NeoSep1

Statistical Analysis Plan for Part 2

An open-label randomised controlled trial comparing novel combination and currently used antibiotic regimens for the empiric treatment of neonatal sepsis with a run-in confirmatory pharmacokinetic phase

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Revision History

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Draft 0.02	W Stöhr	30-Jul-2024	First draft
Draft 0.03	W Stöhr	06-Aug-2024	Incorporating changes following discussion with Sarah Walker
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Note: This Statistical Analysis Plan covers Part 2 of the NeoSep1 trial. A separate Statistical Analysis Plan has been written for Part 1.

1 ABBREVIATIONS

Abbreviation	Expansion
AE	Adverse event
ALT	Alanine Aminotransferase
AMR	Antimicrobial resistance
AST	Aspartate transaminase
BiPAP	Bilevel Positive Airway Pressure
BUN	Blood urea nitrogen
CI	Confidence interval
CPAP	Continuous positive airways pressure
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DMC	Data Monitoring Committee
eDC	Electronic data capture
EOT	End of treatment
FBC	Full blood count
GARDP	Global Antibiotic Research and Development Partnership
HFNC	High flow nasal cannula
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	Intention-to-treat
IV	Intravenous
LFT	Liver function test
LMIC	Low- and middle-income country
MDR	Multi-Drug Resistant
MedDRA	Medical Dictionary for Regulatory Activities
MRC CTU at UCL	Medical Research Council Clinical Trials Unit at University College London
NAESS	Neonatal Adverse Event Severity Scale
NeoOBS	Neonatal observational study
PSBI	Possible serious bacterial infection
PK	Pharmacokinetics
PNA	Post-natal age
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAR	Serious adverse reaction
SD	Standard deviation
SGUL	St Georges University of London
TBV	Total blood volume
TSC	Trial Steering Committee
UCL	University College London
WBC	White blood cells
WHO	World Health Organisation

2 BACKGROUND

While there is a high burden of neonatal sepsis globally, its impact is especially marked in low- and middle-income countries (LMICs), where there are an estimated 6.9 million annual episodes of possible serious bacterial infection and 680,000 related deaths. Increasing antimicrobial resistance (AMR), including a higher prevalence of AMR in isolates from septic neonates, threatens to undermine the effectiveness of World Health Organisation (WHO) recommended antibiotic treatments in these settings. The key threat is multi drug-resistant Gram-negative bacteria, where there are very few neonatal treatment options and increasing use of meropenem, driving carbapenem resistance. Current WHO guidelines, however, continue to recommend empiric first- and second-line regimens for neonatal sepsis that have remained unchanged for nearly 20 years.

Given increasing AMR, the coverage of current WHO-recommended regimens is expected to be low in many high-burden settings, and there is a clear need to re-evaluate the guidance for empiric treatment of neonatal sepsis in the hospital setting and to provide new options for treatment of MDR neonatal sepsis that have global relevance. Relevant regimens for comparison with WHO-recommended regimens should include antibiotics with a neonatal licence and provide good coverage for globally relevant extended-spectrum beta-lactamase producing organisms. Given the lack of evidence supporting much neonatal sepsis treatment and the severity of the condition, it also important to directly compare suitable novel regimens, including off-patent drugs with a neonatal licence but not currently widely used, to currently recommended and widely used regimens.

Therefore, in NeoSep1 three groups of empiric antibiotic regimens will be investigated:

- WHO-recommended regimens: ampicillin (or benzylpenicillin, amoxicillin or cloxacillin) + gentamicin, or the third generation cephalosporins, cefotaxime or ceftriaxone
- Broad spectrum antibiotics in common use in neonatal units with licenced and/or recommended neonatal doses: piperacillin/tazobactam, piperacillin/tazobactam + amikacin, ceftazidime, ceftazidime + amikacin, meropenem
- Older off patent antibiotics which have a licenced neonatal dose but are not currently widely used globally in neonatal units: fosfomycin, flomoxef and amikacin

These older off patent antibiotics will be tested as novel dual combinations, which is fosfomycin + amikacin, flomoxef + amikacin and fosfomycin + flomoxef. Since they have been infrequently used in neonatal populations, a run-in non-randomised pharmacokinetic study of these three combinations of fosfomycin, flomoxef and amikacin has been performed to confirm plasma drug levels at the proposed doses based on dosing recommendations and other studies, as well as to collect safety data (NeoSep1 Part 1) before the start of the main randomised trial (NeoSep1 Part 2).

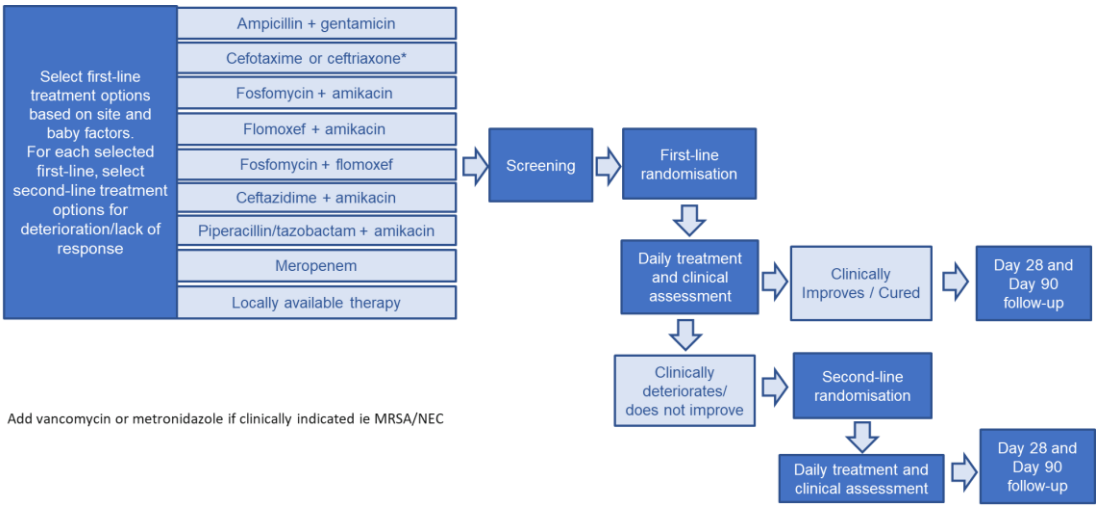
NeoSep1 Part 1 has been completed, and participants (mostly preterm neonates) had fosfomycin and flomoxef plasma concentrations similar to published literature. Although variability was observed shortly after birth, drug exposures support these doses for the larger randomised NeoSep1 Part 2 trial (Bekker on behalf of the NeoSep1 Part1 Study Team, 2024).

3 STUDY METHODS

3.1 TRIAL DESIGN

NeoSep1 Part 2 is a pragmatic clinical trial using a Personalised RAndomised Controlled Trial (PRACTical) (Walker, White et al. 2021) design comparing multiple different novel combination and currently used antibiotic regimens, including a Sequential Multiple Assignment Randomised Trial (SMART) design (Almirall, Nahum-Shani et al. 2014) to allow randomisation to second-line antibiotic treatment where indicated.

Figure 1: Trial entry, randomisation and first and second-line treatment



Note: locally available therapy is only available as second line treatment randomisation; see **section 3.5** for more details.

3.2 STUDY OBJECTIVES

3.2.1 Primary objectives

The primary objective of NeoSep1 Part 2 is to provide a ranking of eight different clinically relevant antibiotic regimens for first-line empiric and second-line (after lack of response/deterioration) treatment in terms of 28-day mortality as the primary outcome measure (see **section 6** for details). It will flexibly compare these multiple different relevant treatment regimens to enable the trial to be run in sites worldwide with very different background rates of different pathogens and resistance and different routine clinical care by randomising each participant to locally relevant antibiotic regimens agreed prior to site initiation.

This trial will also directly address the question as to the potential advantages and disadvantages of using initial broader-spectrum empiric therapy versus narrower-spectrum empiric therapy with prompt switch to broader spectrum for clinical non-response/deterioration. Specifically, neonates randomised to an empiric regimen in the trial will be closely monitored for clinical non response/deterioration, and if this occurs, they will be randomised to a second set of regimens, which will again depend on site appropriateness (particularly resistance phenotype) as well as their first regimen.

3.2.2 Secondary objectives

Secondary objectives are to provide a ranking of clinically relevant antibiotic regimens based on other efficacy and safety secondary outcomes, as well as on health economic measures and the potential selection of resistance. The trial data will provide data to inform the balance between efficacy, safety, cost and propensity for resistance selection that will influence facility-level and national decision-making about adoption of studied regimens, and potential future inclusion in WHO guidelines.

3.3 STUDY DURATION

The study duration for each participating neonate is 90 days from enrolment. Overall, Part 2 of the NeoSep1 trial is expected to take 48 months.

3.4 STUDY POPULATION

3.4.1 Inclusion criteria

1. Currently admitted to hospital
2. Aged ≤28 days (post-natal age)
3. Weight ≥1000g
4. Clinical diagnosis of a new episode of sepsis with two or more of the following clinical signs together with planned treatment with IV antibiotics
    a. Abnormal temperature (<35.5°C Or ≥38°C)
    b. Chest indrawing or increase in oxygen requirement or need for respiratory support
    c. Abdominal distension
    d. Difficulty in feeding or feeding intolerance
    e. Evidence of shock including cold peripheries
    f. Lethargy, or reduced or no spontaneous movement
    g. Cyanosis
    h. Abnormal heart rate (bradycardia <80 bpm; tachycardia >180 bpm)
    i. Convulsions
    j. Irritability

    For making the diagnosis of significant sepsis, the neonate should have no alternative primary explanation for these criteria (such as Hypoxic Ischaemic Encephalopathy, hypothermia, hypoglycemia, prematurity etc).
5. At moderate to high risk of death from this episode of sepsis, based on a neonatal sepsis severity score (NeoSep Severity Score), specifically a NeoSep Severity Score of 5 or higher at presentation for this episode of sepsis (which may be before formal screening).
6. Can receive all potential treatment options on the relevant randomisation list for this neonate at their site, ensuring randomisation is possible (see country-specific appendices)
7. IV antibiotics about to be started OR not received more than 24 hours of IV antibiotics for this episode of neonatal sepsis at the point of randomisation.
8. Parent/guardian willing and able to provide consent (written or, if their neonate is severely ill, verbal consent which must be confirmed by written consent as soon as possible and wherever possible within 48 hours after the first trial specific procedure). Verbal consent allows for administration of first-line antibiotics at no or minimal delay.

3.4.2 Exclusion criteria

1. A known serious, non-infective co-morbidity anticipated to cause death within this admission (including major congenital abnormalities anticipated to cause death within this admission other than prematurity, e.g. known large ventricular septal defect)
2. Previously enrolled in this trial
3. Current participation in any other clinical study of an Investigational Medicinal Product that is a systemic drug, unless it has received prior approval by the NeoSep1 Trial Management Group
4. Known contraindication to any of the trial antibiotics on the randomisation list for the relevant neonatal sub-population in that site (these will vary according to the antibiotics on the specific randomisation list)

3.5 RANDOMISATION

The trial will use a PRACTical design, in which each neonate is randomised only to regimens that are considered clinically acceptable for that specific site and sub-population. The design will also incorporate the use of a SMART design to allow randomisation to second-line treatment where required.

As each sub-population in each site will have a separate randomisation list, simple 1:1 randomisation between all trial treatments in each randomisation list will be used for both first-line and second-line randomisations. Whereas all neonates enrolled will be randomised to first-line treatment, not all participants will be randomised to second-line treatment; randomisation to second-line treatment will only occur if the neonate does not improve clinically or clinically deteriorates and there are two or more treatment regimen options in the relevant randomisation list for that neonate.

3.5.1 First-line treatment options

- Ampicillin (or amoxicillin or benzylpenicillin or cloxacillin or flucloxacillin) + gentamicin
- Cefotaxime or ceftriaxone
- Fosfomycin and amikacin
- Flomoxef and amikacin
- Fosfomycin and flomoxef
- Piperacillin/tazobactam and amikacin
- Ceftazidime and amikacin
- Meropenem

Neonates will be allocated to a first-line treatment option by randomisation. As part of set-up activities, each site will define which of the first-line treatment regimens listed above are clinically appropriate for specific sub-populations of neonates in each participating neonatal unit. Each neonatal unit will define the list of antibiotics that they decide is appropriate to randomise that specific population in that neonatal unit to.

3.5.2 Second-line treatment options

- Cefotaxime or ceftriaxone
- Fosfomycin and amikacin
- Flomoxef and amikacin
- Fosfomycin and flomoxef
- Piperacillin/tazobactam and amikacin
- Ceftazidime and amikacin
- Meropenem
- Locally selected therapy

Specific second-line randomisation lists will vary according to the first-line treatment the neonate received, and will reflect a broadening of antibiotic activity as well as taking into account resistance phenotypes at the site and any susceptibility testing results from a pathogen isolated from the individual neonate.
This list may include specific antibiotics or regimens used in particular sites that are not available in other sites ("locally selected therapy").

3.5.3 Examples for randomisation list choices

Below are **examples** for agreed randomisation lists for specific sub-populations of neonates at Tygerberg Hospital, one of the trial sites:

Randomisation:	First-line	
Sub-population:	Neonates with early onset sepsis without suspicion of meningitis	Neonates with late onset sepsis without suspicion of meningitis
List of regimens:	• Penicillin + Gentamicin • Fosfomycin + Amikacin • Flomoxef + Amikacin • Fosfomycin + Flomoxef	• Fosfomycin + Amikacin • Flomoxef + Amikacin • Fosfomycin + Flomoxef • Ceftazidime + Amikacin • Piperacillin/tazobactam + Amikacin • Meropenem

Randomisation:	Second-line	
Sub-population:	First-line penicillin + gentamicin & low suspicion of meningitis	First-line fosfomycin + amikacin & high suspicion of meningitis
List of regimens:	• Fosfomycin + Amikacin • Flomoxef + Amikacin • Fosfomycin + Flomoxef • Ceftazidime + Amikacin • Piperacillin/tazobactam + Amikacin • Meropenem • Locally selected therapy	• Flomoxef + Amikacin • Fosfomycin + Flomoxef • Ceftazidime + Amikacin • Meropenem • Locally selected therapy

Of note, in the description of the statistical analysis below, a particular randomisation list (i.e. set of acceptable regimens for a sub-population) will be referred to as **treatment pattern,** for each of first- and second-line randomisation. "Treatment pattern" refers to the specific randomisation list – that is, there is a treatment pattern for each participant for the first-line randomisation, and – if randomised to second-line – a separate treatment pattern for the second-line randomisation. Treatment pattern is treated as a stratifier in the primary analysis (see **section 6.1**).

3.6 OUTCOME MEASURES

The following outcomes are described as in the protocol, version 3.0. For details of their operationalisation see **section 7**.

3.6.1 Primary outcome measure

- 28-day mortality

3.6.2 Secondary outcome measure: efficacy

- Clinical status, assessed daily after randomisation through to the earlier of discharge from a trial site or Day 28 using a clinical recovery score based on data from the NeoOBS observational study (NeoSep Recovery Score)
- Additional systemic antibiotics beyond the first randomised treatment through Day 28
- Additional systemic antibiotics beyond the first randomised and second (for failure) treatment through Day 28
- Length of stay during the index hospitalisation
- Systemic antibiotic exposure (days on antibiotics) during the index hospitalisation
- 90-day mortality
- Change in C-reactive protein (CRP) to Day 3 and 7 from baseline
- Re-admission by Day 90 (all-cause)

3.6.3 Secondary outcome measure: safety

- Grade 3/4 adverse events (AEs) graded using a combined LMIC relevant adapted Division of AIDS (DAIDS) and International Neonatal Consortium Neonatal Adverse Event Severity Scale (NAESS) through Day 28
- Adverse events of any grade related to antibiotics through Day 28
- Modification (including discontinuation) of antibiotics for adverse reactions through Day 28
- Neurodevelopment as assessed by the WHO Global Scale for Early Development (GSED) package at Day 28 and Day 90

Note: serious adverse events (SAEs) will be collected for pharmacovigilance but are not trial outcome measures given the severity of illness of the population.

3.7 TIMING OF OUTCOME ASSESSMENTS

Participants will be followed up daily after randomisation through to the earlier of discharge from a trial site or Day 28. This includes a clinical examination (clinical signs and symptoms, vital parameters, calculation of the NeoSep Recovery Score) and collection of treatment administration data, as well as an evaluation of AEs and SAEs. CRP will be assessed in all neonates at baseline, Day 3 and Day 7. Other routine laboratory assessments will only be repeated if abnormal at the previous visit or neonate's condition is not stable. Vital status (alive or deceased) will be ascertained after discharge through contact with the parent/guardian, either by a scheduled hospital visit or telephone call, on Day 14, Day 28 and Day 90.
All data will be collected via the eDC system and monitored centrally.

Table 1. Trial Assessment Schedule for NeoSep1 Part 2 (from protocol version 3.0)

Visit type	Screening	First-line Randomisation	Treatment and Follow-up (counting days from first-line randomisation)						
Timing (window)	**Day -1[2]**	**Day 0[2]**	**Daily whilst in hospital at trial site**	**Day 3[7] (±1 day)**	**Day 7 (±2 days)**	**EOT [8]** (± 3 days)	**Day 14* (± 3 days)**	**Day 28* (± 5 days)**	**Day 90* (± 14 days)**
Informed consent	x[1]								
Verification of eligibility	x	x							
Medical history	x								
Signs and symptoms of sepsis[9]	x	x	x	x	x		x	x	
C-reactive Protein	x[3]			x	x				
Full Blood Count (FBC)	x[3]			x[6]	x[6]		x[6]	x[6]	x[6]
Urea and electrolytes (U&E)	x[3]			x[6]	x[6]		x[6]	x[6]	x[6]
Liver function test (LFT)	x[3]			x[6]	x[6]		x[6]	x[6]	x[6]
Creatinine	x[3]			x[6]	x[6]		x[6]	x[6]	x[6]
Blood culture	x[4]			x[5]					
Administration of antibiotics (if still on antibiotics)		x	(x)	(x)	(x)		(x)		
Microbiology swab (peri-rectal) (sub-study in selected sites only)[10]		x				X[10]			
Adverse events (AE) assessment[11]		x	x	x	x		x	x	
Re-admissions[11]							x	x	x
Global Scale for Early Development								x	x
Concomitant medication		x	x	x	x		x	x	
Health economic assessment[12]		x					x	x	x
Residual CSF storage[13]			(x)	(x)	(x)		(x)		

EOT= end of antibiotic treatment. Last visit will be on Day 90. Day of randomisation counted as Day 0 so that the Day 7 visit is one calendar week later.

Note 1: Blood should be taken for the tests shown wherever possible at the timepoints specified in **Table *1***; however, if not done due to insufficient blood available to draw in the neonate or clinical condition of the neonate, this will not be considered a protocol deviation. The clinical need of the neonate will take

priority and clinical judgement can be applied as needed throughout all trial assessments. Trial related total blood sampling volumes should generally not exceed 3% of the total blood volume during a period of 4 weeks and not exceed 1% at any single time (TBV estimated to be 90 ml/kg body weight).

Note 2: "Randomisation" in **Table 1** refers to the randomisation to first-line treatment regimens. As per **Figure 1**, if the condition of the neonate does not improve or they deteriorate (including after initial response), they may be randomised to a different set of second-line treatment regimens or start another second-line antibiotic regimen. This may happen at any time after the first-line randomisation, hence is not included at a specific timepoint in the table above, but response should be formally assessed at Day 3 (see footnote 7 below). Blood tests at switch to second-line should be performed based on clinical concern in order to minimise blood draws in this vulnerable population, prioritising taking blood for culture (footnote 5).

Note 3: AEs must be assessed for whether they are an SAE that requires expedited reporting daily. Other AEs, signs/symptoms of sepsis, antibiotics and concomitant medications must be recorded in the eDC for every day BUT these records can be completed retrospectively from notes and drug charts at the time points shown in the schedule of assessment.

* follow up by telephone / if clinically indicated, then hospital visit.
[1] Written informed consent to be obtained from parent/guardian; at minimum, verbal consent must be obtained before randomisation and documented in source document, with written informed consent to follow as soon as possible, and wherever possible within 48h of first trial related procedure.
[2] Randomisation and treatment initiation may be on the same day as the screening visit.
[3] Lab results should be available from a sample taken within 72h before randomisation, but these samples can be taken either at screening or randomisation, or values may be used from samples taken as part of clinical management outside of the trial but within 72h before randomisation. Test results are not required to be known at randomisation as not part of eligibility criteria.
Lab tests: Full Blood Count (FBC) including haemoglobin, platelets, white blood count (WBC) and neutrophil count (where available from local laboratory), CRP (where done additionally as part of routine care outside the scheduled timepoints in the Schedule of Assessment above). Urea & Electrolytes: blood urea nitrogen (BUN) or urea, creatinine, sodium, potassium. LFTs: ALT, AST, total bilirubin.
[4] Ideally, blood should be taken for culture within 48h before randomisation; however, if blood for culture has already been taken as part of the standard clinical management within the 48h preceding the screening visit, this test does not need to be repeated and results should be reported on the Culture and Susceptibility electronic Data Capture (eDC) for Day 0. Should the clinical condition of the neonate not allow a blood sample to be taken for culture, this will not be considered a protocol deviation; the neonate may be enrolled in the trial if all eligbility criteria are met. Any microorganisms isolated should be stored.
[5] Repeat blood culture only if neonate switches treatment (at the time of switch) due to clinical deterioration or lack of response. Blood for culture and for CRP testing should be taken before switch of antibiotics except in circumstances outside the responsible clinician's control and/or relating to the clinical condition of the neonate.
[6] Repeat blood tests only if abnormal at previous visit or neonate's condition not stable and/or there is a clinical concern (in order to minimise blood draws in this vulnerable population).
[7] Randomisation to second-line treatment if the neonate fails to respond or clinically deteriorates. Neonates should also be randomised to second-line treatment if they deteriorate after Day 3. In the situation where the neonate's clinical condition deteriorates rapidly between 24 and 48 hours, first-line treatment should also be randomised to second-line immediately. If randomisation is not possible, the neonate should be switched to a clinically appropriate regimen.

[8] Planned duration of treatment at randomisation is expected to generally be up to Day 7±2 days for blood culture-negative sepsis, and to Day 10 [-3,+4] days for blood culture-positive sepsis if there is no switch to second-line. If antibiotics are switched to second-line, the total duration of antibiotic treatment including first- and second- line treatment is expected to generally be up to Day 14 ±7 days depending on the neonate's condition.

[9] Signs and symptoms of sepsis after first-line randomisation (NeoSep Recovery Score) can be recorded on eDC retrospectively from routine clinical notes as part of routine assessment.

[10] Microbiology sub-study assessing colonisation will be conducted in selected sites only. Peri-rectal swabs should be taken at baseline (as soon as possible after randomisation if not possible logistically before randomisation) and at the end of antibiotic treatment, i.e. two swabs per neonate in total.

[11] SAEs that require expedited reporting must be reported daily within 24 hours from becoming aware. All other reportable AEs (SAEs not requiring expedited reporting, AEs related to or causing modification of any antibiotics, or AEs that are Grade 3-4 AEs) should be reported periodically on Day 3, Day 7, Day 14, 28 and 90 (as relevant) as per the Schedule of Assessment above, or on an unscheduled eDC. These AEs/SAEs should be reported if they occur from participant enrolment (the earliest of verbal assent or written consent) up to the later of Day 28 or the last administration of trial antibiotics plus 2 days. Re-admissions to hospital or death will be reported up to Day 90.

[12] At baseline including brief socio-economic history (e.g. parental age, educational level and broad measures of socio-economic status); at follow-up costs incurred by household including out-of-pocket expenditures, costs for transport to facilities, local food and accommodation, and income losses due to absences from work.

[13] Where cerebrospinal fluid (CSF) is taken for clinical reasons (not required for the trial) in a neonate who has received fosfomycin or flomoxef as part of the trial in the preceding 24h, if there is any residual CSF sample remaining after local testing and the site has storage facilities available, then this residual sample should be stored for pharmacokinetic analysis.

3.8 SAMPLE SIZE

Sample size is calculated based on simulations, given the number of different regimens involved.

At the first randomisation, we have assumed that personalised randomisation lists (Walker, White et al. 2021) will be drawn from a list of 8 regimens according to three different treatment patterns (see definition in **section 3.5**), reflecting their acceptability in different sites. At the second randomisation, it is assumed that personalised randomisation lists will be determined by the neonate's first randomised regimen, and include all regimens that are broader spectrum (excluding any regimen used in the first-line treatment).

Sample size calculations are informed by preliminary analyses from the neonatal observational study (NeoOBS). Following treatment under the first-line/second-line strategies available, 28 day mortality is expected to vary from 10-20%. Fixed values for first-line and second-line regimen effects have been selected to achieve this variation. We have assumed an equal split between the three assumed treatment patterns of randomisation, 5% early mortality before second randomisation and 25% of neonates switching to a randomised second-line treatment. Simulations were performed to investigate how much information would be provided by the planned trial design under varying sample sizes.

It is estimated that, compared to assigning a random regimen to each neonate, using "top-ranked" strategies based on results from a trial including 3000 neonates would achieve 65-72% of the maximum possible reduction in mortality across the population, and a 91-93% chance of reducing mortality for each neonate. This would also lead to a 79-86% chance of mortality being within 2% of the best strategy for each neonate. In sensitivity analyses, we varied two assumptions to allow unequal treatment patterns of randomisation and 50% switching to randomised second-line treatment and obtained similar results.

Neonates for whom verbal assent is confirmed by written consent, and neonates that die before verbal consent will contribute to the total sample size (following the approved protocol). This is in order to ensure that these children contribute to the primary outcome (mortality at Day 28) and to SAE pharmacovigilance. If verbal consent is not confirmed by written consent then no further data will be collected and the neonate will not count towards the sample size (will be explicitly counted as verbal consent not confirmed by written consent).

A sample size review will be conducted when 50% of participants have completed the Day 28 follow-up visit, as part of an interim analysis (see **section 6.4.2**). This will not use information about differences between randomised arms but would use the percentages randomised under different first-line treatment patterns and an overall (blinded) estimate of the primary endpoint rate (assumed to range from 10-20%, average 15% in original calculations).

4 DATA AND DEFINITIONS

4.1 DEFINITION OF POST-NATAL AGE

For the purpose of this trial, post-natal age will be calculated by considering the date of birth as the first day of life e.g. the neonate is considered to be 1 day old on the day of birth.

4.2 DEFINITION OF BASELINE

Baseline is defined as the date of randomisation (Day 0, see **Table 1**). Baseline value is defined for lab results as the latest measurement up to 72 hours before randomisation, for blood culture the latest sample up to 48 hours before screening, and for signs and symptoms of sepsis the last assessment between (and including) presentation and randomisation.

4.3 DEFINITION OF FOLLOW-UP

Time will be measured from randomisation (Day 0). For the analysis of the primary endpoint, follow-up will be to Day 28, i.e. 29 days from and including the day of randomisation, based on the ascertainment of mortality.

Re-admissions to hospital or deaths will be reported up to Day 90, the last trial visit (see **Table *1***). If participants are censored earlier due to loss to follow-up or withdrawal of consent, it will be assumed that such censoring is independent of the outcome.

A participant who does not attend their end of trial visit/call (Day 90) will be classified as “lost to follow-up” if they are not known to have died and the clinic has confirmed that they are unable to contact them.

4.4 SAFETY

The adverse event assessment will explicitly record signs and symptoms of untoward medical occurrence, regardless of grade, including possible drug toxicities. AEs (clinical and laboratory) will be graded using a combination of the DAIDS grading scales (Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events) and the clinically based NAESS (Salaets, Turner et al. 2019), with wording adapted to reflect available diagnostic and management options in LMICs and applying the NAESS generic severity grading (which refers to changes in care and monitoring) whenever possible across NAESS and DAIDS specified events relevant to neonatal care.

All adverse events of any grade that lead to modification (including discontinuation) of antibiotics or are considered related to antibiotics will be reported on eDC, as will any Grade 3 or 4 adverse events.

SAEs are not an outcome measure in NeoSep1 because the neonates will be very sick when admitted, however, will be reported for pharmacovigilance purposes. For the same reason, and in contrast to NeoSep1 Part 1, grade 1 and 2 AEs occurring as a result of the participant’s medical condition or standard hospital treatment will not be collected.

4.5 NEOSEP SEVERITY SCORE

The NeoSep Severity Score was developed for predicting 28-day mortality based on clinical information at the start of a new episode of sepsis. It was adapted from the WHO PSBI (possible serious bacterial infection) based scores for the hospital setting and developed from the NeoOBS study as described in the table below. It will be used at screening to enrol only neonates at a moderate to high risk of death from this episode of sepsis (NeoSep Severity Score of 5 or higher at presentation).

Table 2. NeoSep Severity Score

Factor (clinical signs in the 24h preceding start of clinical sepsis episode)	Score value if present
Time in hospital: ≤ 10 days	1
Gestational age: <37 weeks	1
Birth Weight: • >2 kg • 1-2 kg • <1 kg	 0 1 2
Congenital anomalies	2
Temperature • <35.5 °C • 35.5 to 37.9 °C • 38 to 38.9 °C • ≥39 °C	 1 0 1 2
Maximum respiratory support: • Oxygen supplementation • CPAP, BiPAP, HFNC • Invasive ventilation	 2 3 3
Abdominal distension	1
Being fed and difficulty in feeding/feeding intolerance*	1
Evidence of shock including cold peripheries	1
Lethargy / no or reduced movement† • Lethargic but moving spontaneously • No spontaneous movement or movement only on stimulation	 1 2

CPAP = continuous positive airway pressure, BiPAP = Bilevel Positive Airway Pressure, HFNC = high flow nasal cannula.
* Neonates ordered nil by mouth score 0; if intention is to feed and neonate is not feeding, then score 1.
† Note that this should represent an acute change in activity/movements; Neonates with persistent hypotonia, e.g. due to congenital disease or those who have no spontaneous movement due to sedative and/or paralytic medications, score 0.

4.6 NEOSEP RECOVERY SCORE

The NeoSep Recovery Score was developed from daily updated assessments of neonates' status in the NeoOBS study to predict mortality and guide clinical decision making, for example switch to second-line treatment.

Table 3. NeoSep Recovery Score

Factor (clinical signs in the preceding 24h)	Score value if present
Temperature • <35.5°C • 35.5 to 37.9°C • 38 to 38.9 °C • ≥39 °C	 1 0 1 2
Maximum respiratory support: • Oxygen supplementation • CPAP, BiPAP, HFNC • Invasive ventilation	 2 3 3
Abdominal distension	1
Being fed and difficulty in feeding/feeding intolerance*	1
Evidence of shock including cold peripheries	1
Lethargy / no or reduced movement† • Lethargic but moving spontaneously • No spontaneous movement or movement only on stimulation	 1 2
Cyanosis	1

CPAP = continuous positive airway pressure, BiPAP = Bilevel Positive Airway Pressure, HFNC = high flow nasal cannula.
* Neonates ordered nil by mouth score 0; if intention is to feed and neonate is not feeding, then score 1.
† Note that this should represent an acute change in activity/movements; neonates with persistent hypotonia, e.g. due to congenital disease or those who have no spontaneous movement due to sedative and/or paralytic medications, score 0.

4.7 NEURODEVELOPMENTAL DELAY

Neurodevelopmental delay will be assessed using the WHO Global Scale for Early Development (GSED) package at Day 28 and Day 90 (World Health Organization, 2023). This package is an internationally validated open-access tool that was developed to assess childhood development in a culturally neutral and easy to undertake way, that is acceptable and understandable to parents/guardians. It is used to assess the development of children under the age of three and can be based on caregiver self-report (i.e. done over the telephone).

4.8 HEALTH ECONOMICS

This will be described elsewhere.

5 ESTIMANDS FRAMEWORK

Table 4. Estimand framework for the primary analysis of the primary outcome

Treatments	The comparison is between the following first-line antibiotic treatments: **First-line treatment options:** • Ampicillin° and gentamicin • Cefotaxime or ceftriaxone • Fosfomycin and amikacin • Flomoxef and amikacin • Fosfomycin and flomoxef • Ceftazidime and amikacin • Piperacillin/tazobactam and amikacin • Meropenem
Population	The population of interest is hospitalised neonates aged ≤28 days and weighing ≥1000g with clinical signs of sepsis as defined in **section 3.4.1**.
Endpoint	Death by Day 28
Population-level summary measure	Hazard ratio
Intercurrent events • Not providing written consent following verbal consent	Principal stratum (modified intention-to-treat) [§]
• Not starting randomised first-line treatment	Treatment policy (use endpoint regardless of whether or not this intercurrent event had occurred) [¶]
• Any treatment modification including starting second-line treatment	Treatment policy (use endpoint regardless of whether or not this intercurrent event had occurred) [¥]
• Missed doses of treatment	Treatment policy (use endpoint regardless of whether or not this intercurrent event had occurred) [‡]

Notes: °or benzylpenicillin or cloxacillin or flucloxacillin or amoxicillin

[§] Modified ITT meaning excluding from the analysis participants where verbal consent was not confirmed by written consent. The only exception are neonates who die prior to written consent being obtained, when this primary outcome will be used in analysis to ensure accuracy of results.

[¶] If <1% do not initiate randomised first-line, treatment policy will be the only analysis. If 1-5% do not initiate, a secondary analysis of the primary outcome only will use principal stratum (modified ITT) meaning excluding from the analysis participants who did not initiate randomised treatment. This approach is unbiased under the assumption that the intercurrent event (i.e. not starting randomised first-line treatment) is not affected by the assigned treatment, that is participants who do not initiate one particular treatment (for example ampicillin & gentamicin) would also not initiate treatment if assigned to another treatment (for example meropenem). However, if non-initiation is >5%, inverse-probability weighting methods will be used in secondary analyses of the primary endpoint only.

[¥] In secondary analyses, inverse-probability weighting methods will be used, see **section 6.1**.

[‡] Missed doses are expected to be rare because neonates will be hospitalised and hospital staff will be responsible for antibiotic administration.

6 STATISTICAL PRINCIPLES

6.1 GENERAL CONSIDERATIONS

Analysis of the trial data will be carried out by using network meta-analytic methods to compare the first-line/second-line strategies and to rank strategies with respect to each outcome exploiting both the direct randomised comparisons and the indirect information across the network (Lee, Turner et al. 2023). Bootstrapping will be used to estimate uncertainty in the rankings. There is no formal pairwise hypothesis testing because there is no standard of care arm against which to conduct these tests.

In the primary analysis of the primary outcome, we will present hazard ratios comparing each first-line regimen against the control WHO regimen (ampicillin + gentamicin), regardless of any second-line treatment, together with their 95% CI. Analyses will use time-to-event models with regimen, treatment pattern and site as factor variables. To aid clinical interpretation, we will also convert adjusted hazard ratios to differences in percentages by applying them to the baseline estimate of the survival function from the adjusted Cox model. The reason for using a Cox model rather than a logistic model is to incorporate censoring from potential losses to follow-up before Day 28.

In a secondary analysis of the primary endpoint, we will present hazard ratios comparing each strategy (i.e. combination of first- AND second-line regimen) against the control strategy of WHO regimens (ampicillin + gentamicin followed by ceftriaxone/cefotaxime). For participants who do not require a second randomisation, cloned records will be created to represent all possible regimens if randomised a second time. An inverse probability weighting approach will then be taken, with a weight equal to 1/(number of treatments in second-line treatment pattern) for each cloned record ensuring that each unswitched participant has the same weight in the analysis (summed over their cloned records) as each switched participant. Participants who switch to non-randomised second-line therapy will be censored from the time of switching in this secondary analysis and similar participants who switched but were randomised to second-line will be upweighted using a different set of weights.

We will explore heterogeneity in particular subgroups which are used to define personalised randomisation lists tailored by each site (early onset sepsis; late onset sepsis; suspicion of meningitis; no suspicion of meningitis; suspicion of necrotising enterocolitis). Heterogeneity in other subgroups such as culture positive vs culture negative (including specific organisms e.g. Klebsiella pneumoniae, if there are sufficient numbers) and subgroups defined by baseline CRP will also be explored.

Analyses of secondary outcomes will use similar methods (including ranking). Where there is strong evidence for a difference between randomised first-line regimens in a composite secondary outcome (for example Recovery Score), then separate comparative analysis using ranking will be undertaken for its components.

In Part 2, the primary objective is to provide a ranking of eight different clinically relevant antibiotic regimens for first-line empiric and second-line (after lack of response/deterioration) treatment in terms of 28-day mortality as the primary outcome measure using the treatment policy approach for intercurrent events, including starting second-line treatment. In Part 2, a secondary objective is to provide a ranking of clinically relevant antibiotic regimens based on other efficacy and safety secondary outcomes, as well as on health economic measures and the potential selection of resistance. Overall, addressing this secondary objective, the determination of which strategies perform best with respect to mortality, safety, cost and resistance, will be carried out in two steps. As a first step in addressing this secondary objective, rankings will be examined with respect to mortality and safety, to identify a set of antibiotic regimens that dominate the others, i.e. are safer and more effective. Rankings of strategies from best to worst will be presented in a table and also illustrated in a plot showing performance in both dimensions. The rankings of this remaining set of regimens

with respect to resistance either in infecting isolates on carriage in the microbiology substudy will then be examined as a second step to determine how this affects the ranking on mortality and safety. Costs (which may vary by region) will be examined independently in a health economic analysis overall and/or by region.

Results which are not primary or secondary outcomes will be presented without ranking.

6.2 GENERAL STATISTICAL PRINCIPLES

6.2.1 Descriptive statistics

Categorical variables will be summarised using frequencies (n) and percentages; continuous variables will be summarised using the mean and standard deviation (SD) or median, lower quartile, and upper quartile plus minimum and maximum values. Ordinal variables will be described using median, lower and upper quartile plus minimum and maximum values. Descriptive statistics will be reported overall and by treatment group, and percentages will be of non-missing values, with the number (%) of non-missing values given if data are not complete.

6.2.2 Binary outcomes

Chi-squared test and logistic or binomial regression (adjusting for treatment pattern) will be used for the analysis of binary outcomes (specified in detail in **section 7).**

6.2.3 Continuous outcomes

T-tests and normal linear regression (adjusted for baseline values and treatment pattern) will be used for the analysis of continuous outcomes (specified in detail in **section 7**). Appropriate transformations will be applied after inspecting the distribution of the data.

6.2.4 Time to event outcomes

Kaplan-Meier analysis, Cox proportional-hazards models and competing risk regression (adjusting for treatment pattern) will be used for time-to-event outcomes (specified in detail in **section 7).** Where possible time-to-event methods will be used to account for deaths before reaching the secondary endpoint, for example all-cause re-admissions.

6.3 ANALYSIS POPULATIONS

The primary analysis population is intention-to-treat, including all randomised neonates, regardless of whether they received the allocated treatmentor not, excluding those where verbal consent was not confirmed by written consent and the neonate did not die (following the approved protocol). This corresponds to estimating the impact of the effectiveness of the strategies.

6.4 STATISTICAL INTERIM ANALYSES AND STOPPING GUIDANCE

Data will be reviewed by an independent Data Monitoring Committee (DMC). A DMC charter will be drawn up that describes the membership of the DMC, relationships with other committees, terms of reference, decision-making processes. The Charter will also contain a description of stopping guidelines and membership.

The DMC will meet within 6 months after the trial opens; although the DMC will in general meet every 6-9 months, the frequency of subsequent meetings will be determined by the DMC and could be more frequent if deemed necessary. The DMC will review all available data on safety parameters for all antibiotic regimens. The DMC can recommend premature closure or reporting of the trial, or that recruitment to any randomised group be discontinued or modified. Such recommendations would be made if, in the view of the DMC, there is proof beyond reasonable doubt that one of the allocated strategies is better than any other in terms of a difference of clinically significant magnitude in a primary outcome. The guiding statistical criteria for "proof beyond reasonable doubt" is a Haybittle-Peto type rule based on the 99.9% confidence interval.

6.4.1 Feasibility phase

A feasibility phase will enrol approximately 10% of the trial cohort (300 patients) to assess the feasibility of implementing the study at the participating sites. This will focus on:

- Assessing recruitment compliance with first-line treatment options
- Assessing implementation of second randomisation and compliance to second-line treatment options
- Assessing the percentage of culture positive babies at baseline
- Review (by DMC) of sodium levels at baseline, particularly the percentage of neonates with sodium ≥ 150 mmol/L

6.4.2 Sample size review

A sample size review will be conducted when 50% of participants have completed the Day 28 follow up visit, as part of an interim analysis. This will update the sample size calculations in the light of accumulating evidence about the frequency of use of each personalised randomisation list and the overall mortality rate (i.e. will not use information about estimated differences between randomised groups at the time). It will consider whether recruitment should continue to the original target based on the overall mortality rate within the trial (assumed to range between 10-20%, average 15%, for sample size calculations as above) or be modified, or whether for example, the randomised allocation ratio should be varied from 1:1 to randomise more neonates to less represented regimens. Any decision to increase the sample size is a Sponsor decision in collaboration with the TSC.

6.5 TIMING OF FINAL ANALYSIS

The final analysis will be performed after the last randomised participant has reached Day 90, data have been cleaned and database locked.

6.6 MISSING DATA

Every effort will be made to collect all data as per the schedule of assessments, and we expect the number of missing data to be relatively small. Patients who withdraw consent will be excluded from trial analyses from the time they withdraw. When missing data occurs, it is assumed that it will be at random, and all analyses will be based on observed data (for the primary outcome, see below).

6.6.1 Primary outcome

As follow-up for the primary outcome is short (28 days post-randomisation), loss-to-follow-up is expected to be low. The primary analysis will be conducted on observed data using time-to-event methods, assuming not died if censored (lost/withdrawn) prior to Day 28.

6.7 CONFIDENCE INTERVALS AND P VALUES

Estimates from statistical models will be presented with two-sided Wald 95% confidence intervals (CIs). 95% CIs around single percentages will not be presented because first-line groups will not necessarily be balanced by design in terms of disease severity or other risk factors because of the different randomisation lists. Formal statistical tests for hypothesis testing will not be applied in this trial.

6.8 STATISTICAL SOFTWARE

Analyses will be performed using Stata version 18 (or above), unless otherwise specified.

7 ANALYSIS DETAILS

7.1 RECRUITMENT

- Number screened and enrolled by calendar time (by calendar month and cumulative)
- Total screened and enrolled by centre, with dates of first and latest enrolment
- Eligibility: number and reasons for any participants found to be ineligible after enrolment
- Reasons for not enrolling screened subjects will also be described.

Screening, recruitment and retention data will also be presented in a CONSORT flow diagram.

7.2 BASELINE CHARACTERISTICS

If not reporting n (%), characteristics will be described as defined for continuous data (**section 6.2.1**). Of note, first-line groups will not necessarily be balanced by design because of the different randomisation lists. Therefore, testing for imbalance will not be performed. Signs and symptoms of sepsis may be presented separately for the time of first sepsis assessment, in addition to baseline as defined in **section 4.2**.

- Sex: n (%) male, female
- Age (in days from birth)
- Time in hospital at presentation (days)
- Gestational age at birth (weeks): summarised as continuous variable, and n (%) in categories preterm (<37) and term (≥37)
- Birth weight (g): summarised as continuous variable, and n (%) in categories <1 kg, 1-2 kg, >2 kg
- Presence of congenital abnormalities: n (%) in categories none, minor, major
- Temperature (°C): summarised as continuous variable, and n (%) in categories <35.5 °C, 35.5 to 37.9 °C, 38 to 38.9 °C, ≥39 °C
- Respiratory support: n (%) in categories oxygen supplementation, CPAP/BiPAP/HFNC, invasive ventilation
- Abdominal distension: n (%)
- Difficulty in feeding or feeding intolerance: n (%)
- Evidence of shock: n (%)
- Lethargy or reduced/no movement: n (%) in categories lethargy only, movement only on stimulation, no movement
- NeoSep Severity Score (see **section 4.5**): median, first quartile, third quartile, minimum and maximum; may also be grouped, for example into low (score 0-4), medium (5-8) and high (9-16) risk groups, and presented categorically
- Mode of delivery: n (%) in categories elective (planned) caesarean section, emergency caesarean section, vaginal delivery (spontaneous), vaginal delivery (assisted)
- HIV status: n (%) in categories infected, exposed/uninfected, uninfected, not known/not tested
- Any lines used in the past 24 hours: n (%) in categories umbilical venous line, umbilical arterial line, peripheral arterial line, central venous line, PICC line
- Weight (g)
- Length (cm)
- Heart rate (beats per minute)

- Respiratory rate (breaths per minute)
- Oxygen saturation (%)
- Convulsions: n (%)
- Severe chest indrawing: n (%)
- Increase in oxygen requirement (at baseline, reflecting clinical status): n (%)
- Increase of respiratory support (at baseline, reflecting clinical status): n (%)
- Cyanosis: n (%)
- Irritability: n (%)
- Start of IV antibiotics for this episode of sepsis before enrolment: n (%) overall; n (%) per antibiotic, total daily dose (mg or IU) per antibiotic, duration of treatment (hours)
- Systemic antibiotics taken for other reasons in preceding 7 days: n (%) overall, n (%) per antibiotic, time since stop (hours)
- Sodium (mmol/L)
- Potassium (mmol/L)
- Blood Urea Nitrate (mmol/L)
- Creatinine (µmol/L)
- CRP (mg/L)
- ALT (U/L)
- AST (U/L)
- Total bilirubin (µmol/L)
- Haemoglobin (g/dL)
- WBC ($x10^9$ cells/L)
- Neutrophils ($x10^9$ cells/L)
- Platelets ($x10^9$ cells/L)

7.3 FIRST-LINE ANTIBIOTIC TREATMENT

- Allocated first-line treatment: number (%) started / not started (plus reasons if not started); description of antibiotic regimens and treatment patterns

7.4 WITHDRAWAL / FOLLOW-UP

- For each of the follow-up visits on Days 7, 14, 28 and 90: number (%) happened/missed (plus reasons if missed); number (%) attended in clinic or assessed by telephone; n (%) on antibiotic treatment
- Withdrawal from trial participation before a) 28 days, and b) 90 days: number (%); description of reasons
- Lost to follow-up by Day 90: number (%)

7.5 PRIMARY OUTCOME ANALYSIS

28-Day mortality will be analysed using time-to-event methods with regimen, treatment pattern (see **section 3.5**) and site as factor variables (see **section 6.1**). The reason for the primary analysis using time-to-event methods is to account for an unknown percentage of children being lost-to-follow-up before day 28, e.g. post-discharge. As there is a possibility that there could be variation in the relative differences between

intervention arms between early and late deaths, a test of non-proportionality will be conducted, accompanied by visual inspection of Kaplan-Meier curves. If there is evidence of non-proportionality ($p<0.05$ or Kaplan Meier curves crossing), then 28-day mortality will also be analysed as a binary outcome using logistic regression to provide a risk ratio that reflects the "net" mortality difference at 28 days. If missing data due to lost-to-follow-up are <5% then analysis will be complete case. Otherwise, day-28 mortality will be imputed using separate imputation models per arm, and including as factors site, treatment pattern, and NeoSep Severity Score.

7.6 SECONDARY OUTCOME ANALYSIS: EFFICACY

7.6.1 Clinical status

Clinical status will be assessed daily after randomisation through to the earlier of discharge from a trial site or Day 28.

- The NeoSep Recovery Score (see **section 4.6**) will be summarised at Days 3, 7, 14, and 28 as defined for continuous variables, and by n (%) in categories <4, and ≥4 points. Treatments will be analysed (and ranked) using normal linear regression or (ordered) logit models adjusted for treatment pattern and the baseline value (absolute or in categories), as appropriate.
- Individual signs and symptoms of sepsis after enrolment will be summarised as described in **section 6.2.1**; ranking treatment regimens will only be done if there is strong evidence for a difference between randomised first-line regimens in the Recovery Score:
  - Temperature (°C): summarised as continuous variable, and n (%) in categories <35.5 °C, 35.5 to 37.9 °C, 38 to 38.9 °C, ≥39 °C
  - Respiratory support: n (%) in categories oxygen supplementation, CPAP/BiPAP/HFNC, invasive ventilation
  - Abdominal distension: n (%)
  - Difficulty in feeding: n (%)
  - Evidence of shock: n (%)
  - Lethargy or reduced/no movement: n (%) in categories lethargy only, movement only on stimulation, no movement
  - Cyanosis: n (%)
  - Heart rate (beats per minute)
  - Respiratory rate (breaths per minute)
  - Oxygen saturation (%)
  - Convulsions: n (%)
  - Irritability: n (%)
  - Severe chest indrawing: n (%)

7.6.2 Additional systemic antibiotics beyond the first randomised treatment through Day 28

- Number (%) receiving ANY additional systemic antibiotics beyond the first randomised treatment through Day 28. Treatments will be analysed and ranked as defined for time-to-event outcomes with death before receipt of additional systemic antibiotics beyond the first randomised treatment as competing risk.
  - As explorative outcome, the number (%) experiencing the competing risk (death before receipt of additional systemic antibiotics beyond the first randomised treatment) will also be analysed, as will the composite endpoint of death or receipt of additional systemic

antibiotics beyond the first randomised treatment (a proxy for failure of first-line treatment).
    - Further additional systemic antibiotics beyond the first randomised treatment could be before or after stopping the first randomised treatment (defined as no antibiotic doses for ≥48h). The former could occur for toxicity or perceived failure (noting that this is a subjective judgement in an open-label trial and cannot be assumed to reflect genuine treatment failure, particularly in this very sick population with multiple other comorbidities); the latter could occur for a new infection episode. The randomised intervention could, however, have very different effects on these two components, so these will also be considered separately in exploratory competing risks analyses.
    - Note: the reason for proposing both competing risks and composite endpoint analysis is because the percentages receiving additional antibiotics beyond randomised first-line and dying before doing so are unknown. Given the patient population, it is possible that many deaths are not antibiotic-modifiable, in which case the composite endpoint would suffer from dilution bias were there genuine benefits from some antibiotics over others. However, if any benefits were similar for both early deaths and first-line treatment failure, and providing that receiving additional antibiotics is a reasonable proxy for first-line treatment failure (not necessarily the case in an open-label trial) then the composite endpoint could have greater power. Whilst the competing risks analysis will be the primary analysis, we will therefore accompany this with an exploratory composite endpoint analysis.
- Additionally, the following will be summarised:
    - Number (%) receiving randomised second-line treatment; of those randomised, n (%) started randomised 2nd-line regimen; description of randomised second-line antibiotics
    - Number (%) receiving non-randomised second-line treatment; description of second-line antibiotics and reasons for not randomising to second-line
    - Number (%) receiving additional systemic antibiotics for other reasons than presumed failure of the initial regimen (e.g. new episode of sepsis); description of antibiotics and reasons

7.6.3 Additional systemic antibiotics beyond the first randomised and second (for failure) treatment through Day 28

- Number (%) receiving additional systemic antibiotics beyond the first randomised and second (for failure) treatment through Day 28. Treatments will be analysed and ranked as defined for time-to-event outcomes with death before additional systemic antibiotics beyond the first randomised and second (for failure) as competing risk.
    - As explorative outcome, the number (%) experiencing the competing risk (death before receipt of additional systemic antibiotics beyond the first and second randomised treatment) will also be analysed, as will the composite endpoint of death or receipt of additional systemic antibiotics beyond the first randomised and second (for failure) treatment (a proxy for failure of first-line and second-line treatment)
- Additionally, antibiotics and reasons will be described.

Description of all non-allocated antibiotics used per participant: total number, type and class of antibiotics, reason started.

7.6.4 Length of stay during the index hospitalisation

- Length of stay (days) during the index hospitalisation is defined as time from day of randomisation (Day 0) to the day of first discharge. It will be analysed using competing-risks regression models with in-hospital death as competing risk, as defined for time-to-event outcomes. Ranking will be based on the beta coefficients (subhazard ratios) from the model. If participants are transferred to another hospital they will be censored at the time of transfer.

7.6.5 Systemic antibiotic exposure

- Systemic antibiotic exposure (days on antibiotics) during the index hospitalisation: time to stop of all IV antibiotics: cumulative incidence

7.6.6 90-day mortality

- 90-day mortality: number (%); analysed as defined for time-to-event outcomes.

7.6.7 CRP

- CRP at Day 3
- CRP at Day 7
- Change in CRP from baseline to Day 3
- Change in CRP from baseline to Day 7

CRP and change in CRP will be described using means (SD) and analysed using linear regression (adjusted for baseline values and treatment pattern), as defined for continuous outcomes.

7.6.8 Re-admission by Day 90 (all-cause)

- Re-admission by Day 90 (all-cause): number (%); analysed as defined for time-to-event outcomes with death as competing risk, and late entry at the initial discharge. Only the first re-admission per participant will be considered. The number of neonates with more than one re-admission will be tabulated.

7.7 SECONDARY OUTCOME ANALYSIS: SAFETY

Adverse events, including SARs, will be summarised by body system (MedDRA System Organ Class), and within body system by MedDRA Preferred Term.

- Number (%) of participants experiencing a grade 3/4 adverse event through Day 28
- Number (%) of participants experiencing an adverse event of any grade related to antibiotics through Day 28
- Number (%) of participants experiencing a modification (including discontinuation) of antibiotics for adverse reactions through Day 28

These secondary outcomes will be analysed and ranked as defined for binary outcomes.

- Neurodevelopment at Day 28 and Day 90: described and analysed as defined for continuous outcomes.

Although not an outcome, a line listing of all SAEs will be generated.

Of note, adverse event will be collected up to the later of Day 28 or the last administration of trial antibiotics plus 2 days for pharmacovigilance. However, the safety endpoints above will be calculated through Day 28 only to ensure that randomised groups are compared across the same time periods, regardless of changes to antibiotic treatment (following the principles of intention-to-treat).

A sensitivity analysis will consider modification (including discontinuation) of antibiotics for adverse events (rather than reactions) through Day 28 to incorporate the possibility of ascertainment bias in determining relatedness as the trial is open-label. However, this alternative definition will include events unrelated to

antibiotic administration which nevertheless require changes in antibiotics, e.g. due to drug-drug interactions, and hence is a sensitivity analysis.

7.8 MICROBIOLOGY

7.8.1 Baseline blood culture

- Blood culture taken: n (%)
- Blood culture results: n (%) in categories no organisms found, contaminant, pathogen; description of type of pathogen and antimicrobial susceptibility as determined locally by phenotypic methods.

7.8.2 Blood cultures after enrolment

Similar to above, any new culture results post baseline will be described, e.g. prior to starting second-line treatment.

7.8.3 CSF culture results

CSF samples are not mandatory in this trial. However, any CSF culture results will be described similar to blood culture results.

7.9 CONCOMITANT MEDICATION

- Description of all concomitant medication used in the trial, including treatment started prior to but continued beyond randomisation, by drug and class (WHO Anatomical Therapeutic Chemical 1st level). All non-topical medications (other than systemic (i.e. IV and oral) antibiotics which will be analysed as a secondary outcome) for any condition are considered a concomitant medication, regardless of route of delivery, including blood transfusion and vitamin infusions. Topical medications will not be collected and are not considered concomitant medications.

7.10 IMPORTANT PROTOCOL DEVIATIONS

Protocol deviations are defined in the NeoSep1 Quality Management and Monitoring Plan and classified as critical, major, or minor. Critical and major deviations constitute important protocol deviations according to the MRC CTU Protocol Deviations SOP.

- Important protocol deviations: number (%) overall and by type

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NeoSep1

Statistical Analysis Plan for Part 2

An open-label randomised controlled trial comparing novel combination and currently used antibiotic regimens for the empiric treatment of neonatal sepsis with a run-in confirmatory pharmacokinetic phase

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Draft 0.01	Rebecca Turner Ann Sarah Walker	20-Jun-2024	Protocol version 2.0
Draft 0.02	W Stöhr	30-Jul-2024	First draft
Draft 0.03	W Stöhr	06-Aug-2024	Incorporating changes following discussion with Sarah Walker
Draft 0.1	W Stöhr	26-Aug-2024	Incorporating changes following review by Rebecca Turner
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Draft 0.3	W Stöhr	13-Dec-2024	Incorporating changes following review by DMC
1.0	W Stöhr	30-Jan-2025	Reviewed by TSC members (no comment); one minor error corrected.

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Note: This Statistical Analysis Plan covers Part 2 of the NeoSep1 trial. A separate Statistical Analysis Plan has been written for Part 1.

1 ABBREVIATIONS

Abbreviation	Expansion
AE	Adverse event
ALT	Alanine Aminotransferase
AMR	Antimicrobial resistance
AST	Aspartate transaminase
BiPAP	Bilevel Positive Airway Pressure
BUN	Blood urea nitrogen
CI	Confidence interval
CPAP	Continuous positive airways pressure
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DMC	Data Monitoring Committee
eDC	Electronic data capture
EOT	End of treatment
FBC	Full blood count
GARDP	Global Antibiotic Research and Development Partnership
HFNC	High flow nasal cannula
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	Intention-to-treat
IV	Intravenous
LFT	Liver function test
LMIC	Low- and middle-income country
MDR	Multi-Drug Resistant
MedDRA	Medical Dictionary for Regulatory Activities
MRC CTU at UCL	Medical Research Council Clinical Trials Unit at University College London
NAESS	Neonatal Adverse Event Severity Scale
NeoOBS	Neonatal observational study
PSBI	Possible serious bacterial infection
PK	Pharmacokinetics
PNA	Post-natal age
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAR	Serious adverse reaction
SD	Standard deviation
SGUL	St Georges University of London
TBV	Total blood volume
TSC	Trial Steering Committee
UCL	University College London
WBC	White blood cells
WHO	World Health Organisation

2 BACKGROUND

While there is a high burden of neonatal sepsis globally, its impact is especially marked in low- and middle-income countries (LMICs), where there are an estimated 6.9 million annual episodes of possible serious bacterial infection and 680,000 related deaths. Increasing antimicrobial resistance (AMR), including a higher prevalence of AMR in isolates from septic neonates, threatens to undermine the effectiveness of World Health Organisation (WHO) recommended antibiotic treatments in these settings. The key threat is multi drug-resistant Gram-negative bacteria, where there are very few neonatal treatment options and increasing use of meropenem, driving carbapenem resistance. Current WHO guidelines, however, continue to recommend empiric first- and second-line regimens for neonatal sepsis that have remained unchanged for nearly 20 years.

Given increasing AMR, the coverage of current WHO-recommended regimens is expected to be low in many high-burden settings, and there is a clear need to re-evaluate the guidance for empiric treatment of neonatal sepsis in the hospital setting and to provide new options for treatment of MDR neonatal sepsis that have global relevance. Relevant regimens for comparison with WHO-recommended regimens should include antibiotics with a neonatal licence and provide good coverage for globally relevant extended-spectrum beta-lactamase producing organisms. Given the lack of evidence supporting much neonatal sepsis treatment and the severity of the condition, it also important to directly compare suitable novel regimens, including off-patent drugs with a neonatal licence but not currently widely used, to currently recommended and widely used regimens.

Therefore, in NeoSep1 three groups of empiric antibiotic regimens will be investigated:

- WHO-recommended regimens: ampicillin (or benzylpenicillin, amoxicillin or cloxacillin) + gentamicin, or the third generation cephalosporins, cefotaxime or ceftriaxone
- Broad spectrum antibiotics in common use in neonatal units with licenced and/or recommended neonatal doses: piperacillin/tazobactam, piperacillin/tazobactam + amikacin, ceftazidime, ceftazidime + amikacin, meropenem
- Older off patent antibiotics which have a licenced neonatal dose but are not currently widely used globally in neonatal units: fosfomycin, flomoxef and amikacin

These older off patent antibiotics will be tested as novel dual combinations, which is fosfomycin + amikacin, flomoxef + amikacin and fosfomycin + flomoxef. Since they have been infrequently used in neonatal populations, a run-in non-randomised pharmacokinetic study of these three combinations of fosfomycin, flomoxef and amikacin has been performed to confirm plasma drug levels at the proposed doses based on dosing recommendations and other studies, as well as to collect safety data (NeoSep1 Part 1) before the start of the main randomised trial (NeoSep1 Part 2).

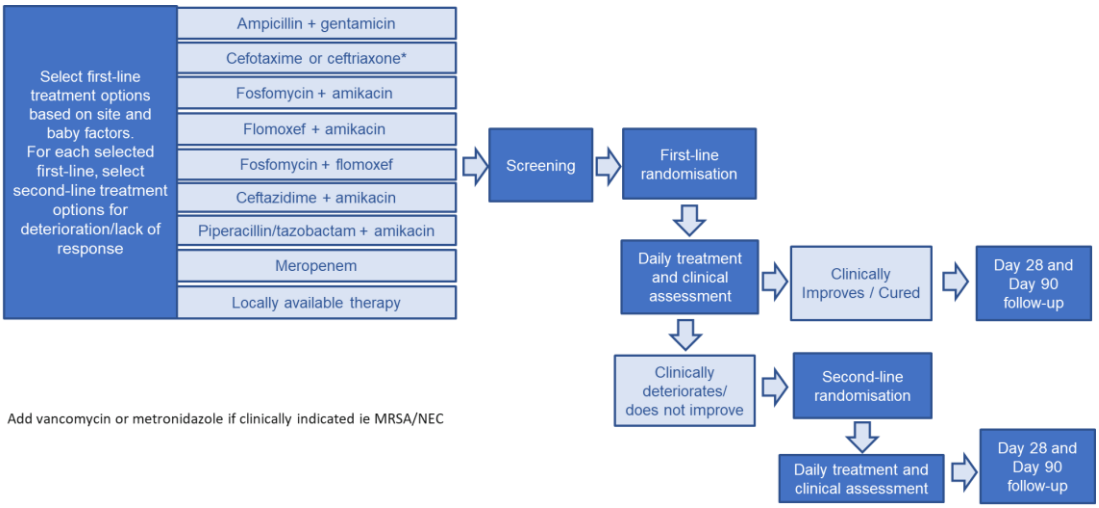
NeoSep1 Part 1 has been completed, and participants (mostly preterm neonates) had fosfomycin and flomoxef plasma concentrations similar to published literature. Although variability was observed shortly after birth, drug exposures support these doses for the larger randomised NeoSep1 Part 2 trial (Bekker on behalf of the NeoSep1 Part1 Study Team, 2024).

3 STUDY METHODS

3.1 TRIAL DESIGN

NeoSep1 Part 2 is a pragmatic clinical trial using a Personalised RAndomised Controlled Trial (PRACTical) (Walker, White et al. 2021) design comparing multiple different novel combination and currently used antibiotic regimens, including a Sequential Multiple Assignment Randomised Trial (SMART) design (Almirall, Nahum-Shani et al. 2014) to allow randomisation to second-line antibiotic treatment where indicated.

Figure 1: Trial entry, randomisation and first and second-line treatment



Note: locally available therapy is only available as second line treatment randomisation; see **section 3.5** for more details.

3.2 STUDY OBJECTIVES

3.2.1 Primary objectives

The primary objective of NeoSep1 Part 2 is to provide a ranking of eight different clinically relevant antibiotic regimens for first-line empiric and second-line (after lack of response/deterioration) treatment in terms of 28-day mortality as the primary outcome measure (see **section 6** for details). It will flexibly compare these multiple different relevant treatment regimens to enable the trial to be run in sites worldwide with very different background rates of different pathogens and resistance and different routine clinical care by randomising each participant to locally relevant antibiotic regimens agreed prior to site initiation.

This trial will also directly address the question as to the potential advantages and disadvantages of using initial broader-spectrum empiric therapy versus narrower-spectrum empiric therapy with prompt switch to broader spectrum for clinical non-response/deterioration. Specifically, neonates randomised to an empiric regimen in the trial will be closely monitored for clinical non response/deterioration, and if this occurs, they will be randomised to a second set of regimens, which will again depend on site appropriateness (particularly resistance phenotype) as well as their first regimen.

3.2.2 Secondary objectives

Secondary objectives are to provide a ranking of clinically relevant antibiotic regimens based on other efficacy and safety secondary outcomes, as well as on health economic measures and the potential selection of resistance. The trial data will provide data to inform the balance between efficacy, safety, cost and propensity for resistance selection that will influence facility-level and national decision-making about adoption of studied regimens, and potential future inclusion in WHO guidelines.

3.3 STUDY DURATION

The study duration for each participating neonate is 90 days from enrolment. Overall, Part 2 of the NeoSep1 trial is expected to take 48 months.

3.4 STUDY POPULATION

3.4.1 Inclusion criteria

1. Currently admitted to hospital
2. Aged ≤28 days (post-natal age)
3. Weight ≥1000g
4. Clinical diagnosis of a new episode of sepsis with two or more of the following clinical signs together with planned treatment with IV antibiotics
    a. Abnormal temperature (<35.5°C Or ≥38°C)
    b. Chest indrawing or increase in oxygen requirement or need for respiratory support
    c. Abdominal distension
    d. Difficulty in feeding or feeding intolerance
    e. Evidence of shock including cold peripheries
    f. Lethargy, or reduced or no spontaneous movement
    g. Cyanosis
    h. Abnormal heart rate (bradycardia <80 bpm; tachycardia >180 bpm)
    i. Convulsions
    j. Irritability

    For making the diagnosis of significant sepsis, the neonate should have no alternative primary explanation for these criteria (such as Hypoxic Ischaemic Encephalopathy, hypothermia, hypoglycemia, prematurity etc).
5. At moderate to high risk of death from this episode of sepsis, based on a neonatal sepsis severity score (NeoSep Severity Score), specifically a NeoSep Severity Score of 5 or higher at presentation for this episode of sepsis (which may be before formal screening).
6. Can receive all potential treatment options on the relevant randomisation list for this neonate at their site, ensuring randomisation is possible (see country-specific appendices)
7. IV antibiotics about to be started OR not received more than 24 hours of IV antibiotics for this episode of neonatal sepsis at the point of randomisation.
8. Parent/guardian willing and able to provide consent (written or, if their neonate is severely ill, verbal consent which must be confirmed by written consent as soon as possible and wherever possible within 48 hours after the first trial specific procedure). Verbal consent allows for administration of first-line antibiotics at no or minimal delay.

3.4.2 Exclusion criteria

1. A known serious, non-infective co-morbidity anticipated to cause death within this admission (including major congenital abnormalities anticipated to cause death within this admission other than prematurity, e.g. known large ventricular septal defect)
2. Previously enrolled in this trial
3. Current participation in any other clinical study of an Investigational Medicinal Product that is a systemic drug, unless it has received prior approval by the NeoSep1 Trial Management Group
4. Known contraindication to any of the trial antibiotics on the randomisation list for the relevant neonatal sub-population in that site (these will vary according to the antibiotics on the specific randomisation list)

3.5 RANDOMISATION

The trial will use a PRACTical design, in which each neonate is randomised only to regimens that are considered clinically acceptable for that specific site and sub-population. The design will also incorporate the use of a SMART design to allow randomisation to second-line treatment where required.

As each sub-population in each site will have a separate randomisation list, simple 1:1 randomisation between all trial treatments in each randomisation list will be used for both first-line and second-line randomisations. Whereas all neonates enrolled will be randomised to first-line treatment, not all participants will be randomised to second-line treatment; randomisation to second-line treatment will only occur if the neonate does not improve clinically or clinically deteriorates and there are two or more treatment regimen options in the relevant randomisation list for that neonate.

3.5.1 First-line treatment options

- Ampicillin (or amoxicillin or benzylpenicillin or cloxacillin or flucloxacillin) + gentamicin
- Cefotaxime or ceftriaxone
- Fosfomycin and amikacin
- Flomoxef and amikacin
- Fosfomycin and flomoxef
- Piperacillin/tazobactam and amikacin
- Ceftazidime and amikacin
- Meropenem

Neonates will be allocated to a first-line treatment option by randomisation. As part of set-up activities, each site will define which of the first-line treatment regimens listed above are clinically appropriate for specific sub-populations of neonates in each participating neonatal unit. Each neonatal unit will define the list of antibiotics that they decide is appropriate to randomise that specific population in that neonatal unit to.

3.5.2 Second-line treatment options

- Cefotaxime or ceftriaxone
- Fosfomycin and amikacin
- Flomoxef and amikacin
- Fosfomycin and flomoxef
- Piperacillin/tazobactam and amikacin
- Ceftazidime and amikacin
- Meropenem
- Locally selected therapy

Specific second-line randomisation lists will vary according to the first-line treatment the neonate received, and will reflect a broadening of antibiotic activity as well as taking into account resistance phenotypes at the site and any susceptibility testing results from a pathogen isolated from the individual neonate.
This list may include specific antibiotics or regimens used in particular sites that are not available in other sites ("locally selected therapy").

3.5.3 Examples for randomisation list choices

Below are **examples** for agreed randomisation lists for specific sub-populations of neonates at Tygerberg Hospital, one of the trial sites:

Randomisation:	First-line	
Sub-population:	Neonates with early onset sepsis without suspicion of meningitis	Neonates with late onset sepsis without suspicion of meningitis
List of regimens:	• Penicillin + Gentamicin • Fosfomycin + Amikacin • Flomoxef + Amikacin • Fosfomycin + Flomoxef	• Fosfomycin + Amikacin • Flomoxef + Amikacin • Fosfomycin + Flomoxef • Ceftazidime + Amikacin • Piperacillin/tazobactam + Amikacin • Meropenem

Randomisation:	Second-line	
Sub-population:	First-line penicillin + gentamicin & low suspicion of meningitis	First-line fosfomycin + amikacin & high suspicion of meningitis
List of regimens:	• Fosfomycin + Amikacin • Flomoxef + Amikacin • Fosfomycin + Flomoxef • Ceftazidime + Amikacin • Piperacillin/tazobactam + Amikacin • Meropenem • Locally selected therapy	• Flomoxef + Amikacin • Fosfomycin + Flomoxef • Ceftazidime + Amikacin • Meropenem • Locally selected therapy

Of note, in the description of the statistical analysis below, a particular randomisation list (i.e. set of acceptable regimens for a sub-population) will be referred to as **treatment pattern,** for each of first- and second-line randomisation. "Treatment pattern" refers to the specific randomisation list – that is, there is a treatment pattern for each participant for the first-line randomisation, and – if randomised to second-line – a separate treatment pattern for the second-line randomisation. Treatment pattern is treated as a stratifier in the primary analysis (see **section 6.1**).

3.6 OUTCOME MEASURES

The following outcomes are described as in the protocol, version 3.0. For details of their operationalisation see **section 7**.

3.6.1 Primary outcome measure

- 28-day mortality

3.6.2 Secondary outcome measure: efficacy

- Clinical status, assessed daily after randomisation through to the earlier of discharge from a trial site or Day 28 using a clinical recovery score based on data from the NeoOBS observational study (NeoSep Recovery Score)
- Additional systemic antibiotics beyond the first randomised treatment through Day 28
- Additional systemic antibiotics beyond the first randomised and second (for failure) treatment through Day 28
- Length of stay during the index hospitalisation
- Systemic antibiotic exposure (days on antibiotics) during the index hospitalisation
- 90-day mortality
- Change in C-reactive protein (CRP) to Day 3 and 7 from baseline
- Re-admission by Day 90 (all-cause)

3.6.3 Secondary outcome measure: safety

- Grade 3/4 adverse events (AEs) graded using a combined LMIC relevant adapted Division of AIDS (DAIDS) and International Neonatal Consortium Neonatal Adverse Event Severity Scale (NAESS) through Day 28
- Adverse events of any grade related to antibiotics through Day 28
- Modification (including discontinuation) of antibiotics for adverse reactions through Day 28
- Neurodevelopment as assessed by the WHO Global Scale for Early Development (GSED) package at Day 28 and Day 90

Note: serious adverse events (SAEs) will be collected for pharmacovigilance but are not trial outcome measures given the severity of illness of the population.

3.7 TIMING OF OUTCOME ASSESSMENTS

Participants will be followed up daily after randomisation through to the earlier of discharge from a trial site or Day 28. This includes a clinical examination (clinical signs and symptoms, vital parameters, calculation of the NeoSep Recovery Score) and collection of treatment administration data, as well as an evaluation of AEs and SAEs. CRP will be assessed in all neonates at baseline, Day 3 and Day 7. Other routine laboratory assessments will only be repeated if abnormal at the previous visit or neonate's condition is not stable. Vital status (alive or deceased) will be ascertained after discharge through contact with the parent/guardian, either by a scheduled hospital visit or telephone call, on Day 14, Day 28 and Day 90.
All data will be collected via the eDC system and monitored centrally.

Table 1. Trial Assessment Schedule for NeoSep1 Part 2 (from protocol version 3.0)

Visit type	Screening	First-line Randomisation	Treatment and Follow-up (counting days from first-line randomisation)						
Timing (window)	**Day -1[2]**	**Day 0[2]**	**Daily whilst in hospital at trial site**	**Day 3[7] (±1 day)**	**Day 7 (±2 days)**	**EOT [8]** (± 3 days)	**Day 14* (± 3 days)**	**Day 28* (± 5 days)**	**Day 90* (± 14 days)**
Informed consent	x[1]								
Verification of eligibility	x	x							
Medical history	x								
Signs and symptoms of sepsis[9]	x	x	x	x	x		x	x	
C-reactive Protein	x[3]			x	x				
Full Blood Count (FBC)	x[3]			x[6]	x[6]		x[6]	x[6]	x[6]
Urea and electrolytes (U&E)	x[3]			x[6]	x[6]		x[6]	x[6]	x[6]
Liver function test (LFT)	x[3]			x[6]	x[6]		x[6]	x[6]	x[6]
Creatinine	x[3]			x[6]	x[6]		x[6]	x[6]	x[6]
Blood culture	x[4]			x[5]					
Administration of antibiotics (if still on antibiotics)		x	(x)	(x)	(x)		(x)		
Microbiology swab (peri-rectal) (sub-study in selected sites only)[10]		x				X[10]			
Adverse events (AE) assessment[11]		x	x	x	x		x	x	
Re-admissions[11]							x	x	x
Global Scale for Early Development								x	x
Concomitant medication		x	x	x	x		x	x	
Health economic assessment[12]		x					x	x	x
Residual CSF storage[13]			(x)	(x)	(x)		(x)		

EOT= end of antibiotic treatment. Last visit will be on Day 90. Day of randomisation counted as Day 0 so that the Day 7 visit is one calendar week later.

Note 1: Blood should be taken for the tests shown wherever possible at the timepoints specified in **Table *1***; however, if not done due to insufficient blood available to draw in the neonate or clinical condition of the neonate, this will not be considered a protocol deviation. The clinical need of the neonate will take

priority and clinical judgement can be applied as needed throughout all trial assessments. Trial related total blood sampling volumes should generally not exceed 3% of the total blood volume during a period of 4 weeks and not exceed 1% at any single time (TBV estimated to be 90 ml/kg body weight).

Note 2: "Randomisation" in **Table 1** refers to the randomisation to first-line treatment regimens. As per **Figure 1**, if the condition of the neonate does not improve or they deteriorate (including after initial response), they may be randomised to a different set of second-line treatment regimens or start another second-line antibiotic regimen. This may happen at any time after the first-line randomisation, hence is not included at a specific timepoint in the table above, but response should be formally assessed at Day 3 (see footnote 7 below). Blood tests at switch to second-line should be performed based on clinical concern in order to minimise blood draws in this vulnerable population, prioritising taking blood for culture (footnote 5).

Note 3: AEs must be assessed for whether they are an SAE that requires expedited reporting daily. Other AEs, signs/symptoms of sepsis, antibiotics and concomitant medications must be recorded in the eDC for every day BUT these records can be completed retrospectively from notes and drug charts at the time points shown in the schedule of assessment.

* follow up by telephone / if clinically indicated, then hospital visit.
[1] Written informed consent to be obtained from parent/guardian; at minimum, verbal consent must be obtained before randomisation and documented in source document, with written informed consent to follow as soon as possible, and wherever possible within 48h of first trial related procedure.
[2] Randomisation and treatment initiation may be on the same day as the screening visit.
[3] Lab results should be available from a sample taken within 72h before randomisation, but these samples can be taken either at screening or randomisation, or values may be used from samples taken as part of clinical management outside of the trial but within 72h before randomisation. Test results are not required to be known at randomisation as not part of eligibility criteria.
Lab tests: Full Blood Count (FBC) including haemoglobin, platelets, white blood count (WBC) and neutrophil count (where available from local laboratory), CRP (where done additionally as part of routine care outside the scheduled timepoints in the Schedule of Assessment above). Urea & Electrolytes: blood urea nitrogen (BUN) or urea, creatinine, sodium, potassium. LFTs: ALT, AST, total bilirubin.
[4] Ideally, blood should be taken for culture within 48h before randomisation; however, if blood for culture has already been taken as part of the standard clinical management within the 48h preceding the screening visit, this test does not need to be repeated and results should be reported on the Culture and Susceptibility electronic Data Capture (eDC) for Day 0. Should the clinical condition of the neonate not allow a blood sample to be taken for culture, this will not be considered a protocol deviation; the neonate may be enrolled in the trial if all eligbility criteria are met. Any microorganisms isolated should be stored.
[5] Repeat blood culture only if neonate switches treatment (at the time of switch) due to clinical deterioration or lack of response. Blood for culture and for CRP testing should be taken before switch of antibiotics except in circumstances outside the responsible clinician's control and/or relating to the clinical condition of the neonate.
[6] Repeat blood tests only if abnormal at previous visit or neonate's condition not stable and/or there is a clinical concern (in order to minimise blood draws in this vulnerable population).
[7] Randomisation to second-line treatment if the neonate fails to respond or clinically deteriorates. Neonates should also be randomised to second-line treatment if they deteriorate after Day 3. In the situation where the neonate's clinical condition deteriorates rapidly between 24 and 48 hours, first-line treatment should also be randomised to second-line immediately. If randomisation is not possible, the neonate should be switched to a clinically appropriate regimen.

[8] Planned duration of treatment at randomisation is expected to generally be up to Day 7±2 days for blood culture-negative sepsis, and to Day 10 [-3,+4] days for blood culture-positive sepsis if there is no switch to second-line. If antibiotics are switched to second-line, the total duration of antibiotic treatment including first- and second- line treatment is expected to generally be up to Day 14 ±7 days depending on the neonate's condition.

[9] Signs and symptoms of sepsis after first-line randomisation (NeoSep Recovery Score) can be recorded on eDC retrospectively from routine clinical notes as part of routine assessment.

[10] Microbiology sub-study assessing colonisation will be conducted in selected sites only. Peri-rectal swabs should be taken at baseline (as soon as possible after randomisation if not possible logistically before randomisation) and at the end of antibiotic treatment, i.e. two swabs per neonate in total.

[11] SAEs that require expedited reporting must be reported daily within 24 hours from becoming aware. All other reportable AEs (SAEs not requiring expedited reporting, AEs related to or causing modification of any antibiotics, or AEs that are Grade 3-4 AEs) should be reported periodically on Day 3, Day 7, Day 14, 28 and 90 (as relevant) as per the Schedule of Assessment above, or on an unscheduled eDC. These AEs/SAEs should be reported if they occur from participant enrolment (the earliest of verbal assent or written consent) up to the later of Day 28 or the last administration of trial antibiotics plus 2 days. Re-admissions to hospital or death will be reported up to Day 90.

[12] At baseline including brief socio-economic history (e.g. parental age, educational level and broad measures of socio-economic status); at follow-up costs incurred by household including out-of-pocket expenditures, costs for transport to facilities, local food and accommodation, and income losses due to absences from work.

[13] Where cerebrospinal fluid (CSF) is taken for clinical reasons (not required for the trial) in a neonate who has received fosfomycin or flomoxef as part of the trial in the preceding 24h, if there is any residual CSF sample remaining after local testing and the site has storage facilities available, then this residual sample should be stored for pharmacokinetic analysis.

3.8 SAMPLE SIZE

Sample size is calculated based on simulations, given the number of different regimens involved.

At the first randomisation, we have assumed that personalised randomisation lists (Walker, White et al. 2021) will be drawn from a list of 8 regimens according to three different treatment patterns (see definition in **section 3.5**), reflecting their acceptability in different sites. At the second randomisation, it is assumed that personalised randomisation lists will be determined by the neonate's first randomised regimen, and include all regimens that are broader spectrum (excluding any regimen used in the first-line treatment).

Sample size calculations are informed by preliminary analyses from the neonatal observational study (NeoOBS). Following treatment under the first-line/second-line strategies available, 28 day mortality is expected to vary from 10-20%. Fixed values for first-line and second-line regimen effects have been selected to achieve this variation. We have assumed an equal split between the three assumed treatment patterns of randomisation, 5% early mortality before second randomisation and 25% of neonates switching to a randomised second-line treatment. Simulations were performed to investigate how much information would be provided by the planned trial design under varying sample sizes.

It is estimated that, compared to assigning a random regimen to each neonate, using "top-ranked" strategies based on results from a trial including 3000 neonates would achieve 65-72% of the maximum possible reduction in mortality across the population, and a 91-93% chance of reducing mortality for each neonate. This would also lead to a 79-86% chance of mortality being within 2% of the best strategy for each neonate. In sensitivity analyses, we varied two assumptions to allow unequal treatment patterns of randomisation and 50% switching to randomised second-line treatment and obtained similar results.

Neonates for whom verbal assent is confirmed by written consent, and neonates that die before verbal consent will contribute to the total sample size (following the approved protocol). This is in order to ensure that these children contribute to the primary outcome (mortality at Day 28) and to SAE pharmacovigilance. If verbal consent is not confirmed by written consent then no further data will be collected and the neonate will not count towards the sample size (will be explicitly counted as verbal consent not confirmed by written consent).

A sample size review will be conducted when 50% of participants have completed the Day 28 follow-up visit, as part of an interim analysis (see **section 6.4.2**). This will not use information about differences between randomised arms but would use the percentages randomised under different first-line treatment patterns and an overall (blinded) estimate of the primary endpoint rate (assumed to range from 10-20%, average 15% in original calculations).

4 DATA AND DEFINITIONS

4.1 DEFINITION OF POST-NATAL AGE

For the purpose of this trial, post-natal age will be calculated by considering the date of birth as the first day of life e.g. the neonate is considered to be 1 day old on the day of birth.

4.2 DEFINITION OF BASELINE

Baseline is defined as the date of randomisation (Day 0, see **Table 1**). Baseline value is defined for lab results as the latest measurement up to 72 hours before randomisation, for blood culture the latest sample up to 48 hours before screening, and for signs and symptoms of sepsis the last assessment between (and including) presentation and randomisation.

4.3 DEFINITION OF FOLLOW-UP

Time will be measured from randomisation (Day 0). For the analysis of the primary endpoint, follow-up will be to Day 28, i.e. 29 days from and including the day of randomisation, based on the ascertainment of mortality.

Re-admissions to hospital or deaths will be reported up to Day 90, the last trial visit (see **Table *1***). If participants are censored earlier due to loss to follow-up or withdrawal of consent, it will be assumed that such censoring is independent of the outcome.

A participant who does not attend their end of trial visit/call (Day 90) will be classified as “lost to follow-up” if they are not known to have died and the clinic has confirmed that they are unable to contact them.

4.4 SAFETY

The adverse event assessment will explicitly record signs and symptoms of untoward medical occurrence, regardless of grade, including possible drug toxicities. AEs (clinical and laboratory) will be graded using a combination of the DAIDS grading scales (Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events) and the clinically based NAESS (Salaets, Turner et al. 2019), with wording adapted to reflect available diagnostic and management options in LMICs and applying the NAESS generic severity grading (which refers to changes in care and monitoring) whenever possible across NAESS and DAIDS specified events relevant to neonatal care.

All adverse events of any grade that lead to modification (including discontinuation) of antibiotics or are considered related to antibiotics will be reported on eDC, as will any Grade 3 or 4 adverse events.

SAEs are not an outcome measure in NeoSep1 because the neonates will be very sick when admitted, however, will be reported for pharmacovigilance purposes. For the same reason, and in contrast to NeoSep1 Part 1, grade 1 and 2 AEs occurring as a result of the participant’s medical condition or standard hospital treatment will not be collected.

4.5 NEOSEP SEVERITY SCORE

The NeoSep Severity Score was developed for predicting 28-day mortality based on clinical information at the start of a new episode of sepsis. It was adapted from the WHO PSBI (possible serious bacterial infection) based scores for the hospital setting and developed from the NeoOBS study as described in the table below. It will be used at screening to enrol only neonates at a moderate to high risk of death from this episode of sepsis (NeoSep Severity Score of 5 or higher at presentation).

Table 2. NeoSep Severity Score

Factor (clinical signs in the 24h preceding start of clinical sepsis episode)	Score value if present
Time in hospital: ≤ 10 days	1
Gestational age: <37 weeks	1
Birth Weight: • >2 kg • 1-2 kg • <1 kg	 0 1 2
Congenital anomalies	2
Temperature • <35.5 °C • 35.5 to 37.9 °C • 38 to 38.9 °C • ≥39 °C	 1 0 1 2
Maximum respiratory support: • Oxygen supplementation • CPAP, BiPAP, HFNC • Invasive ventilation	 2 3 3
Abdominal distension	1
Being fed and difficulty in feeding/feeding intolerance*	1
Evidence of shock including cold peripheries	1
Lethargy / no or reduced movement† • Lethargic but moving spontaneously • No spontaneous movement or movement only on stimulation	 1 2

CPAP = continuous positive airway pressure, BiPAP = Bilevel Positive Airway Pressure, HFNC = high flow nasal cannula.
* Neonates ordered nil by mouth score 0; if intention is to feed and neonate is not feeding, then score 1.
† Note that this should represent an acute change in activity/movements; Neonates with persistent hypotonia, e.g. due to congenital disease or those who have no spontaneous movement due to sedative and/or paralytic medications, score 0.

4.6 NEOSEP RECOVERY SCORE

The NeoSep Recovery Score was developed from daily updated assessments of neonates' status in the NeoOBS study to predict mortality and guide clinical decision making, for example switch to second-line treatment.

Table 3. NeoSep Recovery Score

Factor (clinical signs in the preceding 24h)	Score value if present
Temperature • <35.5°C • 35.5 to 37.9°C • 38 to 38.9 °C • ≥39 °C	 1 0 1 2
Maximum respiratory support: • Oxygen supplementation • CPAP, BiPAP, HFNC • Invasive ventilation	 2 3 3
Abdominal distension	1
Being fed and difficulty in feeding/feeding intolerance*	1
Evidence of shock including cold peripheries	1
Lethargy / no or reduced movement† • Lethargic but moving spontaneously • No spontaneous movement or movement only on stimulation	 1 2
Cyanosis	1

CPAP = continuous positive airway pressure, BiPAP = Bilevel Positive Airway Pressure, HFNC = high flow nasal cannula.
* Neonates ordered nil by mouth score 0; if intention is to feed and neonate is not feeding, then score 1.
† Note that this should represent an acute change in activity/movements; neonates with persistent hypotonia, e.g. due to congenital disease or those who have no spontaneous movement due to sedative and/or paralytic medications, score 0.

4.7 NEURODEVELOPMENTAL DELAY

Neurodevelopmental delay will be assessed using the WHO Global Scale for Early Development (GSED) package at Day 28 and Day 90 (World Health Organization, 2023). This package is an internationally validated open-access tool that was developed to assess childhood development in a culturally neutral and easy to undertake way, that is acceptable and understandable to parents/guardians. It is used to assess the development of children under the age of three and can be based on caregiver self-report (i.e. done over the telephone).

4.8 HEALTH ECONOMICS

This will be described elsewhere.

5 ESTIMANDS FRAMEWORK

Table 4. Estimand framework for the primary analysis of the primary outcome

Treatments	The comparison is between the following first-line antibiotic treatments: **First-line treatment options:** • Ampicillin° and gentamicin • Cefotaxime or ceftriaxone • Fosfomycin and amikacin • Flomoxef and amikacin • Fosfomycin and flomoxef • Ceftazidime and amikacin • Piperacillin/tazobactam and amikacin • Meropenem
Population	The population of interest is hospitalised neonates aged ≤28 days and weighing ≥1000g with clinical signs of sepsis as defined in **section 3.4.1**.
Endpoint	Death by Day 28
Population-level summary measure	Hazard ratio
Intercurrent events • Not providing written consent following verbal consent	Principal stratum (modified intention-to-treat) [§]
• Not starting randomised first-line treatment	Treatment policy (use endpoint regardless of whether or not this intercurrent event had occurred) [¶]
• Any treatment modification including starting second-line treatment	Treatment policy (use endpoint regardless of whether or not this intercurrent event had occurred) [¥]
• Missed doses of treatment	Treatment policy (use endpoint regardless of whether or not this intercurrent event had occurred) [‡]

Notes: °or benzylpenicillin or cloxacillin or flucloxacillin or amoxicillin

[§] Modified ITT meaning excluding from the analysis participants where verbal consent was not confirmed by written consent. The only exception are neonates who die prior to written consent being obtained, when this primary outcome will be used in analysis to ensure accuracy of results.

[¶] If <1% do not initiate randomised first-line, treatment policy will be the only analysis. If 1-5% do not initiate, a secondary analysis of the primary outcome only will use principal stratum (modified ITT) meaning excluding from the analysis participants who did not initiate randomised treatment. This approach is unbiased under the assumption that the intercurrent event (i.e. not starting randomised first-line treatment) is not affected by the assigned treatment, that is participants who do not initiate one particular treatment (for example ampicillin & gentamicin) would also not initiate treatment if assigned to another treatment (for example meropenem). However, if non-initiation is >5%, inverse-probability weighting methods will be used in secondary analyses of the primary endpoint only.

[¥] In secondary analyses, inverse-probability weighting methods will be used, see **section 6.1**.

[‡] Missed doses are expected to be rare because neonates will be hospitalised and hospital staff will be responsible for antibiotic administration.

6 STATISTICAL PRINCIPLES

6.1 GENERAL CONSIDERATIONS

Analysis of the trial data will be carried out by using network meta-analytic methods to compare the first-line/second-line strategies and to rank strategies with respect to each outcome exploiting both the direct randomised comparisons and the indirect information across the network (Lee, Turner et al. 2023). Bootstrapping will be used to estimate uncertainty in the rankings. There is no formal pairwise hypothesis testing because there is no standard of care arm against which to conduct these tests.

In the primary analysis of the primary outcome, we will present hazard ratios comparing each first-line regimen against the control WHO regimen (ampicillin + gentamicin), regardless of any second-line treatment, together with their 95% CI. Analyses will use time-to-event models with regimen, treatment pattern and site as factor variables. To aid clinical interpretation, we will also convert adjusted hazard ratios to differences in percentages by applying them to the baseline estimate of the survival function from the adjusted Cox model. The reason for using a Cox model rather than a logistic model is to incorporate censoring from potential losses to follow-up before Day 28.

In a secondary analysis of the primary endpoint, we will present hazard ratios comparing each strategy (i.e. combination of first- AND second-line regimen) against the control strategy of WHO regimens (ampicillin + gentamicin followed by ceftriaxone/cefotaxime). For participants who do not require a second randomisation, cloned records will be created to represent all possible regimens if randomised a second time. An inverse probability weighting approach will then be taken, with a weight equal to 1/(number of treatments in second-line treatment pattern) for each cloned record ensuring that each unswitched participant has the same weight in the analysis (summed over their cloned records) as each switched participant. Participants who switch to non-randomised second-line therapy will be censored from the time of switching in this secondary analysis and similar participants who switched but were randomised to second-line will be upweighted using a different set of weights.

We will explore heterogeneity in particular subgroups which are used to define personalised randomisation lists tailored by each site (early onset sepsis; late onset sepsis; suspicion of meningitis; no suspicion of meningitis; suspicion of necrotising enterocolitis). Heterogeneity in other subgroups such as culture positive vs culture negative (including specific organisms e.g. Klebsiella pneumoniae, if there are sufficient numbers) and subgroups defined by baseline CRP will also be explored.

Analyses of secondary outcomes will use similar methods (including ranking). Where there is strong evidence for a difference between randomised first-line regimens in a composite secondary outcome (for example Recovery Score), then separate comparative analysis using ranking will be undertaken for its components.

In Part 2, the primary objective is to provide a ranking of eight different clinically relevant antibiotic regimens for first-line empiric and second-line (after lack of response/deterioration) treatment in terms of 28-day mortality as the primary outcome measure using the treatment policy approach for intercurrent events, including starting second-line treatment. In Part 2, a secondary objective is to provide a ranking of clinically relevant antibiotic regimens based on other efficacy and safety secondary outcomes, as well as on health economic measures and the potential selection of resistance. Overall, addressing this secondary objective, the determination of which strategies perform best with respect to mortality, safety, cost and resistance, will be carried out in two steps. As a first step in addressing this secondary objective, rankings will be examined with respect to mortality and safety, to identify a set of antibiotic regimens that dominate the others, i.e. are safer and more effective. Rankings of strategies from best to worst will be presented in a table and also illustrated in a plot showing performance in both dimensions. The rankings of this remaining set of regimens

with respect to resistance either in infecting isolates on carriage in the microbiology substudy will then be examined as a second step to determine how this affects the ranking on mortality and safety. Costs (which may vary by region) will be examined independently in a health economic analysis overall and/or by region.

Results which are not primary or secondary outcomes will be presented without ranking.

6.2 GENERAL STATISTICAL PRINCIPLES

6.2.1 Descriptive statistics

Categorical variables will be summarised using frequencies (n) and percentages; continuous variables will be summarised using the mean and standard deviation (SD) or median, lower quartile, and upper quartile plus minimum and maximum values. Ordinal variables will be described using median, lower and upper quartile plus minimum and maximum values. Descriptive statistics will be reported overall and by treatment group, and percentages will be of non-missing values, with the number (%) of non-missing values given if data are not complete.

6.2.2 Binary outcomes

Chi-squared test and logistic or binomial regression (adjusting for treatment pattern) will be used for the analysis of binary outcomes (specified in detail in **section 7).**

6.2.3 Continuous outcomes

T-tests and normal linear regression (adjusted for baseline values and treatment pattern) will be used for the analysis of continuous outcomes (specified in detail in **section 7**). Appropriate transformations will be applied after inspecting the distribution of the data.

6.2.4 Time to event outcomes

Kaplan-Meier analysis, Cox proportional-hazards models and competing risk regression (adjusting for treatment pattern) will be used for time-to-event outcomes (specified in detail in **section 7).** Where possible time-to-event methods will be used to account for deaths before reaching the secondary endpoint, for example all-cause re-admissions.

6.3 ANALYSIS POPULATIONS

The primary analysis population is intention-to-treat, including all randomised neonates, regardless of whether they received the allocated treatmentor not, excluding those where verbal consent was not confirmed by written consent and the neonate did not die (following the approved protocol). This corresponds to estimating the impact of the effectiveness of the strategies.

6.4 STATISTICAL INTERIM ANALYSES AND STOPPING GUIDANCE

Data will be reviewed by an independent Data Monitoring Committee (DMC). A DMC charter will be drawn up that describes the membership of the DMC, relationships with other committees, terms of reference, decision-making processes. The Charter will also contain a description of stopping guidelines and membership.

The DMC will meet within 6 months after the trial opens; although the DMC will in general meet every 6-9 months, the frequency of subsequent meetings will be determined by the DMC and could be more frequent if deemed necessary. The DMC will review all available data on safety parameters for all antibiotic regimens. The DMC can recommend premature closure or reporting of the trial, or that recruitment to any randomised group be discontinued or modified. Such recommendations would be made if, in the view of the DMC, there is proof beyond reasonable doubt that one of the allocated strategies is better than any other in terms of a difference of clinically significant magnitude in a primary outcome. The guiding statistical criteria for "proof beyond reasonable doubt" is a Haybittle-Peto type rule based on the 99.9% confidence interval.

6.4.1 Feasibility phase

A feasibility phase will enrol approximately 10% of the trial cohort (300 patients) to assess the feasibility of implementing the study at the participating sites. This will focus on:

- Assessing recruitment compliance with first-line treatment options
- Assessing implementation of second randomisation and compliance to second-line treatment options
- Assessing the percentage of culture positive babies at baseline
- Review (by DMC) of sodium levels at baseline, particularly the percentage of neonates with sodium ≥ 150 mmol/L

6.4.2 Sample size review

A sample size review will be conducted when 50% of participants have completed the Day 28 follow up visit, as part of an interim analysis. This will update the sample size calculations in the light of accumulating evidence about the frequency of use of each personalised randomisation list and the overall mortality rate (i.e. will not use information about estimated differences between randomised groups at the time). It will consider whether recruitment should continue to the original target based on the overall mortality rate within the trial (assumed to range between 10-20%, average 15%, for sample size calculations as above) or be modified, or whether for example, the randomised allocation ratio should be varied from 1:1 to randomise more neonates to less represented regimens. Any decision to increase the sample size is a Sponsor decision in collaboration with the TSC.

6.5 TIMING OF FINAL ANALYSIS

The final analysis will be performed after the last randomised participant has reached Day 90, data have been cleaned and database locked.

6.6 MISSING DATA

Every effort will be made to collect all data as per the schedule of assessments, and we expect the number of missing data to be relatively small. Patients who withdraw consent will be excluded from trial analyses from the time they withdraw. When missing data occurs, it is assumed that it will be at random, and all analyses will be based on observed data (for the primary outcome, see below).

6.6.1 Primary outcome

As follow-up for the primary outcome is short (28 days post-randomisation), loss-to-follow-up is expected to be low. The primary analysis will be conducted on observed data using time-to-event methods, assuming not died if censored (lost/withdrawn) prior to Day 28.

6.7 CONFIDENCE INTERVALS AND P VALUES

Estimates from statistical models will be presented with two-sided Wald 95% confidence intervals (CIs). 95% CIs around single percentages will not be presented because first-line groups will not necessarily be balanced by design in terms of disease severity or other risk factors because of the different randomisation lists. Formal statistical tests for hypothesis testing will not be applied in this trial.

6.8 STATISTICAL SOFTWARE

Analyses will be performed using Stata version 18 (or above), unless otherwise specified.

7 ANALYSIS DETAILS

7.1 RECRUITMENT

- Number screened and enrolled by calendar time (by calendar month and cumulative)
- Total screened and enrolled by centre, with dates of first and latest enrolment
- Eligibility: number and reasons for any participants found to be ineligible after enrolment
- Reasons for not enrolling screened subjects will also be described.

Screening, recruitment and retention data will also be presented in a CONSORT flow diagram.

7.2 BASELINE CHARACTERISTICS

If not reporting n (%), characteristics will be described as defined for continuous data (**section 6.2.1**). Of note, first-line groups will not necessarily be balanced by design because of the different randomisation lists. Therefore, testing for imbalance will not be performed. Signs and symptoms of sepsis may be presented separately for the time of first sepsis assessment, in addition to baseline as defined in **section 4.2**.

- Sex: n (%) male, female
- Age (in days from birth)
- Time in hospital at presentation (days)
- Gestational age at birth (weeks): summarised as continuous variable, and n (%) in categories preterm (<37) and term (≥37)
- Birth weight (g): summarised as continuous variable, and n (%) in categories <1 kg, 1-2 kg, >2 kg
- Presence of congenital abnormalities: n (%) in categories none, minor, major
- Temperature (°C): summarised as continuous variable, and n (%) in categories <35.5 °C, 35.5 to 37.9 °C, 38 to 38.9 °C, ≥39 °C
- Respiratory support: n (%) in categories oxygen supplementation, CPAP/BiPAP/HFNC, invasive ventilation
- Abdominal distension: n (%)
- Difficulty in feeding or feeding intolerance: n (%)
- Evidence of shock: n (%)
- Lethargy or reduced/no movement: n (%) in categories lethargy only, movement only on stimulation, no movement
- NeoSep Severity Score (see **section 4.5**): median, first quartile, third quartile, minimum and maximum; may also be grouped, for example into low (score 0-4), medium (5-8) and high (9-16) risk groups, and presented categorically
- Mode of delivery: n (%) in categories elective (planned) caesarean section, emergency caesarean section, vaginal delivery (spontaneous), vaginal delivery (assisted)
- HIV status: n (%) in categories infected, exposed/uninfected, uninfected, not known/not tested
- Any lines used in the past 24 hours: n (%) in categories umbilical venous line, umbilical arterial line, peripheral arterial line, central venous line, PICC line
- Weight (g)
- Length (cm)
- Heart rate (beats per minute)

- Respiratory rate (breaths per minute)
- Oxygen saturation (%)
- Convulsions: n (%)
- Severe chest indrawing: n (%)
- Increase in oxygen requirement (at baseline, reflecting clinical status): n (%)
- Increase of respiratory support (at baseline, reflecting clinical status): n (%)
- Cyanosis: n (%)
- Irritability: n (%)
- Start of IV antibiotics for this episode of sepsis before enrolment: n (%) overall; n (%) per antibiotic, total daily dose (mg or IU) per antibiotic, duration of treatment (hours)
- Systemic antibiotics taken for other reasons in preceding 7 days: n (%) overall, n (%) per antibiotic, time since stop (hours)
- Sodium (mmol/L)
- Potassium (mmol/L)
- Blood Urea Nitrate (mmol/L)
- Creatinine (µmol/L)
- CRP (mg/L)
- ALT (U/L)
- AST (U/L)
- Total bilirubin (µmol/L)
- Haemoglobin (g/dL)
- WBC ($x10^9$ cells/L)
- Neutrophils ($x10^9$ cells/L)
- Platelets ($x10^9$ cells/L)

7.3 FIRST-LINE ANTIBIOTIC TREATMENT

- Allocated first-line treatment: number (%) started / not started (plus reasons if not started); description of antibiotic regimens and treatment patterns

7.4 WITHDRAWAL / FOLLOW-UP

- For each of the follow-up visits on Days 7, 14, 28 and 90: number (%) happened/missed (plus reasons if missed); number (%) attended in clinic or assessed by telephone; n (%) on antibiotic treatment
- Withdrawal from trial participation before a) 28 days, and b) 90 days: number (%); description of reasons
- Lost to follow-up by Day 90: number (%)

7.5 PRIMARY OUTCOME ANALYSIS

28-Day mortality will be analysed using time-to-event methods with regimen, treatment pattern (see **section 3.5**) and site as factor variables (see **section 6.1**). The reason for the primary analysis using time-to-event methods is to account for an unknown percentage of children being lost-to-follow-up before day 28, e.g. post-discharge. As there is a possibility that there could be variation in the relative differences between

intervention arms between early and late deaths, a test of non-proportionality will be conducted, accompanied by visual inspection of Kaplan-Meier curves. If there is evidence of non-proportionality ($p<0.05$ or Kaplan Meier curves crossing), then 28-day mortality will also be analysed as a binary outcome using logistic regression to provide a risk ratio that reflects the "net" mortality difference at 28 days. If missing data due to lost-to-follow-up are <5% then analysis will be complete case. Otherwise, day-28 mortality will be imputed using separate imputation models per arm, and including as factors site, treatment pattern, and NeoSep Severity Score.

7.6 SECONDARY OUTCOME ANALYSIS: EFFICACY

7.6.1 Clinical status

Clinical status will be assessed daily after randomisation through to the earlier of discharge from a trial site or Day 28.

- The NeoSep Recovery Score (see **section 4.6**) will be summarised at Days 3, 7, 14, and 28 as defined for continuous variables, and by n (%) in categories <4, and ≥4 points. Treatments will be analysed (and ranked) using normal linear regression or (ordered) logit models adjusted for treatment pattern and the baseline value (absolute or in categories), as appropriate.
- Individual signs and symptoms of sepsis after enrolment will be summarised as described in **section 6.2.1**; ranking treatment regimens will only be done if there is strong evidence for a difference between randomised first-line regimens in the Recovery Score:
  - Temperature (°C): summarised as continuous variable, and n (%) in categories <35.5 °C, 35.5 to 37.9 °C, 38 to 38.9 °C, ≥39 °C
  - Respiratory support: n (%) in categories oxygen supplementation, CPAP/BiPAP/HFNC, invasive ventilation
  - Abdominal distension: n (%)
  - Difficulty in feeding: n (%)
  - Evidence of shock: n (%)
  - Lethargy or reduced/no movement: n (%) in categories lethargy only, movement only on stimulation, no movement
  - Cyanosis: n (%)
  - Heart rate (beats per minute)
  - Respiratory rate (breaths per minute)
  - Oxygen saturation (%)
  - Convulsions: n (%)
  - Irritability: n (%)
  - Severe chest indrawing: n (%)

7.6.2 Additional systemic antibiotics beyond the first randomised treatment through Day 28

- Number (%) receiving ANY additional systemic antibiotics beyond the first randomised treatment through Day 28. Treatments will be analysed and ranked as defined for time-to-event outcomes with death before receipt of additional systemic antibiotics beyond the first randomised treatment as competing risk.
  - As explorative outcome, the number (%) experiencing the competing risk (death before receipt of additional systemic antibiotics beyond the first randomised treatment) will also be analysed, as will the composite endpoint of death or receipt of additional systemic

antibiotics beyond the first randomised treatment (a proxy for failure of first-line treatment).
    - Further additional systemic antibiotics beyond the first randomised treatment could be before or after stopping the first randomised treatment (defined as no antibiotic doses for ≥48h). The former could occur for toxicity or perceived failure (noting that this is a subjective judgement in an open-label trial and cannot be assumed to reflect genuine treatment failure, particularly in this very sick population with multiple other comorbidities); the latter could occur for a new infection episode. The randomised intervention could, however, have very different effects on these two components, so these will also be considered separately in exploratory competing risks analyses.
    - Note: the reason for proposing both competing risks and composite endpoint analysis is because the percentages receiving additional antibiotics beyond randomised first-line and dying before doing so are unknown. Given the patient population, it is possible that many deaths are not antibiotic-modifiable, in which case the composite endpoint would suffer from dilution bias were there genuine benefits from some antibiotics over others. However, if any benefits were similar for both early deaths and first-line treatment failure, and providing that receiving additional antibiotics is a reasonable proxy for first-line treatment failure (not necessarily the case in an open-label trial) then the composite endpoint could have greater power. Whilst the competing risks analysis will be the primary analysis, we will therefore accompany this with an exploratory composite endpoint analysis.
- Additionally, the following will be summarised:
    - Number (%) receiving randomised second-line treatment; of those randomised, n (%) started randomised 2nd-line regimen; description of randomised second-line antibiotics
    - Number (%) receiving non-randomised second-line treatment; description of second-line antibiotics and reasons for not randomising to second-line
    - Number (%) receiving additional systemic antibiotics for other reasons than presumed failure of the initial regimen (e.g. new episode of sepsis); description of antibiotics and reasons

7.6.3 Additional systemic antibiotics beyond the first randomised and second (for failure) treatment through Day 28

- Number (%) receiving additional systemic antibiotics beyond the first randomised and second (for failure) treatment through Day 28. Treatments will be analysed and ranked as defined for time-to-event outcomes with death before additional systemic antibiotics beyond the first randomised and second (for failure) as competing risk.
    - As explorative outcome, the number (%) experiencing the competing risk (death before receipt of additional systemic antibiotics beyond the first and second randomised treatment) will also be analysed, as will the composite endpoint of death or receipt of additional systemic antibiotics beyond the first randomised and second (for failure) treatment (a proxy for failure of first-line and second-line treatment)
- Additionally, antibiotics and reasons will be described.

Description of all non-allocated antibiotics used per participant: total number, type and class of antibiotics, reason started.

7.6.4 Length of stay during the index hospitalisation

- Length of stay (days) during the index hospitalisation is defined as time from day of randomisation (Day 0) to the day of first discharge. It will be analysed using competing-risks regression models with in-hospital death as competing risk, as defined for time-to-event outcomes. Ranking will be based on the beta coefficients (subhazard ratios) from the model. If participants are transferred to another hospital they will be censored at the time of transfer.

7.6.5 Systemic antibiotic exposure

- Systemic antibiotic exposure (days on antibiotics) during the index hospitalisation: time to stop of all IV antibiotics: cumulative incidence

7.6.6 90-day mortality

- 90-day mortality: number (%); analysed as defined for time-to-event outcomes.

7.6.7 CRP

- CRP at Day 3
- CRP at Day 7
- Change in CRP from baseline to Day 3
- Change in CRP from baseline to Day 7

CRP and change in CRP will be described using means (SD) and analysed using linear regression (adjusted for baseline values and treatment pattern), as defined for continuous outcomes.

7.6.8 Re-admission by Day 90 (all-cause)

- Re-admission by Day 90 (all-cause): number (%); analysed as defined for time-to-event outcomes with death as competing risk, and late entry at the initial discharge. Only the first re-admission per participant will be considered. The number of neonates with more than one re-admission will be tabulated.

7.7 SECONDARY OUTCOME ANALYSIS: SAFETY

Adverse events, including SARs, will be summarised by body system (MedDRA System Organ Class), and within body system by MedDRA Preferred Term.

- Number (%) of participants experiencing a grade 3/4 adverse event through Day 28
- Number (%) of participants experiencing an adverse event of any grade related to antibiotics through Day 28
- Number (%) of participants experiencing a modification (including discontinuation) of antibiotics for adverse reactions through Day 28

These secondary outcomes will be analysed and ranked as defined for binary outcomes.

- Neurodevelopment at Day 28 and Day 90: described and analysed as defined for continuous outcomes.

Although not an outcome, a line listing of all SAEs will be generated.

Of note, adverse event will be collected up to the later of Day 28 or the last administration of trial antibiotics plus 2 days for pharmacovigilance. However, the safety endpoints above will be calculated through Day 28 only to ensure that randomised groups are compared across the same time periods, regardless of changes to antibiotic treatment (following the principles of intention-to-treat).

A sensitivity analysis will consider modification (including discontinuation) of antibiotics for adverse events (rather than reactions) through Day 28 to incorporate the possibility of ascertainment bias in determining relatedness as the trial is open-label. However, this alternative definition will include events unrelated to

antibiotic administration which nevertheless require changes in antibiotics, e.g. due to drug-drug interactions, and hence is a sensitivity analysis.

7.8 MICROBIOLOGY

7.8.1 Baseline blood culture

- Blood culture taken: n (%)
- Blood culture results: n (%) in categories no organisms found, contaminant, pathogen; description of type of pathogen and antimicrobial susceptibility as determined locally by phenotypic methods.

7.8.2 Blood cultures after enrolment

Similar to above, any new culture results post baseline will be described, e.g. prior to starting second-line treatment.

7.8.3 CSF culture results

CSF samples are not mandatory in this trial. However, any CSF culture results will be described similar to blood culture results.

7.9 CONCOMITANT MEDICATION

- Description of all concomitant medication used in the trial, including treatment started prior to but continued beyond randomisation, by drug and class (WHO Anatomical Therapeutic Chemical 1st level). All non-topical medications (other than systemic (i.e. IV and oral) antibiotics which will be analysed as a secondary outcome) for any condition are considered a concomitant medication, regardless of route of delivery, including blood transfusion and vitamin infusions. Topical medications will not be collected and are not considered concomitant medications.

7.10 IMPORTANT PROTOCOL DEVIATIONS

Protocol deviations are defined in the NeoSep1 Quality Management and Monitoring Plan and classified as critical, major, or minor. Critical and major deviations constitute important protocol deviations according to the MRC CTU Protocol Deviations SOP.

- Important protocol deviations: number (%) overall and by type

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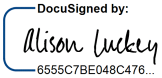
Alison Luckey aluckey@gardp.org Security Level: Email, Account Authentication (Optional)	DocuSigned by:  6555C7BE048C476... Signature Adoption: Pre-selected Style Using IP Address: 85.255.236.20	Sent: 30 January 2025 | 15:32 Viewed: 04 February 2025 | 08:34 Signed: 04 February 2025 | 08:57
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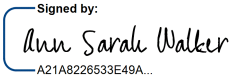
Ann Sarah Walker rmjlasw@ucl.ac.uk Trial Statistician/Project Lead Security Level: Email, Account Authentication (Optional)	Signed by:  A21A8226533E49A... Signature Adoption: Pre-selected Style Using IP Address: 129.222.187.68	Sent: 30 January 2025 | 15:32 Viewed: 31 January 2025 | 09:00 Signed: 31 January 2025 | 09:00
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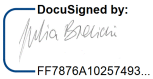
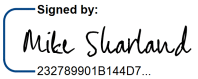
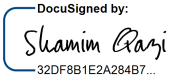
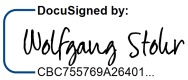
Julia Bielicki jbielick@sgul.ac.uk Security Level: Email, Account Authentication (Optional)	DocuSigned by:  FF7876A10257493... Signature Adoption: Uploaded Signature Image Using IP Address: 131.152.222.185	Sent: 30 January 2025 | 15:32 Viewed: 31 January 2025 | 09:13 Signed: 31 January 2025 | 09:13
Electronic Record and Signature Disclosure: Accepted: 07 May 2021 | 12:54 ID: 17aa40b8-8882-4016-99c6-859cb3126dcc		

Signer Events	Signature	Timestamp
Mike Sharland msharland@sgul.ac.uk Michael Sharland Security Level: Email, Account Authentication (Optional) **Electronic Record and Signature Disclosure:** Accepted: 05 November 2020 | 13:36 ID: 66862256-a905-4c5d-ba30-bb75242d0362	Signed by:  232789901B144D7... Signature Adoption: Pre-selected Style Using IP Address: 92.233.184.76 Signed using mobile	Sent: 30 January 2025 | 15:32 Resent: 09 February 2025 | 13:42 Viewed: 09 February 2025 | 14:57 Signed: 09 February 2025 | 14:57
Shamim Qazi qazis550@gmail.com Security Level: Email, Account Authentication (Optional) **Electronic Record and Signature Disclosure:** Accepted: 03 July 2021 | 12:06 ID: b809a216-483b-4e32-ad61-5dbc1afcb28f	DocuSigned by:  32DF8B1E2A284B7... Signature Adoption: Pre-selected Style Using IP Address: 213.55.246.190	Sent: 30 January 2025 | 15:32 Viewed: 02 February 2025 | 08:31 Signed: 02 February 2025 | 08:34
Tim Peto tim.peto@ndm.ox.ac.uk Security Level: Email, Account Authentication (Optional) **Electronic Record and Signature Disclosure:** Accepted: 22 February 2024 | 17:02 ID: 5c123a4e-cc39-4cb7-9b8b-21379c79a72b	**Uploaded paper with hand signature** Signature Adoption: Signed on Paper Using IP Address: 80.6.251.127	Sent: 30 January 2025 | 15:32 Resent: 09 February 2025 | 13:42 Viewed: 09 February 2025 | 13:53 Signed: 09 February 2025 | 13:55
Wolfgang Stohr w.stohr@ucl.ac.uk Security Level: Email, Account Authentication (Optional) **Electronic Record and Signature Disclosure:** Accepted: 27 June 2023 | 15:19 ID: 0582dc5c-e1eb-4f44-9ffa-bb68396f08a8	DocuSigned by:  CBC755769A26401... Signature Adoption: Pre-selected Style Using IP Address: 144.82.114.236	Sent: 30 January 2025 | 15:32 Viewed: 30 January 2025 | 15:57 Signed: 30 January 2025 | 15:57

In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	30 January 2025 | 15:32
Certified Delivered	Security Checked	30 January 2025 | 15:57
Signing Complete	Security Checked	30 January 2025 | 15:57
Completed	Security Checked	09 February 2025 | 14:57

Payment Events	Status	Timestamps

Electronic Record and Signature Disclosure

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