of illness [APACHE II score as a continuous variable], lung infection and non-fermenting Gram-negative bacilli). It will be assumed that after adjustment for covariates, exposure will relate to outcome in a flexible manner for each infusion method (continuous vs. intermittent), as described below. This relationship will be shown graphically with a 95% confidence interval (CI) band (**Figures 3a** and **3b**).

Adjustment for extreme values:

To limit the effect of outliers, an assumption will be made that for an unbound beta-lactam antibiotic concentration to MIC ratio of ≤ 0.1, outcome does not vary with exposure. The same assumption will be made for an unbound beta-lactam antibiotic concentration to MIC ratio of ≥ 50. Therefore, in the analysis, exposure values < 0.1 will be replaced with 0.1 and exposure values > 50 will be replaced with 50 (variable name: “trimmedexposure”). A log transformed positive exposure variable (tr_exposure) with no extremely low values will then be created as follows: tr_exposure = log(trimmedexposure) - log(0.07). This exposure variable will be modelled, and the best second-degree fractional polynomial model selected for each infusion method (from the default powers in Stata v17).

Additional analyses

1. For Main Objectives 2a) and 2b), the association between beta-lactam exposure and outcomes will be explored within each of the following sub-groups of culture-positive patients#:
   a) (i) Lung infection, and (ii) non-lung infection
   b) (i) Piperacillin-tazobactam, and (ii) meropenem
   c) Severity of illness: (i) APACHE II score < 25, and (ii) APACHE II score ≥ 25[1]
   d) (i) Non-fermenting Gram-negative bacilli, and (ii) other causative organisms

2. Analyses where 'beta-lactam antibiotic exposure' is defined as 24-hour area under the concentration-time curve of unbound beta-lactam antibiotic to MIC ratio ($fAUC_{0-24}$/MIC) will be undertaken for Main Objectives 2a) to 2c). AUC_{0-24} will be estimated by the non-parametric population pharmacokinetic software, Pmetrics.

3. Analyses of culture-positive# vs. culture-negative patients^ will be undertaken for Main Objectives 2a) to 2c).

4. Analyses for all recruited PK-PD sub-study patients (i.e. both culture-positive# and culture-negative patients^) will be undertaken for Main Objectives 2a) to 2c).

5. Additionally, analyses will be undertaken whereby MIC values in culture-negative patients will be based on MIC clinical breakpoints based on *Pseudomonas aeruginosa* susceptibility (i.e. 16 mg/L for piperacillin-tazobactam and 2 mg/L for meropenem based on the European Committee on Antimicrobial Susceptibility Testing's MIC data)[2].

6. *In silico* resistance gene profiling to characterise the resistance gene compliment and resistance associated mutations in the causative organism. The association between predicted non-susceptibility profiles (MDR, XDR, PDR) in organisms resistant to beta-lactam antibiotics and treatment outcomes of the main study will be explored.

7. A comparison between MIC values measured using BMD and E-test® will be undertaken per study antibiotic.

#For culture-positive patients, MIC values of causative organisms will be based on broth microdilution (BMD) measurements.

^For culture-negative patients, median MIC values for piperacillin-tazobactam and meropenem based on culture-positive patient data will be used (if no organism has been grown, then the median MIC value among the culture-positive dataset will be used). If only the Gram stain is available, then the median MIC value among identified causative organisms with the corresponding Gram stain in the culture-positive dataset will be used; if species specific without a measured MIC, then the median value among corresponding isolated species in the same geographical location in the culture-positive dataset will be used, where possible.

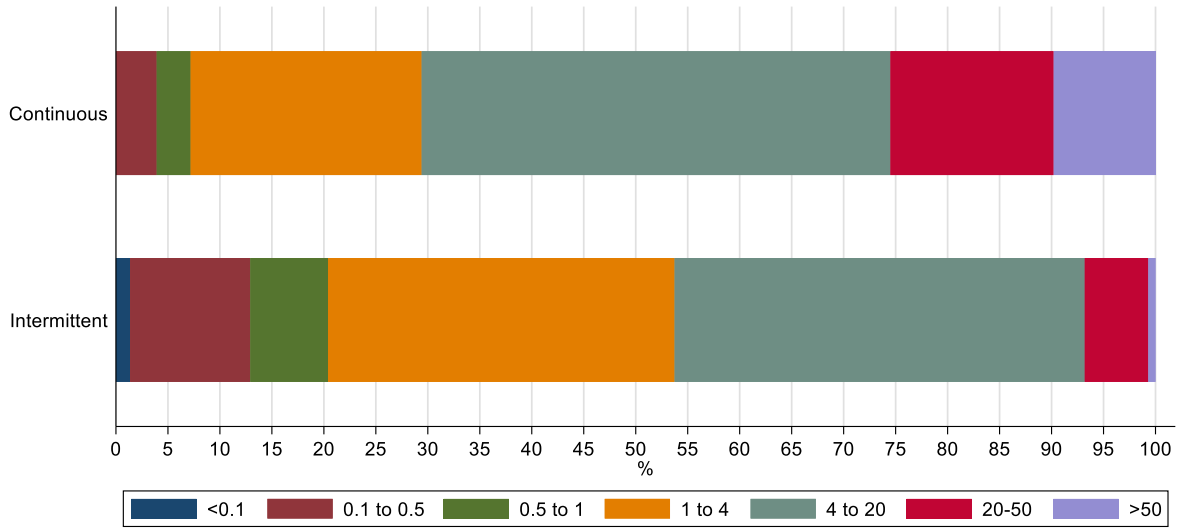
Definition of statistical significance

A two-sided p-value < 0.05 will be considered evidence of a significant association. There will be no multiplicity adjustments made, although the number of analyses undertaken will be considered when interpreting findings.

Proposed Figures and Tables

Figure 2. Based on randomisation status, a stacked bar chart will show proportion of patients in various antibiotic exposure ranges based on unbound beta-lactam antibiotic concentrations* above the MIC (expressed as ratios):

Example:



*For patients randomised to the continuous infusion arm, the mean value of the 3 steady-state concentrations will be used; for patients randomised to the intermittent infusion arm, the measured 'trough' concentration will be used.

Figure 3a. Continuous exposure vs Day 90 mortality

Example:

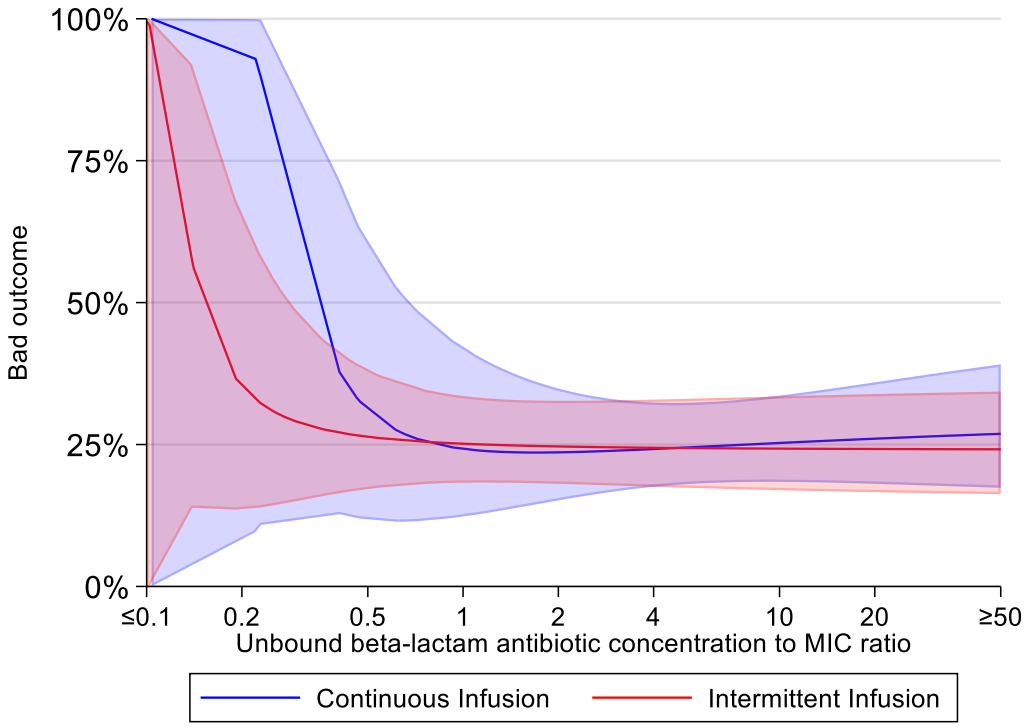


Figure 3b. Continuous exposure vs failure of clinical cure at Day 14
(A graph similar to Figure 3a. will be generated)

Table 1. Baseline characteristics

Characteristic	**Continuous Infusion**			**Intermittent Infusion**			**Total**
	All (n = XXX)	**Culture +VE** (n = XXX)	**Culture -VE** (n = XXX)	**All** (n = XXX)	**Culture +VE** (n = XXX)	**Culture -VE** (n = XXX)	(n = XXX)
Age (years)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Sex, female	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Weight (kg)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Height (cm)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Source of ICU admission							
Accident and Emergency Department	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Hospital floor (i.e. wards)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Transfer from another ICU	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)

Transfer from another hospital	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
From OT following EMERGENCY surgery	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
From OT following ELECTIVE surgery	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Time since ICU admission (hours)	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median
APACHE II score	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median
Lowest PaO_2/FiO_2 ratio in the 24 hours prior to randomisation	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median

Highest creatinine (µmol/L)	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median
Highest bilirubin (µmol/L)	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median
Lowest platelet count ($x10^9$/L)	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median
Lowest MAP in 24 hours prior to randomisation (mmHg)	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median
Worst Glasgow Coma Score (non-sedated) in the 24 hours prior to randomisation	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median	Mean or Median
Receiving inotropes/va	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)

sopressors in the 24 hours prior to randomisation							
Receiving antibiotics in the 24 hours prior to randomisation[1]	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)

[1]Other than piperacillin-tazobactam or meropenem.
APACHE = Acute Physiology and Chronic Health Evaluation. ICU = intensive care unit. MAP = mean arterial pressure. OT = operating theatre. SD = standard deviation.

Table 2. APACHE III diagnosis

Diagnosis	**Continuous Infusion**			**Intermittent Infusion**			**Total**
	All (n = XXX)	**Culture +VE** (n = XXX)	**Culture -VE** (n = XXX)	**All** (n = XXX)	**Culture +VE** (n = XXX)	**Culture -VE** (n = XXX)	(n = XXX)
Non-operative diagnoses	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Cardiovascular	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Respiratory	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Gastrointestinal	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Neurological	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Sepsis	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Trauma	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Metabolic	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Haematological	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Renal/genitourinary	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Musculoskeletal/skin	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Other	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)

Operative diagnoses	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Cardiovascular	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Respiratory	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Gastrointestinal	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Neurological	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Trauma	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Metabolic	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Haematological	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Renal/genitourinary	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Gynaecological	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Musculoskeletal/skin	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)

Table 3. Primary site of infection

Primary site of infection	Continuous Infusion			Intermittent Infusion			Total
	All (n = XXX)	Culture +VE (n = XXX)	Culture -VE (n = XXX)	All (n = XXX)	Culture +VE (n = XXX)	Culture -VE (n = XXX)	(n = XXX)
Pulmonary	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Intra-abdominal	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Blood	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Skin	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Urinary	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Intravenous catheter	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Central nervous system	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Gut	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Endocarditis	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Other	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)

Table 4. Infective organisms identified in culture-positive patients

Organism	**Continuous Infusion** (n = XXX)	**Intermittent Infusion** (n = XXX)	**Total**
Gram positive bacteria	n (%)	n (%)	n (%)
Methicillin-susceptible *Staphylococcus aureus*	n (%)	n (%)	n (%)
Methicillin-Resistant *Staphylococcus aureus*	n (%)	n (%)	n (%)
Streptococcus pneumoniae	n (%)	n (%)	n (%)
Beta-haemolytic streptococci (Group A, B or C)	n (%)	n (%)	n (%)
Viridans group Streptococci	n (%)	n (%)	n (%)
Enterococcus species	n (%)	n (%)	n (%)
Coagulase-negative staphylococci	n (%)	n (%)	n (%)
Nocardia species	n (%)	n (%)	n (%)
Gram negative bacteria	n (%)	n (%)	n (%)
Pseudomonas species	n (%)	n (%)	n (%)

Burkholderia species	n (%)	n (%)	n (%)
Haemophilus species	n (%)	n (%)	n (%)
Acinetobacter species	n (%)	n (%)	n (%)
Klebsiella pneumoniae	n (%)	n (%)	n (%)
Klebsiella aerogenes	n (%)	n (%)	n (%)
Enterobacter species	n (%)	n (%)	n (%)
Escherichia species	n (%)	n (%)	n (%)
Serratia species	n (%)	n (%)	n (%)
Bacteroides species	n (%)	n (%)	n (%)
Neisseria meningitidis	n (%)	n (%)	n (%)
Neisseria gonorrhoeae	n (%)	n (%)	n (%)
Campylobacter species	n (%)	n (%)	n (%)
Citrobacter species	n (%)	n (%)	n (%)
Proteus species	n (%)	n (%)	n (%)
Stenotrophomonas maltophilia	n (%)	n (%)	n (%)
Other	n (%)	n (%)	n (%)

Mycobacterium species	n (%)	n (%)	n (%)
Mixed anaerobes	n (%)	n (%)	n (%)
Other	n (%)	n (%)	n (%)

As there may be more than 1 infective organism per participant, percentages may not add up to 100%.

Table 5. Unadjusted associations between beta-lactam antibiotic exposure ranges (based on unbound concentrations* above the MIC) and patient outcomes in culture-positive patients

Outcome and group	Beta-lactam antibiotic exposure						
	<0.1	0.1 to 0.5	0.5 to 1	1 to 4	4 to 20	20 to 50	>50
All-cause patient mortality at Day 90							
Continuous Infusion	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)
Intermittent Infusion	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)
Total	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)
Failure of clinical cure at Day 14							
Continuous Infusion	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)
Intermittent Infusion	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)
Total	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)

*For patients randomised to the continuous infusion arm, the mean value of the 3 steady-state concentrations will be used; for patients randomised to the intermittent infusion arm, the measured 'trough' concentration will be used.

Table 6. Unadjusted associations between beta-lactam antibiotic exposure ranges (based on unbound concentrations* above the MIC) and antimicrobial resistance outcomes in culture-positive patients

Outcome and group	**Beta-lactam antibiotic exposure**						
	<0.1	0.1 to 0.5	0.5 to 1	1 to 4	4 to 20	20 to 50	>50
New acquisition, colonisation or infection with a multi-resistant organism or *Clostridium difficile* associated diarrhoea up to Day 14							
Continuous Infusion	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)
Intermittent Infusion	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)
Total	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)	n/N (XX %)

*For patients randomised to the continuous infusion arm, the mean value of the 3 steady-state concentrations will be used; for patients randomised to the intermittent infusion arm, the measured 'trough' concentration will be used.

References

1. Billot L, Lipman J, Brett SJ, De Waele JJ, Cotta MO, Davis JS, Finfer S, Hammond N, Knowles S, McGuinness S *et al*: **Statistical analysis plan for the BLING III study: a phase 3 multicentre randomised controlled trial of continuous versus intermittent beta-lactam antibiotic infusion in critically ill patients with sepsis**. *Critical Care and Resuscitation* 2021, **23**(3):273-284.
2. European Committee on Antimicrobial Susceptibility Testing Steering C: **EUCAST Technical Note on linezolid**. *Clin Microbiol Infect* 2006, **12**(12):1243-1245.