Azithromycin to prevent post-discharge morbidity and mortality in Kenyan children

Toto Bora Trial

NCT02414399

Statistical Analysis Plan Version 5.0 (May 29, 2020)

TABLE OF CONTENTS

SECTION 1. ADMINISTRATIVE INFORMATION

Title: Statistical Analysis Plan for Azithromycin to prevent post-discharge morbidity and mortality in Kenyan children: a protocol for a randomized, double-blind, placebo-controlled trial (the Toto Bora trial).

Trial Registration: NCT02414399

SAP Version: 5.0 (29May2020)

Protocol Version: 12Dec2017

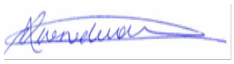
SAP Revision History

SAP Revision history Version (date)	Justification for each SAP revision	Timing of SAP in relation to interim analysis[i]
1.0 (21-Nov-2015)	Original	Before
2.0 (15-Mar-2016)	The DSMC reviewed our SAP and made suggestions which were implemented in an updated version. These changes included: plans for checking proportional hazards assumption, explicit mentioning of informative censoring, and addressing missing data. Specified interim analysis stopping rules and alpha boundary for final analysis. Added statistical analysis plan of secondary aims.	Before
3.0 (24 Aug 2017)	Clarified the primary outcome definition to exclude re-hospitalizations that were a continuation of the management prescribed as part of the original hospitalization or that occur during enrollment procedures (therefore hospitalizations that likely started prior to the investigational product being administered). Added the definition of loss to follow-up and a shell-table demonstrating how this information would be reported in quarterly reports. Updated the shell table for how adverse events would be reported by categories AE's in terms of Grade as opposed to specific AE's.	Before
4.0 (27 Sep 2019)	Re-formatted the SAP to be consistent with published guidelines for SAP (as outlined in JAMA. 2017; 318(23):2337-2343). Incorporated changes that had been discussed (and documented) during DMSC discussions (inclusion of site in ITT model, specifying primary adherence measure)	After
5.0[ii] (29 May 2020)	Clarified how adherence data would be handled among those missing adherence information captured by questionnaire. Defined hospitalization as overnight stay (not previously defined). Specified cause of death would be determined by clinical consensus and secondarily by verbal autopsy. Updated shell tables.	After

i. All study personnel (other than the trial statistician) were blinded during the interim-analysis which included data through 31Oct2018

ii. Updated prior to unblinding

Roles and responsibilities in SAP and Signatures:

Name	Trial Role	SAP Role	Signatures
Patricia Pavlinac, PhD	Project Director	Writing	
Barbra Richardson, PhD	Trial Statistician	Senior statistician responsible	
Judd Walson, MD	Primary Investigator	Chief investigator/clinical lead	

SECTION 2. INTRODUCTION

Background and Rationale

An estimated 3.5 million deaths occur annually in children less than 5 years of age in sub-Saharan Africa, approximately 70% of which are due to infectious causes.[1] One-year mortality rates as high as 15% have been documented following hospital discharge in sub-Saharan Africa, a rate that is 8-fold higher than non-hospitalized children.[2-4] Children being discharged from hospital in Africa may represent an accessible high-risk population in which to target interventions to reduce mortality.

A recent trial of mass drug administration of azithromycin reduced childhood mortality by half among children in Ethiopia in communities receiving the intervention.[5, 6] However, concerns about the potential for the emergence of antimicrobial resistance, possible toxicity, and the feasibility of delivery are all barriers to community-wide distribution of antibiotics.

A short course of azithromycin given to children with recent severe illness being discharged from hospital may optimize benefit while reducing both individual and population level risks. Azithromycin may reduce post discharge morbidity and mortality through infection-related mechanisms such as treating undiagnosed, incompletely treated or nosocomial infections, or by protecting against new or recrudescent infections that occur during recovery. Azithromycin may also act through non-antimicrobial pathways by anti-inflammatory and/or immune-modulatory effects.

Objectives

Aim 1. To compare rates of **re-hospitalization and mortality** in the 6 months following hospital discharge among Kenyan children receiving 5-day azithromycin vs. placebo.

Hypothesis: The provision of a 5-day course of azithromycin provided at discharge will reduce hospital re-admission and death within the 6 months following discharge, as compared to placebo.

Aim 2a. To evaluate possible **mechanism(s)** by which azithromycin may affect morbidity and mortality, by comparing **reasons for re-hospitalization and prevalence of pathogen carriage** between the randomization arms.

Hypothesis: Children treated with azithromycin will experience fewer hospitalizations due to diarrhea, acute respiratory infection, and malnutrition, and will be less likely to have respiratory and gastrointestinal carriage of potentially pathogenic organisms, as compared to children treated with placebo in the 6 months following hospital discharge.

Aim 2b. To determine whether empiric administration of azithromycin at hospital discharge **increases risk of antimicrobial resistance** in commensal *Escherichia coli* (*E. coli*) and *Streptococcus pneumoniae (S. pneumoniae)* isolates from treated children and their household contacts.

Hypothesis: Isolates of commensal E. coli and S. pneumoniae from children treated with azithromycin and their household contacts will have higher levels of macrolide and β-lactam resistance, compared to the placebo group, after 90 days of follow-up, but resistance in the 2 arms will be similar by 6 months.

Aim 3. To identify **correlates and intermediate markers of post-discharge mortality and hospital readmission** among hospitalized Kenyan children.

Hypothesis: Children younger in age, with higher levels of bacterial pathogen carriage, immune dysfunction, and malnutrition will experience more frequent re-hospitalizations and deaths.

Aim 4. To determine the **cost-effectiveness** of post-discharge administration of a 5-day course of azithromycin in settings of varying antibiotic use, re-hospitalization rates, and mortality rates.

Hypothesis: The provision of a 5-day course of azithromycin provided at discharge is cost-effective in settings with moderate to high re-hospitalization and mortality rates and this cost-benefit is sensitive to modest changes in individual and community levels of drug resistance.

SECTION 3. STUDY METHODS

Trial Design

A 2-arm, double-blind, randomized clinical trial (RCT) comparing the efficacy of a 5-day course of azithromycin to placebo among Kenyan children aged 1-59 months.

Randomization

Block randomization (1:1) in random-sized blocks of no more than 10 stratified by site will be used. Primary randomization will include allocation to the contact cohorts at a ratio of 1:5 (resulting in 150 per treatment arm). Each subject will be assigned a patient identification (PID) number, and the randomization code linking each PID to the allocated treatment will be generated by a designated statistician and maintained by the University of Washington Research Pharmacy. Study participants, investigators (other than the statistician), the study staff, hospital clinicians and persons involved in data management or analysis will remain blinded to the allocation group during all data collection phases of the study.

Sample size

The total sample size required (1400 children) was calculated for the primary endpoint of time to death or hospital readmission within the 6-month post-discharge period, assuming an alpha level of 0.05, power of 0.80, a ratio of treatment to placebo random assignment of 1:1, and a cumulative incidence of 22.5% for the combined outcome of death or re-hospitalization among placebo-treated children. Further details of the sample size calculation are outlined in the protocol.

Framework

All hypotheses are tested for superiority.

Statistical Interim Analysis and Stopping Guidance

A single interim analysis for re-hospitalization-free survival will be performed using O'Brien-Fleming boundaries for benefit or harm when 50% of expected person time (350 child-years) has been accrued. Assuming 157 events will be available at half of the person-time accrual, a z-score critical value of 2.797, or *p*-value < 0.005, from a Kaplan Meier log-rank test will determine the cut-off of statistical significance. A symmetric boundary will be used for benefit or harm. The DSMC will review this analysis and make a determination about continuing the study. Futility will not be a basis for stopping rules because of the trials' value in understanding mechanisms of post-discharge worsening and antibiotic resistance. Assuming the DSMC decides to continue the trial after the interim analysis, an alpha of 0.045 will be used as the statistical significance boundary at the final analysis for the primary hypothesis test.

Timing of Final Analysis

The first main report/ publication of the trial will be prepared for the primary aim when every enrolled child has completed their 6 month follow-up visit or is deemed lost to follow-up and all primary endpoint data has been cleaned (anticipated publication in September 2020).

Timing of Outcome Assessments

The schedule of study procedures is outlined in Table 1. Regularly scheduled visits include those at M0 (hospital discharge), M3, and M6. Participants are actively followed for 1 month following their missed visit. If a visit occurs after the one month window, relevant data will be ascertained when possible, but may not be included in the analysis (as described in Table 1). The start date/time for each participant is the date/time of randomization.

Table 1. Allowable windows for each outcome

Outcome	Visit Window
Death or re-hospitalization	Follow-up time censored 180 days after enrollment or at the time of the last follow-up visit (if <180 days). Data collected (including vital status) from visits that occur beyond 180 days will be included but events and person-time will be censored at 180 days.
Cause specific hospitalization	Follow-up time censored 180 days after enrollment or at the time of the last follow-up visit (if <180 days). Data collected (including vital status) from visits that occur beyond 180 days will be included but events and person-time will be censored at 180 days
Prevalence of *Shigella*, *Salmonella*, *Campylobacter*, *Cryptosporidium*, Giardia @ enrollment, day 90, and day 180	Visits that occur within 30-days on either side of 90-days (M3) and 180-days (M6) visits will be included. Therefore, visits occurring outside of 60-120 days post-enrollment for M3 and outside of 150-210 days post enrollment for M6 will be excluded from the primary analysis but included in sensitivity analyses.
Streptococcus pneumoniae (S. pneumoniae) prevalence @ enrollment, day 90, and day 180	Visits that occur within 30-days on either side of 90-days (M3) and 180-days (M6) visits will be included. Therefore, visits occurring outside of 60-120 days post-enrollment for M3 and outside of 150-210 days post enrollment for M6 will be excluded from the primary analysis but included in sensitivity analyses.
Prevalence of resistance to azithromycin in *E.coli* and *S. pneumoniae* and prevalence of ESBL-producing *E.coli* @ enrollment, day 90, and day 180	Visits that occur within 30-days on either side of 90-days (M3) and 180-days (M6) visits will be included. Therefore, visits occurring outside of 60-120 days post-enrollment for M3 and outside of 150-210 days post enrollment for M6 will be excluded from the primary analysis but included in sensitivity analyses.

SECTION 4. STATISTICAL PRINCIPLES

Confidence Intervals and P values

Level of statistical significance
All statistical tests will be 2-sided using a 5% significance level (alpha of 0.05). For the primary aim (aim 1), an alpha of 0.045 will be used for hypothesis tests that were part of the interim analysis to account for alpha spending.

Type I errors

We will not adjust the alpha for multiple testing in the primary aim. Instead we will clearly state primary and secondary analyses and interpret secondary analyses as hypothesis-generating rather than confirmatory. All secondary aims will be adjusted for multiple comparisons using the Benjamini and Hochberg method using a false discovery proportion of 0.05.

Confidence intervals to be reported

For the primary analysis of Aim 1, two-sided 95.5% confidence intervals will be used to account for the 0.045 alpha spent at the interim analysis. All other analyses will utilize two-sided 95% confidence intervals.

Adherence and Protocol Deviations

Definition of adherence to intervention and how this is assessed including extent of exposure

Adherence is measured by questionnaire at the first follow-up visit (M3). If the M3 follow-up visit is missed, adherence is ascertained at the second follow-up visit (M6). Additionally, caregivers are asked to return the study drug bottle, which has tick-marks for the caregiver to mark as the study drug is given (bottle tick-mark). This measure of adherence is considered secondary to the self-report in questionnaire but a comparison of the two methods will be presented.

Description of how adherence to the intervention will be presented

We will assess two coprimary measures of adherence-1) at least one dose vs. 1 dose only and 2) all 5 doses vs. less than 5. We will additionally report the mean/median # of days of adherence and evaluate adherence as a continuous measure to determine whether there is a dose-response relationship.

For those missing self-reported adherence data, such as due to a death occurring prior to the M3 visit, we will assume all adhered and none adhered and present data for both situations to establish the range of adherence to the investigational product.

Definition of protocol deviations for the trial

The following are pre-defined major protocol deviations with a direct bearing on the primary outcome:
1) Unblinding of the study participant or study team to randomization allocation
2) Errors in applying inclusion/exclusion criteria that are discovered after randomization

The following are pre-defined minor protocol deviations:

1) Missed sample collection (blood, stool/rectal swab, nasopharyngeal swab) due to participant refusal or other barrier to sample collection (such as visit occurring over phone).
2) Missed anthropometry assessment due to follow-up visit occurring over the phone

Description of which protocol deviations will be summarized

Protocol deviations will be classified as major and minor prior to unblinding of randomization allocation. The number (and percentage) of participants with major and minor protocol deviations will be summarized by treatment groups in relevant analyses with details of the deviation provided. The patients that are randomized will be used as the denominator to calculate percentages. No statistical tests will be performed.

Analysis Populations

Analysis of primary outcomes will be by modified intention-to-treat (mITT). The mITT population will include all randomized children according to the treatment they were randomized to receive but will exclude those who were deemed ineligible, post-randomization. In per-protocol analyses (secondary to the mITT), we will compare treatment effects in groups defined by self-reported adherence to the 5-day course of azithromycin. Per protocol analyses will also exclude children who were ineligible, those who were lost to follow-up, and those who withdrew consent.

SECTION 5. TRIAL POPULATION

Screening Data

The total number screened will be reported along with summary of reasons for exclusion into the trial.

Eligibility

Children age 1 to 59 months old who weigh at least 2 kg and have been hospitalized and subsequently discharged from hospitals in Kisii or Homa Bay County for conditions other than trauma, injury, poisoning, or a birth defect, will be eligible for the RCT (Aim 1). We will exclude children in whom azithromycin is contraindicated (children taking or prescribed other macrolide antibiotics, such as erythromycin or clarithromycin, or the protease inhibitor lopinavir); those with a known macrolide allergy, those who do not plan to remain in the study site catchment area for at least 6 months, those without a legal guardian or a legal guardian does not provide consent, those with a twin is eligible and enrolled in the trial on the same day, those that are being referred to another facility, and those with a caregiver who is unwilling to participate in contact cohort if selected (willing/able to provide a stool and nasopharyngeal sample at enrollment, 90 days and 6 months of follow-up if randomized to the Contact Cohort).

Recruitment

Per CONSORT guidelines, we will report the number of individuals who:

1. Underwent screening
2. Met inclusion criteria
3. Did not meet inclusion criteria (and reasons)
4. Enrolled in the study and were randomized

5. Were included in the mITT
6. Were included in the per-protocol population

Withdrawal/Follow-Up

Level of withdrawal (e.g. from intervention and/or from follow-up)

Withdrawal of consent will be tabulated using the following categories: withdrawal from follow-up but allow prior collected data/ samples to be used; withdrawal from follow-up and disallow already collected data/samples to be utilized; withdrawal from study intervention but continue with follow-up/data collection; withdrawal from study intervention and discontinue with follow-up/data collection.

Timing of withdrawal/lost to follow-up data

Tabulation of withdrawals will include withdrawals by each follow-up timepoint (M3 and M6).

Reasons and details of how withdrawal/lost to follow-up data will be presented

The numbers and reasons (if available) of losses to follow-up (withdrawals or losses to follow-up) will be summarized by treatment arm.

Baseline Patient Characteristics

Baseline characteristics and how they will be descriptively summarized

We will describe the distribution of baseline characteristics (outlined in Table 2), using summary statistics appropriate for the measurement scale (median and interquartile range [IQR] or n and %). We will present these summary statistics in tables by randomization arm as outlined in Table 2. No statistical tests comparing baseline characteristics by randomization arm will be conducted, but the clinical importance of any appearing imbalance will be noted.

Table 2 Baseline characteristics shell table

Characteristic	All (N=) n(%) Median(IQR)	Azithromycin-treated group (N=) n(%) Median(IQR)	Placebo-treated group (N =) n(%) Median(IQR)
Site			
Kisii Teaching & Referral Hospital			
Homa Bay County Referral Hospital			
St. Paul Mission Hospital			
Kendu Adventist Hospital			
Sociodemographic			
Age			
1m to 5m			
6m to 11m			
12m to 23m			
24m to 59m			
Median age (months)			
Female			
Extreme poverty (household income < $1.90 USD / day)			
Primary caregiver education ≤ primary school			
Household Living Conditions			
Crowding (≥2 people per room living in house)			
Unimproved water source (well/spring/surface water)			
Reports treating drinking water			
Toilet Type			
Flush			
Pit Latrine			
Open Defecation			
Admission History			
Discharge diagnoses as reported in medical record			
Anemia			
Gastroenteritis/diarrhea			
Lower Respiratory Tract Infection			
Malaria			
Malnutrition			
Meningitis			
Sepsis			
Sickle Cell			
Tuberculosis			
Other			
Not recorded in hospital record			
Duration of hospital admission (days)			
Left against medical advice			
Received antibiotics in hospital			
Prescribed antibiotics at discharge			
Nutritional, HIV, and Vaccine Status			
Breastfeeding status (in first 6 months of life)			
Exclusively breastfed			
Partially breastfed			
Never Breastfed			
Unknown			
Stunted (HAZ < -2)			

Underweight (WAZ < -2)			
Acute Malnutrition			
Severe (WHZ < -3 or MUAC <11.5cm or edema)			
Moderate (-3 ≥ WHZ < -2 or 11.5 ≥ MUAC <12.5cm)			
HIV-status			
HIV- infected			
HIV-exposed, uninfected			
HIV-exposed, unknown infection status			
HIV status unknown			
Received all age-appropriate vaccines			

SECTION 6. ANALYSIS

Outcome Definitions

Primary study end-points:

1. **Deaths** will be ascertained by study staff either through community health workers, hospital staff, or determined at follow-up visits. Date of death will be ascertained from the following (in order of prioritization): death certificate, hospital record, caregiver report
2. **Re-hospitalization** will be assessed by questionnaire at day 90 and 180 follow-up visits and medical record review. In cases when both sources are available, information from the medical record will be considered the gold standard. A hospitalization will be defined as an overnight stay, including situations where a child is admitted in the middle of the night (even if after midnight) and discharged the subsequent morning. Re-hospitalizations that are a continuation of management from the previous hospitalization (such as elective blood transfusion) or that occur during enrollment procedures, due to a clinical deterioration post-discharge, will be excluded from the analysis. To operationalize these exclusions, we will exclude re-hospitalizations that occur <2 hours after administration of the study medication and those re-hospitalizations that occur as a result of the child being advised to seek further care for a blood transfusion due to a lack of blood availability at the enrollment facility.
3. **Loss to follow-up** will be defined as non-attendance at both follow-up visits despite up to one month of active tracing and no clear evidence of death.
4. **Death or re-hospitalization** (combined) is the primary endpoint.

Secondary end-points:

5. **Cause-specific deaths** will be determined by consensus among clinical investigators based on all available information (death certificate, medical records, physical exams performed as part of study procedures, and verbal autopsies). Verbal autopsies will also be performed using the Population Health Metrics Research Consortium Shortened Verbal Autopsy Questionnaire. Secondary to the cause-assignment by consensus, we will additionally determine cause using the SmartVA-Analyze tool applied to the Verbal Autopsy form which utilizes the Tariff 2.0 Method to assign cause.[7] Causes assigned by the verbal autopsy tool will be reported secondary to the consensus-assigned cause because the information utilized for cause of death by consensus is likely to exceed that captured by the verbal autopsy questionnaire asked to the caregivers.
6. **Cause-specific re-hospitalizations** will be assessed by questionnaire (maternal recall of diagnosis) at day 90 and 180 follow-up visits and medical record review (discharge diagnosis).

In cases when both sources are available, information from the medical record will be prioritized. Because children can have more than one diagnosis, each diagnosis (diarrhea, pneumonia, malnutrition, or malaria) will be considered in separate analyses. Additionally, we will consider a combined diagnosis of diarrhea, pneumonia, malnutrition and/or malaria to capture the diagnoses we hypothesize to be more affected by azithromycin.

1. **Bacterial pathogen carriage** will be assessed based on microbiologic testing of stool/rectal swabs and nasopharyngeal swabs at 90 and 180-day follow-up visits. *Shigella*, *Salmonella*, and *Campylobacter* will be assessed in the stool and *Streptococcus pneumoniae (S. pneumoniae)* will be assessed in nasopharyngeal swabs.
2. **Antimicrobial resistance** will be assessed in a subset of isolates of *E. coli* and *S. pneumoniae* from stool and nasopharyngeal swabs, respectively, collected at baseline, 90-days and 180-days. Isolates are selected based on a random selection of enrolled children (irrespective of whether or not an isolate is available) to maintain randomization. Zone sizes from antibiotic susceptibility testing will be used to categorize susceptible, intermediate and resistant using the most up to date Clinical and Laboratory Standards Institute CLSI interpretative standards at the time of analysis. In primary analyses, we will consider intermediate and resistant together as "non-susceptible" and in secondary analyses will consider only resistant in the numerator.

Analysis Methods

Analysis of study end-points

Death or re-hospitalization: Cumulative incidence of death or first re-hospitalization will be compared between treatment groups using Cox proportional hazards regression. Participants will be censored at the date of their first re-hospitalization, or at the date of death. Site will be included as an indicator variable in all statistical models including in the intention to treat analysis. If the baseline assessment of randomization reveals an imbalance in characteristics (other than site) between the treatment groups, we will evaluate these variables as potential confounders in a sub-analysis secondary to the ITT. Potential baseline confounders (as outlined in the baseline characteristics table) will be added stepwise in a multivariable Cox model and maintained in the model if adjustment changes the hazard ratio by more than 10%. In addition, we will conduct Cox regression for time to mortality and time to re-hospitalization as separate endpoints to understand intervention effects on these outcomes individually. Table 3 demonstrates how primary endpoint data will be reported by intervention arm.

The assumption of proportional hazards will be checked using graphical methods including plotting a ln(-ln(S(t))) plot for each treatment group and assessing the parallelism of the two lines and by plotting Schoenfeld residuals over time. The assumption of non-informative censoring cannot be assessed explicitly, however every effort will be made to limit losses to follow-up.

Table 3. Outcome shell table (unblinded) to be accompanied by Kaplan Meier Figure by intervention group

	Azithromycin-treated group (N=)			Placebo-treated group (N=)			Effect Estimate		
Outcome	n	Person-time	Incidence rate (/100)	n	Person-time	Incidence rate (/100)	Hazard ratio	(95% CI)	*p*-value
Death or re-hospitalization									

Death								
First re-hospitalization								

In subgroup analyses, we will compare treatment effects in children whose caregivers report no additional antibiotic use over follow-up and separately, who report no additional azithromycin use specifically and in subsets of children defined by the following baseline characteristics: age, county of recruitment, HIV-exposure status, nutritional status, and discharge diagnosis. We will not conduct formal testing of interaction in these analyses. We will report within sub-group p-values but interpret as secondary sub-group analyses. Also in secondary analyses, we will allow more than one hospitalization and model the incidence of re-hospitalization by randomization arm using Anderson-Gill models.

We will report causes of death (and predisposing conditions in those deaths) by randomization arm (without statistical testing) as presented in Table 4.

Table 4. Causes of death by randomization arm

	Azithromycin-treated group (N=)	**Placebo-treated group (N=)**
Primary cause		
LRTI/Pneumonia		
Diarrhea		
Malaria		
Tuberculosis		
Other Infection		
Other non-infectious		
Predisposing conditions		
HIV		
Sickle cell disease		
Confirmed acquired condition		
Confirmed congenital condition		
None		

Statistical Analysis of Secondary Aims

In addition to the primary aim of the study to determine whether a short-course of azithromycin reduces risk of post-discharge morbidity and mortality, secondary aims include determining potential mechanisms and intermediate markers of an intervention effect, the impact of the antibiotic course on antimicrobial resistance in gut and nasopharyngeal pathogens, and correlates of post-discharge morbidity and mortality. The below tables outline these planned analyses.

Aim 2a. To evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality, by comparing reasons for re-hospitalization and prevalence of pathogens between the randomization arms.

Population	**Dependent variable**	**Independent Variable**	**Covariates**	**Statistical model**

Reasons for re-hospitalization				
All enrolled children	Time to re-hospitalization (due to diarrhea, pneumonia, malaria, malnutrition)	Randomization arm (ITT primary and per-protocol secondary)	1) Site 2) adjusting for imbalanced baseline factors	Anderson-Gill
Enteric and nasopharyngeal pathogens				
All enrolled children	Prevalence of *Shigella*, *Salmonella*, *Campylobacter*, *Cryptosporidium*, *Giardia* @ 0, 3, & 6	Randomization arm (ITT primary and per-protocol secondary)	1) Site 2) adjusting for imbalanced baseline factors & baseline presence of pathogen	GEE with Poisson link & exchangeable correlation structure
	Streptococcus pneumoniae prevalence @ 0, 3, & 6	Randomization arm (ITT primary and per-protocol secondary)	1) Site 2) adjusting for imbalanced baseline factors & baseline presence of pathogen	GEE with Poisson link & exchangeable correlation structure

Aim 2b. To determine whether empiric administration of azithromycin at hospital discharge increases risk of antimicrobial resistance in commensal E. coli and pneumococcal isolates from treated children and their household contacts.

Population	**Dependent variable**	**Independent Variable**	**Covariates**	**Statistical model**
Resistance in enrolled children				
1. Random subset of enrolled children selected to be in the AMR sub-study 2. Children in AMR sub-study in whom *E.coli* was isolated	Prevalence of resistance to azithromycin and presence of extended spectrum beta-lactamase-producing *E.coli*. In secondary analyses, prevalence of ampicillin, chloramphenicol, ciprofloxacin, ceftriaxone, ceftazidime, gentamicin, trimethoprim-sulfamethoxazole @ 0, 3, & 6 months	Randomization arm (ITT primary and per-protocol secondary)	1) Site 2) Adjusting for imbalanced baseline factors & baseline presence of resistance 3) Adjusting for propensity score made up of factors predictive of likelihood that *E. coli* is isolated	GEE with Poisson link & exchangeable correlation structure. Wald tests to compare between arms and to test for time-interaction (waning in resistance from 3 to 6 months in AZM arm)
1. Random subset of enrolled children selected to be in the AMR sub-study 2. Children in AMR sub-study in whom *S. pneumoniae* was isolated	Prevalence of resistance to azithromycin. In secondary analyses, prevalence of amoxicillin/ K clavulanate, ampicillin, chloramphenicol, clarithromycin, erythromycin, penicillin, trimethoprim-sulfamethoxazole @ 0, 3, & 6 months	Randomization arm (ITT primary and per-protocol secondary)	1) Site 2) Adjusting for imbalanced baseline factors & baseline presence of resistance 3) Adjusting for propensity score made up of factors predictive of likelihood that *S. pneumoniae* is isolated	GEE with Poisson link & exchangeable correlation structure. Wald tests to compare between arms and to test for time-interaction (waning in resistance from 3 to 6 months in AZM arm)
Resistance in household contacts				

1. Randomly selected adult contacts who are participating in AMR sub-study 2. Random subset of adult AMR sub-study participants in whom *E.coli* was isolated	Prevalence of resistance to azithromycin and presence of extended spectrum beta-lactamase-producing *E.coli*. In secondary analyses, prevalence of ampicillin, chloramphenicol, ciprofloxacin, ceftriaxone, ceftazidime, gentamicin, trimethoprim-sulfamethoxazole @ 0, 3, & 6 months	Randomization arm (ITT primary and per-protocol secondary)	1) Site 2) Adjusting for imbalanced baseline factors & baseline presence of resistance 3) Adjusting for propensity score made up of factors predictive of likelihood that *E. coli* is isolated	GEE with Poisson link & exchangeable correlation structure. Wald tests to compare between arms and to test for time-interaction (waning in resistance from 3 to 6 months in AZM arm)
1. Randomly selected adult contacts who are participating in AMR sub-study 2. Random subset of adult AMR sub-study participants in whom *S. pneumoniae* [illegible]	Prevalence of resistance to azithromycin. In secondary analyses, prevalence of amoxicillin/ K clavulanate, ampicillin, chloramphenicol, clarithromycin, erythromycin, penicillin, trimethoprim-sulfamethoxazole @ 0, 3, & 6 months	Randomization arm (ITT primary and per-protocol secondary)	1) Site 2) Adjusting for imbalanced baseline factors & baseline presence of resistance 3) Adjusting for propensity score made up of factors predictive of likelihood that *S. pneumoniae* is isolated	GEE with Poisson link & exchangeable correlation structure. Wald tests to compare between arms and to test for time-interaction (waning in resistance from 3 to 6 months in AZM arm)

Aim 3. To identify correlates and intermediate markers of post-discharge mortality and hospital-readmission among hospitalized Kenyan children.

Population	Dependent variable	Independent Variable(s)	Covariates	Statistical model
All enrolled children	Time to death or re-hospitalization	• Child nutritional status • HIV status (child & mother) • Vaccination status • Abnormal discharge • Discharge diagnosis • Length of hospital admission • Infection presence at discharge • Antibiotic resistance at discharge • Feeding practices • Socio-economic status • Caregiver education and nutritional status	Randomization arm, site	Cox proportional hazards
	Time to re-hospitalization		Randomization arm, site	Cox proportional hazards and Andersen-Gill for >1 hospitalization
	Time to death		Randomization arm, site	Cox proportional hazards

Aim 4. To determine the cost-effectiveness of post-discharge administration of a 5-day course of azithromycin in settings of varying antibiotic use, re-hospitalization rates, and mortality rates.

Costs analysis: We will assess the costs of all supplies, services and equipment necessary to implement the intervention (direct medical costs). The perspective will be that of the healthcare provider, i.e. Kenya's Ministry of Health. Using WHO guidelines and its ingredients approach, we will quantify the resources and associated unit costs required to deliver a 5-day course of azithromycin, organized in

standard expenditure categories: personnel (salaries), supplies including drugs, equipment, services, space and overhead. We will also measure the costs of severe child hospitalizations, the costs for the different types of personnel employed (e.g. nurses/doctors) and the time demanded from them for conducting the intervention. When data are missing, they will be complemented by data extracted from the literature and other available sources. Full incremental costs will be derived, with estimation of the potential healthcare cost-offset realized in avoiding severe hospitalizations. Costs will be measured in local currency (Kenyan Shilling) and converted into US$. Our main metric will be cost per child treated.

Cost-effectiveness analysis (CEA): we will develop a CEA mathematical model, and estimate incremental costs and cost-effectiveness for implementation of the intervention. The model will include two components: costs (described immediately above) and health benefits. The study will provide clinical outcomes (mortality/morbidity) over a 6-month follow-up period. Subsequently, deaths averted, life-years saved and disability-adjusted life years (DALYs) averted by the intervention will be estimated. We will estimate: a) incremental costs and b) incremental cost-effectiveness of the intervention vs. status quo. *Incremental costs* are the net sum of the costs to implement the intervention compared with status quo, and the costs averted due to the decrease in severe child hospitalizations. *Incremental cost-effectiveness* ratios (ICERs) will be estimated as cost per death averted, cost per life-year saved, and cost per DALY averted. We will use recent estimates of disability weights for estimation of DALYs. Short-term (over study follow-up i.e. 6 months) and longer-term time horizons (extrapolated to 1, 5, and 10 years) will be used. DALYs and costs will be discounted at 3% per year, consistent with CEA guidelines (undiscounted results will also be presented). Sources of uncertainty in the results will be explored in univariate and probabilistic sensitivity analysis. Finally, we will compare our findings to CEA estimates for other health interventions in sub-Saharan Africa.

Missing Data

If there is substantial missing covariate data (>10%), multiple imputation using the Markov chain Monte Carlo (MCMC) method will be used to impute covariate information. Missing outcome data (death or re-hospitalization) will not be imputed, but participants will be censored at the last follow-up visit therefore contributing some person-time to the analysis.

Harms

Adverse and severe adverse events (SAEs) will be descriptively presented by randomization arm as described in Table 5:

Table 5. Adverse events by randomization arm among mITT population

	Azithromycin-treated group (n=)			**Placebo-treated group(n=)**		
		Days since enrollment			Days since enrollment	
AE Grade[i]	**N**[ii]**(%)**	0-7	35-180	**N**[ii]**(%)**	0-7	8-180
Serious AE Total						
Death						
Life Threatening						
Non-Serious AE totals						
3: Severe						
2: Moderate						
1: Mildi[v]						

i. Defined according to 2014 Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events

ii. Events are placed in age categories based on age at enrolled, reports generated prior to July 2018 were based on age at event.
iii. Relatedness for SAEs and serious AEs are determined by clinical team after full review of the case. Non-serious AEs are considered to be possibly related to the study drug if a recognized side effect of azithromycin (diarrhea, vomiting, rash, facial/airway swelling, difficulty breathing, jaundice, abdominal swelling) occurred within 72 hours of a scheduled drug administration (i.e. within 7 days of study enrollment)
iv. Does not include minor complaints (runny nose and bee sting)

Statistical software

All analyses will be conducted using STATA or R and the software used reported in all analysis write-ups

References

References to be provided for non-standard statistical methods

All methods being proposed are standard.

Data Management Plan

Procedures related to data entry and data management are outlined in Trial Standard Operating Procedures #27 (Data Management) and 28 (Electronic Data Entry) version 3.0 (06Aug2018).

Trial Master File and Statistical Master File

The Statistical Master File is maintained by the Study Statistician only.