Supplemental Appendix for the Protocol summary and statistical analysis plan for the Selective Decontamination of the Digestive Tract in Intensive Care patients cross-over, cluster randomised controlled trial. (SuDDICU).

The SuDDICU Investigators

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1 Contents

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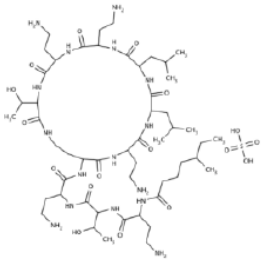
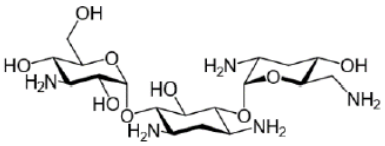
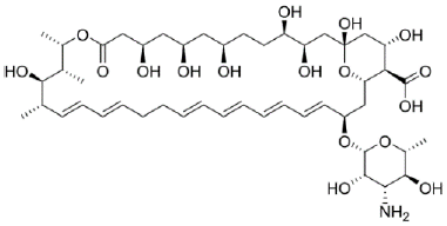
3 SuDDICU Study Drug Preparations

3.1 Composition and characteristics of SDD paste

International nomenclature	The paste is a semi-solid dosage form containing finely dispersed solids, with a stiff consistency, and is intended for topical application. Active pharmaceutical ingredients: 1. Colistin sulphate 2. Tobramycin (as sulphate) 3. Nystatin
Sponsor name	The George Institute for Global Health

Chemical abstract service numbers	Colistin sulphate: 1264-72-8 Tobramycin: 49842-07-01 Nystatin:1400-61-9
Chemical structure	Colistin Sulphate  Tobramycin  Nystatin 
Molecular formula	Colistin sulphate: $2(C_{52}H_{98}N_{16}O_{13}).5(H_2SO4)$ Tobramycin: $C_{18}H_{37}N_5O_9$ Nystatin:$C_{47}H_{75}NO_{17}$
Molecular weight	Colistin sulphate: 2801 g/mol Tobramycin: 467.5 g/mol Nystatin: 926.1 g/mol
Description	SDD paste is a mixture of antimicrobial powders – colistin sulphate, tobramycin sulphate and nystatin - with mineral oil light, petrolatum white and methocel E4M premium in an oral syringe.
Odour	Not applicable
Solubility	Not applicable
Properties	A smooth, yellow paste of uniform consitency

3.2 Composition and characteristics of SDD suspension

International nomenclature	The suspension is a liquid preparation containing drug substances and consisting of solid particles dispersed throughout a liquid phase in which the particles are present in excess of the solubility. The suspensions is supplied as a solid mixture intended for constitution before use with purified/distilled water.
Sponsor name	As per SDD paste

Chemical abstract service numbers	As per SDD paste
Chemical structure	As per SDD paste
Molecular formula	As per SDD paste
Molecular weight	As per SDD paste
Description	SDD powder for suspension is a blend of antimicrobial powders - colistin sulphate, tobramycin sulphate and nystatin - with a suspending agent (Syrspend pH 4 dry), a preservative (potassium sorbate) and citric acid monohydrate in a bottle. Before use, each bottle is reconstituted with purified/distilled water to the required volume and then shaken.
Odour	Not applicable
Solubility	Part of the powder will be dissolved and part suspended
Properties	After suspending: an opaque, straw coloured liquid

3.3 Analysis and characterisation of study drug

The identity of each container within each batch of raw material destined for study drug is confirmed by full pharmacopoeia analysis by validated high-performance liquid chromatography (HPLC) and microbiology methodology (in accordance with Annex 8 of the Pharmaceutical Inspection Co-operation Scheme Guide to GMP Manufacturing).

Homogeneity and potency of each finished product batch of investigational product is confirmed via similar methodology.

Impurities will be assessed by HPLC and other analytical methods during on-going stability studies.

3.4 Stability

The manufacturer stability testing program is focused on determining allowable shelf-life and ensuring the product maintains compliance with the claims made on the SDD paste and SDD suspension labels when exposed to routine usage and storage conditions.

Stability testing uses HPLC and microbiology analyses to ensure that study drugs remains homogenous, with potency within specifications and exhibits normally expected physical characteristics, when stored for defined durations, at the ranges of temperature and subjected to reasonably expected temperature or other excursions.

3.5 Container and packaging

Each SDD study drug kit contains, twenty 1ml BD oral syringes SDD paste, a single sealed PET bottle of SDD suspension (powder form) and a syringe filling adaptor and shipped under refrigerated/cold chain 2-8˚C (35°- 46°F) conditions to the clinical trial site.

3.6 Storage and handling

The SDD study drug kits are to be stored at 2–8°C in a secure area. When SDD powder is reconstituted into suspension the drug kit (paste and suspension) can be stored in a temperature-controlled room of less than 25 °C for up to 1 week.

Each pre-filled syringe is to be discarded after use (i.e. single use only).

The PET bottle is to be returned to the SDD study drug kit after each dose. Hospitals will need to provide their own 10mL oral/enteral syringe to access and measure each 10mL dose from the PET bottle (which contains a syringe filling adaptor).

4 Canadian Consent Process

In Canada, the laws of the jurisdiction do not allow a waiver of consent for this trial, so a delayed model consent by the substitute decision maker will be sought as soon as possible for after intervention commencement in all patients recruited into the intervention arm of the trial.

Informed consent is a process that is initiated prior to the individual agreeing to participate in the RCT and continues throughout the study participation. Given that the study intervention is time sensitive and the participant does not have capacity to provide consent, deferred consent will be used for participating sites in Canada. Deferred consent will be sought from the substitute decision maker (SDM) after the participant has been enrolled into the study and has started to receive the intervention.

Participants in both the SDD and control arms will receive an information sheet after being enrolled into the study. Initial data collection will be conducted under a waiver of consent. A member of the study staff will deliver an Information Sheet informing the SDM of the participant's enrolment into the study. The information sheet will include contact

information for the SDM to reach a member of the study staff if they have any questions or do not want to participate in ongoing data collection for the study.

For participants in the SDD arm, deferred consent will be sought once an SDM has been identified. Continuous attempts (see Consent Process) will be made to approach the SDM to provide them with an informed consent form (ICF) describing in detail the study procedures and associated risks. A member of the study staff will explain the research study to the SDM and answer any questions that may arise. The SDM will sign the ICF to allow the participant to continue with the treatment. The original ICF in its entirety will be maintained by the site, and a copy of the signed ICF will be provided to the SDM. The rights and welfare of the participants will be protected by emphasizing to the SDM that the quality of the participant's clinical care will not be adversely affected if they decline to participate in this study.

If attempts to contact the SDM continue to be unsuccessful and the participant has been discharged from the ICU, or if the participant dies within 48 hours of being enrolled into the study information recorded for the participant, along with outcome data, will be used for the study.

Consent Process:

1. As patients are enrolled into the study, an information sheet informing SDM of patient's enrolment into the study and collection of their study data will be provided.
    a. This information sheet will also provide the SDM with information on how they can contact a member of the study team to discuss consent.
2. A member of the study team will review the patient enrolment log to see which patients have been enrolled/started treatment and therefore requires consent.
3. A member of the study team, designated on the task delegation log to obtain consent, will approach the patient's nurse to confirm if the information sheet was provided to the patient and if an SDM has been identified to be approached for consent. Alternatively, the study team member will check the patient records to identify the SDM.
4. The study team member will conduct regular bedside checks to find and gain consent from the SDM.
5. The study team member will make a minimum of 3 phone calls to the SDM if they cannot be identified at the bedside.
6. The study team member will document all attempts to obtain consent.

5 Study timelines

5.1 Australia (actual timelines)

Date	Milestone
July 2015-December 2016	Start-up phase, including site feasibility and selection, and agreement by sites to follow study protocols (intensive care and infectious diseases
August 2016	Protocol finalised
August-October 2016	Case report form / study documents finalised
November 2016	Ethics approvals and regulatory requirements met

May 2017	3-month pre-trial data collection period commenced (surveillance ecology); site randomisation and initiation
March 2018	Completion of study drug acquisition and commencement of distribution to sites
April 2018	First trial recruitment period (SDD or control) and 3-month surveillance ecology data collection period during recruitment period commenced
April 2019	3-month inter-period data collection (surveillance ecology) and site initiation for crossover commenced
July 2020	Second trial recruitment period (control or SDD after crossover) and 3-month surveillance ecology data collection period during recruitment period commenced
August 2020	3-month post-trial data collection period (surveillance ecology) commenced
August 2021	Completion of post-trial collection period (surveillance ecology) final site
October-December 2021	Database lock, analysis and publication of primary (mortality) and secondary (ecology) endpoints of Australian dataset
June-December 2023	Merger with Canada/UK database, analysis and publication of primary (mortality) and secondary (ecology) endpoints of combined dataset, subject to funding

5.2 Canada / UK (actual and indicative timelines)

Date	Milestone
July 2015-December 2018	Start-up phase, including site feasibility and selection, and agreement by sites to follow study protocols (intensive care and infectious diseases
August 2016	Protocol finalised
May 2019	Ethics approvals and regulatory requirements met
May 2019	Case report form / study documents finalised
May 2019	3-month pre-trial data collection period commenced (surveillance ecology); site randomisation and initiation
July 2019	Completion of Research Collaborative Agreements
August 2019	Completion of study drug acquisition and commencement of distribution to sites
September 2019	First trial recruitment period (SDD or control) and 3-month surveillance ecology data collection period during recruitment period commenced
March 2020	Suspension of recruitment due to COVID-19 at all sites
May 2020	Potential restart after COVID-19 at selected sites
March 2021	Start of 3-month inter-period data collection (surveillance ecology) and site initiation for crossover commenced
June 2021	Start of second trial recruitment period (control or SDD after crossover) and 3-month surveillance ecology data collection period during recruitment period

July 2022	Start of 3-month post-trial data collection period (surveillance ecology)
December 2022	Potential completion of post-trial collection period (surveillance ecology) final site
June-December 2023	Database lock, analysis and publication of Canada/UK dataset. This will be followed by merger with Australian database, analysis and publication of combined primary (mortality) and secondary (ecology) endpoints of combined dataset, subject to funding

6 Analysis Models

6.1 Explanation and justification of revised power calculations

The original intention in 2009 was to design and conduct a parallel-group c-RCT that would recruit a study population of 22 500 patients from 100 international sites in Canada, the United Kingdom, Australia and New Zealand.

The projected study population at that time was informed by metrics from completed large-scale pragmatic RCTs done by our group at that time and pilot data from selected sites in Australia, New Zealand, UK and Canada.

For a number of financial, logistical and operational reasons that are summarised in the paper, the study design was modified to a crossover c-RCT in 2015 that would be conducted solely in 19 Australian sites with a projected study population of 12000-14000 patients.

An additional 10 sites recruiting 6000-10000 patients from Canada/UK were subsequently added to the trial.

Given the reduced numbers of clusters and patient from the original design, adjustment to the power calculations were made considering feasible recruitment projections to detect a biologically plausible effect size. These align with the original design with respect to key outcomes and indices of internal validity.

The decision to split the trial into two studies in 2020 to report the Australian and the Canada/UK cohorts was done primarily due to additional delays in commencing recruitment Canada and the UK. These delays have been compounded by the impact of COVID-19 in Canada and the UK.

Given the projected gap in timelines between Australia and Canada/UK that exceeded 18 months – 2 years, we considered that there was an ethical and scientific imperative to present the SuDDICU x-cRCT in 3 stages – the Australia cohort that will be completed by the end of 2021 with over 14 000 patients , the combined Canada/UK cohort with 6 000 and a combined analysis of 20 000 patients from both regions.

The table below presents power calculations for a range of plausible absolute reductions in mortality. This is done for 10, 20 and 30 clusters to represent the 10 sites from Canada/UK, the 19 sites from Australia and the total.

Given that we expect the final cluster size to be between 150 and 200 patients per period, we have done the calculations for both these numbers.

Cluster size per period	Number of clusters	Absolute difference 3.0%	3.5%	4.0%	4.5%	5.0%
150	30	67%	80%	90%	>90%	>90%
	20	50%	64%	76%	85%	>90%
	10	<50%	<50%	<50%	58%	67%
200	30	74%	86%	>90%	>90%	>90%
	20	56%	70%	81%	89%	>90%
	10	<50%	<50%	52%	62%	72%

Common assumptions:
Mortality in the control arm – 29%
ICC=0.01, IPC=0.05
2-sided alpha=5%

The projected power calculations for each cohort align with the original study population.

The revised design has sufficient statistical power to detect a plausible effect size for the primary outcome (hospital mortality) for each regional cohort.

6.2 Analysis model of the primary outcome

The primary outcome is hospital mortality analysed as the proportion of patients who died during the index hospital admission (up to day 90).

The primary statistical hypotheses are as follows:

- Null hypothesis: no difference in the proportion of patients who died during the index admission between those randomised to SDD (p1) and Usual Care (p0) i.e. p1 = p0
- Alternative hypothesis (2-sided): p1 ≠ p0, with the expectation that the proportion would be lower in those randomised to SDD.

The primary intervention effect will be estimated as the odds ratio (OR) of mortality between the SDD arm and the control arm obtained from a hierarchical logistic model (defined below). Other analyses of hospital mortality will include a survival analysis of time to death obtained via a Cox model.

Main analysis

The primary analysis of hospital mortality will be conducted using a hierarchical logistic regression which allows us to directly model all levels of clustering. To account for clustering of participants within sites as well as the cross-over design, the model will include both a random cluster effect and a random cluster-period effect (see "random-random" model (equation 11) in Morgan et al[1]). The corresponding logistic model can be written as follows:

$$\text{logit}(P\{Y_{ijk}=1\}) = \mu + \beta_\tau \tau_{ij} + \beta_\pi \pi_j + c_i + p_{ij} + e_{ijk} \quad (1)$$

where:

Y_{ijk} denotes the outcome (0 or 1) of subject k (k=1,…,m_{ij}) in period j (j=1,2) within cluster i (i=1,…, N) where m_{ij} indicates the number of subjects in period j within cluster i

μ is the overall intercept

τ_{ij} is a dummy variable indicating group allocation during period j for cluster i with τ_{ij} = 1 for intervention and τ_{ij} = 0 for control

β_τ is the parameter of interest estimating the fixed effect of the SDD intervention and where exp(β_τ) is the odds ratio (OR)

π_j is a dummy variable indicating the period with with π_j = 1 for the second period and $\pi_j = 0$ for the first period

β_π is a fixed period effect

c_i is a random cluster effect with $c_i \sim N(0,\sigma_c^2)$

p_{ij} is a random cluster-period effect with $p_{ij} \sim N(0,\sigma_p^2)$

e_{ijk} are individual-level errors following a binomial distribution

The effect of the intervention will be presented as the OR of death and its 95% confidence interval (CI) using the control arm as the reference (i.e. where an OR greater than unity corresponds to an increase in hospital mortality in the SDD arm compared to the control arm). The Kenward-Rodger correction[2] will be applied to estimate the number of degrees of freedom as it has been shown to improve estimation with a small number of clusters.[3] Based on this model, two intra-cluster correlation coefficients (ICC) will be estimated, the first one (ρ_c) corresponding to the correlation between two outcomes from the same cluster-period, the second one (ρ_p) corresponding to the additional correlation between two outcomes from the same cluster-period compared to two outcomes from the same cluster but different periods.

The two ICCs, ρ_c and ρ_p, will be estimated as follow:

$$\rho_c = \{ \sigma^2_c + \sigma^2_p \} / \{ \sigma^2_c + \sigma^2_p + \pi^2/3\}$$

$$\rho_P = \{ \sigma^2_p \} / \{ \sigma^2_c + \sigma^2_p + \pi^2/3\}$$

where:

σ^2_c is the variance between the cluster means

σ^2_p is the variance between the cluster-period means conditional on the cluster mean

π is the mathematical constant (3.1415…).

The inter-period correlation (IPC), that is the correlation between two outcomes from the same cluster but different periods will be estimated as:

$$\eta = \{ \sigma^2_c \} / \{ \sigma^2_c + \sigma^2_p + \pi^2/3\}$$

In our sample size calculations, we assumed values of 0.01 for ρ_c and 0.005 for η (ρ_P = 0.005). With 19 sites recruited in Australia and a total of about 30 sites, we expect the random-random model described above to provide reasonable control of the type-I error rate; however, to assess the robustness to potential departures from our assumptions, especially around the two ICCs, we will be conducting the following sensitivity analyses:

1. Re-running the same model as model (1) but without using the Kenward-Roger correction

2. Linear regression on summary measures: we will fit a linear regression to model the proportion of events in each cluster-period using the following model (see equation 13 in Morgan et al[1]:

$$P_{ij} = \alpha + \beta_\tau \tau_{ij} + \beta_\pi \pi_j + \gamma_i + e_{ij} \quad (2)$$

where:

P_{ij} is the proportion of events in period j of cluster i (Y_{ij}/n_{ij})

α is the overall intercept

β_τ is the treatment effect corresponding to the average difference in proportions and τ_{ij} is an indicator variable for treatment of interest

β_π is a fixed period effect and π_j is an indicator variable for period

γ_i is a fixed effect of cluster

e_{ij} is a normally distributed residual error term with mean zero and variance $\sigma^2 e$

The regression will be weighted proportionally to the inverse of the binomial variance for each cluster-period with each weight calculated as $\{ n_{ij} / [p_{ij} (1 - p_{ij})] \}$ (i.e. using the third set of weights in Morgan et al[1].

Treatment of missing data

The primary analysis will use all available data with no imputation. In addition, we will perform sensitivity analyses to assess the potential impact of missing data on the study conclusions. We will start by rerunning Model (1) under the two following most extreme scenarios:

1. Extreme scenario 1 ('worst' case): all patients with missing data assumed as dead in the SDD arm and alive in the control arm
2. Extreme scenario 2 ('best' case): all patients with missing data assumed as alive in the SDD arm and dead in the control arm

We will check whether the study conclusion changes as indicated by the resulting p-values. In the case where both extremes result in the same statistical significance as the main analysis, no further tipping point analysis will be performed. In case the conclusions disagree, we will perform a tipping point search by identifying combinations where the level of significance switches from significant to non-significant (or vice versa) as described by Yan et al. [4]

6.3 Analysis of secondary outcomes

Secondary clinical outcomes include duration of mechanical ventilation, ICU length of stay, hospital length of stay and ICU mortality.

Duration outcomes will be analysed as the number of days alive and free of outcome (e.g. days alive and free of mechanical ventilation or days alive and out of ICU) up to Day 90. Outcomes will be analysed using a model similar to Model 1 but using a linear model (normal distribution and identity link) instead of a logistic one (binomial distribution and logit link). The effect of the intervention will be reported as the adjusted mean difference and its 95% confidence interval. Only the main version of Model 1 using the Kenward-Roger correction will be applied to these outcomes. We are not planning adjusted or subgroup analyses of these outcomes.

Mechanical ventilation is only expected to occur while in ICU. Therefore, once discharged from ICU, patients will be assumed to be free of mechanical ventilation. Similarly, once discharged from hospital, patients will be assumed to be alive up to Day 90. While in ICU, a missing daily mechanical ventilation status will be handled as follows:

1. For intermittent missing values i.e., missing values surrounded by non-missing values both before and after, we will replace the missing value with the closest (in time) non-missing value. In case the time-interval before and after are the same, the missing value will be replaced with the most pessimistic value of the two.
2. For missing values following the baseline assessment i.e. occurring on Day 1 onwards, the mechanical ventilation status at the time of randomisation will be used to guide the imputation. In case of a missing baseline value, the patient will be assumed to be free of mechanical ventilation at baseline. After imputation of the baseline value, missing values will be imputed by following Step 1 above.
3. For missing values preceding ICU discharge, the patient will be assumed to be free of mechanical ventilation on the day of discharge if discharged alive and not if she/he died within a day of ICU discharge. After imputation of the value on the day of discharge, missing values will be imputed by following Step 1 above.

As a sensitivity analysis, we will allocate zero 'free days' to patients who die by Day 90.

Time to alive discharge from index ICU and time to alive discharge from index hospital admission will be summarized using cumulative incidence functions treating mortality as a competing risk and with censoring at Day 90. Medians and quartiles of time to discharge will be obtained from the cumulative incidence functions. The effect of the intervention will be estimated as the hazard ratio (SDD divided by Usual care) and its 95% confidence interval obtained from a Cox model of the cause-specific hazard which estimates the risk of discharge in subjects who are still alive and have not yet been discharged.[5] To model potential within-site correlations due to stratification, we will use a shared-parameter frailty Cox model with a fixed effect of treatment and a random site effect.[6]

ICU mortality will be analysed in the same way as hospital mortality using Model 1.

6.4 Analysis of unit level ecology surveillance data

The study includes five unit-level ecology surveillance periods as shown on the diagram below. During these periods, data on ecology outcomes are collected on all patients admitted to the ICU for one full week during each calendar month, regardless of the patient's ventilation status.

Pre-trial	Study Period 1		Washout	Study Period 2		Post-trial
Ecology 1		Ecology 2	Ecology 3		Ecology 4	Ecology 5

Data collected during these periods will be used to evaluate the effect of the SDD intervention on the following outcomes:

- The incidence of antibiotic resistant organisms isolated from sterile or non-sterile sites
- The incidence of all new positive blood cultures

- The incidence of new *Clostridioides difficile* infections

A flow chart will include details of subjects included in the ecology surveillance in each period (pre-trial, Study Period 1, washout, Study Period 2, and post-trial). Baseline characteristics including sex, age, time since admission and APACHE score will be described together with their vital status at discharge.

Incidence of AROs, bloodstream infections and *Clostridioides difficile* infections will first be described as the number and proportion of patients within each of the five ecology periods (pre-trial, period 1, washout, period 2 and post-trial). This will be done according to the randomisation sequence, that is, separately for sites randomised to control→ SDD and sites randomised to SDD→Control.

The main hypothesis to be tested is whether rates are the same with and without the SDD intervention. This will be assessed using a non-inferiority comparison and with a non-inferiority margin set at 2%.

- *Null hypothesis*: The proportion of patients with the outcome is higher with the SDD intervention than with usual care
- *Alternative hypothesis:* The proportion of patients with the outcome with the SDD intervention is not inferior to the one with usual care

To reject the null hypothesis and 'declare' non-inferiority of SDD compared to usual care, the upper bound of the 95% confidence interval around the absolute risk difference (SDD - usual care) will need to be lower than 2%. We will analyse data from all 5 periods using linear regression to model the proportion of events in each cluster and each period as in the second sensitivity analysis of the primary outcome (see Model (2)) and using the same weighting method.

To test whether the proportion of patients with the SDD intervention is non inferior to the one with usual care, we will group periods 2 and 3 and, within each arm (SDD or usual care), will estimate the change between period 1 (baseline) and periods 2/3 combined. We will then estimate the difference (SDD – standard care) in change from baseline to assess whether the change with SDD is similar (non-inferior) to the change with usual care.

Using the same model, to further assess the effect of SDD over time including potential withdrawal effects (i.e. whether rates change after withdrawing SDD), we will compare the change between period 3 and periods 4/5 combined in units randomised to use SDD in the first intervention period.

Other exploratory tests may be conducted depending on the results of these two pre-specified comparisons.

7 References

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