Statistical Analysis Plan

TRIAL FULL TITLE	PRE-DELIVERY ADMINISTRATION OF AZITHROMYCIN TO PREVENT NEONATAL SEPSIS AND DEATH: A PHASE III DOUBLE-BLIND RANDOMIZED CLINICAL TRIAL
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2 Abbreviations and Definitions

AE	Adverse Event
CRF	Case Report Form
CSF	Cerebrospinal fluid
GAS	Group A streptococcus
GBS	Group B streptococcus
IMP	Investigational Medical Product
HPS	Hypertrophic pyloric stenosis
HAZ	Height-for-age z-score
LBW	Low birth weight (<2.5kg)
MUAC	Mid upper arm circumference
RR	Risk ratio
SAP	Statistical Analysis Plan
SD	Standard deviation
VLBW	Very low birth weight (<1.5kg)
WAZ	Weight-for-age z-score
WHZ	Weight-for-height z-score

3 Introduction

3.1 Preface

The study aims to assess the impact of azithromycin treatment during labour on neonatal mortality as well as maternal and neonatal sepsis and infant growth. If successful, this simple intervention could be implemented through the health system at the most peripheral level of care. It has the potential to achieve wide coverage in sub-Saharan Africa where low-cost interventions to reduce neonatal mortality are urgently needed.

3.2 Purpose of the analyses

These analyses will assess the efficacy and safety of the intervention and will be included in peer-reviewed publications of the trial results.

3.3 Study Objectives

(ICH E3; 8)

Primary objective

To assess the effect of giving one oral dose of AZI (2g) during labour on the combined endpoint of neonatal sepsis and mortality. Deaths due to severe birth asphyxia, very low birth weight (VLBW) and major congenital malformations will be excluded from the composite endpoint.

Secondary objectives

To examine the effect of the intervention on other clinical endpoints and microbiological endpoints in the neonate and mother.

Safety objectives

To evaluate the safety of the intervention through active and passive surveillance for adverse events (AE) in mothers and newborns during the 28-day follow-up period. All newborns with history of projectile vomiting will be thoroughly assessed for hypertrophic pyloric stenosis (HPS).

3.4 Endpoints

(ICH E9; 2.2.2)

Primary endpoint:

Neonatal sepsis or death within 28 days of birth, with the latter excluding deaths due to severe birth asphyxia, very low birth weight (VLBW) or a major congenital malformation.

Secondary endpoints:

Neonates (first 28 days of life)

1. Sepsis alone
2. Neonatal deaths, excluding those due to severe birth asphyxia, VLBW and major congenital malformations
3. Culture-confirmed sepsis
4. All-cause hospitalisation
5. All-cause mortality among VLBW

6. Skin infections
7. Bacterial conjunctivitis
8. Umbilical cord infections
9. Clinical malaria
10. Use of antibiotics during the neonatal period

Anthropometric cohort (1,000 infants) – follow-up for 12 months

1. All-cause mortality
2. Malnutrition (day 28, 6 and 12 months) - Height-for-age z-score (HAZ), weight-for-age z-score (WAZ), weight-for-height z-score (WHZ), body mass index-for-age, head circumference-for-age, and mid-upper arm circumference for age (MUAC)

Mothers (during the 28 days follow-up)

1. Post-partum sepsis
2. Post-partum mastitis
3. Post-partum malaria
4. Post-partum fever
5. Post-partum antibiotic use
6. Post-partum hospitalisation
7. Post-partum mortality

Hospitalised neonates

1. *Streptococcus pneumoniae* in nasopharyngeal swabs
2. *Klebsiella* species in nasopharyngeal swabs
3. *Escherichia coli* in rectal swabs
4. *Pseudomonas* species in rectal swabs
5. *Staphylococcus aureus* in oropharyngeal swabs
6. Group B Streptococcus in oropharyngeal swabs
7. Group A Streptococcus in oropharyngeal swabs

Carriage cohort (500 mothers and their children -250 pairs per country)

1. *S. pneumoniae* in infant nasopharyngeal swabs
2. *Klebsiella* in infant nasopharyngeal swabs
3. *E. coli* in infant rectal swabs
4. *Klebsiella* in infant rectal swabs
5. *Pseudomonas* in infant rectal swabs
6. *S. aureus* in infant oropharyngeal swabs

7. GBS in infant oropharyngeal swabs
8. GAS in infant oropharyngeal swabs
9. *S. aureus* in breast milk samples
10. GBS in breast milk samples
11. GAS in breast milk samples
12. *E. coli* in breast milk samples
13. *Pseudomonas* in breast milk samples
14. *Klebsiella* in breast milk samples
15. *E. coli* in NPS collected from women
16. *S. pneumoniae* in NPS collected from women
17. *Klebesiella* in NPS collected from women
18. *S. aureus* in OPS collected from women
19. GBS in OPS collected from women
20. GAS in OPS collected from women
21. Resistance to AZI, oxacillin and amoxicillin among isolates of *S. aureus*
22. Resistance to AZI, oxacillin and amoxicillin among isolates of GBS
23. Resistance to AZI, oxacillin and amoxicillin among isolates of GAS
24. Resistance to AZI, oxacillin and amoxicillin among isolates of *S. pneumoniae*
25. Resistance to AZI, oxacillin and amoxicillin among isolates of *Klebsiella*

Safety endpoints

1. Solicited and unsolicited AEs observed or reported in mothers in the 28 days after treatment.
2. Solicited and unsolicited AEs in newborns observed or reported in the first 28 days after birth.
3. AEs reported among infants followed up to 1 year.

4 Study Methods

4.1 General Study Design and Plan

(ICH E3; 9)

PregnAnZI-2 was a phase III, double-blinded, placebo-controlled randomized clinical trial in which women in labour were randomized to either a single 2g dose of oral AZI or placebo. The study recruited 12,000 women (6750 in The Gambia and 5250 in Burkina Faso).

In The Gambia, women were recruited in two health facilities—Bundung Maternal and Child Health Hospital and Serrekunda Health Centre. In Burkina Faso, recruitment took place in a number of health facilities in Nanoro and Yako health districts.

Women were consented during the antenatal care visits but the study number (randomisation number) was only assigned after screening during labour, provided the woman met the necessary inclusion and exclusion criteria for participation.

Before March 23rd 2020, women and their newborns were visited at 28 day (either at home or at the health facility). After March 23rd 2020, women were not visited at home because of the risk of SARS-CoV-2 transmission, instead the day 28 interview was conducted by telephone. Cases of maternal and neonatal sepsis were identified through passive case detection at the study health facilities during the 28-day follow up period.

Additional scheduled visits at 6 and 12 months were performed for the first 2,000 women recruited (half in The Gambia and half in Burkina Faso) to assess infant anthropometrical measurements, hospitalization and all-cause mortality.

Carriage samples (recto-vaginal swabs, NPS, OPS, rectal swabs and breast milk samples) were collected from 500 randomly selected mother-newborn dyads (or triads in the case of twins). The samples were collected at day 0, day 6, day 28 and at 4 months.

4.2 Inclusion-Exclusion Criteria

(ICH E3; 9.3. ICH E9; 2.2.1)

Inclusion criteria

- Pregnant women in labour
- Attending the study health facilities for delivery
- Aged ≥16 years
- Previously given consent and willing to continue participation

Exclusion criteria

- Known HIV infection
- Any chronic or acute conditions that might interfere with the study as judged by the research clinician

- Planned travel out of the catchment area during the following 28 days
- Planned caesarean section or known referral
- Known severe congenital malformation
- Intrauterine death confirmed before randomisation
- Known allergy to macrolides
- Known to have taken drugs that prolong QT interval—such as chloroquine, quinine, piperaquine, erythromycin—during the last 2 weeks
- Already participating in another trial

4.3 Blinding and randomisation

(ICH E3; 9.4.3, 9.4.6. ICH E9; 2.3.1, 2.3.2)

A list of randomization numbers was prepared by an independent statistician at the IDIFARMA contract development and manufacturing company using block randomisation. A five-digit number + control number was used, with the first digit denoting country (The Gambia = 1 and Burkina Faso =2). For example, in The Gambia a randomisation number is of the form |1||X||X||X||X|/|X|.

The randomisation list was used to number blister packs of 4 blisters of AZI or placebo (the dose of AZI or placebo was administered as 4 x 0.5g tablets) which were stored in boxes of 25-50 blister packs. Within each box, the blister packs were ordered sequentially.

Boxes were allocated to health facilities; therefore, the randomisation is stratified by health facility.

Note that the randomization numbers are not ordered by recruitment date because each box was assigned to a specific health facility, and women were recruited in several health facilities simultaneously.

Sealed envelopes containing the treatment allocation are kept at the study sites for emergency cases. In addition, a copy of the randomisation is being held by the DSMC.

4.4 Study Variables

(ICH E3; 9.5.1. ICH E9; 2.2.2)

The study activities for all mother-newborn dyads/triads enrolled in the study are summarised in the table below (Table 1). In the subset enrolled in the carriage sub-study (n=250 per country), additional home visits were conducted at day 6 and 4 months, and in the subset enrolled in the anthropometrical sub-study (n=1,000 per country) additional home visits were conducted at 6 and 12 months. Surveillance for deaths, sepsis and adverse events was up to day 28 for all participants. In addition, for participants in the carriage sub-study, surveillance for deaths and serious adverse events extended to 4 months, and for participants in the anthropometric sub-study surveillance extended to 12 months.

Note that day 0 is the date of birth rather than date of randomisation as in the study protocol. We have chosen to re-define follow up time as time from birth rather than time from randomisation to ensure that the definition of neonatal sepsis—one of the components of the primary outcome—is consistent with the standard definition.

Table 1 Summary of study activities

	Pregnancy	Day 0[1] or Day -1 or Day 1	Day 28 (±4days)
Sensitisation & consent	X		
Health facility visit for delivery		X	
Review of inclusion & exclusion criteria		X	
Randomisation & treatment		X	
Discharge review		X	
Active follow-up (home visit)			X
Passive surveillance[2]		→	

[1]Day 0 is defined to be the day of birth. Note that randomisation and discharge might not occur on the same day as the delivery.

[2]Passive surveillance for adverse events, mortality and sepsis. The surveillance period extends to 4 months for mothers and children participating in the carriage sub-study and to 12 months for children participating in the anthropometrical sub-study.

Table 2 Carriage samples collected from mother-newborn pairs (n=500; 250/country) enrolled in the carriage sub-study

	Day 0[1] (or Day -1)	Day 6 (±2 days)	Day 28 (±4 days)	4 Months (±2 weeks)
Mother				
Recto-Vaginal	X[2]			
Nasopharyngeal	X[2]	X		
Oropharyngeal	X[2]	X		
Breast milk		X	X	X
Newborn				
Nasopharyngeal	X[3]	X	X	X
Oropharyngeal	X[3]	X	X	X
Rectal	X[3]	X	X	X

[1]Samples collected on the day of birth (baby) or randomisation (mother) were collected at the health facility. Note that randomisation often occurred the day before delivery.
[2]Samples collected before treatment
[3]Samples collected within 4 hours of delivery/birth

Baseline variables

The following variables will be used to characterise the study population. The list includes variables that are measured post-randomisation and are therefore not, strictly speaking, baseline variables. We have chosen to include these variables in the list of baseline variables because they are unlikely to be affected by the intervention.

Maternal Ethnicity: Mandinka, Wollof, Fula, Jola, in The Gambia and Mossi, Gourounsi and Peulh in Burkina Faso.

Maternal age: median and interquartile range

Apgar score (1-minute): ≤3, 4-7, 8-10

Birthweight (kg): <1.5, 1.5-2.4, 2.5-3.9, 4.0+

Sex of newborn: male, female

Mode of delivery: vaginal, caesarean section (CS)

Congenital malformation (visible): yes, no

Birth outcome: live birth, fresh still birth, macerated still birth

Primary endpoint

The primary endpoint is a composite endpoint of neonatal sepsis or death within 28 days of birth, excluding deaths (but not cases of sepsis) due to severe birth asphyxia, very low birth weight (VLBW) or a major congenital malformation.

We will use the following definitions of sepsis, severe birth asphyxia, very low birth weight (VLBW) and major congenital malformation:

Neonatal Sepsis

Cases of early- and late-onset sepsis will be identified using the clinical and laboratory criteria listed in Table 3. The list of criteria is the same as that used in Cutland et al. (2009), except that it does not include hypotension, because blood pressure is not routinely measured in babies in The Gambia and Burkina Faso. In addition, any hospitalised newborn who has a positive non-contaminated blood or CSF culture will be classified as a case. Note that the blood samples used to identify cases must have been collected during the same admission or at an OPD visit $\leq$5 days before the admission.

Early-onset sepsis (days 0-3)

At least one of the laboratory criteria in Table 3 and either: respiratory distress (one criterion required) or at least two clinical criteria recorded during an admission.

OR

A positive non-contaminated blood or CSF culture.

Late-onset sepsis (days 4 - 28)

At least one laboratory criterion and either: respiratory distress (two criteria required), OR one feature of respiratory distress and one other clinical criterion OR at least two other clinical criteria.

OR

A positive non-contaminated blood or CSF culture.

Table 3 Criteria used in the definition of neonatal sepsis.

Clinical criteria	**Definition**
Respiratory distress	Respiratory rate >60 breaths/min or cyanosis or chest wall indrawing or grunting on expiration or respiratory distress noted in medical records
Pyrexia or hypothermia	Axillary temperature >38.0 ^{o}C, not attributable to external warming, or axillary temperature <36.0^{o}C
Abdominal/feeding problems	Abdominal distension OR feeding intolerance (>20% residual over 24 hours), or poor feeding after feeding well, or 2 episodes of emesis
Bleeding diathesis	Defined as petechiae, echymosis, mucous membrane bleeding, pulmonary haemorrhage, or excessive oozing from venipunture sites
Lethargy or irritability	Noted by medical staff in the absence of other central nervous system symptoms
Central nervous system	Seizures, or bulging fontanelle, or single witnessed episode of apnoea
Laboratory criteria	
White blood cell count (WCC)	WCC <5x10^{9}/L **OR** >25x10^{9}/L in the absence of receiving corticosteroids
Absolute neutrophil count (ANC)	ANC<1.75x10^{9}/L or >15x10^{9}/L
Platelet count	<150x10^{9}/L
C-reactive protein	>10 mg/L (early-onset sepsis) >40 mg/L (late-onset sepsis)
Elevated CSF white blood cell (WBC) count	>30x10^{6}/L WBC in the absence of significant red blood cells

Severe birth asphyxia

1-minute Apgar score ≤3.

Very low birth weight

Weight <1.5kg within 24 hours of birth.

Congenital malformation

Congenital malformations are identified by a study clinician and recorded either at delivery or discharge. Examples include: congenital heart defects, Down syndrome, craniofacial and musculoskeletal abnormalities such as encephalocoele, cleft lip, cleft palate, talipes equinovarus. Only major congenital malformations (e.g. congenital heart defects, Down syndrome) will be excluded from the primary endpoint.

Secondary endpoints - neonate

Culture-confirmed sepsis

A case of culture-confirmed neonatal sepsis will be recorded if a micro-organism that is not a common contaminant is isolated from either a blood or CSF sample. Culture-confirmed neonatal sepsis will be classified as either early-onset (0-3 days) or late-onset (4-28 days). The following bacteria will be considered contaminants: Coagulase-negative staphylococci, Micrococcus spp, Bacillus spp, Anthrobacter spp, Rhodococcus spp, Diphtheroids, Viridans streptococci.

Malaria

A child will be considered positive for malaria parasitaemia if he/she tests positive by microscopy or rapid diagnostic test during a hospital admission or OPD visit.

Skin infection

A skin infection will be recorded if either: i) a skin swab is taken during a hospital admission or OPD visit, or ii) any of the following diagnoses are recorded on a solicited AE (day 28), unsolicited AE, OPD visit, admission or discharge review form.

The Gambia:

“SKIN SEPSIS” or “SKIN INFECTION” or “PUSTULE(S)” or “IMPETIGO” or “FURUNCLE(S)” or “BOIL(S)” or “TREPONEMATOSIS” or “YAWS” or “CARBUNCLE(S)” or “ABSCESS(ES)” or “CELLULITIS” or “COLLECTION(S)” or “INFECTED ULCER(S)” or “WEN(S)” or “CANKER(S)” or “CYST(S)” or “ERYSIPELAS” or “NECROTISING INFECTIONS” or “CUTANEOUS ANTHRAX” or “CHANCRE”

Burkina Faso:

“FURONCLE” or "SEPTICEMIE CUTANEE" or "SEPSIS DE LA PEAU" or "POSTULE" or "IMPÉTIGO" or "TREPINOMATOSE” or "ESCARBOUCLE" or "INFECTION DE LA PEAU"

Eye infection

An eye infection will be recorded if either: i) an eye swab is done during a hospital admission or OPD visit, or ii) “PURULENT EYE DISCHARGE” (The Gambia) or “ECOULEMENT OCULAIRE PURULENTE” (Burkina Faso) is recorded on a solicited AE (day 28), unsolicited AE, OPD visit, admission or discharge review form.

Umbilical infection

An umbilical infection will be recorded if: i) an umbilical swab is done during a hospital admission or OPD visit or ii) “UMBILICAL CORD DISCHARGE” (The Gambia) or “OMPHALITIS” (The Gambia) or “ECOULEMENT AU NIVEAU DU CORDON OMBILICAL” (Burkina Faso) is recorded on a solicited AE (day 28), unsolicited AE, OPD visit or admission form.

Ear infection

An ear infection will be recorded if: i) an ear swab is done during a hospital admission or OPD visit or ii) "EAR DISCHARGE" or “OTITIS MEDIA” (The Gambia) or “OTITE” or “ECOULEMENT OREILLE” (Burkina Faso) is recorded on a solicited AE (day 28), unsolicited AE, OPD visit or admission form.

Culture-confirmed non-invasive infections

Skin, eye, umbilical and ear infections will be considered “bacterially-confirmed” if a swab is done during a hospital admission or OPD visit and a micro-organism that is not a common contaminant is isolated from the swab.

Fever

A case of fever will be recorded if an axillary temperature $\geq$38.0 ◦C, not attributable to external warming, is recorded in the neonate during an admission, OPD visit, or at the discharge review.

Malnutrition

Height, weight, MUAC and head circumference measurements at 6 and 12 months of age will be used to identify malnutrition in the anthropometrical cohort. WHO 2006 child-growth standards will be used to calculate Z-scores height-for-age (HAZ), weight-for-age (WAZ), weight-for-height (WHZ), head circumference-for-age, and

MUAC for age. Children with z-scores <-2SD and <-3SD will be classified as malnourished and severely malnourished, respectively.

Antibiotic use

Records on concomitant medications will be searched for use of any of the following drugs during the 28-day follow up period: ampicillin, amoxicillin/amox, septrin (cotrimoxazole), flagyl (metronidazole), gentamicin/gentamycin, ciprofloxacin, cloxacillin, cephalosporin (ceftriaxone, cephalexin), ampiclox, augmentin/co-amoxiclav, penicillin, chloramphenicol, erythromycin.

Because tetracycline and chloramphenicol are given prophylactically at birth as part of routine care, we will exclude these records from this outcome.

Bacterial carriage

Swabs will be tested for particular bacteria as outlined in Table 4. Each swab will be classified as positive or negative for each bacteria.

Table 4 Bacteria isolated from each swab type collected in infants

Swab	Samples collected	Bacteria
NPS	day 0, day 6, day 28, 4 months	*S. pneumoniae* and *Klebsiella*
Rectal	day 0, day 6, day 28, 4 months	*E. coli* and *Pseudomonas, Klebsiella*
OPS	day 0, day 6, day 28, 4 months	*S. aureus*, GBS and GAS

Secondary endpoints - mother

Post-partum sepsis

A case will be recorded if either:

≥2 of the following criteria are recorded during an admission:

i) uterine tenderness, ii) foul smelling lochia, iii) abnormal vaginal discharge iv) temperature ≥38°C.

OR

≥2 of the above criteria are recorded at an OPD visit and the mother is admitted within 24 hours of the visit

OR

The mother is diagnosed with post-partum sepsis at admission AND 1 of the above clinical criteria is recorded

The case will be considered culture-confirmed if, in addition, a positive CSF, blood, or wound culture is recorded.

Clinical Mastitis

A case will be recorded if "Breast pain/Engorgement" or "mastitis" is recorded on an admission, unsolicited AE, or on an OPD form or solicited AE (day 28) form. The case will be classified as culture-confirmed if it is associated with a culture-positive breast swab.

Malaria

A mother will be considered positive for malaria parasitaemia if she tests positive by microscopy or rapid diagnostic test during a hospital admission or OPD visit.

Antibiotic use

Records on concomitant medications will be used to identify maternal antibiotic use (see definition of antibiotic use in neonate for the (non-exhaustive) list).

Fever

A case of fever will be recorded if an axillary temperature $\geq$38.0 ^{o}C, not attributable to external warming, is recorded in the mother during an admission or OPD visit.

Bacterial carriage

Swabs will be tested for particular bacteria as outlined in Table 5. Each swab will be classified as positive or negative for each bacteria.

Table 5 Bacteria isolated from each swab type collected in mothers

Swab	Sampling times	Bacteria
NPS	day 0*, day 6	*E. coli, S. pneumoniae* and *Klebsiella spp*
Recto-vaginal swab	day 0*	*E. coli* and *Pseudomonas, Klebsiella*
OPS	day 0*, day 6	*S. aureus*, GBS and GAS

Breast milk	day 6, day 28, 4 months	*S. aureus*, GBS, GAS, *E. coli*, *Pseudomonas* and *Klebsiella*

*Samples collected before treatment. The samples may have been collected on day -1 rather than day 0.

5 Sample Size

(ICH E3; 9.7.2. ICH E9; 3.5)

The study was originally designed to assess the effect of the intervention on mortality. We assumed mortality in the placebo arm would be at least 1.4% and that the intervention would reduce mortality by 40%. Based on these parameter values, and allowing for 8% loss to follow-up, it was estimated that a total of 12,500 women would need to be recruited to achieve 80% power to detect such an effect.

However, during the course of the study data from the MORDOR study was published (Keenan et al., 2018) which suggested that the intervention was unlikely to achieve a 40% reduction. Study power was also negatively impacted by the lower than anticipated incidence of mortality (the observed mortality up to Jan 2020 was lower than anticipated (0.7%) probably because of the improved care associated with participating in the trial), and by the recruitment freeze between March and August 2020, which resulted in the target sample size being reduced from 12,500 to 12,000.

Because of this loss of study power, we decided to change the primary outcome from neonatal mortality to the composite endpoint of neonatal mortality + neonatal Sepsis. The revised power calculations are shown in Table 6. The calculations are based on mortality and sepsis data collected up to the end of Jan 2020 and effect size of 25% (RR=0.75) for mortality and 30% (RR=0.70) for sepsis. The revised study power is 82% power.

Table 6 Revised study power calculation

Gambia parameters		**Gambia**	Control	AZI	Power
Total N	7000	N	3500	3500	
Incidence sepsis	1.9%	Sepsis	2.2%	1.6%	0.54
Incidence mortality	0.7%	Mortality	0.8%	0.6%	0.17
Proportion deaths due to sepsis	31%	Combined	2.8%	2.0%	0.60
Burkina Faso parameters		**Burkina Faso**			

Total N	5000	N	2500	2500	
Incidence sepsis	1.8%	Sepsis	2.1%	1.5%	0.39
Incidence mortality	0.7%	Mortality	0.8%	0.6%	0.13
Proportion deaths due to sepsis	17%	Combined	2.8%	2.0%	0.46
RR sepsis	0.7	**Overall**			
RR mortality	0.75	N	6000	6000	
		Sepsis	2.2%	1.5%	0.76
Significance level (z-value)	1.96	Mortality	0.8%	0.6%	0.26
		Combined	**2.8%**	**2.0%**	**0.82**

6 General Considerations

6.1 Timing of Analyses

The final analysis of the primary endpoint will be performed when the recruitment target has been reached, and data up to day 28 has been entered into the main database (see data management plan) and verified for all participants.

6.2 Analysis Populations

(ICH E3; 9.7.1, 11.4.2.5. ICH E9; 5.2)

Primary analysis cohort

The cohort will include all mother-newborn dyads (or triads in the case of twins) who have been randomised to one of the study arms and where the day-28 survival status is known for all members of the dyad/triad. Dyads/triads where the survival status of all members cannot be determined from the day 28 visit or previous death records, or who have withdrawn from the study, will be excluded from the cohort.

We do not anticipate significant non-compliance and therefore do not intend to conduct a per protocol analysis. In the unlikely event that >10% of the study women do not ingest the study pills then we will conduct an additional analysis of the primary outcome restricted to mother-newborn dyads/triads where the mother is known to have ingested the medication.

Each subject's inclusion or exclusion status in the primary analysis population will be assigned prior to breaking the code.

Carriage cohort

The cohort will include all mother-newborn dyads/triads who meet the following criteria:

- Selected to be in the carriage cohort
- Included in the primary analysis cohort
- Contributed swabs at day 0 and day 6 (but not necessarily at day 28 or 4 months)
- All members of the dyad/triad alive and participating in the study at the time of the 4-month visit

Anthropometric cohort

The cohort will include all infants who meet the following criteria:

- Selected to be in the anthropometric cohort
- Included in the primary analysis cohort
- Alive and participating in the study at day 28

6.3 Covariates and Subgroups

(ICH E3; 9.7.1, 11.4.2.1. ICH E9; 5.7)

Subgroups

Exploratory analyses in which the data are stratified by country will be done for all outcomes analysed in the Primary cohort. In addition, the primary outcome will be analysed in the following subgroups:

- Deliveries >2 hours after administration of the study pills
- Deliveries ≤2 hours after administration of the study pills
- Deliveries in the wet season (June-October)
- Deliveries in the dry season (November-May)
- Overweight and obese mothers (BMI≥25.0kg/m^2)
- Normal weight and underweight mothers (BMI<25.0kg/m^2)
- Newborns delivered via caesarean
- Vaginal deliveries

Covariate adjustment

Where possible, regression models will be used to improve statistical power (Kahan, 2014). We will use linear regression for continuous outcomes and logistic regression for binary outcomes.

In the analysis of outcomes in the Primary and Anthropometric cohorts, the models will include country as a binary predictor, and 1-minute Apgar score and birthweight as continuous linear terms.

In the analysis of data from the Carriage cohort, logistic regression will be used to adjust for day 0 maternal carriage (day 0 neonatal carriage cannot be adjusted for because it is recorded after treatment).

No covariate adjustment will be used in the analysis of the aetiologies of sepsis (Primary cohort) because the prevalence of the outcomes in this analysis is anticipated to be low and logistic regression may be unreliable in this situation (Peduzzi et al., 1996).

6.4 Missing Data

(ICH E3; 9.7.1, 11.4.2.2. ICH E9; 5.3. EMA Guideline on Missing Data in Confirmatory Clinical Trials)

Primary cohort

Missing data will not be explicitly recorded for the outcomes in the Primary cohort because the outcomes that will be analysed in this cohort are measured through passive surveillance.

Carriage and Anthropometric cohorts

Since outcomes are actively recorded in these cohorts, missing data may be recorded for some of the outcomes analysed in the Carriage and Anthropometric cohorts.

We anticipate some missing data at the 4-month time point because of the interruption due to the COVID-19 pandemic. Therefore, an available case analysis will be conducted in the Carriage cohort. The occurrence of missing data is unlikely to be associated with any particular characteristic of the women/neonates, and the risk of bias due to missing data is small.

The anthropometric cohort was recruited prior to the COVID-19 pandemic. Nevertheless, we expect some missing data due to loss to follow up, and will therefore conduct an available case analysis in this cohort. We will assess the risk of selection bias by comparing the baseline characteristics of children who are lost to follow up with those who remain in the cohort throughout follow up.

Baseline covariates

To avoid having to exclude individuals because of missing covariate data, we will impute missing values of birthweight and 1-minute Apgar score with mean values. In the Carriage cohort, imputation will be unnecessary because having baseline carriage data is an inclusion criteria for this cohort.

6.5 Interim Analyses and Data Monitoring

Data on baseline characteristics, incidence of primary outcome, numbers recruited, numbers under follow up, have been presented at DSMB meetings.

No further interim analyses are planned.

6.6 Accounting for differences between countries

(ICH E3; 9.7.1, 11.4.2.4. ICH E9; 3.2)

The regression models used to analyse outcomes in the Primary and Anthropometric cohorts will include country. Hence these analyses will be adjusted for country (in addition to 1-minute Apgar score and birthweight). In the analysis of the Primary cohort, we will do separate analyses in each country using a Wald test to test the treatment x country interaction. This analysis will be considered to be exploratory. No adjustment for country will made in the analysis of the Carriage cohort because of the smaller size of this cohort.

7 Summary of Study Data

See dummy tables and consort diagram.

8 Efficacy Analyses

8.1 Primary analysis cohort

Primary outcome

We will report the proportion that meet the criteria for the primary outcome (death or sepsis within 28 days of birth) in each arm.

The null hypothesis of no difference between the arms will be tested by fitting a logistic regression model that, in addition to study arm, includes 1-minute Apgar score and birthweight as continuous, linear covariates. The p-value, odds ratio and

confidence interval associated with study arm will be reported, with the confidence interval and p-value being based on a Wald test.

Mortality and sepsis

Mortality and sepsis will be analysed separately, as secondary outcomes, as well as being part of the primary outcome.

They will be analysed as binary outcomes and as time to event outcomes using Kaplan Meier curves. In the K-M analysis of sepsis, death will be treated as a censoring event. There will be no censoring in the analysis of mortality because any neonate whose day 28 survival status is unknown is excluded from the Primary cohort.

A concern in the analysis of composite endpoints is that of competing risks. In this analysis, death is a competing risk for sepsis – an individual who has died is not at risk of sepsis. Although some bias due to the competing risk of death is expected in the K-M analysis of sepsis, the bias will be small because death is a rare outcome.

Secondary outcomes

The secondary outcomes are all binary and will therefore be analysed using logistic regression as described for the primary outcome (except the specific aetiologies of sepsis which will be analysed using Fisher's exact test because these are rare outcomes).

8.2 Carriage cohort

Maternal carriage

Maternal bacterial carriage will be analysed by estimating the proportion of women positive at day 6 and the proportion positive at one or more of the post-intervention time points (i.e. day 6, day 28 or 4 months). The latter will be estimated using women who have data available at all time points, i.e. women missing day 28 or 4-month data will be excluded from this analysis. However, a secondary analysis will be done using a multiply imputed dataset in which missing day 28 and 4-month carriage data are imputed using day 0 and day 6 data. For bacteria that are isolated from multiple sample types an individual will be identified as positive if bacteria are isolated from any of the samples (Table 5). For example,

women will be considered positive for *Klebsiella* at day 6 if bacteria are isolated from either the NPS or breast milk sample. Besides presenting the prevalence of individual bacteria, the bacteria will be grouped into gram positives (*S. pneumoniae, S. aureus*, GBS, GAS) and gram negatives (*E. coli, pseudomonas, Klebsiella*).

We will use logistic regression to compare the proportion positive by study arm adjusting for carriage at day 0. For example, *S. aureus* carriage on day 6 will be compared by calculating an odds ratio adjusted for *S. aureus* carriage on day 0. If the prevalence of a carriage outcome is <4% then an unadjusted odds ratio will be presented instead of the adjusted OR and Fisher's exact test will be used to calculate the p-value. The cut-off of 4% has been chosen to ensure that logistic regression is not used when there are fewer than 10 events per parameter as recommended by Peduzzi et al (1996).

A longitudinal analysis of carriage will be conducted using data from breast milk samples collected on day 6, day 28 and at 4 months. As with the other analyses, logistic regression will be used to adjust for carriage at day 0 where possible (i.e. provided the prevalence is ≥4%). Two versions of the analysis will be done: the first will be restricted to women with complete data at all three time points, and the second will be done using the multiply imputed dataset. The prevalence at each time point and in each arm will be displayed graphically.

Infant carriage

With the exception of *Klebsiella*, which is tested for in NPS and RS, in samples collected from infants each bacteria is only tested for in one sample type (e.g. *S. pneumoniae* is tested for in NPS and *E.coli* is tested for in rectal swabs). Therefore, in contrast to the analysis of maternal carriage, there is no need to combine results across different sample types. Each bacteria will be compared between study arms at day 0, day 6, day 28 and 4 months (Table 4). Additionally, we will compare the proportion positive at any time point. We will use logistic regression to adjust for maternal carriage at day 0 if the prevalence is ≥4%, if the prevalence is less than this then we will calculate an unadjusted OR and use Fisher's exact test. Note that we have chosen to adjust for maternal carriage at day 0 rather than neonatal carriage because neonatal samples are collected after treatment and may, therefore, be affected by treatment. The prevalence at each time point and in each arm will be displayed graphically. As with the analysis of maternal carriage, the analyses will be

done in children with complete data and then repeated using multiply imputed data (missing day 28 and 4-month outcomes will be imputed from day 0 and day 6 outcomes).

Carriage of resistant isolates

The analysis of resistant strains in the mother and neonate will mirror the analysis of overall carriage. Resistance will be defined using the E-test resistance cut-offs defined in Table 7 below. The prevalence of resistance can be defined either in terms as the proportion of resistant isolates among carriers or as the proportion among all individual (carriers + non-carriers). In this analysis, we will use the number of individuals tested as the denominator.

Table 7 E-test cut-offs used to define resistance to azithromycin

Bacteria	Cut-off (µg/mL)		
	Sensitive	Intermediate	Resistant
E. coli	≤16	16	≥32
Klebsiella spp	≤16	16	≥32
Pseudomonas	≤16	16	≥32
S. aureus	≤2	4	≥8
S. pneumoniae	≤0.5	0.5	≥1
GBS	≤0.5	0.5	≥1
GAS	≤0.5	0.5	≥1

8.3 Anthropometric cohort

Standardised anthropometric Z-scores (WHZ, WAZ, HAZ and MUAC-for-age, head-circumference-for-age) based on WHO 2006 growth charts will be calculated using the Stata package "zanthro" (Vidmar et al., 2013).

As recommended in the WHO report on data quality in anthropometric studies https://www.who.int/nutrition/publications/anthropomentry-data-quality-report-chapter3.pdf, a flag system will be used to recode implausible z-score values as missing:

- height-for-age: < -6 or > +6
- weight-for-length/height: < -5 or > +5
- weight-for-age: < -6 or > +5

- body mass index-for-age: < -5 or > +5

For each anthropometric measure, we will compare between study arms the mean score at 6 months, 12 months, and growth velocity, i.e. difference between 6 and 12-month scores. Linear regression will be used to calculate a difference between arms and p-value adjusted for country, birth weight and 1-minute Apgar score. The continuous variables (birth weight and Apgar score) will be included in the model as linear terms.

In addition, we will dichotomise the Z-scores using < -2 SD as the cut-off for malnutrition and < -3 SD as the cut-off for severe malnutrition. We will use logistic regression to adjust for country, birth weight and 1-minute Apgar score.

9 Safety Analyses

We will report the numbers of severe adverse, hospitalisations and deaths in mothers and neonates in the Primary cohort and number of deaths in the Carriage and Anthropometric cohorts. When calculating the incidence, each subject will be counted only once and any repetitions will be ignored; the denominator will be the total population size. P-values will be calculated using Fisher's exact test.

10 Technical Details

Data management and statistical analyses will be done using Stata version 15 and R version 3.6.1.

Anthropometric Z-score will be calculated using the Stata package "zanthro" (Vidmar et al. 2013).

11 References

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