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Azithromycin to prevent post-discharge morbidity and mortality in Kenyan children: A protocol for a randomized, double-blind, placebo-controlled trial

Journal:	BMJ Open
Manuscript ID	bmjopen-2017-019170
Article Type:	Protocol
Date Submitted by the Author:	17-Aug-2017
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Primary Subject Heading :	Global health
Secondary Subject Heading:	Paediatrics, Infectious diseases
Keywords:	Child mortality, Antibiotic prophylaxis, Post-discharge interventions

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Azithromycin to prevent post-discharge morbidity and mortality in Kenyan children: A protocol for a randomized, double-blind, placebo-controlled trial
AZM to prevent post-discharge morbidity and mortality
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This trial is sponsored by the National Institutes of Health Eunice Kennedy Shriver National Institute of Child Health & Human Development (R01 HD079695) and drug donation by Pfizer IIR #WI201906.
child mortality, antibiotic prophylaxis, post-discharge interventions

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ABSTRACT

Introduction: Child mortality due to infectious diseases remains unacceptably high in much of sub-Saharan Africa. Children who are hospitalized represent a particularly vulnerable population, both during and following hospitalization. Children being discharged from hospital represent an accessible high-risk population in which targeted use of antibiotics could offer clinical benefit.

Methods and analysis: In this randomized, double-blind, placebo-controlled trial, 1400 children aged 1 to 59 months discharged from 2 hospitals in western Kenya, in Kisii and Homa Bay, will be randomized to either a 5-day course of azithromycin or placebo to determine whether a short-course of azithromycin reduces rates of re-hospitalization and/or death in the subsequent 6-month period. The primary analysis will be modified intention-to-treat and will compare the rates of re-hospitalization or death in children treated with azithromycin or placebo using Cox proportional hazard regression. The trial will also explore mechanistic questions including the effect of a short course of azithromycin on enteric and nasopharyngeal infections and cause-specific re-hospitalizations. We will also identify clinical and host risk determinants of post-discharge morbidity and mortality. The emergence of antibiotic resistance among treated individuals and in a random subset of their primary caregivers will also be assessed and cost-effectiveness analyses performed to inform policy decisions.

Ethics and dissemination: Study procedures were reviewed and approved by the institutional review boards of the Kenya Medical Research Institute, the University of Washington, and the Kenyan Pharmacy and Poisons Board. The study is being externally monitored by Westat[®] and a data safety and monitoring committee has been assembled to monitor patient safety and evaluate the efficacy of the intervention. The results of this trial will be published in peer-reviewed scientific journals and presented at relevant academic conferences and to key stakeholders.

Trial registration number: NCT02414399

Key words: child mortality, antibiotic prophylaxis, post-discharge interventions

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Randomized, placebo-controlled, double-blinded design and intention-to-treat analysis plan will ensure unbiased treatment effect measure
- Comprehensive data are collected, including biological specimens for all child participants and a subset of adult caregivers, for analyses of mechanisms of post-discharge morbidity and mortality, as well as assessments of antibiotic resistance and cost-effectiveness
- Results will likely be generalizable due to the limited exclusion criteria, large sample size, and multiple study sites
- Causes of death and re-hospitalization may not be accurate due to limited availability of medical records and recall bias in caregiver report
- The primary endpoint of this study is a combined outcome of re-hospitalization and death, which, while improving statistical power, may present challenges for interpretation

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2 BACKGROUND 3

An estimated 3.5 million deaths occur annually in children less than 5 years of age in sub-Saharan Africa (SSA), 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 SSA, a rate that is 8-fold higher than similarlyaged children in the community.[2-4] Children being discharged from hospital in SSA may represent an accessible high-risk population in which to target interventions to reduce mortality.

A single dose of azithromycin halved mortality rates in among Ethiopian children living in communities randomized to receive the antibiotic as part of a mass drug administration program.[5, 6] However, concerns 10 about the potential for the emergence of antimicrobial resistance, possible toxicity, and feasibility of delivery 11 are barriers to community-wide distribution of antibiotics. Targeted antimicrobial interventions, including the 12 use of cotrimoxazole among HIV-infected children and the use of amoxicillin or cefdinir among malnourished 13 children, have been shown to reduce mortality in these specific vulnerable populations.[7-10] Children who 14 have been recently hospitalized are a high-risk population in which targeted azithromycin distribution may 15 optimize benefit while reducing both individual and population level risks. 16

Among high-risk pediatric populations with history of recent illness, azithromycin may treat residual 17 disease not eliminated during inpatient therapy, may provide prophylaxis against infectious exposures during a 18 time of immune vulnerability following illness, and may reduce carriage of pathogenic organisms, including 19 20 those associated with mucosal surface disruption, inflammation, and immune activation. 21

22 **OBJECTIVE**

23 The primary objective of this double-blind, placebo-controlled, randomized controlled trial (RCT) is to 24 determine whether a 5-day course of azithromycin in children age 1 to 59 months discharged from hospital in 25 western Kenya reduces rates of re-hospitalizations and/or death in the subsequent six months. The secondary 26 objectives are (1) to evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality, 27 by comparing reasons for re-hospitalization and prevalence of enteric and nasopharyngeal infections between 28 the randomization arms; (2) to determine whether empiric administration of azithromycin at hospital discharge 29 increases risk of antimicrobial resistance in commensal Escherichia coli (E. coli) and Streptococcus 30 pneumoniae (S. pneumoniae) isolates from treated children and their primary caregiver; (3) to identify 31 correlates and intermediate markers of post-discharge mortality and hospital readmission; (4) to determine the 32 cost-effectiveness of post-discharge administration of a 5-day course of azithromycin in settings of varying 33 antibiotic use, re-hospitalization rates, and mortality rates; and (5) to create a repository of stool, 34 nasopharyngeal, and blood specimens from highly characterized, recently discharged children to be used to 35 address future research questions. 36 37

38 **METHODS** 39

Reporting of this study protocol has been verified in accordance with the SPIRIT (Standard Protocol Items for Randomized Trials) recommendations.

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43 Children age 1 to 59 months old weighing at least 2 kg and have been hospitalized and subsequently 44 discharged and who are willing to participate will be eligible for inclusion. Children will be excluded if: 45 azithromycin is contraindicated (children taking or prescribed other macrolide antibiotics, such as erythromycin 46 or clarithromycin, or the protease inhibitor, lopinavir); they were admitted to hospital for a trauma, injury, or a 47 birth defect; they do not plan to remain in the study site catchment area for at least 6 months; the legal 48 guardian does not provide consent; or if a sibling was enrolled in the Toto Bora Trial on the same day of 49 discharge. Caregivers of potentially eligible children must be at least 18 years of age or classified as an 50 emancipated minor and be willing to participate in the Adult Contact Cohort if randomly selected. 51

52 Recruitment

53 Children will be recruited from the inpatient wards of Kisii Teaching & Referral and Homa Bay County 54 Hospitals where study staff will accompany hospital staff on ward rounds to identify children being discharged 55 each day. All discharged children, as determined by the onsite hospital clinicians, will be screened by study 56 staff during working hours. If the caregiver is interested in participating and indicates consent for screening, the 57 study staff will screen the child for eligibility, and if eligible, will obtain informed consent for study participation. 58

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Informed consent includes an explanation of the potential risks and benefits of the study and additional provision for use of participant data and samples for future studies, and will be conducted in the language of the respondent's choosing (English, Kiswahili, Kisii, or Luo). The parent or guardian (primary caregiver) must sign written informed consent (or provide a witnessed thumbprint if not literate) prior to enrollment.

Enrollment

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Children will be enrolled at the time of discharge by the clinical staff. At enrollment, primary caregivers will be interviewed to assess demographic information, medical history, and detailed contact information for the 10 child. Medical records will also be used to abstract information from the hospitalization (including presenting 11 diagnosis, medical management, length of stay, procedures performed, relevant medical history, physical 12 examination, and laboratory data). All enrolled participants will undergo a physical examination performed by 13 the study clinician, including measurement of height/length, weight, and mid-upper arm circumference (MUAC), 14 each of which will be measured three times. The height, weight, and MUAC of the caregiver will also be 15 collected. HIV status will be obtained from medical records or from performed testing if records are not 16 available. Detailed home location and contact information will be collected to enable patient tracing. 17

18 **Specimen collection** 19

Specimens will be collected at enrollment (prior to study medication administration, as well as at 3 and 20 6-month follow-up visits). All children will also be asked to provide a whole stool for enteric pathogen 21 identification and storage. Stool samples/swabs will be divided within one hour of collection for the following 22 23 1) placed in Cary-Blair for eventual bacterial culture (FecalSwab Cary-Blair Collection and purposes: Transport Systemt[™], Copan Diagnostics), 2) immediately tested for *Giardia* and *Cryptosporidium* using the 24 immunoassay (Quik Chek[™], Alere) and 3) placed in -80°C storage for future molecular determination of 25 26 pathogen or commensal flora and markers of gut function (whole stool for these purposes will be divided into 27 two separate vials). If a child cannot produce whole stool, two flocked rectal swabs (Pedatric FLOQswabTM, 28 Copan Diagnostics) will be collected and one placed in Cary-Blair and the other stored in -80°C for future 29 analyses.

30 One flocked swab (Nylon Flocked Dry Swabs, Copan Diagnostics) nasopharyngeal swab will also be 31 collected from all enrolled children at each time point, immediately placed in skim milk, tryptone, glucose, and 32 glycerine (STGG) media, and frozen (-80°C) within 1 hour of collection for future S. pneumoniae culture. [11, 33 12] Primary caregivers in the Contact Cohort will also be asked to provide a stool sample (or 2 rectal swabs) 34 and nasopharyngeal sample at each visit for testing and storage as described above. 35

Venous blood (up to 1 teaspoon [5mL]) will be collected from all enrolled children and caregivers 36 enrolled in the Contact Cohort at each at each time point into EDTA tube and separated for the following 37 purposes: 1) 0.5mL for immediate HIV-testing (if indicated according to Kenyan Ministry of Health guidelines), 38 2) 0.4mL for a thin malaria smear which will be stored at room temperature, 3) 0.4mL for a dried blood spot 39 whole blood -80°C storage and eventual sickle cell testing and 4) 2-4mL for plasma and buffy coat isolation 40 and -80°C storage. 41

42 Randomization 43

Block randomization (1:1) in random sized blocks of no more than 10, stratified by site, will be used. 44 Primary randomization will include allocation to the Contact Cohorts at a ratio of 1:5 (resulting in 150 per 45 treatment arm). Each subject will be assigned a Patient Identification Number (PID), and the randomization 46 47 code linking each PID to the allocated treatment will be maintained by the University of Washington Research 48 Pharmacy. Study participants, investigators, the study staff, hospital clinicians, and persons involved in data 49 management or analysis will remain blinded to the allocation group during all data collection phases of the 50 study. 51

52 Intervention

53 Caregivers of enrolled children will be provided a 5-day course of oral suspension formulation 54 azithromycin (Zithromax® from Pfizer, 10 mg/kg on day 1, followed by 5mg/kg/day on days 2-5) or identically 55 appearing and tasting placebo at discharge. Dosing ranges were determined such that a given child would 56 never be under-dosed and not over-dosed by more than 20% that the weight-specific intended dose (Table 1). 57 The day 1 dose will be split in half and the first half administered first by study clinician (to be observed by the 58

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caregiver) followed by the second half administered by the caregiver under careful observation of the study staff. Caregivers will be provided with visual instructions in the language of their choosing (English, Kiswahili, Luo, Kisii).

Automated daily text message reminders will be sent for the four days following discharge and caregivers asked to respond with whether or not the child took the daily dose. The response text message will be free of charge to caregivers and caregivers will be 10 reimbursed for each response at the final study visit. Caregivers are 11 also asked to record each administered dose on the bottle and to 12 return bottles at the 3 month follow-up visit. 13

14 **Follow-up Procedures** 15

All enrolled children and primary caregivers will be 16 scheduled to return to the health facility at 3 and 6 months following 17 enrollment collect clinical information and samples. to 18 Anthropometric measurements will be obtained from all children and 19 20 caregivers at both follow up visits (height/length, weight, MUAC) 21 and caregivers will be asked about any hospitalizations occurring 22 since the last time the child was seen by study staff. A flowchart of 23 follow-up and sample collection is shown in Figure 5. Caregivers will 24 be provided with 400KSH (approximately \$4USD) to cover the cost 25 of their round-trip transportation. 26

Transportation cost will be reimbursed at each follow-up visit. If the participant does not return at their scheduled time, study

Table 1. Azithromycin dosing chart by child weight Day 1 dose Day 2-5 dose Weight (kg) (mL) (mL) 2.0 0.25 x 2 0.25 2.1-2.4 0.30 x 2 0.30 2.5-2.8 0.35 x 2 0.35 0.40 2.9-3.2 0.40 x 2 0.45 3.3-3.6 0.45 x 2 0.50 3.7-4.0 0.50 x 2 4.1-4.8 0.60 x 2 0.6 0.7 4.9-5.6 0.70 x 2 0.85 5.7-6.8 0.85 x 2 6.9-8.0 1.0 1.0 x 2 1.2 8.1-9.6 1.2 x 2 9.7-11.2 1.4 1.4 x 2 1.6 11.3-13.6 1.6 x 2 2.0 13.7-16.0 2.0 x 2 2.4 16.1-19.2 2.4 x 2 2.9 19.3-23.2 2.9 x 2 3.2 23.3-25.0 3.2 x 2

28 staff will attempt to make contact with the primary caregiver via cell phone; if no telephone number is provided, 29 or if the participant cannot be reached, study staff will trace the child to the household within 2 weeks of the 30 scheduled follow-up time. 31

During scheduled follow-up visits, study staff will use a standardized questionnaire to ascertain history 32 of recent illness/morbidity, post-discharge medication use including antibiotic treatment, and current condition 33 of the child (any hospitalizations, admission and discharge date of any hospitalization, alive or dead, date of 34 death if applicable). If caregivers report a hospitalization, causes of admission, medication administration, and 35 length of stay will be ascertained from both caregivers and medical records, when available. 36

Caregivers will be encouraged, at enrollment and at each subsequent contact, to bring the child to the 37 38 study health facility at any time the child is sick. Study staff will triage children to the appropriate health facility 39 staff and will conduct a brief unscheduled visit questionnaire to ascertain adverse event information. If the 40 unscheduled visit leads to a hospitalization, this will trigger the completion of a hospital admission form.

41 If at any point during follow-up a child dies, a verbal autopsy using the shortened Population Health 42 Metrics Research Consortium questionnaire will be performed[13] If the death occurred in a hospital, data from 43 the hospital records, including cause of death, if available, will be abstracted. If a death certificate is available, 44 cause(s) and timing of death will be abstracted. 45

Final causes of re-hospitalization and death will be determined after data collection is complete by an 46 independent adjudication committee comprised of clinicians specializing in pediatrics and infectious disease. 47 Sources of cause of re-hospitalization (medical records and caregiver report) and causes of death (causes 48 automatically assigned from the verbal autopsy using SmartVA-Analyze [Tariff 2.0 Method][14], hospital 49 records, or death certificates) will be presented to the adjudication committee for final cause assignment. 50

51 Laboratory procedures and specimen collection and storage 52

Stool (rectal swabs), nasopharyngeal swabs and blood will be collected as described above and 53 undergo either immediate or future laboratory testing as described in Table 2. All biological samples will be 54 collected by staff trained in biosafety and Good Clinical Laboratory Practice (GCLP). Samples will be 55 processed in Kenya when technology is available at one of the following laboratories: Kenya Medical Research 56 Institute (KEMRI) (Wellcome Trust or Centre for Microbiology Research [CMR]) or at the University of Nairobi 57 (Microbiology Department). Metagenomic analyses and/or analyses that require technology not available in 58 59

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Kenya will be performed at the University of Washington. If stool culture results report Shigella or Salmonella infection, the study staff will contact the child's caregiver and encourage the caregiver to bring the child back for an evaluation and treatment if the child is symptomatic.

Table 2. Sample processing description.		Table 2.	Sample	processing	description.
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7 8	Specimen	Purpose	Tests Performed
	Collected	Desta del ID es l	
9	Stool/ flocked	Bacterial ID and	Fresh samples/rectal swabs will be cultured to identify Shigella, Salmonella, Campylobacter,
10	rectal swabs	storage for AST	and Escherichia coli using standard microbiologic methods and biochemically confirmed
11			using bioMérieux's API [®] strips. All <i>Shigella</i> , <i>Salmonella</i> , and <i>Campylobacter</i> isolates, as well as a random subset of <i>E.coli</i> isolates will undergo antimicrobial resistance testing using disc
12			diffusion for the following antibiotics: amoxicillin-clavulanic acid (augmentin), ampicillin,
13			azithromycin, chloramphenicol, ciprofloxacin, ceftriaxone, ceftazidime, cefoxitin, gentamicin,
14			imipenem, trimethoprim-sulfamethoxazole, ceftazidime/clavulanate (ESBL),
15			cefotaxime/clavulanate (ESBL). Categorizations of susceptible, intermediate, and resistant
16			will be determined using zone-size cut-offs outlined in CLSI interpretive standards (M100-S24
17			2014).
18		Parasite detection	Fresh stool and rectal swabs will be tested for Giardia and Cryptosporidium using the
19			immunoassay Giardia/ Cryptosporidium QUIK CHEK [™] .
20		Storage	Stool/ flocked swabs and colonies of E.coli, Shigella spp., Salmonella spp., and
20			Campylobacter spp. will be stored at -80°C.
	Nasopharyngeal	Bacterial isolation,	Streptococcus pneumoniae (S. pneumoniae) colonies will be isolated using standard
22	Swabs	storage, and	microbiologic or molecular diagnostic protocols and susceptibility testing performed using
23		resistance testing	standard microbiologic or molecular techniques. A random subset of <i>S. pneumoniae</i> isolates
24			will undergo antimicrobial resistance testing using disc diffusion for the following antibiotics: amoxicillin-clavulanic acid (Augmentin), ampicillin, azithromycin, chloramphenicol,
25			ciprofloxacin, ceftriaxone, ceftazidime, cefoxitin, gentamicin, imipenem, and trimethoprim-
26			sulfamethoxazole. Categorizations of susceptible, intermediate, and resistant will be
27			determined using zone-size cut-offs outlined in CLSI interpretive standards M100-S24 2014.
28		Storage	Back-up sample and S.pneumoniae colonies will be will be stored at -80°C.
29	Blood	HIV and malaria	HIV testing will be performed per Kenyan National Guidelines and malaria microscopy
30		testing	performed using standard methods.
31		Storage	Plasma and buffy coat will be stored at -80°C. Dried blood spots will be stored at room
32		Ŭ	temperature.
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Data Management and Confidentiality

34 Personal information about the participants, including medical records and data ascertained per 35 caregiver interview, will be securely stored in files in the study offices at the study sites. Only pre-designated 36 study staff will have access to the files. Data will be entered into an electronic database (Dacima® Electronic 37 Data Capture) regularly by study staff. Access to the electronic database will be secured using password 38 protected accounts for study staff. Data reports of screening, enrollment, and exclusion totals will be 39 disseminated to the study team on a weekly basis; reports including baseline demographic characteristics. 40 41 laboratory results, adherence data, and serious adverse event summaries will be distributed to study co-42 investigators and data monitors guarterly. Data will be regularly gueried to facilitate ongoing data cleaning. 43

44 **Data Analysis** 45

46 Primary endpoints:

47 The primary study endpoint is a combined outcome of mortality and hospital readmission, as re-hospitalization 48 is highly associated with risk of subsequent poor outcome. Re-hospitalizations that are a continuation of 49 management from the previous hospitalization (such as elective blood transfusion) or that occur during 50 enrollment procedures, due to a clinical deterioration post-discharge, will be excluded from the analysis. Loss 51 to follow-up will be defined as non-attendance at both follow-up visits despite up to one month of active tracing 52 and no clear evidence of death. 53

54 Secondary endpoints include: 55

1. Cause-specific re-hospitalizations assessed by questionnaire (maternal recall of diagnosis) at day 90 and 56 day 180 follow-up visits and medical record review (discharge diagnosis). In cases when both sources are 57

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available, information from the medical record will be considered superior. Separate analyses will be performed for each diagnosis: diarrhea, acute respiratory infection, malnutrition, or malaria.

- 2. <u>Enteric pathogen carriage</u>, operationalized as presence of a bacterial pathogen-Shigella species (spp.),
 Campylobacter spp., or Salmonella spp., or parasite- Giardia or Cryptosporidium in stool or rectal swabs assessed at day 90 and day 180 follow-up visits.
- 3. <u>Streptococcus pneumoniae (S. pneumoniae)</u> isolated from nasopharyngeal swab cultures at 90 and 180day follow-up visits.
- **4.** <u>Antimicrobial resistance</u>, specifically resistance to azithromycin, ampicillin, augmentin, trimethoprimsulfamethoxazole, in *E.coli* and *S. pneumoniae* isolates, and presence of ESBL in *E.coli* isolates, from day 90 and day 180 samples.

To compare rates of re-hospitalization and mortality in the 6 months following hospital discharge among 14 Kenyan children receiving 5-day azithromycin vs. placebo. Primary analyses will be modified intent-to-treat 15 (mITT) based on randomization allocation to the 5-day course of azithromycin versus placebo. Cumulative 16 incidence of death or first re-hospitalization will be compared between treatment groups using Cox proportional 17 hazards regression. Participants will be censored at the date of their first re-hospitalization, or at the date of 18 death. Median time to hospitalization-free survival will be compared between randomization groups using 19 20 Kaplan-Meier (K-M) survival analysis and associated log-rank test. If the baseline assessment of 21 randomization reveals an imbalance in characteristics between the treatment groups, we will evaluate these 22 variables as potential confounders in a sub-analysis secondary to the mITT. Potential baseline confounders will 23 be added stepwise in a multivariable Cox model and maintained in the model if adjustment changes the hazard 24 ratio by more than 10%. In per protocol analyses also secondary to the mITT, we will compare treatment 25 effects in groups defined by self-reported adherence to the 5-day course of azithromycin (5 doses vs. < 5 26 doses; ≥3 doses vs. <3 doses; >1 dose vs. 1 dose only). In addition, we will conduct Cox regression and K-M 27 survival analyses for time to mortality and time to re-hospitalization as separate endpoints to understand 28 intervention effects on these outcomes individually. The assumption of proportional hazards will be checked in 29 all models using graphical methods including plotting a $\ln(-\ln(S(t)))$ plot for each treatment group and assessing 30 the parallelism of the two lines and by plotting Schoenfeld residuals over time. If there is substantial missing 31 covariate data, multiple imputation using the Markov chain Monte Carlo (MCMC) method will be used to impute 32 covariate information. Missing outcome data (death or re-hospitalization) will not be imputed, but participants 33 will be censored at the last follow-up visit therefore contributing some person-time to the analysis. 34

To evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality, by 35 comparing reasons for re-hospitalization and change in prevalence of pathogen carriage between the 36 randomization arms. To evaluate the association between azithromycin and the rates of cause-specific re-37 38 hospitalizations (hospitalization due to diarrhea, acute respiratory infection, malnutrition, or malaria) we will use 39 Anderson-Gill proportional hazards modeling with previous re-hospitalizations included as time-dependent 40 covariates in the model to capture the dependent structure of recurrence times. Because we will not have 41 granularity in the time points other than 90-days and 6-months for assessment of pathogen carriage, we will 42 compare the prevalence of a bacterial and parasitic pathogens (Shigella, Salmonella, Campylobacter, 43 Cryptosporidium, Giardia) at 90-days and 6 months by randomization arm using generalized estimating 44 equations (GEE) with a Poisson link, exchangeable correlation structure, and will adjust for baseline presence 45 of a bacterial pathogen. To determine whether an observed association between the intervention and pathogen 46 carriage wanes over time, we will test the hypothesis that the prevalence ratios comparing carriage in 47 intervention arms are the same at the two follow-up time points using a chi-squared test. 48

To determine whether empiric administration of azithromycin at hospital discharge increases risk of 49 antimicrobial resistance in commensal E. coli and pneumococcal isolates from treated children and their 50 household contacts. Among children and adult household contacts in whom commensal E. coli and/or S. 51 pneumoniae are isolated, we will compare the proportion of isolates resistant to azithromycin, ampicillin, 52 augmentin, and trimethoprim-sulfamethoxazole, between randomization arms and Contact Cohorts for each 53 arm, at 90-days and 6 months using GEE with a Poisson link and exchangeable correlation structure. A chi-54 squared test will be used to determine whether the association between intervention arm and antimicrobial 55 resistance wanes over time. Because the likelihood of having a bacterial pathogen isolated may depend on 56 baseline factors, including intervention arm, we will conduct secondary analyses utilizing propensity scores to 57

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account for the potential differential likelihood of having antimicrobial susceptibility testing performed, which will allow us to make inference to the entire study population and their contacts.

<u>To identify correlates and intermediate markers of post-discharge mortality and hospital-readmission</u> <u>among hospitalized children.</u> Enrollment hospital admission diagnosis, indicators of malnutrition, age, HIVexposure and HIV-infection status, sickle cell anemia, and randomization arm will be assessed in a multivariable Cox regression model to identify correlates of the primary endpoint of death and/or hospitalreadmission independent of the treatment effect. In addition, Cox regression models will also be built for correlates of mortality and correlates of re-admission individually to understand distinct cofactors for each of these outcomes.

11 To determine the cost-effectiveness of post-discharge administration of a 5-day course of azithromycin 12 in settings of varying antibiotic use, re-hospitalization rates, and mortality rates. Costs analysis: We will assess 13 the costs of all supplies, services and equipment necessary to implement the intervention (direct medical 14 costs). The perspective will be that of the healthcare provider, i.e. Kenya's Ministry of Health. Using WHO 15 guidelines and its ingredients approach, we will quantify the resources and associated unit costs required to 16 deliver a 5-day course of azithromycin, organized in standard expenditure categories: personnel (salaries), 17 supplies including drugs, equipment, services, space and overhead. We will also measure the costs of severe 18 child hospitalizations, the costs for the different types of personnel employed (e.g. nurses/doctors) and the time 19 20 demanded from them for conducting the intervention.[15] When data are missing, they will be complemented 21 by data extracted from the literature and other available sources. Full incremental costs will be derived, with 22 estimation of the potential healthcare cost-offset realized in avoiding severe hospitalizations. Costs will be 23 measured in local currency (Kenyan Shilling) and converted into US\$. Our main metric will be cost per child 24 treated. Cost-effectiveness analysis (CEA): we will develop a CEA mathematical model, and estimate 25 incremental costs and cost-effectiveness for implementation of the intervention. The model will include two 26 components: costs (described immediately above) and health benefits. The study will provide clinical outcomes 27 (mortality/morbidity) over a 6-month follow-up period. Subsequently, deaths averted, life-years saved and 28 disability-adjusted life years (DALYs) averted by the intervention will be estimated. We will estimate: a) 29 incremental costs and b) incremental cost-effectiveness of the intervention vs. status quo. Incremental costs 30 are the net sum of the costs to implement the intervention compared with status quo, and the costs averted 31 due to the decrease in severe child hospitalizations. Incremental cost-effectiveness ratios (ICERs) will be 32 estimated as cost per death averted, cost per life-year saved, and cost per DALY averted. We will use recent 33 estimates of disability weights for estimation of DALYs.[16, 17] Short-term (over study follow-up i.e. 6 months) 34 and longer-term time horizons (extrapolated to 1, 5, and 10 years) will be used. DALYs and costs will be 35 discounted at 3% per year, consistent with CEA guidelines (undiscounted results will also be presented). 36 Sources of uncertainty in the results will be explored in univariate and probabilistic sensitivity analysis. [18, 19] 37 38 Finally, we will compare our findings to CEA estimates for other health interventions in sub-Saharan Africa.[20, 39 21] 40

41 Data and Safety Monitoring

A Data Safety and Monitoring Committee (DSMC) was established at study initiation to monitor severe 42 43 adverse events (SAEs) and to evaluate the statistical analysis plan and associated stopping rules. The DSMC 44 includes expertise in clinical trials, statistics, child mortality assessment, ethics, and pediatric care in resource 45 limited settings. Adverse events will be monitored by the DSMC. Monthly adverse event summaries will be sent 46 to the DSMC safety officer and individual-child SAE forms, which include detailed medication history to 47 evaluate possible drug interactions, will be sent to the safety officer per request. Each SAE will be assigned the 48 plausibility of relatedness to study drug by study PIs. The data will not be presented by intervention group 49 unless requested by DSMB safety officer. These reports will be descriptive (no statistical analyses). The DSMC 50 will make recommendations regarding any imbalances in safety outcomes.

A single interim analysis for re-hospitalization-free survival will be prepared by the study statistician using O'Brien-Fleming boundaries for <u>benefit</u> and <u>harm</u> 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 and harm. The DSMC will review this analysis and make a determination about study continuation. 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

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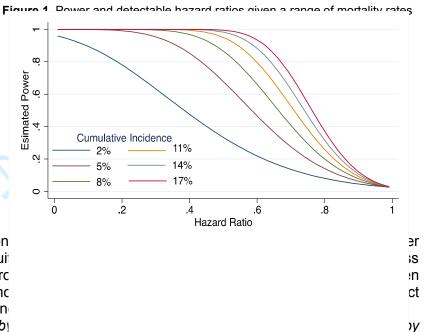
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DSMC decides to continue the trial after the interim analysis, an alpha of 0.045 will be used as the statistical significance boundary at the final analysis.

Statistical Power

6 To compare rates of re-hospitalization and mortality in the 6 months following hospital discharge among 7 Kenyan children receiving 5-day azithromycin vs. placebo. The total sample size required was calculated for 8 the primary endpoint of time to death or hospital re-admission within the 6 month post-discharge period. 9 assuming an alpha level of 0.05, power of 0.80, and a ratio of treatment to placebo random assignment of 1:1. 10 In SSA, it is estimated that 2-15% of children aged less than 5 years died within 6 months of hospital discharge 11 and 15.5% of children who survived discharge from the district hospital were re-admitted with the same 12 diagnosis within 6-months[2, 4, 22] Assuming that an additional 5-10% of children are re-admitted for other 13 conditions, we expect that re-hospitalizations will occur in 20.5 to 30.5% of children enrolled in the study. 14

Combined with our expected fatality rate (2-15 15%), we expect the cumulative incidence 16 of the combined endpoint to range from 22.5 17 to 45.5%.[22] Based on a previous trial of 18 mass drug administration of a single dose of 19 azithromycin in which a single dose of the 20 antibiotic was associated with a 49% 21 reduction in risk of death, we calculated 22 23 sample sizes using estimates of reduction in 24 risk ranging from 30-50% with the cumulative 25 incidence range of 22.5 to 45.5%, and found 26 the sample size required ranged from 90 to 27 550 children per treatment arm.[5] Using the 28 most conservative estimates of a hazard ratio 29 of 0.70 and 22.5% prevalence of re-admission 30 arm) to achieve adequate power. We will recruit 31 to follow-up, resulting in a total planned enrc 32 considering mortality alone, and estimated mc 33 hazard ratios ≤ 0.5 for mortality rates of $\geq 8\%$ and 34 To evaluate possible mechanism(s) by



35 comparing reasons for re-hospitalization and prevalence of pathogen carriage between the randomization 36 arms. We calculated the minimum detectable association between treatment arm and cause-specific re-37 hospitalizations (hospitalization due to diarrhea, acute respiratory infection, malnutrition, or malaria vs. any 38 other) among enrolled children, assuming an alpha level of 0.05, power of 0.80, and a ratio of treatment to 39 placebo of 1:1. Based on data from Kenya, re-hospitalization rates due to specific causes ranged from 40 approximately 0.5% to 5.7% in the 6 month post-discharge period.[4] By not conditioning on the child having 41 the same diagnosis as the initial hospitalization, we expect the cumulative incidence of cause-specific re-42 43 hospitalizations to range from 2.5% to 10%. With this range of outcome rates, we will be able to detect hazard 44 ratios of 0.48 to 0.70 for the effect of azithromycin on specific severe morbidities.

We expect 56% of children in the placebo group to have *S. pneumoniae* isolated from nasopharyngeal swabs, providing \geq 80% power to detect a prevalence ratio of 0.85 (or 1.15) between the two treatment arms at each time point.[23-25] Based on prevalences of *Shigella*, *Salmonella*, *Campylobacter*, *Cryptosporidium*, *Giardia* among asymptomatic children in Western Kenya, we expect 10% of children in the placebo group to have a bacterial pathogen isolated at each time point, resulting in \geq 80% power to detect differences in enteric pathogen prevalences of 0.67 (1.49) at each time point.[26]

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. We will select a random selection of 400 E. coli and 400 S. pneumoniae isolates (200 per arm) for β -lactam and macrolide resistance testing at each timepoint. We will also store all S. pneumoniae, E. coli isolates and other isolated bacteria from stool for potential future testing in the event that resistance prevalence is lower than expected. As shown in Table 3, we will have > 80% power to detect prevalence ratios

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> 1.1, with an ability to detect the smallest effect sizes when the prevalence of resistance in the placebo group is highest.

5 We will enroll 300 adults in the Contact Cohort for E. 6 coli and S. pneumoniae isolation. We expect E.coli to 7 be isolated from all adults and S.pnuemoniae isolated 8 from between 5-55%.[23, 27, 28] Assuming an alpha of 9 .05, a 1:1 ratio of testable isolates, and a prevalence of 10 resistance of 50% in the placebo arm, we will have 80% 11 power to detect a 1.4-fold higher prevalence to 1.9-fold 12 higher resistance prevalence in the contacts of 13 azithromycin-treated children. 14

<u>To identify correlates and intermediate markers</u> of post-discharge mortality and hospital-readmission

16of post-discharge mortality and hospital-readmission| 80 | >99 | >99 | >99 | >99 | >99 | >99 | 99 | 6417among hospitalized Kenyan children.Conservatively estimating a 20% loss-to-follow-up rate in the RCT and a18cumulative incidence of death or re-hospitalization of 22.5%, we will have >80% power to detect hazard ratios19≥1.3 between correlates and the outcome with exposure prevalences of ≥20% or more and hazard ratios ≥1.520for exposure prevalences <20%.</td>

22 Study timeline

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The trial began on June 28, 2016 and participant recruitment and follow-up will continue over a 36month period, with anticipated final follow-up visit(s) occurring in June 2019. Primary analyses will be complete by February 2020.

27 **Potential Challenges and Limitations**

28 In order to ensure adequate power to detect a discernable clinically relevant difference between study 29 groups in the primary outcome, we have combined hospital readmission with death. Preliminary studies 30 suggest that sufficient numbers of children will reach this combined outcome. However, we have incorporated 31 an interim analysis by the DSMB to review the accrued data and an adapted sample size could be considered 32 if the combined event frequency is less than predicted. It is possible that since most children receive antibiotics 33 during hospitalization, the benefit anticipated with the use of azithromycin based on previous trials of mass 34 drug administration will not be observed. However, most hospitalized children are treated with penicillins, 35 cephalosporins, gentamicin, or cotrimoxazole while in hospital and the broad spectrum of activity (including 36 malaria prevention) and long half-life of azithromycin suggest that there may be additive treatment and/or 37 prophylactic benefit. Interim analysis will allow us to determine whether children receiving specific agents 38 during inpatient treatment are less likely to benefit and will allow us to adapt our study design, sample size, or 39 approach if necessary. After discharge, it is difficult to ensure adherence with the full 5-day treatment course. 40 We will measure adherence using three different measures (text message responses, bottle check boxes, and 41 caregiver-report at follow-up visits). In addition, the mortality benefit of azithromycin observed in Ethiopia was 42 from a single dose and in this study the first dose will be directly observed.[5] While relying on caregiver report 43 of mortality and morbidity may lead to bias due to outcome misclassification, this misclassification should not 44 differ between randomization arms and therefore will be non-differential. Further hospital records will be used 45 when available to determine diagnoses. Finally, resistance prevalence may be lower than predicted, limiting 46 47 power to detect clinically relevant differences in resistance prevalence between the intervention arms. We will 48 store all isolates in the event that a greater number of isolates are needed for antimicrobial resistance testing. 49

⁵⁰ **Regulatory Authorities**

This study has received IRB approval by the University of Washington Human Subjects Division (HSD), KEMRI Scientific and Ethics Review Unit (SERU), and the Kenya Pharmacy and Poisons Board. The clinical trial is also registered with clinicaltrials.gov (NCT02414399). Any modifications to the study protocol or consent materials will be submitted for approval all regulatory authorities before implementation. Westat[®] will provide external clinical, pharmacy, and laboratory monitoring.

Dissemination

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Table 3. Power (%) to detect prevalence ratios of macrolide and β -lactamase resistance in 200 *E.coli* and 200 *S. pneumoniae* isolates per treatment group

	Resistance prevalence (%) in placebo group						
Resistance Prevalence (%)	10	20	30	40	50	60	70
10							
20	80						
30	>99	64					
40	>99	99	55				
50	>99	>99	98	48			
60	>99	>99	>99	>99	52		
70	>99	>99	>99	>99	98	55	
80	>99	>99	>99	>99	>99	99	64

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Results of this study will be disseminated by publication in a peer-reviewed scientific journal, presented at relevant academic conferences, and amongst participating partners and health facilities in Kenya.

⁵ Author's contributions

JLW, PBP, GJS, BAR, BOS, and RN conceived of this trial and developed of this study protocol. JLW and BOS are study co-Principal Investigators and PBP is the Project Director; BAR oversaw the statistical analyses plans; JBB developed the CEA plan; KDT developed procedures for ascertaining and reporting SAEs; CJM developed procedures related to blood specimen procedures and drug adherence measurement. GJS, CJM, RN, and PBP provide scientific expertise. RLB and MA are involved in collection and management of the data. MA and PBP coordinate and oversee implementation of all clinical study procedures and SK with assistance from DR oversees all laboratory procedures. All authors contributed to the development of this manuscript and/or study procedures, and to reading and approving the final version for publication.

15Funding statement

This work was funded by the National Institutes of Health Eunice Kennedy Shriver National Institute of Child Health & Human Development, grant number R01 HD079695.

20 Acknowledgements

Pfizer donated the Zithromax® to be used in this clinical trial, and Copan Diagnostics donated all rectal swabs and Cary-Blair media. Investigators from KEMRI-Wellcome Trust Kilifi, Jay Berkley, Anthony Scott, Joseph Waichungo, Angela Karani, Donald Akech provided microbiology expertise trainings in nasopharyngeal swab collection, STGG media preparation, and overall microbiologic quality assurance and control. Alex Awuor and Caleb Okonji, with the support of Richard Omore, provided training in anthropometric measurement. We are extremely thankful to Dr. Phillip Walson who developed azithromycin dosing regimens. Hannah Atlas and Stephanie Belanger contributed to the standard operating procedure and case report form development and implementation. Gillian Levine played an invaluable role in the proposal development.

31 Competing interests statement

None of the authors or study co-investigators have any competing interests to declare.

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ltem No	Description	Addressed or page number
Administrative inf	ormation		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	
Roles and	5a	Names, affiliations, and roles of protocol contributors	
responsibilities	5b	Name and contact information for the trial sponsor	
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

1 2	Introduction			
3 4 5	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	
6 7		6b	Explanation for choice of comparators	
8 9	Objectives	7	Specific objectives or hypotheses	
10 11 12 13	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	
14 15	Methods: Participa	nts, inte	erventions, and outcomes	
16 17 18	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	
19 20 21	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	
22 23 24 25	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	
26 27 28		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	
29 30 31		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	
32 33		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	
34 35 36 37 38 39	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
40 41 42	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	
43 44 45 46			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	2

1 2 3	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	
4 5	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	
6 7	Methods: Assignm	ent of i	nterventions (for controlled trials)	
8 9	Allocation:			
10 11 12 13 14 15	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	
16 17 18 19	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered,	
20 21 22 23	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	
24 25 26	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	
27 28 29		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
30 31	Methods: Data coll	ection,	management, and analysis	
32 33	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related	
34 35 36 37	methods		processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
38 39 40 41		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be	
42 43 44 45			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	3

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1 2 3 4	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	
5 6 7	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	
8 9		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
10 11 12 13		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	
14 15	Methods: Monitorir	ng		
16 17 18 19 20 21	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
22 23 24		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
25 26 27	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
28 29 30	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
31 32	Ethics and dissemi	nation		
33 34 35 36	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	
37 38 39 40 41	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	
42 43 44 45 46			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	4

1 2 3	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and	_
4 5 6		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary	-
7 8 9	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained	-
10 11 12	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	-
13 14 15	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that	-
16 17 18 19	Ancillary and post- trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trialparticipation	-
20 21 22 23	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	_
24 25		31b	Authorship eligibility guidelines and any intended use of professional writers	_
26 27 28		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_
29 30	Appendices			
31 32 33	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-
34 35 36	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_
37 38 39 40 41	Amendments to the p	protocol	that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the item should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons - <u>NoDerivs 3.0 Unported</u> " license.	S.
42 43 44 45			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	5

BMJ Open

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)

Journal:	BMJ Open
Manuscript ID	bmjopen-2017-019170.R1
Article Type:	Protocol
Date Submitted by the Author:	09-Oct-2017
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Primary Subject Heading :	Global health
Secondary Subject Heading:	Paediatrics, Infectious diseases
Keywords:	Child mortality, Post-discharge interventions, Toto Bora Trial, Targeted empiric antibiotic therapy

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Title: 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) Running head: AZM to prevent post-discharge morbidity and mortality Pavlinac PB¹, Singa BO^{7,8}, John-Stewart GC¹⁻⁴, Richardson BA^{1,5}, Brander RL², Authors: McGrath CJ¹, Tickell KD^{1,8}, Amondi M⁷, Rwigi D⁷, Babigumira JB^{1,6}, Kariuki S⁷, Nduati R⁹, Walson JL^{1-4,8} Departments of ¹Global Health, ²Epidemiology, ³Pediatrics, ⁴Allergy and Affiliations: Infectious Disease, ⁵Biostatistics, ⁶Pharmacy, University of Washington, Seattle, WA, USA; ⁷Kenya Medical Research Institute, Nairobi, Kenya; ⁸The Childhood Acute Illness & Nutrition Network, Nairobi, Kenya; ⁹ University of Nairobi, Nairobi, Kenva Patricia Pavlinac, 325 9th Ave, Box 359931, Seattle, WA 98104 (ppav@uw.edu) **Correspondence:** Sponsored by: This trial is sponsored by the National Institutes of Health Eunice Kennedy Shriver National Institute of Child Health & Human Development (R01 HD079695) and drug donation by Pfizer IIR #WI201906. JLW is supported by the Childhood Acute Illness and Nutrition Network (CHAIN, OPP1131320). GJS is supported by a National Institute of Health mentoring award (grant number K24-HD054314) and PBP and GJS are supported by the International Core of the University of Washington Center for AIDS Research (CFAR; Seattle, WA, USA), an NIH funded program (P30 Al027757). Word count: 5,315 (excluding tables and figures)

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ABSTRACT

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Introduction: Child mortality due to infectious diseases remains unacceptably high in much of sub-Saharan Africa. Children who are hospitalized represent an accessible population at particularly high-risk of death, both during and following hospitalization. Hospital discharge may be a critical time point at which targeted use of antibiotics could reduce morbidity and mortality in high-risk children.

10 Methods and analysis: In this randomized, double-blind, placebo-controlled trial (Toto Bora), 1400 children 11 aged 1 to 59 months discharged from 2 hospitals in western Kenya, in Kisii and Homa Bay, will be randomized 12 13 to either a 5-day course of azithromycin or placebo to determine whether a short-course of azithromycin 14 reduces rates of re-hospitalization and/or death in the subsequent 6-month period. The primary analysis will be 15 modified intention-to-treat and will compare the rates of re-hospitalization or death in children treated with 16 azithromycin or placebo using Cox proportional hazard regression. The trial will also evaluate the effect of a 17 short course of azithromycin on enteric and nasopharyngeal infections and cause-specific morbidities. We will 18 also identify risk factors for post-discharge morbidity and mortality and subpopulations most likely to benefit 19 from post-discharge antibiotic use. Antibiotic resistance in Escherichia coli and Streptococcus pneumoniae 20 among enrolled children and their primary caregivers will also be assessed and cost-effectiveness analyses 21 performed to inform policy decisions. 22

Ethics and dissemination: Study procedures were reviewed and approved by the institutional review boards of the Kenya Medical Research Institute, the University of Washington, and the Kenyan Pharmacy and Poisons Board. The study is being externally monitored and a data safety and monitoring committee has been assembled to monitor patient safety and to evaluate the efficacy of the intervention. The results of this trial will be published in peer-reviewed scientific journals and presented at relevant academic conferences and to key stakeholders.

Trial registration number: NCT02414399

Key words: child mortality, Toto Bora, targeted empiric antibiotic therapy, post-discharge interventions

STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- Randomized, placebo-controlled, double-blinded design and modified intention-to-treat analysis will
 ensure unbiased treatment effect measure
- Comprehensive data are collected, including biological specimens for all child participants and a subset of adult caregivers, for analyses of mechanisms of post-discharge morbidity and mortality, subsets of children most likely to benefit from the antibiotic, as well as assessments of antibiotic resistance and cost-effectiveness
- Results will likely be generalizable due to the limited exclusion criteria, large sample size, and multiple study sites
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Limitations

- Causes of death and re-hospitalization may be misclassified due to limited availability of medical records and recall bias in caregiver report
- The primary endpoint of this study is a combined outcome of re-hospitalization and death, which, while improving statistical power, may present challenges for interpretation
- Children in both intervention arms may receive other antibiotics over the course of follow-up

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2 BACKGROUND 3

An estimated 3.5 million deaths occur annually in children less than 5 years of age in sub-Saharan Africa (SSA), approximately 70% of which are due to infectious causes. Children who were recently hospitalized have mortality rates 6 to 8-fold higher than similarly-aged children from the same community.¹⁻³ Post-discharge mortality rates as high as 15% have been documented in the 12 months following discharge, with mortality risk remaining elevated up to two years post-discharge.⁴⁻⁸ Children who are very young, malnourished, or HIV-infected are at particularly high risk of post-discharge mortality within the 3 months following discharge. ^{1-4 6-8} Children being discharged from hospital in SSA may represent an accessible high-10 risk population in which to target interventions to reduce mortality and morbidity. 11

Targeted antibiotic interventions, including the use of cotrimoxazole among HIV-infected children and 12 13 the use of amoxicillin or cefdinir among children with severe acute malnutrition (SAM), have been shown to 14 reduce morbidity and mortality in these specific vulnerable populations.⁹⁻¹² Other trials of targeted antibiotic use 15 in vulnerable populations, including cotrimoxazole in HIV-exposed uninfected (HEU) children and in children 16 with SAM, have failed to demonstrate a mortality benefit.^{13 14} In contrast, non-targeted mass drug 17 administration of a single dose of azithromycin halved mortality rates among Ethiopian children living in 18 communities randomized to receive the antibiotic.^{15 16} Concerns about the potential emergence of antibiotic 19 resistance, possible toxicity, and feasibility of delivery are barriers to the non-targeted antibiotic distribution 20 strategies. 21

A short-course of azithromycin given to children with recent severe illness being discharged from 22 hospital may optimize benefit while reducing both individual and population level risks. Azithromycin may 23 24 reduce post-discharge morbidity and mortality through infection related mechanisms such as treating 25 undiagnosed, incompletely treated or nosocomial infections or by protecting against new or recrudescent 26 infections that occur during recovery. Azithromycin may also act through non antimicrobial pathways such as 27 by anti-inflammatory and/or immunomodulatory effects. 28

OBJECTIVE

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30 The primary objective of this double-blind, placebo-controlled, randomized controlled trial (RCT) is to 31 determine whether a 5-day course of azithromycin in children age 1 to 59 months discharged from hospital in 32 western Kenya reduces rates of re-hospitalizations and/or death in the subsequent six months. The secondary 33 objectives are (1) to evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality. 34 by comparing reasons for re-hospitalization and prevalence of enteric and nasopharyngeal infections between 35 the randomization arms; (2) to determine whether empiric administration of azithromycin at hospital discharge 36 increases risk of antibiotic resistance in commensal Escherichia coli (E. coli) and Streptococcus pneumoniae 37 38 (S. pneumoniae) isolates from treated children and their primary caregiver; (3) to identify correlates and 39 intermediate markers of post-discharge mortality and hospital readmission; (4) to determine the cost-40 effectiveness of post-discharge administration of a 5-day course of azithromycin in settings of varying antibiotic 41 use, re-hospitalization rates, and mortality rates; and (5) to create a repository of stool, nasopharyngeal, and 42 blood specimens from highly characterized, recently discharged children, half of whom are treated with 43 azithromycin, to be used to address future research questions. 44

METHODS

Reporting of this study protocol has been verified in accordance with the SPIRIT (Standard Protocol Items for Randomized Trials) recommendations.

Eligibility

Children age 1 to 59 months old weighing at least 2 kg who have been hospitalized, and subsequently 51 52 discharged, will be eligible for inclusion. Caregivers of potentially eligible children must be at least 18 years of 53 age or classified as an emancipated minor and be willing to participate in the Contact Cohort if randomly 54 selected. Children will be excluded if: azithromycin is contraindicated (children taking or prescribed other 55 macrolide antibiotics, such as erythromycin or clarithromycin, or the protease inhibitor, lopinavir); they were 56 admitted to hospital for a trauma, injury, or a birth defect; they do not plan to remain in the study site catchment 57 area for at least 6 months; the legal guardian does not provide consent; or if a sibling was enrolled in the trial 58 on the same day of discharge. 59

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Recruitment

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Children will be recruited from the inpatient wards of Kisii Teaching & Referral and Homa Bay County Hospitals where study staff will accompany hospital staff on ward rounds to identify children being discharged each day. All discharged children, as determined by the onsite hospital clinicians, will be screened by study staff during working hours. If the caregiver is interested in participating and indicates consent for screening, the study staff will screen the child for eligibility, and if eligible, will obtain informed consent for study participation. Informed consent includes an explanation of the potential risks and benefits of the study and additional provision for use of participant data and samples for future studies, and will be conducted in the language of the respondent's choosing (English, Kiswahili, Kisii, or Luo). The parent or guardian (primary caregiver) must sign written informed consent (or provide a witnessed thumbprint if not literate) prior to enrollment.

Enrollment

Children will be enrolled at the time of discharge by the clinical staff. At enrollment, primary caregivers will be interviewed to assess demographic information, medical history, and detailed contact information for the child (Table 1). Medical records will also be used to abstract information from the hospitalization (including presenting diagnosis, medical management, length of stay, procedures performed, relevant medical history, physical examination, and laboratory data). All enrolled participants will undergo a physical examination performed by the study clinician, including measurement of height (in children ≥ 24 months), length (in children <24 months), weight, and mid-upper arm circumference (MUAC), each of which will be measured three times. HIV status will be obtained from medical records or from performed testing if records are not available. Detailed home location and contact information will be collected to enable patient tracing.

	Enrollment visit (hospital discharge)	3 month follow up visit		6 month follow up visit		Unscheduled visits
•	Questionnaire of sociodemographic, clinical history, treatments prescribed in hospital and at discharge, hospitalization costs, dietary factors, household factors, and environmental exposures Physical exam Anthropometry Abstraction of medical records (if re-hospitalized) Verbal autopsy (or abstracted medical records) Heel/finger prick (HIV and malaria) Stool collection (<i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> , <i>Escherichia coli</i> , <i>Cryptosporidium</i> , and Giardia) Nasopharyngeal swab collection (<i>Streptococcus</i> <i>pneumoniae</i>)	 Questionnaire of study dru administration, and reporte illnesses, hospitalization costs, change in clinical history, and treatments sin last visit Physical exam Anthropometry Abstraction of medical records (if re-hospitalized) Verbal autopsy (or abstract medical records) Heel/finger prick (HIV and malaria) Stool collection (<i>Shigella</i>, <i>Salmonella</i>, <i>Campylobacte</i> <i>Escherichia coli</i>, <i>Cryptosporidium</i>, and Giardia) Nasopharyngeal swab collection (<i>Streptococcus</i> <i>pneumoniae</i>) 	ed	Questionnaire of reported illnesses, hospitalization costs, change in clinical history, and treatments since last visit Physical exam Anthropometry Abstraction of medical records (if re-hospitalized) Verbal autopsy (or abstracted medical records) Heel/finger prick (HIV and malaria, sickle-cell) Stool collection (<i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> , <i>Escherichia coli</i> , <i>Cryptosporidium</i> , and Giardia) Nasopharyngeal swab collection (<i>Streptococcus</i> <i>pneumoniae</i>)	•	Questionnaire of reported illnesses since last schedule visit, change in clinical history, and treatments since la visit Abstraction of medical records (if re-hospitalized) Verbal autopsy (or abstracted medical records)

Specimen collection

52 Specimens will be collected at enrollment (prior to study medication administration, as well as at 3 and 53 6-month follow-up visits). All children will also be asked to provide a whole stool for enteric pathogen 54 identification and storage. Stool samples will be divided within one hour of collection for the following purposes: 55 1) placed in Cary-Blair for eventual bacterial culture (FecalSwab Cary-Blair Collection and Transport 56 Systemt[™], Copan Diagnostics), 2) immediately tested for *Giardia* and *Cryptosporidium* using the 57 immunoassay (Quik Chek[™], Alere) and 3) placed in -80°C storage for future molecular determination of 58 pathogen or commensal flora and markers of gut function (whole stool for these purposes will be divided into 59 two separate vials). If a child cannot produce whole stool, two flocked rectal swabs (Pedatric FLOQswab[™], 60

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Copan Diagnostics) will be collected and one placed in Cary-Blair and the other stored in -80°C for future analyses.

One flocked dry nasopharyngeal swab (Copan Diagnostics) will also be collected from all enrolled children at each time point, immediately placed in skim milk, tryptone, glucose, and glycerine (STGG) media, and frozen (-80°C) within 1 hour of collection for future S. pnuemoniae culture.^{17 18} Primary caregivers in the Contact Cohort will also be asked to provide a stool sample (or 2 rectal swabs) and nasopharyngeal sample at each visit for testing and storage as described above.

Venous blood (up to 1 teaspoon [5mL]) will be collected from all enrolled children and caregivers 10 enrolled in the Contact Cohort at each at each time point into EDTA tube and separated for the following 11 purposes: 1) 0.5mL for immediate HIV-testing (if indicated according to Kenyan Ministry of Health guidelines), 12 13 2) 0.4mL for a thin malaria smear which will be stored at room temperature, 3) 0.4mL for a dried blood spot, 14 and 4) 2-4mL for plasma and buffy coat isolation and -80°C storage. 15

16 Randomization

17 Block randomization (1:1) in random sized blocks of no more than 10, stratified by site, will be used. 18 Primary randomization will include allocation to the Contact Cohorts at a ratio of 1:5 (resulting in 150 per 19 treatment arm). Each subject will be assigned a Patient Identification Number (PID), and the randomization 20 code linking each PID to the allocated treatment will be generated by a designated statistician and maintained 21 by the University of Washington Research Pharmacy. Study participants, investigators (other than the 22 statistician), the study staff, hospital clinicians, and persons involved in data management or analysis will 23 remain blinded to the allocation group during all data collection phases of the study. 24

Intervention

26 Caregivers of enrolled children will be provided a 5-day course of oral suspension formulation 27 azithromycin (Zithromax® from Pfizer, 10 mg/kg on day 1, followed by 5mg/kg/day on days 2-5) or identically 28 appearing and tasting placebo at discharge. Identically appearing bottles will be pre-labelled with the PID. 29 Dosing ranges were determined such that a given child would never be under-dosed or over-dosed by more 30 31 than 20% of the weight-specific intended dose (Table 2). The day 1 dose will be split in half and the first half 32

administered by the study clinician (to be observed by the 33 caregiver) followed by the second half administered by the 34 caregiver under careful observation of the study staff. Caregivers 35 will be provided with visual instructions in the language of their 36 choosing (English, Kiswahili, Luo, Kisii). 37

Automated daily text message drug administration 38 reminders will be sent for the four days following discharge and 39 caregivers asked to respond with whether or not the child took 40 the daily dose. The response text message will be free of charge 41 42 to caregivers and caregivers will be reimbursed for each response at the final study visit. Caregivers are also asked to 43 44 record each administered dose on the bottle and to return bottles 45 at the 3 month follow-up visit. 46

47 Follow-up Procedures

48 All enrolled children and primary caregivers will be 49 scheduled to return to the health facility at 3 and 6 months 50 following enrollment to collect clinical information and samples. 51 Anthropometric measurements will be obtained from all children 52 and caregivers at both follow up visits (height/length, weight, 53 MUAC) and caregivers will be asked about any hospitalizations 54 occurring since the last time the child was seen by study staff. 55 Caregivers will be provided with 400KSH (approximately \$4USD) 56 57 to cover the cost of their round-trip transportation.

58 Transportation cost will be reimbursed at each follow-up 59 visit. If the participant does not return at their scheduled time, 60

Weight (kg)	Day 1 dose (mL)	t by child weight Day 2-5 dose (mL)	
2.0	0.25 x 2	0.25	
2.1-2.4	0.30 x 2	0.30	
2.5-2.8	0.35 x 2	0.35	
2.9-3.2	0.40 x 2	0.40	
3.3-3.6	0.45 x 2	0.45	
3.7-4.0	🔷 0.50 x 2	0.50	
4.1-4.8	0.60 x 2	0.6	
4.9-5.6	0.70 x 2	0.7	
5.7-6.8	0.85 x 2	0.85	
6.9-8.0	1.0 x 2	1.0	
8.1-9.6	1.2 x 2	1.2	
9.7-11.2	1.4 x 2	1.4	
11.3-13.6	1.6 x 2	1.6	
13.7-16.0	2.0 x 2	2.0	
16.1-19.2	2.4 x 2	2.4	
19.3-23.2	2.9 x 2	2.9	
23.3-25.0	3.2 x 2	3.2	

Table 2 Azithromycin dosing chart by child weight

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study staff will attempt to make contact with the primary caregiver via cell phone; if no telephone number is provided, or if the participant cannot be reached, study staff will trace the child to the household within 2 weeks of the scheduled follow-up time.

During scheduled follow-up visits, study staff will use a standardized questionnaire to ascertain history of recent illness/morbidity, post-discharge medication use including antibiotic treatment, and current condition of the child (any hospitalizations, admission and discharge date of any hospitalization, vital status, date of death if applicable). If caregivers report a hospitalization, causes of admission, medication administration, cost of admission, and length of stay will be ascertained from both caregivers and medical records, when available.

Caregivers will be encouraged, at enrollment and at each subsequent contact, to bring the child to the study health facility at any time the child is sick. Study staff will triage children to the appropriate health facility staff and will conduct a brief unscheduled visit questionnaire to ascertain adverse event information. If the unscheduled visit leads to a hospitalization, this will trigger the completion of a hospital admission form.

¹⁵ If at any point during follow-up a child dies, a verbal autopsy using the Population Health Metrics Research Consortium Shortened Verbal Autopsy Questionnaire.¹⁹ If the death occurred in a hospital, data from the hospital records, including cause of death, if available, will be abstracted. If a death certificate is available, cause(s) and timing of death will be abstracted.

Final causes of re-hospitalization and death will be determined after data collection is complete by an independent adjudication committee comprised of clinicians specializing in pediatrics and infectious disease. Sources of cause of re-hospitalization (medical records and caregiver report) and causes of death (causes automatically assigned from the verbal autopsy using SmartVA-Analyze [Tariff 2.0 Method]²⁰, hospital records, or death certificates) will be presented to the adjudication committee for final cause assignment.

26 Laboratory Procedures

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Stool (rectal swabs), nasopharyngeal swabs and blood will be collected as described above and 27 undergo either immediate or future laboratory testing as described in Table 3. All biological samples will be 28 29 collected by staff trained in biosafety and Good Clinical Laboratory Practice (GCLP). Samples will be 30 processed in Kenya when technology is available at the Kenya Medical Research Institute (KEMRI) Wellcome 31 Trust or Centre for Microbiology Research [CMR]. Metagenomic analyses and/or analyses that require 32 technology not available in Kenya will be performed at the University of Washington. If stool culture results 33 report Shigella or Salmonella infection, the study staff will contact the child's caregiver and encourage the 34 caregiver to bring the child back for an evaluation and potential treatment if the child is symptomatic. 35

Specimen Collected	Purpose	Tests Performed
Stool/ flocked rectal swabs	Bacterial ID and storage for AST	Fresh samples/rectal swabs will be cultured to identify <i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> , and <i>Escherichia coli</i> (<i>E.coli</i>) using standard microbiologic methods and biochemically confirmed using bioMérieux's API [®] strips. All <i>Shigella</i> , <i>Salmonella</i> , and <i>Campylobacter</i> isolates, as well as a random subset of <i>E.coli</i> isolates will undergo antibiotic resistance testing using disc diffusion for the following antibiotics: amoxicillin-clavulanic acid (augmentin), ampicillin, azithromycin, chloramphenicol, ciprofloxacin, ceftriaxone, ceftazidime, cefoxitin, gentamicin, imipenem, trimethoprim-sulfamethoxazole, ceftazidime/clavulanate (ESBL), cefotaxime/clavulanate (ESBL). Categorizations of susceptible, intermediate, and resistant will be determined using zone-size cut-offs outlined in Clinical and Laboratory Standards Institute (CLSI) interpretive standards.
	Parasite detection	Fresh stool and rectal swabs will be tested for <i>Giardia</i> and <i>Cryptosporidium</i> using the immunoassay Giardia/ Cryptosporidium QUIK CHEK [™] .
	Storage	Stool/ flocked swabs and colonies of <i>E.coli</i> , <i>Shigella</i> spp., <i>Salmonella</i> spp., and <i>Campylobacte</i> r spp. will be stored at -80°C.
Nasopharyngeal Swabs	Bacterial isolation, storage, and resistance testing	Streptococcus pneumoniae (S. pneumoniae) colonies will be isolated using standard microbiologic or molecular diagnostic protocols and susceptibility testing performed using standard microbiologic or molecular techniques. A random subset of <i>S. pneumoniae</i> isolates will undergo antimicrobial resistance testing using disc diffusion for the following antibiotics: amoxicillin-clavulanic acid (Augmentin), ampicillin, azithromycin, chloramphenicol, ciprofloxacin, ceftriaxone, ceftazidime, cefoxitin, gentamicin, imipenem, and trimethoprim-sulfamethoxazole. Categorizations of susceptible, intermediate, and resistant will be determined using zone-size cut-offs outlined in CLSI interpretive standards.
	Storage	Back-up sample and S.pneumoniae colonies will be will be stored at -80°C.

Table 3. Sample storage and processing descriptions

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2 3	Blood	HIV and malaria testing	HIV testing will be performed per Kenyan National Guidelines and malaria microscopy performed using standard methods.
4		Storage	Plasma and buffy coat will be stored at -80°C. Dried blood spots will be stored at room
5			temperature.
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Data Management and Confidentiality

Personal information about the participants, including medical records and data ascertained per caregiver interview, will be securely stored in files in the study offices at the study sites. Only pre-designated 10 study staff will have access to the files. Data will be entered into an electronic database (Dacima® Electronic 11 Data Capture) regularly by study staff. Access to the electronic database will be secured using password 12 protected accounts for study staff. Data reports of screening, enrollment, and exclusion totals will be 13 disseminated to the study team on a weekly basis; reports including baseline demographic characteristics, 14 laboratory results, adherence data, and serious adverse event summaries will be distributed to study co-15 investigators and data monitors guarterly. Data will be regularly gueried to facilitate ongoing data cleaning. 16

Data Analysis

19 Primary endpoints: 20

The primary study endpoint is a combined outcome of mortality and hospital readmission, as re-hospitalization 21 is highly associated with risk of subsequent poor outcome. Re-hospitalizations that are a continuation of 22 management from the previous hospitalization (such as elective blood transfusion) or that occur during 23 24 enrollment procedures, due to a clinical deterioration post-discharge, will be excluded from the analysis. Loss 25 to follow-up will be defined as non-attendance at both follow-up visits despite one month of active tracing and 26 no clear evidence of death. 27

Secondary endpoints include:

- 29 1. Cause-specific re-hospitalizations assessed by guestionnaire (maternal recall of diagnosis) at month 3 and 30 month 6 follow-up visits and medical record review (discharge diagnosis). In cases when both sources are 31 available, information from the medical record will be considered as the primary source. Separate analyses 32 will be performed for each diagnosis: diarrhea, acute respiratory infection, malnutrition, or malaria. 33
- 34 2. Mild, moderate, and severe events that did not result in re-hospitalization, including diarrhea, vomiting, skin 35 rash, lip swelling, difficulty breathing/wheeze, and seizure will be ascertained by caregivers identified by the 36 study clinicians during clinical exams at scheduled follow-up visits or during unscheduled visits. Severity 37 (grade 1-3) will be defined according to 2014 Division of AIDS (DAIDS) Table for Grading the Severity of 38 Adult and Pediatric Adverse Events.
- 39 3. Enteric pathogen carriage, operationalized as presence of a bacterial pathogen-Shigella species (spp.), 40 Campylobacter spp., or Salmonella spp., or parasite- Giardia or Cryptosporidium in stool or rectal swabs 41 assessed at month 3 and month 6 follow-up visits. 42
- 4. Streptococcus pneumoniae (S. pneumoniae) isolated from nasopharyngeal swab cultures at month 3 and 43 month 6 follow-up visits. 44
- 5. Antibiotic resistance, specifically resistance to azithromycin, ampicillin, augmentin, ciprfrofloxacin, 45 trimethoprim-sulfamethoxazole, in E.coli and S. pneumoniae isolates, and presence of ESBL in E.coli 46 47 isolates, from month 3 and month 6 follow-up visits. 48

49 To compare rates of re-hospitalization and mortality in the 6 months following hospital discharge among 50 Kenyan children receiving 5-day azithromycin vs. placebo. Primary analyses will be modified intent-to-treat 51 (mITT) based on randomization allocation to the 5-day course of azithromycin versus placebo. Cumulative 52 incidence of death or first re-hospitalization will be compared between treatment groups using Cox proportional 53 hazards regression. Participants will be censored at the date of their first re-hospitalization, or at the date of 54 death. Median time to hospitalization-free survival will be compared between randomization groups using 55 Kaplan-Meier (K-M) survival analysis and associated log-rank test. If the baseline assessment of 56 randomization reveals an imbalance in characteristics between the treatment groups, we will evaluate these 57 variables as potential confounders in a sub-analysis secondary to the mITT. Potential baseline confounders will 58 be added stepwise in a multivariable Cox model and maintained in the model if adjustment changes the hazard 59 60

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ratio by more than 10%. In per protocol analyses also secondary to the mITT, we will compare treatment effects in groups defined by self-reported adherence to the 5-day course of azithromycin (5 doses vs. < 5 doses; ≥3 doses vs. <3 doses; >1 dose vs. 1 dose only). In addition, we will conduct Cox regression and K-M survival analyses for time to mortality and time to re-hospitalization as separate endpoints to understand intervention effects on these outcomes individually. The assumption of proportional hazards will be checked in all models 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. If there is substantial missing covariate data, multiple imputation using the Markov chain Monte Carlo (MCMC) method will be used to impute 10 covariate information. Missing outcome data (death or re-hospitalization) will not be imputed, but participants 11 will be censored at the last follow-up visit therefore contributing some person-time to the analysis. In sensitivity 12 13 analyses, we will compare treatment effects in children whose caregivers report no additional antibiotic use 14 over follow-up and separately, who report no additional azithromycin use specifically, and in subsets of children 15 defined by age, site, and discharge diagnosis.

16 To evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality, by 17 comparing reasons for re-hospitalization and change in prevalence of pathogen carriage between the 18 randomization arms. To evaluate the association between azithromycin and the rates of cause-specific re-19 hospitalizations (hospitalization due to diarrhea, acute respiratory infection, malnutrition, or malaria) we will use 20 Anderson-Gill proportional hazards modeling with previous re-hospitalizations included as time-dependent 21 covariates in the model to capture the dependent structure of recurrence times. Because we will not have 22 granularity in the time points other than 3 months and 6 months for assessment of pathogen carriage, we will 23 compare the prevalence of a bacterial and parasitic pathogens (Shigella, Salmonella, Campylobacter, 24 25 Cryptosporidium, Giardia) at 3 and 6 months by randomization arm using generalized estimating equations 26 (GEE) with a Poisson link, exchangeable correlation structure, and will adjust for baseline presence of a 27 bacterial pathogen. To determine whether an observed association between the intervention and pathogen 28 carriage wanes over time, we will test the hypothesis that the prevalence ratios comparing carriage in 29 intervention arms are the same at the two follow-up time points using a chi-squared test. 30

To determine whether empiric administration of azithromycin at hospital discharge increases risk of 31 antibiotic resistance in commensal E. coli and pneumococcal isolates from treated children and their household 32 contacts. Among children and adult household contacts in whom commensal E. coli and/or S. pneumoniae are 33 isolated, we will compare the proportion of isolates resistant to azithromycin, ampicillin, augmentin, 34 ciprofloxacin, and trimethoprim-sulfamethoxazole, between randomization arms and Contact Cohorts for each 35 arm, at 3 and 6 months using GEE with a Poisson link and exchangeable correlation structure. A chi-squared 36 test will be used to determine whether the association between intervention arm and antibiotic resistance 37 wanes over time. Because the likelihood of having a bacterial pathogen isolated may depend on baseline 38 39 factors, including intervention arm, we will conduct secondary analyses utilizing propensity scores to account 40 for the potential differential likelihood of having antibiotic susceptibility testing performed, which will allow us to 41 make inference to the entire study population and their contacts. Also we will compare resistance proportions 42 among children (as opposed to among isolates) where absence of an isolated bacteria is considered not 43 resistant. 44

To identify correlates and intermediate markers of post-discharge mortality and hospital-readmission 45 among hospitalized children. Enrollment hospital admission diagnosis, indicators of malnutrition, age, HIV-46 exposure and HIV-infection status, sickle cell anemia, and randomization arm will be assessed in a 47 multivariable Cox regression model to identify correlates of the primary endpoint of death and/or hospital-48 readmission independent of the treatment effect. In addition, Cox regression models will also be built for 49 correlates of mortality and correlates of re-admission individually to understand distinct cofactors for each of 50 51 these outcomes.

52 To determine the cost-effectiveness of post-discharge administration of a 5-day course of azithromycin 53 in settings of varying antibiotic use, re-hospitalization rates, and mortality rates. Costs analysis: We will assess 54 the costs of all supplies, services and equipment necessary to implement the intervention (direct medical 55 costs). The perspective will be that of the healthcare provider, i.e. Kenya's Ministry of Health. Using WHO 56 guidelines and its ingredients approach, we will quantify the resources and associated unit costs required to 57 deliver a 5-day course of azithromycin, organized in standard expenditure categories: personnel (salaries), 58 supplies including drugs, equipment, services, space and overhead. We will also measure the costs of severe 59 child hospitalizations, the costs for the different types of personnel employed (e.g. nurses/doctors) and the time 60

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2 demanded from them for conducting the intervention.²¹ When data are missing, they will be complemented by 3 data extracted from the literature and other available sources. Full incremental costs will be derived, with 4 estimation of the potential healthcare cost-offset realized in avoiding severe hospitalizations. Costs will be 5 measured in local currency (Kenyan Shilling) and converted into US\$. Our main metric will be cost per child 6 treated. Cost-effectiveness analysis (CEA): we will develop a CEA mathematical model, and estimate 7 incremental costs and cost-effectiveness for implementation of the intervention. The model will include two 8 components: costs (described immediately above) and health benefits. The study will provide clinical outcomes 9 (mortality/morbidity) over a 6-month follow-up period. Subsequently, deaths averted, life-years saved and 10 disability-adjusted life years (DALYs) averted by the intervention will be estimated. We will estimate: a) 11 12 incremental costs and b) incremental cost-effectiveness of the intervention vs. status quo. Incremental costs 13 are the net sum of the costs to implement the intervention compared with status quo, and the costs averted 14 due to the decrease in severe child hospitalizations. Incremental cost-effectiveness ratios (ICERs) will be 15 estimated as cost per death averted, cost per life-year saved, and cost per DALY averted. We will use recent 16 estimates of disability weights for estimation of DALYs.^{22 23} Short-term (over study follow-up i.e. 6 months) and 17 longer-term time horizons (extrapolated to 1, 5, and 10 years) will be used. DALYs and costs will be discounted 18 at 3% per year, consistent with CEA guidelines (undiscounted results will also be presented). Sources of 19 uncertainty in the results will be explored in univariate and probabilistic sensitivity analysis. ^{24 25} Finally, we will 20 compare our findings to CEA estimates for other health interventions in sub-Saharan Africa.^{26 27} 21

Data and Safety Monitoring

23 A Data Safety and Monitoring Committee (DSMC) will be established at study initiation to monitor 24 severe adverse events (SAEs) and to evaluate the statistical analysis plan and associated stopping rules. The 25 DSMC includes expertise in clinical trials, statistics, child mortality assessment, ethics, and pediatric care in 26 resource limited settings. Adverse events will be monitored by the DSMC. Monthly adverse event summaries 27 28 will be sent to the DSMC safety officer and individual-child SAE forms, which include detailed medication 29 history to evaluate possible drug interactions, will be sent to the safety officer per request. Each SAE will be 30 assigned the plausibility of relatedness to study drug by study PIs. The data will not be presented by 31 intervention group unless requested by DSMB safety officer. These reports will be descriptive (no statistical 32 analyses). The DSMC will make recommendations regarding any imbalances in safety outcomes.

33 A single interim analysis for re-hospitalization-free survival will be prepared by the study statistician 34 using O'Brien-Fleming boundaries for benefit and harm when 50% of expected person time (350 child-years) 35 has been accrued. Assuming 157 events will be available at half of the person-time accrual, a z-score critical 36 value of 2.797, or p-value < 0.005, from a Kaplan Meier log-rank test will determine the cut-off of statistical 37 significance. A symmetric boundary will be used for benefit and harm. The DSMC will review this analysis and 38 make a determination about study continuation. Futility will not be a basis for stopping rules because of the 39 trials' value in understanding mechanisms of post-discharge worsening and antibiotic resistance. Assuming the 40 41 DSMC decides to continue the trial after the interim analysis, an alpha of 0.045 will be used as the statistical 42 significance boundary at the final analysis. 43

44 Statistical Power

45 To compare rates of re-hospitalization and mortality in the 6 months following hospital discharge among 46 Kenyan children receiving 5-day azithromycin vs. placebo. The total sample size required was calculated for 47 the primary endpoint of time to death or hospital re-admission within the 6 month post-discharge period. 48 assuming an alpha level of 0.05, power of 0.80, and a ratio of treatment to placebo random assignment of 1:1. 49 In SSA, it is estimated that 2-15% of children aged less than 5 years died within 6 months of hospital discharge 50 and 15.5% of children who survived discharge from the district hospital were re-admitted with the same 51 diagnosis within 6-months.^{1 3 8} Assuming that an additional 5-10% of children are re-admitted for other 52 conditions, we expect that re-hospitalizations will occur in 20.5 to 30.5% of children enrolled in the study. 53 Combined with our expected fatality rate (2-15%), we expect the cumulative incidence of the combined 54 endpoint to range from 22.5 to 45.5%.⁸ Based on a previous trial of mass drug administration of a single dose 55 of azithromycin in which a single dose of the antibiotic was associated with a 49% reduction in risk of death, we 56 57 calculated sample sizes using estimates of reduction in risk ranging from 30-50% with the cumulative incidence 58 range of 22.5 to 45.5% in the placebo-treated group, and found the sample size required ranged from 90 to 59 550 children per treatment arm.¹⁵ Using the most conservative estimates of a hazard ratio of 0.70 and 22.5% 60

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prevalence of re-admission/death, we need to enroll 1100 children in the study (550 per arm) to achieve adequate power. We will recruit an additional 300 children (\approx 20%) to account for possible loss to follow-up, resulting in a total planned enrollment of 1400 children, or 700 per treatment group. When considering mortality alone, and estimated mortality ranges of 2-17% among place-treated children, we will have >80% power to detect hazard ratios ≤0.5 for mortality rates of ≥ 8% and hazard ratios ≤0.6 for mortality rates ≥11% (Figure 1).

<u>To evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality, by</u> <u>comparing reasons for re-hospitalization and prevalence of pathogen carriage between the randomization</u> <u>arms.</u> We calculated the minimum detectable association between treatment arm and cause-specific rehospitalizations (hospitalization due to diarrhea, acute respiratory infection, malnutrition, or malaria vs. any other) among enrolled children, assuming an alpha level of 0.05, power of 0.80, and a ratio of treatment to placebo of 1:1. Based on data from Kenya, re-hospitalization rates due to specific causes ranged from approximately 0.5% to 5.7% in the 6 month post-discharge period.³ By not conditioning on the child having the same diagnosis as the initial hospitalization, we expect the cumulative incidence of cause-specific rehospitalizations to range from 2.5% to 10%. With this range of outcome rates, we will be able to detect hazard ratios of 0.48 to 0.70 for the effect of azithromycin on specific severe morbidities.

¹⁸ We expect 56% of children in the placebo group to have *S. pneumoniae* isolated from nasopharyngeal ¹⁹ swabs, providing \geq 80% power to detect a prevalence ratio of 0.85 (or 1.15) between the two treatment arms at ²⁰ each time point.²⁸⁻³⁰ Based on prevalences of *Shigella, Salmonella, Campylobacter, Cryptosporidium, Giardia* ²¹ among asymptomatic children in Western Kenya, we expect 10% of children in the placebo group to have a ²³ bacterial pathogen isolated at each time point, resulting in \geq 80% power to detect differences in enteric ²⁴ pathogen prevalences of 0.67 (1.49) at each time point.³¹

<u>To determine whether empiric administration of azithromycin at hospital discharge increases risk of</u> antibiotic resistance in commensal E. coli and S. pneumoniae isolates from treated children and their

26 27 household contacts. We will select a random selection of 28 400 E. coli and 400 S. pneumoniae isolates (200 per arm) 29 for β -lactam and macrolide resistance testing at each 30 timepoint. We will also store all S. pneumoniae, E. coli 31 isolates and other isolated bacteria from stool for 32 potential future testing in the event that resistance 33 prevalence is lower than expected. As shown in Table 4, 34 we will have > 80% power to detect prevalence ratios > 35 1.1, with an ability to detect the smallest effect sizes 36 when the prevalence of resistance in the placebo group is 37 highest. We will enroll 300 adults in the Contact Cohort 38 39 for E. coli and S. pneumoniae isolation. We expect E.coli 40 to be isolated from all adults and S.pnuemoniae isolated 41 from between 5-55%.^{28 32 33} Assuming an alpha of .05, a 42 1:1 ratio of testable isolates, and a prevalence of

Table 4. Power (%) to detect prevalence ratios of macrolide and β -lactamase resistance in 200 *E.coli* and 200 *S.pneumoniae* isolates per treatment group

0.0.0		Resistance prevalence (%) in placebo							
		group							
(%		10	20	30	40	50	60	70	
e (°	10								
prevalence (%) mycin group	20	80							
valc	30	>99	64						
pre my	40	>99	99	55					
Resistance preval in azithromycin	50	>99	>99	98	48				
star azit	60	>99	>99	>99	>99	52			
esis	70	>99	>99	>99	>99	98	55		
R	80	>99	>99	>99	>99	>99	99	64	

resistance of 50% in the placebo arm, we will have 80% power to detect a 1.4-fold higher prevalence to 1.9fold higher resistance prevalence in the contacts of azithromycin-treated children.

To identify correlates and intermediate markers of post-discharge mortality and hospital-readmission among hospitalized Kenyan children. Conservatively estimating a 20% loss-to-follow-up rate in the RCT and a cumulative incidence of death or re-hospitalization of 22.5%, we will have >80% power to detect hazard ratios ≥ 1.3 between correlates and the outcome with exposure prevalences of $\geq 20\%$ or more and hazard ratios ≥ 1.5 for exposure prevalences <20%.

51 52 Study timeline

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The trial began on June 28, 2016 and participant recruitment and follow-up will continue over a 36month period, with anticipated final follow-up visit(s) occurring in June 2019. Primary analyses will be complete by February 2020.

57 **Potential Challenges and Limitations**

⁵⁸ In order to ensure adequate power to detect a discernable clinically relevant difference between study ⁵⁹ groups in the primary outcome, we have combined hospital readmission with death. Preliminary studies

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2 suggest that sufficient numbers of children will reach this combined outcome. However, we have incorporated 3 an interim analysis by the DSMB to review the accrued data and an adapted sample size could be considered 4 if the combined event frequency is less than predicted. It is possible that since most children receive antibiotics 5 during hospitalization, the benefit anticipated with the use of azithromycin based on previous trials of mass 6 drug administration will not be observed. However, most hospitalized children are treated with penicillins, 7 cephalosporins, gentamicin, or cotrimoxazole while in hospital and the broad spectrum of activity (including 8 malaria prevention) and long half-life of azithromycin suggest that there may be additive treatment and/or 9 prophylactic benefit. Similarly, children may receive azithromycin during follow up - either as treatment for an 10 illness or because the caregiver sought out azithromycin upon learning of the hypothesis - and this 11 azithromycin use may lead to contamination in the placebo-arm. After discharge, it is difficult to ensure 12 13 adherence with the full 5-day treatment course. We will measure adherence using three different measures 14 (text message responses, bottle check boxes, and caregiver-report at follow-up visits) although all are limited 15 by caregiver-report. In addition, the mortality benefit of azithromycin observed in Ethiopia was from a single 16 dose and in this study the first dose will be directly observed.¹⁵ While relying on caregiver report of mortality 17 and morbidity may lead to bias due to outcome misclassification, this misclassification should not differ 18 between randomization arms and therefore will be non-differential. Further hospital records will be used when 19 available to determine diagnoses. Finally, resistance prevalence may be lower than predicted, limiting power to 20 detect clinically relevant differences in resistance prevalence between the intervention arms. We will store all 21 isolates in the event that a greater number of isolates are needed for antibiotic resistance testing. 22

2324 Regulatory Authorities

This study has received IRB approval by the University of Washington Human Subjects Division (HSD), KEMRI Scientific and Ethics Review Unit (SERU), and the Kenya Pharmacy and Poisons Board. The clinical trial is also registered with clinicaltrials.gov (NCT02414399). Any modifications to the study protocol or consent materials will be submitted for approval all regulatory authorities before implementation. Westat[®] will provide external clinical, pharmacy, and laboratory monitoring.

Dissemination

Results of this study will be disseminated by publication in a peer-reviewed scientific journal, presented at relevant academic conferences, and amongst participating partners and health facilities in Kenya.

35 36 **Author's contributions**

JLW, PBP, GJS, BAR, BOS, and RN conceived of this trial and developed of this study protocol. JLW and BOS 37 are study co-Principal Investigators and PBP is the Project Director; BAR oversaw the statistical analyses 38 39 plans; JBB developed the CEA plan; KDT developed procedures for ascertaining and reporting SAEs; CJM 40 developed procedures related to blood specimen procedures and drug adherence measurement. GJS, CJM, 41 RN, and PBP provide scientific expertise. RLB and MA are involved in collection and management of the data. 42 MA and PBP coordinate and oversee implementation of all clinical study procedures and SK, with assistance 43 from DR, oversees all laboratory procedures. All authors contributed to the development of this manuscript 44 and/or study procedures, and to reading and approving the final version for publication. 45

46 47 Funding statement

This work was funded by the National Institutes of Health Eunice Kennedy Shriver National Institute of Child Health & Human Development, grant number R01 HD079695.

5051 Acknowledgements

52 Pfizer donated the Zithromax® to be used in this clinical trial, and Copan Diagnostics donated all rectal swabs 53 and Cary-Blair media. Investigators from KEMRI-Wellcome Trust Kilifi, Jay Berkley, Anthony Scott, Joseph 54 Waichungo, Angela Karani, Donald Akech, and Horace Gumba provided microbiology expertise and training in 55 nasopharyngeal swab collection, STGG media preparation, and laboratory quality assurance and control. Liru 56 Meshack Wekesa and George Bogonko provide clinical expertise and facilitate the study's integration into 57 pediatric wards at the two hospitals. Alex Awuor and Caleb Okonji, with the support of Richard Omore, 58 provided training in anthropometric measurement. We are extremely thankful to Dr. Philip Walson who 59 developed azithromycin dosing regimens. Hannah Atlas and Stephanie Belanger contributed to the standard 60

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operating procedure and case report form development and implementation. Gillian Levine played an invaluable role in the proposal development.

Competing interests statement

None of the authors or study co-investigators have any competing interests to declare.

Figure Legend

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Figure 1. Power and detectable hazard ratios assuming a range of mortality rates from 2-17%

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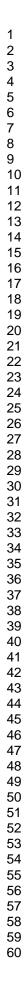
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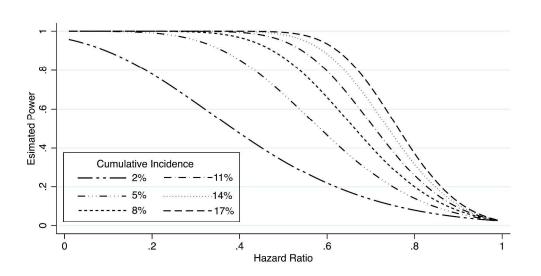


Figure 1. Power and detectable hazard ratios assuming a range of mortality rates from 2-17%

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ltem No	Description	Addressed on page number
Administrative inf	ormatior		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	
Roles and	5a	Names, affiliations, and roles of protocol contributors	
responsibilities	5b	Name and contact information for the trial sponsor	
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	

1 2	Introduction			
3 4 5	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	
6 7		6b	Explanation for choice of comparators	
8 9 10 11 12 13	Objectives	7	Specific objectives or hypotheses	
	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	
14 15	Methods: Participa	nts, inte	erventions, and outcomes	
16 17 18 19	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	
20 21 22	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	
23 24 25	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	
26 27 28 29		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	
30 31 32		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence(eg, drug tablet return, laboratory tests)	
33 34		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	
35 36 37 38 39 40 41 42 43	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	
44 45 46 47			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

Page	e 17 of 19		BMJ Open						
1 2 3	Sample size 14		Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations						
4 5 6 7 8 9 10 11 12 13 14 15	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size						
	Methods: Assignment of interventions (for controlled trials)								
	Allocation:								
	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions						
16 17 18 19 20	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered,						
21 22 23	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to						
24 25 26	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcomeassessors, data analysts), and how						
27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial						
	Methods: Data collection, management, and analysis								
	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol						
		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols						
			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml						

1 2 3 4	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	
5 6 7	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of thestatistical analysis plan can be found, if not in the protocol	
8 9		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
10 11 12 13		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	
14 15	Methods: Monitorir	ng		
16 17 18 19 20 21	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of	
22 23 24 25		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim	
25 26 27 28	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
29 30 31	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent	
32 33	Ethics and dissemi	nation		
34 35 36 37	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	
38 39 40 41 42	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	
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1 2	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	-
3 4 5 6		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary	-
7 8 9	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained	-
10 11 12 13	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	-
14 15 16	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that	-
17 18 19	Ancillary and post- trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trialparticipation	-
20 21 22 23 24	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	-
25 26		31b	Authorship eligibility guidelines and any intended use of professional writers	-
27 28 29		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
0	Appendices			
1 2 3 4	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-
85 86 87	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular	-
38 39 40 41 42	Amendments to the p	rotocol	that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons NoDerivs 3.0 Unported" license.	<u> </u>
43 44 45 46			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	5

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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)

Journal:	BMJ Open
Manuscript ID	bmjopen-2017-019170.R2
Article Type:	Protocol
Date Submitted by the Author:	08-Nov-2017
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Primary Subject Heading :	Global health
Secondary Subject Heading:	Paediatrics, Infectious diseases
Keywords:	Child mortality, Post-discharge interventions, Toto Bora Trial, Targeted empiric antibiotic therapy

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Title: 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) Running head: AZM to prevent post-discharge morbidity and mortality Pavlinac PB¹, Singa BO^{7,8}, John-Stewart GC¹⁻⁴, Richardson BA^{1,5}, Brander RL², Authors: McGrath CJ¹, Tickell KD^{1,8}, Amondi M⁷, Rwigi D⁷, Babigumira JB^{1,6}, Kariuki S⁷, Nduati R⁹, Walson JL^{1-4,8} Departments of ¹Global Health, ²Epidemiology, ³Pediatrics, ⁴Allergy and Affiliations: Infectious Disease, ⁵Biostatistics, ⁶Pharmacy, University of Washington, Seattle, WA, USA; ⁷Kenya Medical Research Institute, Nairobi, Kenya; ⁸The Childhood Acute Illness & Nutrition Network, Nairobi, Kenya; ⁹ University of Nairobi, Nairobi, Kenva **Correspondence:** Patricia Pavlinac, 325 9th Ave, Box 359931, Seattle, WA 98104 (ppav@uw.edu) Sponsored by: This trial is sponsored by the National Institutes of Health Eunice Kennedy Shriver National Institute of Child Health & Human Development (R01 HD079695) and drug donation by Pfizer IIR #WI201906. JLW is supported by the Childhood Acute Illness and Nutrition Network (CHAIN, OPP1131320). GJS is supported by a National Institute of Health mentoring award (grant number K24-HD054314) and PBP and GJS are supported by the International Core of the University of Washington Center for AIDS Research (CFAR; Seattle, WA, USA). an NIH funded program (P30 Al027757). 5,730 (excluding tables and figures) Word count:

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ABSTRACT

Introduction: Child mortality due to infectious diseases remains unacceptably high in much of sub-Saharan Africa. Children who are hospitalized represent an accessible population at particularly high-risk of death, both during and following hospitalization. Hospital discharge may be a critical time point at which targeted use of antibiotics could reduce morbidity and mortality in high-risk children.

Methods and analysis: In this randomized, double-blind, placebo-controlled trial (Toto Bora Trial), 1400 children aged 1 to 59 months discharged from hospitals in western Kenya, in Kisii and Homa Bay, will be randomized to either a 5-day course of azithromycin or placebo to determine whether a short-course of azithromycin reduces rates of re-hospitalization and/or death in the subsequent 6-month period. The primary analysis will be modified intention-to-treat and will compare the rates of re-hospitalization or death in children treated with azithromycin or placebo using Cox proportional hazard regression. The trial will also evaluate the effect of a short course of azithromycin on enteric and nasopharyngeal infections and cause-specific morbidities. We will also identify risk factors for post-discharge morbidity and mortality and subpopulations most likely to benefit from post-discharge antibiotic use. Antibiotic resistance in *Escherichia coli* and *Streptococcus pneumoniae* among enrolled children and their primary caregivers will also be assessed and cost-effectiveness analyses performed to inform policy decisions.

Ethics and dissemination: Study procedures were reviewed and approved by the institutional review boards of the Kenya Medical Research Institute, the University of Washington, and the Kenyan Pharmacy and Poisons Board. The study is being externally monitored and a data safety and monitoring committee has been assembled to monitor patient safety and to evaluate the efficacy of the intervention. The results of this trial will be published in peer-reviewed scientific journals and presented at relevant academic conferences and to key stakeholders.

Trial registration number: NCT02414399

Key words: child mortality, Toto Bora, targeted empiric antibiotic therapy, post-discharge interventions

STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- Randomized, placebo-controlled, double-blinded design and modified intention-to-treat analysis will
 ensure unbiased treatment effect measure
- Comprehensive data are collected, including biological specimens for all child participants and a subset of adult caregivers, for analyses of mechanisms of post-discharge morbidity and mortality, subsets of children most likely to benefit from the antibiotic, as well as assessments of antibiotic resistance and cost-effectiveness
- Results will likely be generalizable due to the limited exclusion criteria, large sample size, and multiple study sites

Limitations

- Causes of death and re-hospitalization may be misclassified due to limited availability of medical records and recall bias in caregiver report
- The primary endpoint of this study is a combined outcome of re-hospitalization and death, which, while improving statistical power, may present challenges for interpretation
- Children in both intervention arms may receive other antibiotics over the course of follow-up

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2 BACKGROUND 3

Close to 3 million deaths occur annually in children less than 5 years of age in sub-Saharan Africa 4 (SSA), over half of which are attributed to infectious causes.¹ Children who were recently hospitalized have mortality rates 6 to 8-fold higher than similarly-aged children from the same community.²⁻⁴ Post-discharge mortality rates as high as 15% have been documented in the 12 months following discharge, with mortality risk 7 remaining elevated up to two years post-discharge.⁵⁻⁹ Children who are very young, malnourished, or HIV-8 infected are at particularly high risk of post-discharge mortality within the 3 months following discharge.^{2-5 7-9} 9 Children being discharged from hospital in SSA may represent an accessible high-risk population in which to 10 target interventions to reduce mortality and morbidity. 11

Targeted antibiotic interventions, including the use of cotrimoxazole among HIV-infected children and 12 13 the use of amoxicillin or cefdinir among children with severe acute malnutrition (SAM), have been shown to 14 reduce morbidity and mortality in these specific vulnerable populations.¹⁰⁻¹³ Other trials of targeted antibiotic 15 use in vulnerable populations, including cotrimoxazole in HIV-exposed uninfected (HEU) children and in 16 children with SAM, have failed to demonstrate a mortality benefit.^{14 15} In contrast, non-targeted mass drug 17 administration of a single dose of azithromycin halved mortality rates among Ethiopian children living in 18 communities randomized to receive the antibiotic.^{16 17} Concerns about the potential emergence of antibiotic 19 resistance, possible toxicity, and feasibility of delivery are barriers to community-wide antibiotic distribution 20 strategies. 21

A short-course of azithromycin given to children with recent severe illness being discharged from 22 hospital may optimize benefit while reducing both individual and population level risks. Azithromycin may 23 24 reduce post-discharge morbidity and mortality through infection related mechanisms such as treating 25 undiagnosed, incompletely treated or nosocomial infections or by protecting against new or recrudescent 26 infections that occur during recovery. Azithromycin may also act through non-antimicrobial pathways such as 27 by anti-inflammatory and/or immune-modulatory effects. 28

OBJECTIVE

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30 The primary objective of this double-blind, placebo-controlled, randomized controlled trial (RCT) is to 31 determine whether a 5-day course of azithromycin in children age 1 to 59 months discharged from hospital in 32 western Kenya reduces rates of re-hospitalizations and/or death in the subsequent six months. The secondary 33 objectives are (1) to evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality. 34 by comparing reasons for re-hospitalization and prevalence of enteric and nasopharyngeal infections between 35 the randomization arms; (2) to determine whether empiric administration of azithromycin at hospital discharge 36 increases risk of antibiotic resistance in commensal Escherichia coli (E. coli) and Streptococcus pneumoniae 37 (S. pneumoniae) isolates from treated children and their primary caregiver; (3) to identify correlates and 38 39 intermediate markers of post-discharge mortality and hospital readmission; (4) to determine the cost-40 effectiveness of post-discharge administration of a 5-day course of azithromycin in settings of varying antibiotic 41 use, re-hospitalization rates, and mortality rates; and (5) to create a repository of stool, nasopharyngeal, and 42 blood specimens from highly characterized, recently discharged children, half of whom are treated with 43 azithromycin, to be used to address future research questions. 44

METHODS

Reporting of this study protocol has been verified in accordance with the SPIRIT (Standard Protocol Items for Randomized Trials) recommendations.

Eligibility

51 Children age 1 to 59 months old weighing at least 2 kg who have been hospitalized, and subsequently 52 discharged, will be eligible for inclusion. Caregivers of potentially eligible children must be at least 18 years of 53 age or classified as an emancipated minor and be willing to participate in the Contact Cohort if randomly 54 selected. Children will be excluded if: azithromycin is contraindicated (children taking or prescribed other 55 macrolide antibiotics, such as erythromycin or clarithromycin, or the protease inhibitor, lopinavir); they were 56 admitted to hospital for a trauma, injury, or a birth defect; they do not plan to remain in the study site catchment 57 area for at least 6 months; the legal guardian does not provide consent; or if a sibling was enrolled in the trial 58 on the same day of discharge. 59

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Recruitment

Children will be recruited from the inpatient wards of health facilities in Kisii and Homa Bay Counties where study staff will accompany hospital staff on ward rounds to identify children being discharged each day. All discharged children, as determined by the onsite hospital clinicians, will be screened by study staff during working hours. If the caregiver is interested in participating and indicates consent for screening, the study staff will screen the child for eligibility, and if eligible, will obtain informed consent for study participation. Informed consent includes an explanation of the potential risks and benefits of the study and additional provision for use of participant data and samples for future studies, and will be conducted in the language of the respondent's choosing (English, Kiswahili, Kisii, or Luo). The parent or guardian (primary caregiver) must sign written informed consent (or provide a witnessed thumbprint if not literate) prior to enrollment.

Enrollment

Children will be enrolled at the time of discharge by the clinical staff. At enrollment, primary caregivers will be interviewed to assess demographic information, medical history, and detailed contact information for the child (Table 1). Medical records will also be used to abstract information from the hospitalization (including presenting diagnosis, medical management, length of stay, procedures performed, relevant medical history, physical examination, and laboratory data). All enrolled participants will undergo a physical examination performed by the study clinician, including measurement of height (in children ≥ 24 months), length (in children <24 months), weight, and mid-upper arm circumference (MUAC), each of which will be measured three times. HIV status will be obtained from medical records or from performed testing if records are not available. Detailed home location and contact information will be collected to enable patient tracing.

	Enrollment visit (hospital discharge)	3 month follow up vis	it	6 month follow up visit		Unscheduled visits
• • • • •	Questionnaire of sociodemographic, clinical history, treatments prescribed in hospital and at discharge, hospitalization costs, dietary factors, household factors, and environmental exposures Physical exam Anthropometry Abstraction of medical records (if re-hospitalized) Verbal autopsy (or abstracted medical records) Heel/finger prick (HIV and malaria) Stool collection (<i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> , <i>Escherichia coli</i> , <i>Cryptosporidium</i> , and Giardia) Nasopharyngeal swab collection (<i>Streptococcus</i> <i>pneumoniae</i>)	 Questionnaire of study administration, and rep illnesses, hospitalizatio costs, change in clinica history, and treatments last visit Physical exam Anthropometry Abstraction of medical records (if re-hospitaliz Verbal autopsy (or abs medical records) Heel/finger prick (HIV a malaria) Stool collection (<i>Shigel Salmonella, Campylob</i> <i>Escherichia coli, Cryptosporidium,</i> and Giardia) Nasopharyngeal swab collection (<i>Streptococco pneumoniae</i>) 	orted since ed) racted a, acter,	Questionnaire of reported illnesses, hospitalization costs, change in clinical history, and treatments since last visit Physical exam Anthropometry Abstraction of medical records (if re-hospitalized) Verbal autopsy (or abstracted medical records) Heel/finger prick (HIV and malaria, sickle-cell) Stool collection (<i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> , <i>Escherichia coli</i> , <i>Cryptosporidium</i> , and Giardia) Nasopharyngeal swab collection (<i>Streptococcus</i> <i>pneumoniae</i>)	•	Questionnaire of reported illnesses since last schedule visit, change in clinical history, and treatments since la visit Abstraction of medical records (if re-hospitalized) Verbal autopsy (or abstracted medical records)

Specimen collection

Specimens will be collected at enrollment (prior to study medication administration, as well as at 3 and 6-month follow-up visits). All children will also be asked to provide a whole stool for enteric pathogen identification and storage. Stool samples will be divided within one hour of collection for the following purposes: 1) placed in Cary-Blair for eventual bacterial culture (FecalSwab Cary-Blair Collection and Transport Systemt[™], Copan Diagnostics), 2) immediately tested for *Giardia* and *Cryptosporidium* using the immunoassay (Quik Chek[™], Alere) and 3) placed in -80 °C storage for future molecular determination of pathogen or commensal flora and markers of gut function (whole stool for these purposes will be divided into two separate vials). If a child cannot produce whole stool, two flocked rectal swabs (Pedatric FLOQswab[™],

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Copan Diagnostics) will be collected and one placed in Cary-Blair and the other stored in -80°C for future analyses.

One flocked dry nasopharyngeal swab (Copan Diagnostics) will also be collected from all enrolled children at each time point, immediately placed in skim milk, tryptone, glucose, and glycerine (STGG) media, and frozen (-80°C) within 1 hour of collection for future S. pnuemoniae culture.^{18 19} Primary caregivers in the Contact Cohort will also be asked to provide a stool sample (or 2 rectal swabs) and nasopharyngeal sample at each visit for testing and storage as described above.

Venous blood (up to 1 teaspoon [5mL]) will be collected from all enrolled children at each time point into 10 EDTA tubes and separated for the following purposes: 1) 0.5mL for immediate HIV-testing (if indicated 11 according to Kenyan Ministry of Health guidelines), 2) 0.4mL for a thin malaria smear which will be stored at 12 13 room temperature, 3) 0.4mL for a dried blood spot, and 4) 2-4mL for plasma and buffy coat isolation and -80 ℃ 14 storage. Blood will also be collected from primary caregivers for HIV-testing if indicated. 15

16 Randomization

17 Block randomization (1:1) in random sized blocks of no more than 10, stratified by site, will be used. 18 Primary randomization will include allocation to the Contact Cohorts at a ratio of 1:5 (resulting in 150 per 19 treatment arm). Each subject will be assigned a Patient Identification Number (PID), and the randomization 20 code linking each PID to the allocated treatment will be generated by a designated statistician and maintained 21 by the University of Washington Research Pharmacy. Study participants, investigators (other than the 22 statistician), the study staff, hospital clinicians, and persons involved in data management or analysis will 23 remain blinded to the allocation group during all data collection phases of the study. 24

Intervention

26 Enrolled children will be prescribed a 5-day course of oral suspension formulation azithromycin 27 (Zithromax® from Pfizer, 10 mg/kg on day 1, followed by 5mg/kg/day on days 2-5) or identically appearing and 28 tasting placebo at discharge. Identically appearing bottles will be pre-labelled with the PID. Dosing ranges were 29 determined such that a given child would never be under-dosed or over-dosed by more than 20% of the 30 31 weight-specific intended dose (Table 2). The day 1 dose will be split in half and the first half administered by 32 the study clinician (to be observed by the

33 caregiver) followed by the second half administered by the 34 caregiver under careful observation of the study staff. Day 2-5 35 doses will be administered by caregivers at their home. 36 Caregivers will be provided with visual instructions in the 37 language of their choosing (English, Kiswahili, Luo, Kisii). 38

Automated daily text message drug administration 39 reminders will be sent for the four days following discharge and 40 caregivers asked to respond with whether or not the child took 41 42 the daily dose. The response text message will be free of charge to caregivers and caregivers will be reimbursed for each 43 44 response at the final study visit. Caregivers are also asked to 45 record each administered dose on the bottle and to return bottles 46 at the 3-month follow-up visit. The questionnaire administered 47 during the 3-month follow up visit also includes guestions about 48 how many doses of the study drug the child received. 49

50 Follow-up Procedures

51 All enrolled children and primary caregivers will be 52 scheduled to return to the health facility at 3 and 6 months 53 following enrollment to collect clinical information and samples. 54 Anthropometric measurements will be obtained from all children 55 and caregivers at both follow up visits (height/length, weight, 56 57 MUAC) and caregivers will be asked about any hospitalizations 58 occurring since the last time the child was seen by study staff. 59

Weight (kg)	Day 1 dose (mL)	Day 2-5 dose (mL)
2.0	0.25 x 2	0.25
2.1-2.4	0.30 x 2	0.30
2.5-2.8	0.35 x 2	0.35
2.9-3.2	0.40 x 2	0.40
3.3-3.6	0.45 x 2	0.45
3.7-4.0	🔷 0.50 x 2	0.50
4.1-4.8	0.60 x 2	0.6
4.9-5.6	0.70 x 2	0.7
5.7-6.8	0.85 x 2	0.85
6.9-8.0	1.0 x 2	1.0
8.1-9.6	1.2 x 2	1.2
9.7-11.2	1.4 x 2	1.4
11.3-13.6	1.6 x 2	1.6
13.7-16.0	2.0 x 2	2.0
16.1-19.2	2.4 x 2	2.4
19.3-23.2	2.9 x 2	2.9
23.3-25.0	3.2 x 2	3.2

 Table 2
 Azithromycin dosing chart by child weight

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Caregivers will be provided with 400KSH (approximately \$4USD) to cover the cost of their round-trip transportation.

Transportation cost will be reimbursed at each follow-up visit. If the participant does not return at their scheduled time, study staff will attempt to make contact with the primary caregiver via cell phone; if no telephone number is provided, or if the participant cannot be reached, study staff will trace the child to the household within 2 weeks of the scheduled follow-up time.

During scheduled follow-up visits, study staff will use a standardized questionnaire to ascertain history of recent illness/morbidity, post-discharge medication use including antibiotic treatment, and current condition of the child (any hospitalizations, admission and discharge date of any hospitalization, vital status, date of death if applicable). If caregivers report a hospitalization, causes of admission, medication administration, cost of admission, and length of stay will be ascertained from both caregivers and medical records, when available.

Caregivers will be encouraged, at enrollment and at each subsequent contact, to bring the child to the study health facility at any time the child is sick. Study staff will triage children to the appropriate health facility staff and will conduct a brief unscheduled visit questionnaire to ascertain adverse event information. If the unscheduled visit leads to a hospitalization, this will trigger the completion of a hospital admission form.

If at any point during follow-up a child dies, a verbal autopsy using the Population Health Metrics Research Consortium Shortened Verbal Autopsy Questionnaire.²⁰ If the death occurred in a hospital, data from the hospital records, including cause of death, if available, will be abstracted. If a death certificate is available, cause(s) and timing of death will be abstracted.

Final causes of re-hospitalization and death will be determined after data collection is complete by an independent adjudication committee comprised of clinicians specializing in pediatrics and infectious disease. Sources of cause of re-hospitalization (medical records and caregiver report) and causes of death (causes automatically assigned from the verbal autopsy using SmartVA-Analyze [Tariff 2.0 Method]²¹, hospital records, or death certificates) will be presented to the adjudication committee for final cause assignment.

29 Laboratory Procedures

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30 Stool (rectal swabs), nasopharyngeal swabs and blood will be collected as described above and 31 undergo either immediate or future laboratory testing as described in Table 3. All biological samples will be 32 collected by staff trained in biosafety and Good Clinical Laboratory Practice (GCLP). Samples will be 33 processed in Kenya when technology is available at the Kenya Medical Research Institute (KEMRI) Wellcome 34 Trust or Centre for Microbiology Research [CMR]. Metagenomic analyses and/or analyses that require 35 technology not available in Kenya will be performed at the University of Washington. If stool culture results 36 report Shigella or Salmonella infection, the study staff will contact the child's caregiver and encourage the 37 caregiver to bring the child back for an evaluation and potential treatment if the child is symptomatic. 38

3940 Table 3. Sample storage and processing descriptions

41 42	Specimen Collected	Purpose	Tests Performed
42 43 44 45 46 47 48 49 50 51 52 53 54	Stool/ flocked rectal swabs	Bacterial ID and storage for AST	Fresh samples/rectal swabs will be cultured to identify <i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> , and <i>Escherichia coli</i> (<i>E.coli</i>) using standard microbiologic methods and biochemically confirmed using bioMérieux's API [®] strips. All <i>Shigella</i> , <i>Salmonella</i> , and <i>Campylobacter</i> isolates, as well as a random subset of <i>E.coli</i> isolates will undergo antibiotic resistance testing using disc diffusion for the following antibiotics: amoxicillin-clavulanic acid (augmentin), ampicillin, azithromycin, chloramphenicol, ciprofloxacin, ceftriaxone, ceftazidime, cefoxitin, gentamicin, imipenem, trimethoprim-sulfamethoxazole, ceftazidime/clavulanate (ESBL), cefotaxime/clavulanate (ESBL). Categorizations of susceptible, intermediate, and resistant will be determined using zone-size cut-offs outlined in Clinical and Laboratory Standards Institute (CLSI) interpretive standards.
		Parasite detection	Fresh stool and rectal swabs will be tested for <i>Giardia</i> and <i>Cryptosporidium</i> using the immunoassay Giardia/ Cryptosporidium QUIK CHEK TM .
		Storage	Stool/ flocked swabs and colonies of <i>E.coli</i> , <i>Shigella</i> spp., <i>Salmonella</i> spp., and <i>Campylobacte</i> r spp. will be stored at -80 ℃.
55 56 57 58 59 60	Nasopharyngeal Swabs	Bacterial isolation, storage, and resistance testing	Streptococcus pneumoniae (S. pneumoniae) colonies will be isolated using standard microbiologic or molecular diagnostic protocols and susceptibility testing performed using standard microbiologic or molecular techniques. A random subset of <i>S. pneumoniae</i> isolates will undergo antimicrobial resistance testing using disc diffusion for the following antibiotics: amoxicillin-clavulanic acid (Augmentin), ampicillin, azithromycin, chloramphenicol, ciprofloxacin, ceftriaxone, ceftazidime, cefoxitin, gentamicin, imipenem, and trimethoprim-

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		sulfamethoxazole. Categorizations of susceptible, intermediate, and resistant will be determined using zone-size cut-offs outlined in CLSI interpretive standards.
	Storage	Back-up sample and <i>S.pneumoniae</i> colonies will be will be stored at -80 ℃.
Blood	HIV and malaria testing	HIV testing will be performed per Kenyan National Guidelines and malaria microscopy performed using standard methods.
	Storage	Plasma and buffy coat will be stored at -80 °C. Dried blood spots will be stored at room temperature.

Data Management and Confidentiality

10 Personal information about the participants, including medical records and data ascertained per 11 caregiver interview, will be securely stored in files in the study offices at the study sites. Only pre-designated 12 study staff will have access to the files. Data will be entered into an electronic database (Dacima® Electronic 13 14 Data Capture) regularly by study staff. Access to the electronic database will be secured using password 15 protected accounts for study staff. Data reports of screening, enrollment, and exclusion totals will be 16 disseminated to the study team on a weekly basis; reports including baseline demographic characteristics, 17 laboratory results, adherence data, and serious adverse event summaries will be distributed to study co-18 investigators and data monitors quarterly. Data will be regularly queried to facilitate ongoing data cleaning. 19

20 **Data Analysis** 21

22 Primary endpoints

23 The primary study endpoint is a combined outcome of mortality and hospital readmission, as re-hospitalization 24 is highly associated with risk of subsequent poor outcome.² Re-hospitalizations that are a continuation of 25 management from the previous hospitalization (such as elective blood transfusion) or that occur during 26 enrollment procedures, due to a clinical deterioration post-discharge, will be excluded from the analysis. Loss 27 to follow-up will be defined as non-attendance at both follow-up visits despite one month of active tracing and 28 no clear evidence of death. 29

Secondary endpoints

- 32 1. Cause-specific re-hospitalizations assessed by questionnaire (maternal recall of diagnosis) at month 3 and 33 month 6 follow-up visits and medical record review (discharge diagnosis). In cases when both sources are 34 available, information from the medical record will be considered as the primary source. Separate analyses 35 will be performed for each diagnosis: diarrhea, acute respiratory infection, malnutrition, or malaria. 36
 - 2. Mild, moderate, and severe events that did not result in re-hospitalization, including diarrhea, vomiting, skin rash, lip swelling, difficulty breathing/wheeze, and seizure will be ascertained by caregivers identified by the study clinicians during clinical exams at scheduled follow-up visits or during unscheduled visits. Severity (grade 1-3) will be defined according to 2014 Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events.
- 42 **3.** Enteric pathogen carriage, operationalized as presence of a bacterial pathogen-Shigella species (spp.), 43 Campylobacter spp., or Salmonella spp. - or parasite- Giardia or Cryptosporidium-in stool or rectal swabs 44 assessed at month 3 and month 6 follow-up visits. 45
 - 4. Streptococcus pneumoniae (S. pneumoniae) isolated from nasopharyngeal swab cultures at month 3 and month 6 follow-up visits.
- 47 5. Antibiotic resistance, specifically resistance to azithromycin, ampicillin, augmentin, ciprofloxacin, 48 trimethoprim-sulfamethoxazole, in E.coli and S. pneumoniae isolates, and presence of ESBL in E.coli 49 isolates, from month 3 and month 6 follow-up visits. 50

Statistical analysis

54 To compare rates of re-hospitalization and mortality in the 6 months following hospital discharge among 55 Kenyan children receiving 5-day azithromycin vs. placebo

56 Primary analyses will be modified intent-to-treat (mITT) based on randomization allocation to the 5-day course 57 of azithromycin versus placebo. Cumulative incidence of death or first re-hospitalization will be compared 58 between treatment groups using Cox proportional hazards regression. Participants will be censored at the date 59 of their first re-hospitalization, or at the date of death. Median time to hospitalization-free survival will be 60

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compared between randomization groups using Kaplan-Meier (K-M) survival analysis and associated log-rank test. If the baseline assessment of randomization reveals an imbalance in characteristics between the treatment groups, we will evaluate these variables as potential confounders in a sub-analysis secondary to the mITT. Potential baseline confounders 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 per protocol analyses also secondary to the mITT, we will compare treatment effects in groups defined by self-reported adherence to the 5-day course of azithromycin (5 doses vs. < 5 doses; ≥3 doses vs. <3 doses; >1 dose vs. 1 dose only). In addition, 9 we will conduct Cox regression and K-M survival analyses for time to mortality and time to re-hospitalization as 10 separate endpoints to understand intervention effects on these outcomes individually. The assumption of 11 proportional hazards will be checked in all models using graphical methods including plotting a ln(-ln(S(t))) plot 12 13 for each treatment group and assessing the parallelism of the two lines and by plotting Schoenfeld residuals 14 over time. If there is substantial missing covariate data, multiple imputation using the Markov chain Monte 15 Carlo (MCMC) method will be used to impute covariate information. Missing outcome data (death or re-16 hospitalization) will not be imputed, but participants will be censored at the last follow-up visit therefore 17 contributing some person-time to the analysis. In sensitivity analyses, we will compare treatment effects in 18 children whose caregivers report no additional antibiotic use over follow-up and separately, who report no 19 additional azithromycin use specifically, and in subsets of children defined by age, site, and discharge 20 diagnosis. 21

22 To evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality, by comparing 23 reasons for re-hospitalization and change in prevalence of pathogen carriage between the randomization arms 24

25 To evaluate the association between azithromycin and the rates of cause-specific re-hospitalizations 26 (hospitalization due to diarrhea, acute respiratory infection, malnutrition, or malaria) we will use Anderson-Gill 27 proportional hazards modeling with previous re-hospitalizations included as time-dependent covariates in the 28 model to capture the dependent structure of recurrence times. Because we will not have granularity in the time 29 points other than 3 months and 6 months for assessment of pathogen carriage, we will compare the 30 prevalence of a bacterial and parasitic pathogens (Shigella, Salmonella, Campylobacter, Cryptosporidium, 31 Giardia) at 3 and 6 months by randomization arm using generalized estimating equations (GEE) with a Poisson 32 link, exchangeable correlation structure, and will adjust for baseline presence of a bacterial pathogen. To 33 determine whether an observed association between the intervention and pathogen carriage wanes over time. 34 we will test the hypothesis that the prevalence ratios comparing carriage in intervention arms are the same at 35 the two follow-up time points using a chi-squared test. 36

38 To determine whether empiric administration of azithromycin at hospital discharge increases risk of antibiotic 39 resistance in commensal E. coli and pneumococcal isolates from treated children and their household contacts 40 Among children and adult household contacts in whom commensal E. coli and/or S. pneumoniae are

41 isolated, we will compare the proportion of isolates resistant to azithromycin, ampicillin, augmentin, 42 ciprofloxacin, and trimethoprim-sulfamethoxazole, between randomization arms and Contact Cohorts for each 43 arm, at 3 and 6 months using GEE with a Poisson link and exchangeable correlation structure. A chi-squared 44 test will be used to determine whether the association between intervention arm and antibiotic resistance 45 wanes over time. Because the likelihood of having a bacterial pathogen isolated may depend on baseline 46 factors, including intervention arm, we will conduct secondary analyses utilizing propensity scores to account 47 for the potential differential likelihood of having antibiotic susceptibility testing performed, which will allow us to 48 make inference to the entire study population and their contacts. Also we will compare resistance proportions 49 among children (as opposed to among isolates) where absence of an isolated bacteria is considered not 50 51 resistant. 52

53 To identify correlates and intermediate markers of post-discharge mortality and hospital-readmission among 54 hospitalized children

55 Enrollment hospital admission diagnosis, indicators of malnutrition, age, HIV-exposure and HIV-56 infection status, sickle cell anemia, and randomization arm will be assessed in a multivariable Cox regression 57 model to identify correlates of the primary endpoint of death and/or hospital-readmission independent of the 58 treatment effect. In addition, Cox regression models will also be built for correlates of mortality and correlates 59 of re-admission individually to understand distinct cofactors for each of these outcomes. 60

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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

4 Costs analysis: We will assess the costs of all supplies, services and equipment necessary to 5 implement the intervention (direct medical costs). The perspective will be that of the healthcare provider, i.e. 6 Kenya's Ministry of Health. Using WHO guidelines and its ingredients approach, we will quantify the resources 7 and associated unit costs required to deliver a 5-day course of azithromycin, organized in standard expenditure 8 categories: personnel (salaries), supplies including drugs, equipment, services, space and overhead. We will 9 also measure the costs of severe child hospitalizations, the costs for the different types of personnel employed 10 (e.g. nurses/doctors) and the time demanded from them for conducting the intervention.²² When data are 11 missing, they will be complemented by data extracted from the literature and other available sources. Full 12 13 incremental costs will be derived, with estimation of the potential healthcare cost-offset realized in avoiding 14 severe hospitalizations. Costs will be measured in local currency (Kenyan Shilling) and converted into US\$. 15 Our main metric will be cost per child treated. Cost-effectiveness analysis (CEA): we will develop a CEA 16 mathematical model, and estimate incremental costs and cost-effectiveness for implementation of the 17 intervention. The model will include two components: costs (described immediately above) and health benefits. 18 The study will provide clinical outcomes (mortality/morbidity) over a 6-month follow-up period. Subsequently, 19 deaths averted, life-years saved and disability-adjusted life years (DALYs) averted by the intervention will be 20 estimated. We will estimate: a) incremental costs and b) incremental cost-effectiveness of the intervention vs. 21 status quo. Incremental costs are the net sum of the costs to implement the intervention compared with status 22 guo, and the costs averted due to the decrease in severe child hospitalizations. Incremental cost-effectiveness 23 ratios (ICERs) will be estimated as cost per death averted, cost per life-year saved, and cost per DALY 24 25 averted. We will use recent estimates of disability weights for estimation of DALYs.^{23 24} Short-term (over study 26 follow-up i.e. 6 months) and longer-term time horizons (extrapolated to 1, 5, and 10 years) will be used. DALYs 27 and costs will be discounted at 3% per year, consistent with CEA guidelines (undiscounted results will also be 28 presented). Sources of uncertainty in the results will be explored in univariate and probabilistic sensitivity analysis. ^{25 26} Finally, we will compare our findings to CEA estimates for other health interventions in sub-29 30 Saharan Africa.27 28 31

Data and Safety Monitoring

33 A Data Safety and Monitoring Committee (DSMC) will be established at study initiation to monitor 34 severe adverse events (SAEs) and to evaluate the statistical analysis plan and associated stopping rules. The 35 DSMC includes expertise in clinical trials, statistics, child mortality assessment, ethics, and pediatric care in 36 resource limited settings. Adverse events will be monitored by the DSMC. Monthly adverse event summaries 37 will be sent to the DSMC safety officer and individual-child SAE forms, which include detailed medication 38 history to evaluate possible drug interactions, will be sent to the safety officer per request. Each SAE will be 39 40 assigned the plausibility of relatedness to study drug by study PIs. The data will not be presented by 41 intervention group unless requested by DSMB safety officer. These reports will be descriptive (no statistical 42 analyses). The DSMC will make recommendations regarding any imbalances in safety outcomes.

43 A single interim analysis for re-hospitalization-free survival will be prepared by the study statistician 44 using O'Brien-Fleming boundaries for benefit and harm when 50% of expected person time (350 child-years) 45 has been accrued. Assuming 157 events will be available at half of the person-time accrual, a z-score critical 46 value of 2.797, or *p*-value < 0.005, from a Kaplan Meier log-rank test will determine the cut-off of statistical 47 significance. A symmetric boundary will be used for benefit and harm. The DSMC will review this analysis and 48 make a determination about study continuation. Futility will not be a basis for stopping rules because of the 49 trials' value in understanding mechanisms of post-discharge worsening and antibiotic resistance. Assuming the 50 DSMC decides to continue the trial after the interim analysis, an alpha of 0.045 will be used as the statistical 51 significance boundary at the final analysis. 52

53 54 **Statistical Power**

⁵⁶ To compare rates of re-hospitalization and mortality in the 6 months following hospital discharge among ⁵⁷ Kenyan children receiving 5-day azithromycin vs. placebo ⁵⁸ The total sample size required was calculated for the primary endpoint of time to death or hospital re-

The total sample size required 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, and a

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ratio of treatment to placebo random assignment of 1:1. In SSA, it is estimated that 2-15% of children aged less than 5 years died within 6 months of hospital discharge and 15.5% of children who survived discharge from the district hospital were re-admitted with the same diagnosis within 6-months.^{2 4 9} Assuming that an additional 5-10% of children are re-admitted for other conditions, we expect that re-hospitalizations will occur in 20.5 to 30.5% of children enrolled in the study. Combined with our expected fatality rate (2-15%), we expect the cumulative incidence of the combined endpoint to range from 22.5 to 45.5%.⁹ Based on a previous trial of mass drug administration of a single dose of azithromycin in which a single dose of the antibiotic was associated with a 49% reduction in risk of death, we calculated sample sizes using estimates of reduction in 10 risk ranging from 30-50% with the cumulative incidence range of 22.5 to 45.5% in the placebo-treated group, 11 and found the sample size required ranged from 90 to 550 children per treatment arm.¹⁶ Using the most 12 13 conservative estimates of a hazard ratio of 0.70 and 22.5% prevalence of re-admission/death, we need to 14 enroll 1100 children in the study (550 per arm) to achieve adequate power. We will recruit an additional 300 15 children (≈20%) to account for possible loss to follow-up, resulting in a total planned enrollment of 1400 16 children, or 700 per treatment group. When considering mortality alone, and estimated mortality ranges of 2-17 17% among place-treated children, we will have >80% power to detect hazard ratios ≤0.5 for mortality rates of 18 \geq 8% and hazard ratios \leq 0.6 for mortality rates \geq 11% (Figure 1). 19

20 To evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality, by comparing 21 reasons for re-hospitalization and prevalence of pathogen carriage between the randomization arms 22

We calculated the minimum detectable association between treatment arm and cause-specific re-23 24 hospitalizations (hospitalization due to diarrhea, acute respiratory infection, malnutrition, or malaria vs. any 25 other) among enrolled children, assuming an alpha level of 0.05, power of 0.80, and a ratio of treatment to 26 placebo of 1:1. Based on data from Kenya, re-hospitalization rates due to specific causes ranged from 27 approximately 0.5% to 5.7% in the 6 month post-discharge period.⁴ By not conditioning on the child having the 28 same diagnosis as the initial hospitalization, we expect the cumulative incidence of cause-specific re-29 hospitalizations to range from 2.5% to 10%. With this range of outcome rates, we will be able to detect hazard 30 ratios of 0.48 to 0.70 for the effect of azithromycin on specific severe morbidities. 31

We expect 56% of children in the placebo group to have *S. pneumoniae* isolated from nasopharyngeal 32 swabs, providing \geq 80% power to detect a prevalence ratio of 0.85 (or 1.15) between the two treatment arms at 33 each time point.²⁹⁻³¹ Based on prevalences of *Shigella*, *Salmonella*, *Campylobacter*, *Cryptosporidium*, *Giardia* 34 among asymptomatic children in Western Kenya, we expect 10% of children in the placebo group to have a 35 bacterial pathogen isolated at each time point, resulting in $\geq 80\%$ power to detect differences in enteric 36 pathogen prevalences of 0.67 (1.49) at each time point.³² 37 38

39 To determine whether empiric administration of azithromycin at hospital discharge increases risk of antibiotic 40 resistance in commensal E. coli and S. pneumoniae isolates from treated children and their household 41 contacts

42 We will select a random selection of 400 E. coli 43 and 400 S. pneumoniae isolates (200 per arm) for ß-44 lactam and macrolide resistance testing at each 45 timepoint. We will also store all S. pneumoniae, E. coli 46 isolates and other isolated bacteria from stool for 47 potential future testing in the event that resistance 48 prevalence is lower than expected. As shown in Table 4, 49 we will have > 80% power to detect prevalence ratios > 50 1.1, with an ability to detect the smallest effect sizes 51 52 when the prevalence of resistance in the placebo group is 53 highest. We will enroll 300 adults in the Contact Cohort 54 for E. coli and S. pneumoniae isolation. We expect E.coli 55 to be isolated from all adults and S.pnuemoniae isolated 56 from between 5-55%.^{29 33 34} Assuming an alpha of .05, a 57

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S.pheumoniae isolales per treatment group										
		Resistance prevalence (%) in placebo								
					group					
(%		10	20	30	40	50	60	70		
e (9	10									
prevalence (%) mycin group	20	80								
valo	30	>99	64							
pre my	40	>99	99	55						
Resistance preval in azithromycin	50	>99	>99	98	48					
star azit	60	>99	>99	>99	>99	52				
esi in	70	>99	>99	>99	>99	98	55			
Н	80	>99	>99	>99	>99	>99	99	64		

 Table 4. Power (%) to detect prevalence ratios of macrolide
 and β-lactamase resistance in 200 E.coli and 200 S preumoniae isolates per treatment group

1:1 ratio of testable isolates, and a prevalence of resistance of 50% in the placebo arm, we will have 80% 58 power to detect a 1.4-fold higher prevalence to 1.9-fold higher resistance prevalence in the contacts of 59 azithromycin-treated children. 60

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13 14 To identify correlates and intermediate markers of post-discharge mortality and hospital-readmission among hospitalized Kenyan children

Conservatively estimating a 20% loss-to-follow-up rate in the RCT and a cumulative incidence of death or re-hospitalization of 22.5%, we will have >80% power to detect hazard ratios ≥1.3 between correlates and the outcome with exposure prevalences of $\geq 20\%$ or more and hazard ratios ≥ 1.5 for exposure prevalences <20%.

Study timeline

The trial began on June 28, 2016 and participant recruitment and follow-up will continue over a 36month period, with anticipated final follow-up visit(s) occurring in June 2019. Primary analyses will be complete by February 2020.

15 Potential Challenges and Limitations 16

In order to ensure adequate power to detect a discernable clinically relevant difference between study 17 18 groups in the primary outcome, we have combined hospital readmission with death. Preliminary studies 19 suggest that sufficient numbers of children will reach this combined outcome. However, we have incorporated 20 an interim analysis by the DSMB to review the accrued data and an adapted sample size could be considered 21 if the combined event frequency is less than predicted. It is possible that since most children receive antibiotics 22 during hospitalization, the benefit anticipated with the use of azithromycin based on previous trials of mass 23 drug administration will not be observed. However, most hospitalized children are treated with penicillins, 24 cephalosporins, gentamicin, or cotrimoxazole while in hospital and the broad spectrum of activity (including 25 malaria prevention) and long half-life of azithromycin suggest that there may be additive treatment and/or 26 prophylactic benefit. Similarly, children may receive azithromycin during follow up - either as treatment for an 27 illness or because the caregiver sought out azithromycin upon learning of the hypothesis - and this 28 azithromycin use may lead to contamination in the placebo-arm. After discharge, it is difficult to ensure 29 adherence with the full 5-day treatment course. We will measure adherence using three different measures 30 31 (text message responses, bottle check boxes, and caregiver-report at follow-up visits) although all are limited 32 by caregiver-report. In addition, the mortality benefit of azithromycin observed in Ethiopia was from a single 33 dose and in this study the first dose will be directly observed.¹⁶ While relying on caregiver report of mortality 34 and morbidity may lead to bias due to outcome misclassification, this misclassification should not differ 35 between randomization arms and therefore will be non-differential. Further hospital records will be used when 36 available to determine diagnoses. Finally, resistance prevalence may be lower than predicted, limiting power to 37 detect clinically relevant differences in resistance prevalence between the intervention arms. We will store all 38 isolates in the event that a greater number of isolates are needed for antibiotic resistance testing. 39

40 Ethics and Dissemination 41

This study has received IRB approval by the University of Washington Human Subjects Division (HSD), KEMRI 42 Scientific and Ethics Review Unit (SERU), and the Kenva Pharmacy and Poisons Board. The clinical trial is 43 44 also registered with clinicaltrials.gov (NCT02414399). Any modifications to the study protocol or consent 45 materials will be submitted for approval all regulatory authorities before implementation. The study is being 46 externally monitored and a data safety and monitoring committee has been assembled to monitor patient 47 safety and to evaluate the efficacy of the intervention. Results of this study will be disseminated by publication 48 in a peer-reviewed scientific journal, presented at relevant academic conferences, and amongst participating 49 partners and health facilities in Kenya. 50

51 Author's contributions 52

JLW, PBP, GJS, BAR, BOS, and RN conceived of this trial and developed of this study protocol. JLW and BOS 53 are study co-Principal Investigators and PBP is the Project Director: BAR oversaw the statistical analyses 54 plans; JBB developed the CEA plan; KDT developed procedures for ascertaining and reporting SAEs; CJM 55 developed procedures related to blood specimen procedures and drug adherence measurement. GJS, CJM, 56 57 RN, and PBP provide scientific expertise. RLB and MA are involved in collection and management of the data. 58 MA and PBP coordinate and oversee implementation of all clinical study procedures and SK, with assistance 59

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from DR, oversees all laboratory procedures. All authors contributed to the development of this manuscript and/or study procedures, and to reading and approving the final version for publication.

Funding statement

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38 39 This work was funded by the National Institutes of Health Eunice Kennedy Shriver National Institute of Child Health & Human Development, grant number R01 HD079695.

9 10 Acknowledgements

Pfizer donated the Zithromax® to be used in this clinical trial, and Copan Diagnostics donated all rectal swabs 11 and Cary-Blair media. Investigators from KEMRI-Wellcome Trust Kilifi, Jay Berkley, Anthony Scott, Joseph 12 13 Waichungo, Angela Karani, Donald Akech, and Horace Gumba provided microbiology expertise and training in 14 nasopharyngeal swab collection, STGG media preparation, and laboratory guality assurance and control. Liru 15 Meshack Wekesa and George Bogonko provide clinical expertise and facilitate the study's integration into 16 pediatric wards at two of the study hospitals, Homa Bay District and Kisii Teaching & Referral Hospital, 17 respectively. Alex Awuor and Caleb Okonji, with the support of Richard Omore, provided training in 18 anthropometric measurement. We are extremely thankful to Dr. Philip Walson who developed azithromycin 19 dosing regimens. Hannah Atlas and Stephanie Belanger contributed to the standard operating procedure and 20 case report form development and implementation. Gillian Levine played an invaluable role in the proposal 21 development. 22

Competing interests statement

None of the authors or study co-investigators have any competing interests to declare.

Figure Legend

Figure 1. Power and detectable hazard ratios assuming a range of mortality rates from 2-17%

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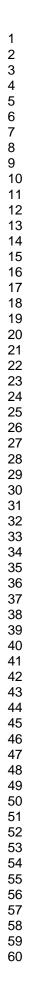
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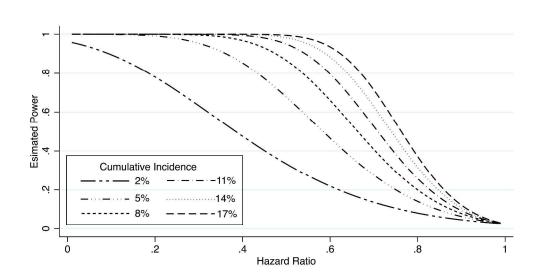


Figure 1. Power and detectable hazard ratios assuming a range of mortality rates from 2-17%

644x332mm (300 x 300 DPI)





SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ltem No	Description	Addressed on page number
Administrative inf	ormatior		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	
Roles and	5a	Names, affiliations, and roles of protocol contributors	
responsibilities	5b	Name and contact information for the trial sponsor	<u> </u>
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	

1 2	Introduction			
3 4 5	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	
6 7		6b	Explanation for choice of comparators	
8 9	Objectives	7	Specific objectives or hypotheses	
10 11 12 13	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	
14 15	Methods: Participa	nts, inte	erventions, and outcomes	
16 17 18 19	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	
20 21 22	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	
23 24 25	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	
26 27 28 29		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	
30 31 32		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	
33 34		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	
35 36 37 38 39 40	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
41 42 43	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	
44 45 46 47			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	2

1 2 3 4 5 6 7 8 9 10 11 23 4 5 6 7 8 9 10 11 23 14 5 6 7 8 9 10 11 2 3 4 5 6 7 8 9 10 11 2 3 4 5 6 7 8 9 10 11 2 3 4 5 6 7 8 9 10 11 2 3 4 5 6 7 8 9 10 11 12 10 10 10 10 10 10 10 10 10 10 10 10 10	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations					
	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size					
	Methods: Assignment of interventions (for controlled trials)							
	Allocation:							
	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions					
	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned					
21 22 23	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to					
24 25 26 27	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcomeassessors, data analysts), and how					
27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial					
	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol					
		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols					
			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	3				

Page	19	of	20
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$\begin{array}{c}1&2&3&4&5&6&7\\8&9&10&1&12&1&1&1&1&1&1&1&1&1&1&1&1&1&1&1&1$	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality(eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol				
	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol				
		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)				
		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)				
	Methods: Monitoring						
	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of				
		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim				
	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverseevents and other unintended effects of trial interventions or trial conduct				
	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor				
	Ethics and dissemi	nation					
	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval				
	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)				
			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	4			

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and	_			
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary	_			
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained	_			
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_			
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that	_			
Ancillary and post- trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_			
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	_			
	31b	Authorship eligibility guidelines and any intended use of professional writers	_			
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_			
Appendices						
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_			
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_			
It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported" license.						
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