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Protocol

Pre-delivery administration of azithromycin to prevent neonatal sepsis and death: a phase iii double-blind randomized clinical trial (PregnAnZI-2 trial)

Bully Camara[1], Joel D. Bognini[2], Usman N. Nakakana[1], Athanase M. Some[2], Isatou Jagne[1], Marc C. Tahita[2], Nathalie Beloum[1], Fatoumata Sillah[1], Madikoi Danso[1], Joquina C. Jones[1], Shashu Graves[1], Pauline Getanda[1], Toussaint Rouamba[2], Ebrahim Ndure[1], Hien S. Franck[2], Sawadogo Y. Edmond[2], Yusupha Njie[1], Bai L. Dondeh[1], Nassa G. J. Wilfried[2], Abdoulie Bojang[1], Garba Zakaria[2], Christian Bottomley[3], Umberto D'Alessandro[1], Halidou Tinto[2], Anna Roca[1]*

[1]Medical Research Council Unit, The Gambia at London School of Hygiene and Tropical Medicine (MRCG at LSHTM), The Gambia
[2]Clinical Research Unit of Nanoro (CRUN), Burkina Faso, West Africa
[3]London School of Hygiene and Tropical Medicine, London, United Kingdom

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***Correspondence:**
Anna Roca,
E-mail: aroca@mrc.gm

ABSTRACT

Background: Despite reduction in the risk of under-5 mortality in the last decade and a half, neonatal deaths have remained stable globally. Gram-positive bacterial infections are leading causes of neonatal sepsis and death. Because the mother is an important source for bacterial transmission to babies during the perinatal period, interventions that lower risk of transmission can potentially reduce invasive bacterial infections. The primary objective of the trial will assess the effect of intrapartum azithromycin on neonatal sepsis and mortality. Secondary objectives include the impact of the intervention on prevalence of carriage and resistance, puerperal infections, and infant growth.
Methods: This is a phase III, double-blinded, placebo-controlled randomized trial in which 12,000 women in labour are randomized to either a single dose of 2 g of oral azithromycin (AZI) or placebo in The Gambia and Burkina Faso. Mother/newborn pairs are followed-up at 28-days post-delivery to assess health and mortality. Passive visits are conducted to collect adverse events including hospitalizations. When clinically indicated, samples are collected for assessment of neonatal and puerperal sepsis. A cohort of 250 mother/newborn pair per country have been included in the carriage sub-study to assess bacterial colonization at day 0, 6, 28 and 4 months. Children of the first 1000 mothers recruited in each country are followed-up at 6 and 12 months for anthropometric assessments.
Conclusions: If successful, this simple implementable intervention has the potential to achieve wide coverage in Sub-Saharan Africa (SSA) where low-cost interventions to reduce neonatal sepsis and mortality and morbidity in mothers are urgently needed.
Trial registration: The trial was registered at ClinicalTrials.gov: NCT03199547.

Keywords: Azithromycin, Neonatal sepsis, Neonatal mortality, Bacterial infections, Randomized clinical trial, Bacterial carriage, Sub-Saharan Africa

INTRODUCTION

Background and rationale

The last decade witnessed significant reduction in the risk of under-5 child mortality.[1] The greatest reduction occurred in children older than 1 month, while death in children 0-28 days (neonatal mortality) has remained stable. Nearly 4 million neonates die each year accounting for 40% of <5 deaths globally.[1]

Neonatal sepsis account for 1 out of 3 deaths in this age group.[2] In Sub-Saharan Africa (SSA), neonatal sepsis is caused by several bacterial pathogens; being the most common *Staphylococcus aureus*, group B *Streptococcus* and *Streptococcus pneumoniae* among gram positives, and *Klebsiella pneumoniae* and *Escherichia coli* among gram negatives.[3]

New context-specific interventions to decrease neonatal sepsis and associated mortality are urgently needed in countries with highest mortality burden, like most countries in SSA.

Azithromycin (AZI) is a macrolide with a wide antimicrobial spectrum.[4] As part of the World Health Organization (WHO)-recommended trachoma control strategy, mass AZI treatment campaigns in endemic countries, have been shown to decrease nasopharyngeal pneumococcal carriage.[5-8] Prophylactic AZI using MDA reduces under-5 mortality with the highest efficacy among infants <6 months.[9]

We conducted the proof-of-concept double-blind randomized-trial (PregnAnZI) to assess the effect of 2 g of oral intra-partum AZI on bacterial carriage in mothers and neonates; considering carriage as a necessary step towards sepsis.[10] The primary outcome assessed impact on the gram-positive bacteria during the neonatal period. The trial showed a consistent reduction of bacterial carriage in both the mother and their newborns and a decrease in the occurrence of disease.[11,12]

This larger trial evaluates the impact of one oral dose (2 g) of intra-partum AZI on neonatal sepsis and associated death in The Gambia and Burkina Faso.

Explanation for the choice of comparators

To ensure a double-blinded intervention, women in the control group receive placebo during labour that look identical to the intervention.

Objectives

The primary objective was to assess the efficacy of the intervention on neonatal sepsis (from birth to day 28) and mortality (excluding mortality from severe birth asphyxia, major congenital malformation and very low birth weight - VLBW). This is a modification from the initial primary objective that was restricted to neonatal mortality.

Secondary objectives include assessing the effect of the intervention on a range of clinical and microbiological endpoints for neonates and mothers, including mortality.

The trial has three different sub-studies with specific objectives.

Anthropometric sub-study

1,000 infants per country are followed up for 1-year to evaluate the efficacy on malnutrition through z-scores.

Carriage sub-study

250 participant pairs randomly selected per country, are sampled to evaluate the efficacy of the intervention on the prevalence of bacterial colonization and antibiotic resistance over 4 months post-birth.

Recto-vaginal swab (RVS) sub-study

This will evaluate the efficacy of the intervention on maternal and neonatal outcomes according to the vaginal bacterial carriage status of women before the intervention is given. This study was discontinued because of COVID-19.

Trial design

This is a phase III, double-blinded, placebo-controlled randomized clinical trial in which women in labour are randomized to receive 2 g of oral AZI or placebo (ratio 1:1).

METHODS

Study setting

Trial countries

The study is being conducted in The Gambia and Burkina Faso. The Gambia is the smallest country in mainland Africa.[13,14] Neonatal mortality declined from 49 to 26 per 1000 live births between 1990 and 2018, respectively.[15] Approximately 15% of maternal deaths are associated with puerperal infections.[16]

Burkina Faso is another West African country. Neonatal mortality is 30.4 per 100,000 live-births, and >10% of maternal deaths are related to puerperal infections.[16,17]

Recruitment settings

In The Gambia, women are recruited in a peri-urban region from two government health facilities, Bundung Maternal and Child Health Hospital (BMCHH) and Serekunda

Health Centre (SHC) (Figure 1a). Recruitment in Burkina Faso takes place in the rural central districts of Nanoro and Yako, and participants are recruited in eight health facilities (Figure 1b).

Eligibility criteria

A designated study nurse or research clinician assesses eligibilty at the study sites.

Inclusion criteria

Pregnant women in labour ≥16 years with previously signed consent and willing to continue participation.

Exclusion criteria

Known HIV infection; any chronic or acute conditions; planned caesarean section or known required referral; known severe congenital malformation, intrauterine death or allergy to macrolides; and drugs known to prolong QT interval taken during the last 2 weeks, such as chloroquine, quinine, piperaquine, and erythromycin were excluded.

Interventions

Intervention description

The intervention is 2 grams of AZI tablets (4 tablets, each of 0.5 grams). AZI is a broad-spectrum macrolide antibiotic, with a long half-life, a high degree of tissue penetration and is used for treatment of several clinical conditions.

Criteria for discontinuing or modifying allocated interventions

Withdrawal from the study is allowed at any time. Safety assessments are done if allowed and previously collected information and stored samples will only be analysed if given permission. A study termination form is completed for participants once the study is discontinued.

Relevant concomitant care permitted or prohibited during the trial

Standard care and treatment is permitted in case of clinical need.

Outcomes

Trial endpoints

Primary endpoint

This is a composite of neonatal sepsis or neonatal death, excluding deaths from severe birth asphyxia, VLDW or severe congenital malformations.

Definitions used for neonatal sepsis are given below.[18]

Early-onset of neonatal sepsis is defined as (within 3 days of life)

Early-onset clinical sepsis

Neonate hospitalised who in the absence of another infection had at least one laboratory criteria (Table 1) and either respiratory distress or at least two clinical criteria (Table 1).

OR

Early-onset of culture-confirmed sepsis

If a micro-organism is isolated from a normally sterile body site (or seen in the gram stain for CSF) . Contaminants are excluded (Table 2).

Late-onset of sepsis will be defined as (between 3-28 days)

Late-onset clinical sepsis

Neonate hospitalised with at least one laboratory criteria (Table 1) and respiratory distress (two criteria required), OR one feature of respiratory distress and one other clinical criterion or at least two other clinical criteria (Table 1).

OR

Late-onset culture confirmed sepsis

If a micro-organism is isolated from a normally sterile body site (or seen in the gram stain for CSF). Contaminants are excluded (Table 2).

A neonatal death is part of the primary endpoint excluding - severe birth asphyxia (SBA): Apgar score $\leq$3 at 1 minute of age; very low birth weight: <1.5 kg; and major congenital malformation: detectable malformations either life threatening or require surgical correction or affect quality-of-life.

Secondary endpoints for neonates (from birth to day 28)

Neonatal sepsis and neonatal mortality as defined above; culture-confirmed sepsis; fever; and clinical bacterial skin infections: skin infection will be diagnosed if a child develops an abscess, cellulitis, impetigo, furuncles (boils), wen, cyst, erysipelas, necrotising infections, cutaneous anthrax, treponematoses, chancre (confirmed bacterial skin infection if the isolation of bacteria from a skin swab (excluding contaminants); clinical bacterial conjunctivitis: redness of the eye and irritation with purulent secretions, eyelids stuck together on waking, may be unilateral at onset (confirmed if isolation of bacteria from an eye swab (excluding contaminants); clinical bacterial umbilical

infection (omphalitis): purulent or malodorous discharge, periumbilical erythema, oedema and tenderness (confirmed if isolation of bacteria from an umbilical swab (excluding contaminants); malaria: clinical suspicion with either a positive blood film for malaria or a rapid diagnostic test (RDT); use of any prescribed antibiotics; and all cause neonatal hospitalization excluding hospitalizations due to injuries.

Secondary endpoints for children include: all-caused hospitalization during the follow up period; infant mortality: all-caused mortality excluding injuries to end of follow-up; malnutrition: anthropometric measurements at 6 and 12 months of age, we will use WHO child-growth standards to calculate z-scores [WHO Anthro version 3.2.2, January 2011], children with z-scores <-2SD and <-3SD will be classified as malnourished and severely malnourished, respectively.

Secondary endpoints for mothers include: post-partum sepsis: maternal hospitalizations within 28 days of delivery for endometritis (defined below) or culture-confirmed infection of sterile site (i.e. blood or CSF) or wound infection (see definition below); endeometritis: at least two of the following signs uterine tenderness, fever, foul-smelling-purulent lochia, or vaginal discharge; wound infection: perineal or caesarean wound infection; mastitis: breast pain, redness, tenderness and localised mass with or without fluctuance (confirmed if it is associated with a culture-positive breast swab); malaria: clinical suspicion (such as fever) confirmed by either positive blood film test for malaria or a RDT for malaria; fever: axillary temperature $\geq 38.0^{o}$ C of two readings with 10 minutes interval between readings; use of antibiotics during the post-partum follow-up; hospitalizations: any hospital admission; and mortality during the 4-weeks of follow-up.

Microbiological secondary endpoints

For samples collected from the carriage sub-study: prevalence in study infants of *S. pneumoniae* and *Klebsiella spp* in the nasopharynx; *E. coli, Pseudomonas spp, Klebsiella spp* from rectal swab and *S. aureus, GBS, GAS* in the oropharynx.

For mothers, microbiological endpoints include: prevalence of *S. aureus, GBS, GAS, E. coli, Pseudomonas spp, Klebsiella* in BM; *S. pneumoniae, Klebsiella spp* in the nasopharynx and *S. aureus, GBS, GAS* in the oropharynx.

Resistance to AZI, oxacillin and amoxicillin will be determined for bacterial isolates.

For pre-intervention RVS samples collected from mothers, incidence of neonatal endpoints will be stratified according to bacterial RSV carriage.

Safety endpoints include the number of AEs in mothers and newborns observed during the follow-up.

Participant timeline

Table 3 shows summary of study activities, Table 4 shows sampling timelines and Figure 2 shows length of follow-up of study participants.

Sample size

Sample size determination

Sample size was calculated considering neonatal mortality as the primary endpoint based on data from our proof-of-concept trial where 6 neonatal deaths (1.4%) were recorded in the placebo arm compared to 2 (0.4%) in the intervention arm.[12] Based on these, we assumed 1.4% mortality in the placebo arm and 40% reduction in the intervention arm. 5,800 women per arm would be required to show a significant difference at 80% power and 5% significance level. Allowing for 8% loss to follow-up, the recruitment target was set at 12,500 women.

However, during the trial we observed a lower mortality than anticipated and after the results from the MORDOR trial, we considered that a 40% reduction was too optimistic. With the approval of the data safety monitoring board (DSMB) and trial steering committee (TSC), the primary endpoint was changed to a composite of neonatal sepsis or mortality.[9]

A revised sample size calculation was done for the new composite endpoint using unblinded trial data on outcomes collected up to January 2020. We assumed the intervention reduces sepsis by 30% and mortality by 25% and predicted a 2.8% incidence of the outcome in the control arm(further details provided in the statistical analysis plan). Based on this a sample size of 12,000 women would be required for 80% power. No adjustment for loss to follow up was made in the calculation.

Recruitment

In The Gambia, the study health facilities run busy antenatal clinics and conduct over 7000 deliveries annually (approximately 5000 in BMCHH and 2000 in SHC). Assuming 35% of the annual deliveries recruited per annum, up to 7000 participants can be recruited in 3-years.

In Burkina Faso, all 8 health facilities provide antenatal care and conduct over all 3800 deliveries annually. Estimating up to 50% of the annual deliveries recruited; the study population of 5250 to 5500 can be recruited within the 3-years.

The COVID-19 pandemic adversely disrupted trial activities by slowing down recruitment rates and prolonging the recruitment period.

Methods: assignment of interventions

Allocation: sequence generation

A list of randomization numbers was prepared by an independent statistician using block randomisation. A five-digit number plus control number was used, with the first digit denoting country (The Gambia=1 and Burkina Faso=2). Boxes included 25-50 blister packs. Within each box, the blister packs were ordered sequentially. IDIFARMA packaged and labelled the interventional medicinal products (IMP) according to the list provided.

Allocation concealment mechanism

The allocation codes will be provided to the chief investigator by the DSMB statistician at the end of the trial.

Implementation

When a pregnant woman arrives at the study health facility for delivery, a randomization nurse confirms consent and if the expectant woman confirms willingness to participate in the study, the rest of eligibility is re-assessed before she is administered the study drug.

Blinding (masking)

Who will be blinded

This is a double-blind trial. All participants and members of the research team are blinded to the study arms.

Procedure for unblinding if needed

Sealed envelopes containing the treatment allocation are kept at the study sites. In the event of a serious adverse event (SAE) that cannot be treated without knowing the treatment arm, the code can be revealed to the attending staff by opening the envelope corresponding to the participant's randomization number. The steps to unblind are detailed in a study specific procedure.

Data collection methods

Plans for assessment and collection of outcomes

Women and babies are seen by study staff before hospital discharge and are also visited at day-28.

Plans to promote participant retention and complete follow-up

At discharge review, a study identification (ID) card containing the woman's study and demographic information are issued to the participant. The full names of the woman including nick name/s (if any), spouse names, description of residence and telephone number (three mobile numbers if possible) are recorded on a worksheet. The information is used to locate the woman during scheduled study visits.

In The Gambia, women and their newborns were initially visited at 28 days by a study nurse until the 23rd of March 2020, when visits were done via telephone because of the risk of SARS-CoV-2 transmission. This change will affect approximately 14.6% of recruitments in The Gambia.

In Burkina Faso, women return with their infants to the study health facilities for the end of follow-up visit; however, if the participant does not present at the scheduled date, a home visit is then conducted by a field worker.

Data management

Data collection and processing were detailed in the data management plan prior to the study start. Data is collected in the field using a GCP compliant research electronic data capture (REDCap) system with the use of encrypted and reliable mobile devices (Samsung tablets) and computers at the study sites.

Statistical methods

Statistical methods for primary and secondary outcomes

We will report the proportion that meet the criteria for the primary outcome in each arm. To test the null hypothesis of no difference between the arms we will fit 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 will be analysed separately as secondary outcomes as binary outcomes and as time to event outcomes using Kaplan Meier curves. In the Kaplan Meier analysis of sepsis, death will be treated as a censoring event. The other secondary outcomes are all binary and will therefore be analysed using logistic regression as described above. A full statistical analysis plan is provided as supplementary information. Deviations from this plan will be documented and approved by the study team and DSMB, and any changes will be documented in trial reports.

Data monitoring

Composition of the data monitoring committee (DMC), its role and reporting structure

The DMC also referred to in this manuscript as the DSMB, comprises 4 members with complementary expertise in statistics (DSMB Chair), neonatology, public health and trials on AZI. The board provides independent advice on the quality of the data produced and the procedures followed including completeness, protocol compliance

and evidence for treatment harm. It can also recommend for the trial to be discontinued if there are safety concerns.

Potential harms

Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events

The sponsor reviews trial data for accuracy, completeness, and safety during monitoring exercises. The local safety monitor (LSM) and sponsor critically reviews all serious adverse event (SAE) reports and protocol deviations (PDs). All SAEs that culminate in the death of participants are reviewed by the medicines cotrol agency (MCA) and the local ECs. SAEs which have been determined to be related to the intervention by the research clinician or the PI are also reported to the ECs within 15 days; if fatal, these SAEs are reported within 7 days. The study monitoring plan outlines schedule of monitoring visits in both countries including remote monitoring of the REDCap database. The trial is considered a medium risk study, and the monitoring activities are undertaken accordingly.

Other safety considerations

Hypertrophic pyloric stenosis (HPS) though very rare, is the main safety consideration with the administration of AZI. Data from our proof-of-concept showed that AZI reached the baby during the entire neonatal period, however no cases of HPS were observed throughout the 8 weeks study follow-up period.[12] Nevertheless, to ensure the safety of participants, active surveillance for HPS is being undertaken in the current study.

Research ethics approval

The study was approved by The Gambia Government/MRCG (Medical Research Council Unit The Gambia) Joint Ethics Committee, the Comité d'Ethique pour la Recherche en Santé (CERS) of Burkina Faso and the LSHTM Ethics Committee.

Protocol amendments

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees)

Changes to the protocol are approved by the ethics commmittee before they are implemented.

Consent

Who will obtain informed consent?

Community sensitization

In both The Gambia and Burkina Faso, community leaders were informed about the study prior to the start of recruitment. In The Gambia, open day events were held at both health facilities to share study information with the community (Figure 3), while in Burkina Faso, meetings with Regional Director of Health, the mayor, traditional chiefs and community leaders were conducted.

Identification of pregnant women (target group sensitization)

In suitable local languages, a field worker explains the main aspects of the study after routine standard health education by the health facility staff during antenatal clinics and interested women are invited to a one-on-one individual sensitisation session with a designated field staff in a private study office.

Individual sensitization

Using the approved study informed consent document (ICD) and the study specific procedure on sensitisation and consent, the study staff gives a detailed explanation on all aspects of the trial to the pregnant women in their preferred language at the health facility (Figure 3) in the presence of an impartial witness if a woman is unable to read and understand the ICD in English or French. If a woman wish to join the study, she is assigned a unique study sensitization number which is placed on her antenatal card. The sensitisation logbook is then completed with the woman's relevant biodata and her sensitization number. She is also given a copy of the ICD and encouraged to discuss with her spouse and or a family member(s) before giving consent.

Consenting

After sensitization, women are encouraged to ask questions. If a woman agrees to participate, her understanding of the ICD is tested using the 'assessment of informed consent understanding' (AICU) questionnaire. A maximum of two attempts is allowed (Figure 3). Once a woman's assessment is successful, she and the attending field worker sign two copies of the consent form (one for her and one for the trial team). If the woman is illiterate, she provides a thumbprint on the consent form and the impartial witness signs the form.

Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies

Additional information is provided to women about the anthropometric, carriage and RVS sub-studies during sensitization and consent and those who wish to be part of any of the sub-studies were included.

Confidentiality

A combined trial master file (TMF) that contain the essential documents for the trial was set up and maintained by the investigator. Besides members of the study team,

only authorised representatives of the ethics committee(s) and regulatory authorities and the trial monitors can access the content of the TMF.

Declaration of interests

The investigators have no financial or other competing interests to declare.

Access to data

None of the study data will be disclosed or material provided to any party not directly involved in the study without written permission from the sponsor.

Provisions for post-trial care

Adverse events (AE) that occurr during the follow-up period of the participants are followed until the AE resolves, improves, or stabilizes, even if this period was beyond the follow-up period of the study.

Dissemination policy – trial results

Dissemination plans

Our communication strategy is guided by the vision and mission of MRCG. This strategy has been prepared by our communications department who will provide full and dedicated support to this study's communications needs.

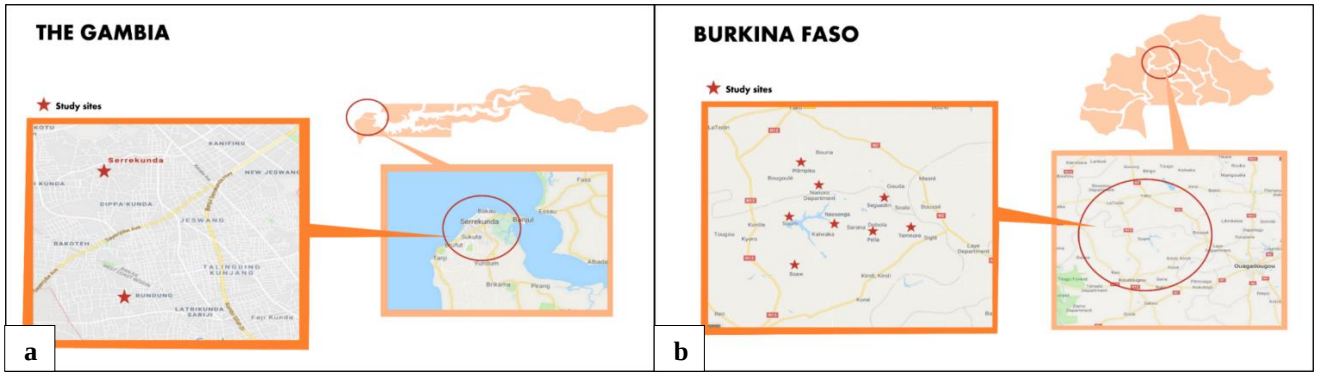

Figure 1: (a) Map of The Gambia, area of recruitment in location of recruitment study sites; and (b) Map of Burkina Faso area of recruitment and situation of recruitment study sites.

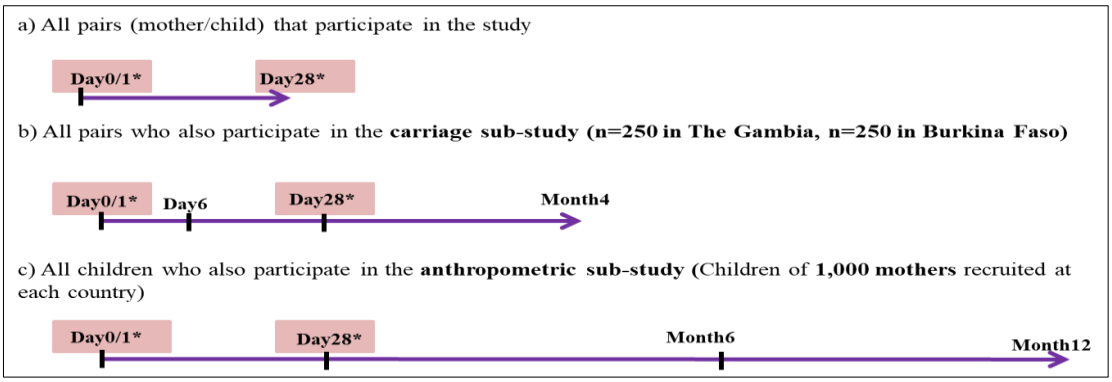

Figure 2: Length of follow-up of study participants and visits.

*Visit at day 0/1 (recruitment at the health facility and discharge from the health facility) and visit at day 28 is the same for all the study participants. The additional active visits are only for participants included in one of the sub-studies

Table 1: Neonatal sepsis criteria.

Clinical criteria	Definition
Respiratory distress	Respiratory rate >60 breaths/min, cyanosis, chest wall indrawing, grunting on expiration, respiratory distress noted in medical records
Pyrexia or hypothermia	Axillary temperature >38.0° C, not attributable to external warming, or axillary temperature <36.0° 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

Continued.

Clinical criteria	Definition
Bleeding diathesis	Defined as petechiae, ecchymosis, mucous membrane bleeding, pulmonary haemorrhage, or excessive oozing from venepuncture 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 $<5\times10^9$/l OR $>25\times10^9$/l in the absence of receiving corticosteroids
Absolute neutrophil count (ANC)	ANC $<1.75\times10^9$/l or $>15\times10^9$/l
Platelet count	$<150\times10^9$/l
C-reactive protein	>10.0 mg/l (early-onset sepsis) OR >40 mg/l (late-onset sepsis)
Elevated CSF white blood cell (WBC) count	$> 30\times10^6$/l WBC in the absence of significant red blood cells

Table 2: Bacterial contaminants isolated from blood cultures.

S. no.	Contaminants
1	Coagulase negative *Staphylococcus*
2	*Micrococci* species
3	*Bacillus* species
4	Diphtheroids
5	*Viridans streptococci* although for diagnosis such as endocarditis it may be considered a pathogen)
6	*Anthrobacter* spp
7	*Rhodococcus* spp

Table 3: Participant timelines.

Visit number	Pre-intervention		Post-intervention				
	Pregna -ncy	**Day 0[2] (delivery)**	**Day 0[2]/1 (delivery)**	**Day 6[3] (±2 days)**	**Day 28[4] (±4 days)**	**4 months[5] (±2 weeks)**	**6 and 12 months[6] (±2 weeks)**
Sensitisation and consent	X						
Health facility visit for labour		X					
Review of inclusion and exclusion criteria		X					
Randomisation and treatment		X					
Discharge review			X				
Active follow-up (home visits)				X	X	X	X
Passive follow-up[1]	Throughout study follow-up period						
Adverse events[1]	Throughout study follow-up period						
Eye swabs[1]	Throughout study follow-up period						
Mortality[1]	Throughout study follow-up period						

[1]Passive case detection, adverse events, eye swabs and mortality are conducted during the follow-up period i.e., 28 days for all babies, 12 months for the anthropometric sub-study and 4 months for the carriage sub-study; [2]day 0 is considered to be the day of delivery, in most cases treatment and delivery occur on the same day (day 0), but delivery may occur the day after treatment; [3]active follow-up visits done only for the carriage sub-study participants per country; [4]active follow-up visits are done for all study participants; [5]passive follow-up visits, adverse events and mortality recording are done only for the carriage sub-study participants per country; [6]passive follow-up visits, adverse events and mortality recording are done only for the anthropometric sub-study participants

Table 4: Sampling timelines for the 250 mother/baby pairs included in the carriage sub-study.

Parameters	Day 0[1] (day 0 or 1)	Day 6 (±2 days)	Day 28 (±4 days)	4 months (±2 weeks)
Mother				
RVS	X[2]			
NPS	X[2]	X		

Continued.

Parameters	Day 0[1] (day 0 or 1)	Day 6 (±2 days)	Day 28 (±4 days)	4 months (±2 weeks)
OPS	X[2]	X		
BM		X	X	X
Newborn				
NPS	X[3]	X	X	X
OPS	X[3]	X	X	X
RS	X[3]	X	X	X

[1]Samples at day 0 will always be collected at the health facilities; [2]for the women, samples will be collected before treatment; [3]within 4 hours after delivery/birth

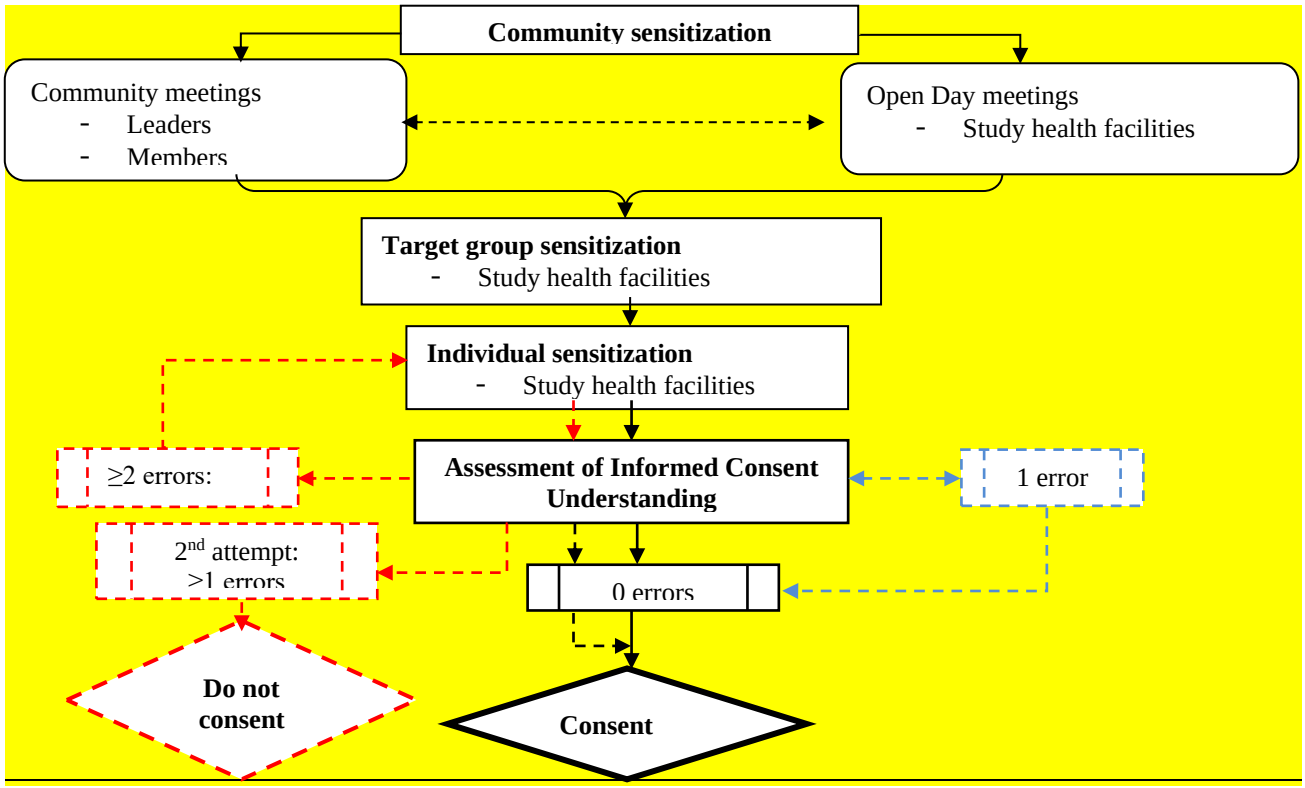

Figure 3: Sensitization and consent process.

Reproducible research

Researchers may request access with the Gambia ethics committee. The release of data will be facilitated by MRC through the Head of Governance at MRCG.

Biological specimens

Sample collection and laboratory processing

All samples for the carriage sub-studies are processed at the MRCG Laboratories in Fajara, The Gambia. Clinical samples are processed on site in the respective countries. Samples are collected following procedures described before.[10]

Once a swab is collected, the inoculated swab is immediately placed into a vial containing skim milk-tryptone-glucose-glycerol (STGG) transport medium. The samples are transferred in a cold box and transported to MRCG Laboratories in The Gambia, or CRUN Laboratories in Burkina Faso within eight hours of collection. Inoculated vials are vortexed briefly before being stored at -70ºC until tested.

Bacterial isolation from swabs

Frozen samples are thawed on ice then vortexed for 20 seconds before 50 µl aliquot is directly introduced onto different selective media for the detection and identification. 50 µl of samples will be placed in respective agar plates following standard procedures described before for *S. pneumoniae*, GBS, GAS, *S. aureus*, *E. coli* and *Klebsiella spp.*[10,19]

Antibiotic susceptibility

The antibiotic susceptibility will be screened using the disk-diffusion and confirmed by MIC using E-strips (AB

Biodisk), in accordance with the manufacturer's instructions.[10]

DISCUSSION

Over 80% of neonatal deaths happen in resource-constrained settings, mainly in SSA and Southern Asia. The sustainable development goal number 4[20] sets the target of neonatal mortality to <12 per 100,000 live-births by 2030 in all countries. To achieve this, context appropriate interventions that focus on neonates are urgently needed in SSA.

Sepsis is a prevalent cause of neonatal mortality with several bacteria involved. Therefore, an intervention to prevent neonatal sepsis and associated mortality in Africa should ideally be based on a wide-spectrum oral antibiotic which can easily be given to women in labour and which is unlikely to generate signifcant antimicrobial resistance. In our proof-of-concept we found that AZI reduced neonatal colonization by gram positive bacteria by over 50%.[12] This effect is likely attributable to the combination of reduced bacterial colonization in the mother and the direct effect of AZI reaching the baby through breast milk during the entire neonatal period.[21] The intervention also showed an effect on clinical outcomes.[11] Because the former trial was not designed to examine severe clinical outcomes, the current trial was necessary.

CONCLUSION

This trial evaluates whether the effects of AZI on maternal and neonatal colonization observed in the proof-of-concept study translate into a reduction of severe neonatal sepsis and mortality. Whatever the results, the trial will generate important information on bacteria causing infection and sepsis in The Gambia and Burkina Faso. The trial will also generate important data on macrolide resistance, which will be central to the overall evaluation of the intervention.

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