**Azithromycin (AZM) to prevent post-discharge
Morbidity and mortality in Kenyan children
(Toto Bora Trial)**

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ABBREVIATIONS & ACRONYMS

AZM	Azithromycin
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
CRP	C-reactive protein
DBS	Dried Blood Spot
ERC	Ethical Review Committee
EQA	External quality assurance
GCLP	Good Clinical and Laboratory Practice
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
IRIS	Immune reconstitution inflammatory syndrome
KEMRI	Kenya Medical Research Institute
MCH	Maternal Child Health
PBMC	Peripheral blood mononuclear cell
PITC	Provider-initiated testing and counseling
PMTCT	Prevention of Mother-to-Child Treatment
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
TB	Tuberculosis
UW	University of Washington
VL	Viral load (HIV-1 RNA copies/ml)
WHO	World Health Organization

SUMMARY

An estimated 3.5 million deaths occur annually in children less than 5 years of age in sub-Saharan Africa, approximately 70% of which are due to infectious causes.[6] One-year mortality rates as high as 15% have been documented following hospital discharge in sub-Saharan Africa, a rate that is 8-fold higher than non-hospitalized children.[7-9] Children being discharged from hospital in Africa may represent an accessible high-risk population in which to target interventions to reduce mortality.

A recent trial of mass drug administration of azithromycin reduced childhood mortality by half among children in Ethiopia in communities receiving the intervention.[10, 11] However, concerns about the potential for the emergence of antimicrobial resistance, possible toxicity, and the feasibility of delivery are all barriers to community-wide distribution of antibiotics.

Targeted chemotherapeutic interventions, including the use of cotrimoxazole among HIV-infected children and the use of amoxicillin or cefdinir among malnourished children, have been shown to reduce mortality in these specific vulnerable populations.[12-15] Children who have been recently hospitalized are a high-risk population in which a similar targeted approach to azithromycin distribution may optimize benefit while reducing both individual and population level risks.

The mechanisms by which azithromycin may impact morbidity and mortality have not been well described. Among high-risk pediatric populations with history of recent illness, azithromycin may act by treating residual disease not eliminated during inpatient therapy, by providing prophylaxis from future infectious exposures during a time of immune suppression and vulnerability following illness, by treating underlying enteric dysfunction and associated mucosal immune/gut barrier disruption and inflammation, and/or by clearing asymptomatic carriage of potentially pathogenic organisms.

We propose a randomized, double-blind, placebo-controlled trial of a 5-day course of azithromycin in children age 1 to 59 months discharged from health facilities in Kisii and Homa Bay Counties in Western Kenya to reduce post-discharge re-hospitalizations and mortality, to explore possible mechanisms by which azithromycin has benefit and risk, and to identify correlates and intermediate markers of re-hospitalization and death in the post-discharge period.

The proposed Toto Bora study will examine a pragmatic post-discharge intervention that could be used in low resource settings to lower mortality and re-hospitalization rates in high-risk children. In addition the trial will also explore a number of scientific questions including the effect of antibiotics on enteric and nasopharyngeal infections, inflammation, and the causes of post-discharge mortality. Kisii and Homabay counties are the ideal areas in Western Kenya to test this intervention because the child mortality rate is high in Nyanza province yet the health facilities in which this Toto Bora study will be conducted have established pediatric research infrastructure.

BACKGROUND

Interventions to reduce child mortality in sub-Saharan Africa (SSA) are not achieving necessary impact. Mortality in children under 5 remains extremely high in SSA, exceeding 100 deaths per 1,000 live births.[12] Three quarters of these deaths (73.2%) are due to preventable and treatable infectious causes, including diarrhea, pneumonia, and malaria.[12, 13] Although some progress has been made in decreasing child mortality over the last decade in parts of SSA, it is unlikely that the global community will meet child mortality targets stated in the Millennium Development Goals without additional effective interventions.[6, 13, 14] Novel interventions targeting highest risk individuals are urgently needed to reach these goals.

The health care encounter is an important point of intervention for reducing morbidity and mortality. The health care encounter represents a point of critical opportunity, where children have already accessed health care services and interventions targeting severe illness can save lives (Figure 1).[3-5] The majority of deaths in children under 5 in SSA are due to infectious diseases, including respiratory infections, diarrheal disease, and malaria.[13-15] Interventions to improve survival from these diseases have largely focused on improving community diagnosis and management of these illnesses and on increasing access to effective medical care.[16, 17] While a substantial portion of children with these illnesses die prior to seeking care, significant numbers of children in SSA with severe illness do encounter the health care system. Studies of care seeking

Figure 1. Mortality due to malaria, pneumonia and diarrhea[3-5]

have shown that up to one-half of children with acute respiratory illness and diarrheal disease do seek health care in most SSA countries and that care seeking rates may be significantly higher (up to 80%) among children with severe disease.[18, 19] Interventions to improve health care seeking are important, but are limited by the availability and affordability of transportation, access to health care providers, cost of care, and social and cultural barriers.[20] Expanding access to clinical services has been shown to be the most effective method to reduce under-5 mortality; resulting in larger reductions in mortality than outreach, family or community-based service programs.[21, 22]

Many children in sub-Saharan Africa who seek medical care suffer recurrent illness and/or death in the immediate period after the health care encounter. Although accessing health care interventions can reduce immediate risk, recurrent morbidity and mortality among those children who survive hospitalization remains extremely high. The risk of death among children with history of recent hospitalization is 6 to 8-fold higher than similarly aged children in the community. While this increased risk is highest in the period immediately following discharge (up to 30 days), mortality risk remains elevated up to two years period post-discharge (Tables 1a and 1b).[7-9, 23-25] Re-hospitalizations are also associated with as much as a 23.6-fold increased risk of death in SSA settings.[7] In addition, the cost of a hospitalization for diarrhea or malaria often exceeds the average household monthly income ($60 USD) in Western Kenya and this impact on the economic stability of a household further contributes to malnutrition and poor health.[26-28]
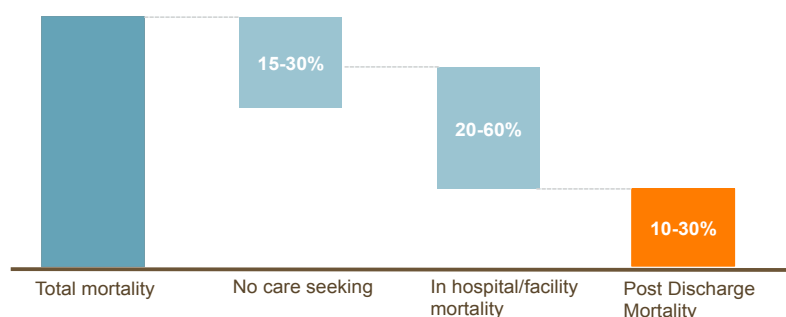
Table 1a. Post-discharge mortality in low-resource settings

Study	Location	Population	Post-discharge mortality	Risk factors
Moisi 2011[7]	Kilifi, Kenya	Children <15 years discharged from hospital	3.3% within 12 months	Age <5 years, hospital stay >13 days, previous discharges, severe malnutrition, meningitis, pneumonia
Snow 2000[9]	Kilifi, Kenya	Children followed in surveillance cohort with a hospital admission	1.4% within 3 months	Gastroenteritis
Zucker 1996[23]	Western Kenya	Children <5 years admitted to hospital	15% within 8 weeks	Young age, severe anemia
Veirum 2007[8]	Guinea-Bissau	Children admitted to hospital	7.5% within 12 months	Young age, ethnicity, self- discharge, poor housing quality, low maternal education, anemia, diarrhea
Phiri 2008[29]	Malawi	Children 6-60 months hospitalized with severe anemia	12.6% within 18 months	HIV, younger age
Roy 1983[24]	Bangladesh	Children 3 months-3 years discharged following treatment for diarrhea	4.2% within 12 months	Age 24-35 months, moderate and severe malnutrition
Villamor 2005[30]	Tanzania	Children 6-60 months hospitalized with pneumonia	10.4% within 24 months	Age <24 months, HIV, stunting, low MUAC, anemia, and lack of water supply in the household
Islam 1996[31]	Bangladesh	Children 1-23 months hospitalized with diarrhea	7.5% within 3 months	Age < 6 months, malnutrition, not breastfeeding, lack of immunization

Table 1b. Hospital re-admission in low-resource settings

Study	Location	Population	Re-admission
Moisi 2011[7]	Kilifi, Kenya	Children <15 years discharged from hospital	17.7% over follow-up period (avg 2.8 years)
Snow 2000[9]	Kilifi, Kenya	Children followed in surveillance cohort with a hospital admission	40.9% over total follow-up period (9% within 3 months)
Veirum 2007[8]	Guinea-Bissau	Children admitted to hospital	20.1% re-admitted over 1 year follow-up
Phiri 2008[29]	Malawi	Children 6-60 months hospitalized with severe anemia	9.4% within 18 months (17.2% among anemia cases)

Illness following discharge from hospital is often the result of preventable and treatable infectious diseases. Infectious morbidity and subsequent mortality following hospitalization may be due to incompletely or inadequately treated infection, nosocomial infections that present following discharge, or new infections resulting from repeated exposure once the child returns to the community. In a large study of 1148 children admitted to hospital in Kenya, more than a quarter (27%) were readmitted following discharge, with over half of those admission occurring within 6 months. Of these admissions, almost half (47.8%) were due to malaria, 26.4% due to acute respiratory disease, and 8.2% due to gastroenteritis.[9] In Western Kenya, over three quarters of all early deaths following discharge occurred among children who were originally hospitalized with malaria or bacteremia, while in Bangladesh, all children who died following hospitalization for diarrhea had persistent symptoms of gastroenteritis preceding death.[17, 23, 24] Nosocomial infections also contribute significantly to recurrent illness following hospitalization and are often unrecognized at discharge. In Kenya, nosocomial bacteremia was documented in almost 6/1000 admissions, with a mortality rate that was more than twice that seen for community-acquired bacteremia (53% vs. 24%).[32] Finally, children discharged from hospital usually return to the communities and exposures from which they originally came. As a result, repeated exposure to community-acquired infections (including malaria, acute respiratory infection, and diarrhea) may contribute to post-hospitalization morbidity and mortality. For example, in Kenya, diarrheal disease and malaria are the most significant diseases contributing to mortality within three months of discharge.[9, 23] Targeted antimicrobial interventions to cover residual infection, reduce carriage of pathogens, and provide prophylaxis against common exposures may significantly impact infectious morbidity and mortality in the post-discharge period.

Underlying conditions associated with increased risk of recurrent infectious disease are common among hospitalized children in Africa. Many children hospitalized in Africa have underlying malnutrition, HIV-infection or -exposure, and/or enteric dysfunction, placing them at high risk of recurrent illness and death from infectious causes.[33-38] In Kenya, two thirds or more of all pediatric hospital admissions, and up to half of all pediatric deaths among hospitalized children, are attributable to malnutrition.[39, 40] Malnourished children have a 14-fold increased risk of death following hospitalization for diarrhea, compared with well-nourished children.[40] In addition, enteric dysfunction as a result of malnutrition, environmental enteropathy, HIV, or parasitic infection affects many children in SSA and may significantly impact risk of diarrheal disease and invasive infection.[41, 42] Despite increased efforts to improve the in-hospital diagnosis of these conditions, many children remain undiagnosed despite being hospitalized.[43-45]

Targeted antimicrobial interventions have demonstrated benefit in these high-risk pediatric populations. Antimicrobial prophylaxis has been shown to decrease mortality in selected high-risk pediatric populations including children with HIV and those with malnutrition. Both amoxicillin and cefdinir have been shown to reduce risk of death among children with severe malnutrition who did not have evidence of systemic infection.[46] Similarly, prophylactic cotrimoxazole has dramatically reduced mortality among HIV infected children in SSA.[36] Many hospitalized children with underlying comorbidities are not being diagnosed with these comorbidities or experience delays in the initiation of care and treatment. The administration of antibiotics to all children at hospital discharge may be particularly effective among those children with underlying comorbid conditions.[47]

Community mass drug administration of antimicrobials has been shown to reduce child mortality in SSA. The mass drug administration of azithromycin to communities in Ethiopia with endemic trachoma was recently shown to reduce mortality among children 1-9 years of age by almost half (49%).[10, 11] Child mortality in these communities was largely due to infectious causes, including malaria, fever, diarrhea or respiratory infection.[10] In another study, a single oral dose of azithromycin provided to children in the context of trachoma control resulted in an 80% reduction in infectious mortality among children 1-5 years of age.[11] Single-dose community-administered azithromycin was associated with decreased mortality over one year of follow-up. Although the mechanism for this observation is not well defined, the broad-spectrum antibiotic coverage and potential effect on pathogen carriage suggest potential benefit in children at risk of re-hospitalization or death.

Mass administration of azithromycin leads to the rapid emergence of widespread macrolide resistance within communities. In a randomized comparison of resistance in nasopharyngeal pneumococcus isolates, communities randomized to azithromycin had increased resistance at 12 months (46.9%) compared to those receiving no azithromycin (9.2%). However, administration of azithromycin did not increase resistance to clindamycin, penicillin, or tetracycline.[48] Although resistance to azithromycin among pneumococcal isolates appears to subside when treatment programs are stopped, the potential impact of community-wide antibiotic administration on prevalence of resistance and treatment failure is of concern.[49, 50] The risk of developing resistance is lower with targeted administration of antibiotics than with community-wide administration. By targeting subgroups of high-risk individuals for empiric antibiotic administration, such as children recently discharged from hospital, the risk of community level resistance can be reduced considerably.

JUSTIFICATION

Many children in sub-Saharan Africa who seek medical care suffer recurrent illness and/or death in the immediate period after the health care encounter. Although accessing health care interventions can reduce immediate risk, recurrent morbidity and mortality among those children who survive hospitalization remains extremely high. Illness following discharge from hospital is often the result of preventable and treatable infectious diseases. Additionally, many hospitalized children with underlying comorbidities are not being diagnosed with these comorbidities or experience delays in the initiation of care and treatment. As such, the administration of antibiotics to all children at hospital discharge may be particularly effective among those children with underlying comorbid conditions. Therefore, targeted antimicrobial interventions to cover residual infection, reduce carriage of pathogens, and provide prophylaxis against common exposures may significantly impact infectious morbidity and mortality in the post-discharge period.

Although some progress has been made in decreasing child mortality over the last decade in parts of SSA, it is unlikely that the global community will meet child mortality targets stated in the Millennium Development Goals without additional effective interventions.[6, 13, 14]. Therefore, novel interventions targeting highest risk individuals are urgently needed to reach these goals. Our study aims to reduce post-discharge re-hospitalizations and mortality, to explore possible mechanisms by which azithromycin has benefit and risk, and to identify correlates and intermediate markers of re-hospitalization and death in the post-discharge period as a novel intervention towards decreasing child mortality.

STUDY OBJECTIVES

Primary Aims
Aim 1. To compare rates of **re-hospitalization and mortality** in the 6 months following hospital discharge among Kenyan children receiving 5-day azithromycin vs. placebo.
   Hypothesis: The provision of a 5-day course of azithromycin provided at discharge will reduce hospital re-admission and death within the 6 months following discharge, as compared to placebo.
Aim 2a. To evaluate possible **mechanism(s)** by which azithromycin may affect morbidity and mortality, by comparing **reasons for re-hospitalization, prevalence of pathogen carriage, and markers of enteric dysfunction** between the randomization arms.
   Hypothesis: Children treated with azithromycin will experience fewer hospitalizations due to diarrhea, acute respiratory infection, and malnutrition, will be less likely to have respiratory and gastrointestinal carriage of potentially pathogenic organisms, and will have less evidence of enteric dysfunction, as compared to children treated with placebo in the 6 months following hospital discharge.
Aim 2b. To determine whether empiric administration of azithromycin at hospital discharge **increases risk of antimicrobial resistance** in commensal *Escherichia coli* (*E. coli*) and *Streptococcus pneumoniae (S. pneumoniae)* isolates from treated children and their household contacts.
   Hypothesis: Isolates of commensal E. coli and S. pneumoniae from children treated with azithromycin and their household contacts will have higher levels of macrolide and β-lactam resistance, compared to the placebo group, after 90 days of follow-up, but resistance in the 2 arms will be similar by 6 months.
Aim 3. To identify **correlates and intermediate markers of post-discharge mortality and hospital readmission** among hospitalized Kenyan children.
   Hypothesis: Children younger in age, with enteric dysfunction, higher levels of bacterial pathogen carriage, immune dysfunction, and malnutrition will experience more frequent re-hospitalizations and deaths.
Aim 4. To determine the **cost-effectiveness** of post-discharge administration of a 5-day course of azithromycin in settings of varying antibiotic use, re-hospitalization rates, and mortality rates.

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

METHODS

Study Design

A randomized, double-blind, placebo-controlled clinical trial. Table 4 outlines outcomes, eligibility and follow-up proposed for the randomized trial cohort and Figure 3 outlines the longitudinal design.

Table 4. Study summary

Study Design:	Randomized, double-blind, placebo-controlled clinical trial
Primary Outcome:	Mortality or hospital re-admission
Inclusion criteria:	Children (age 1-59 months) admitted to hospital for non-trauma conditions and subsequently discharged who weigh 2 kg or more and plan to remain in study area for at least 6 months.
Exclusion criteria:	Contraindication to azithromycin use (the use of another macrolide antibiotic [i.e. erythromycin or clarithromycin]); known macrolide allergy; current use of Lopinavir; residence in an orphanage or children's home; previous participation in the Toto Bora Trial; sibling enrolled in Toto Bora Trial on same day.
Target enrollment:	1400 (700/arm)
Intervention	5-day course of azithromycin or placebo
Duration of follow-up:	6 months (enrollment, 90 days, and 6 months) (Figure 3)
Sampling framework:	All children hospitalized for non-traumatic cause, and subsequently discharged, from study sites during recruitment period

Eligibility

Children age 1 to 59 months old who weigh at least 2 kg and have been hospitalized and subsequently discharged from hospitals in Kisii or Homa Bay County for non-traumatic conditions will be eligible for the RCT (Aim 1). We will exclude children in whom azithromycin is contraindicated, such as those children currently taking another macrolide antibiotic, such as erythromycin or clarithromycin, those with a known macrolide allergy, and those who do not plan to remain in the study site catchment area for at least 6 months. A random subset of 300 children (150 from each arm) will be selected into the **Enteric Function Cohort**

Figure 3. Proposed study design
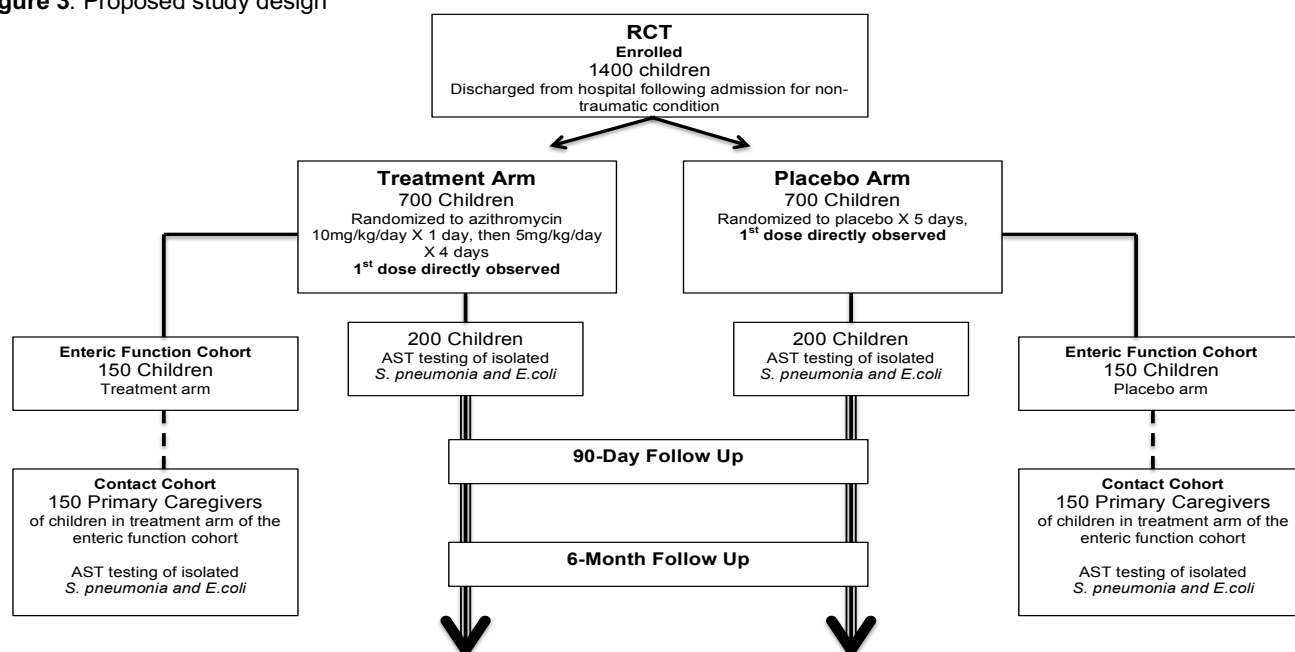
(Aims 2 and 3). We will also enroll a primary caregiver of each the 300 children enrolled in the **Enteric Function Cohort** in the **Contact Cohort** (Aim 2b). These caregivers must be at least 18 years of age or classified as an emancipated minor by Kenyan law, plan to remain in study site catchment area for at least 6

months and be willing/able to provide a stool and nasopharyngeal sample at enrollment, 90 days and 6 months of follow-up if randomized to the **Contact Cohort**.

Sample Size

Aim 1. To compare rates of re-hospitalization and mortality in the 6 months following hospital discharge among children receiving 5-day azithromycin vs. placebo. The total sample size required was calculated for the primary endpoint of time to death or hospital re-admission within the 6 month post-discharge period, assuming an alpha level of 0.05, power of 0.80, and a ratio of treatment to placebo random assignment of 1:1. In sub-Saharan Africa, it is estimated that 2-15% of children aged less than 5 years died within 6 months of hospital discharge 15.5% of children who survived discharge from the district hospital re-admitted with the same diagnosis within 6-months[7, 9, 25] Assuming

Figure 6. Power and detectable hazard ratios given a range of mortality rates

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%.[25] 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 risk ranging from 30-50% with the cumulative incidence range of 22.5 to 45.5%, and found the sample size required ranged from 90 to 550 children per treatment arm.[10] Using the most conservative estimates of a hazard ratio of 0.70 and 22.5% 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 (≈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 2-15%, 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 6). Although the ability to determine differences in mortality between arms will require either a high mortality rate or a large measure of effect, in Kenya, re-hospitalization is associated with as much as a 23.6-fold increased risk of death, indicating that this outcome is itself a severe outcome and an intermediate marker of death.[7]

Aim 2a. To evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality, by comparing reasons for re-hospitalization, prevalence of pathogen carriage, and markers of enteric dysfunction between the randomization arms. We calculated the minimum detectable association between treatment arm and cause-specific re-hospitalizations (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, with the lowest rate reported for gastroenteritis and the highest for malaria.[9] By not conditioning on the child having the same diagnosis at initial hospitalization, we expect the cumulative incidence of cause-specific re-hospitalizations 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.

Among the 1400 children in whom nasopharyngeal swabs and stool samples are cultured, we expect 56% of children in the placebo group to have *S. pneumoniae* identified based on data from Kenya, providing ≥80% power to detect a prevalence ratio of 0.85 (or 1.15) between the two treatment arms at each time point.[51-53] Because there is a strong link between carriage of *S. Pneumoniae* and development of associated disease, and because empiric azithromycin has been shown to prevent reduce clinical pneumonia by as much as 75% in an outbreak setting, we believe that our ability to detect a 15% reduction in risk is appropriately sensitive.[54, 55] Based on prevalence of *Salmonella spp.*, *Shigella spp.*, *Campylobacter spp.*, or diarrheagenic *E.coli* (ETEC, EHEC, EPEC, and EAEC) among asymptomatic children in Western Kenya, we expect 20% of children in the placebo group to have a bacterial pathogen isolated at each time point, resulting in ≥80% power to detect differences in enteric pathogen prevalence of 0.68 (1.36) at each time point.[48]
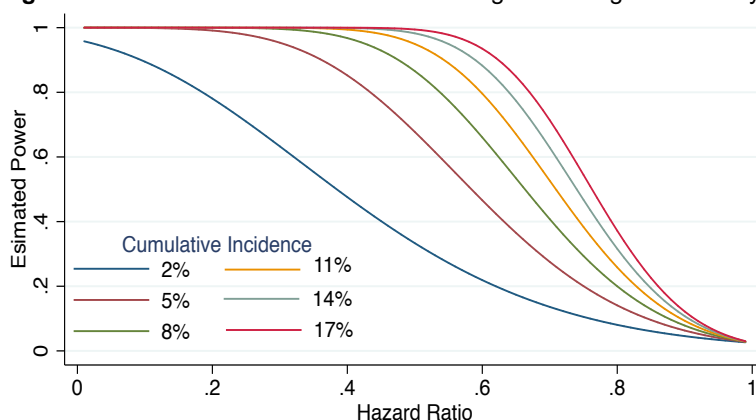
To determine the number of children needed to evaluate the effect of azithromycin on biomarkers of enteric inflammation children in the **Enteric Function Cohort,** we assumed an alpha level of 0.05, power of 0.80, and a ratio of treatment to placebo of 1:1. For the outcome of enteric inflammation, and assuming a clinically relevant unit mean difference of 2.8mg/L, and a standard deviation of 7.8mg/L, 250 individuals would need to be enrolled to achieve adequate power to detect associations between the intervention groups.[56, 57] We will enroll 300 children (150 per arm) in this cohort in order to account for 20% potential loss to follow-up.

Aim 2b. To determine whether empiric administration of azithromycin at hospital discharge increases risk of antimicrobial resistance in commensal Escherichia coli (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. 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 7, we will have > 80% power to detect prevalence ratios > 1.1, with an ability to detect the smallest effect sizes when the prevalence of resistance in the placebo group is highest.

While we expect that all 300 adults in the Contact Cohort will have *E. coli* isolated and available for resistance testing, ranges for adult *S. pneumoniae* carriage in Sub-Saharan Africa range from 5-55%. Therefore, we expect 15 to 165 *S. pneumoniae* isolates to be available for resistance testing.[51, 58, 59] Assuming an alpha of .05, a 1:1 ratio of testable isolates, and a prevalence of resistance of 50% in the placebo arm, we will have 80% power to detect a 1.4-fold higher prevalence to 1.9-fold higher resistance prevalence in the contacts of azithromycin-treated children.

Aim 3. To identify correlates and intermediate markers of post-discharge mortality and hospital-readmission among hospitalized 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 prevalence of ≥20% or more and hazard ratios ≥1.5 for exposure prevalence <20%.

Table 7. 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 (%)	Resistance prevalence (%) in placebo group						
	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

PROCEDURES

Recruitment

We will recruit children who have been hospitalized, and subsequently discharged, from health facilities in Kisii County, including Kisii Teaching & Referral Hospital, Christamarriane Hospital, Tabaka Mission Hospital and Homa Bay County, including Homa Bay District Hospital, Kendu Adventist Hospital, and St. Paul Mission Hospital. Study staff will work closely with hospital staff to determine when a potentially eligible child is going to be discharged from the hospital. After a child has been scheduled for discharge from hospital, the study nurse will approach the primary caregiver of the child and describe the study procedures. A script will be read to the caregiver to obtain consent for pre-screening of medical records and verbal screening. Study staff will explain that the child/caregiver might be randomly selected to provide additional samples (stool/rectal swab and nasopharyngeal swab) and therefore all caregivers who agree to participate will have agreed to take part in the **Enteric Function** and **Contact Cohorts** if randomized to these cohorts. 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 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. We expect to enroll approximately 40 children in the RCT cohort per month from each of the two proposed study sites to reach the target enrollment of 1400 children within 36 months. Each of the proposed study sites records approximately 120 non-trauma pediatric admissions per month. A sub-set of 150 children from each randomization arm (**Enteric Function Cohort)** and their primary caregiver (**Contact Cohort**) will be randomly selected to participate in the nested cohorts. We will enroll approximately 10 children and 10 primary caregivers per month into these cohorts to reach a target enrollment of 300 children and 300 primary caregivers within 24-36 months.

Enrollment

At enrollment, primary caregivers will be interviewed to assess demographic information, medical history, and detailed contact information for the child. 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/length, weight, and mid-upper arm circumference (MUAC). The height, weight, and MUAC of the caregiver will also be collected. If laboratory results are not available from the hospitalization, HIV testing will be performed as indicated by Kenya NASCOP Guidelines (HIV testing should be performed in all children presenting to a healthcare facility).[60] Any child newly diagnosed with HIV will be referred to the HIV Care Clinics at the study sites for follow-up care and treatment. Because enrollment procedures may take up to an hour or more and on days of multiple enrollments, enrolled caregivers/children may have to wait to complete the enrollment procedures, caregivers will be provided with 200-500KSH ($2-5USD) to cover the cost of their transportation home.

Specimen collection

Enrollment, 90-days, and 6 months

All children will also be asked to provide a whole stool for enteric pathogen identification and storage. If a child cannot produce a bowel movement, flocked rectal swabs will be collected. Stool samples/swabs will be divided as follows: 1) Sample for transport to microbiology lab for bacterial culture and AST testing 2) Used immediately for point-of-care parasite testing 3) placed in sample stabilizing reagent for shipment to UW/KEMRI freezers for -80°C storage for future determination of pathogen or commensal flora using standard culture and sensitivity or molecular identification. One nasopharyngeal swab will also be collected from all enrolled children at each time point for pathogen detection using bacterial culture or molecular-based methods. All children randomized to the **Enteric Function Cohort** (300 children) will have an additional portion of stool, or additional swabs collected, shipped to UW/KEMRI in Nairobi for -80°C storage for eventual enteric inflammation assessment. 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 as described above and for future determination of pathogen or commensal flora using standard culture and sensitivity or molecular identification. Venous blood (up to 1 teaspoon [5mL]) will be collected from enrolled children at each time point. This blood will be used for malaria and HIV testing, if not already performed during the hospitalization, and will be stored for future testing of inflammatory, immune, intestinal biomarkers, and sickle-cell disease.

Randomization

Block randomization (1:1) in random sized blocks of no more than 10, stratified by site, will be used. Primary randomization will also include allocation to the **Enteric Function** and **Contact Cohorts.** Randomization for selection into the nested cohort will occur within the treatment arms, at a ratio of 1:5 (resulting in 150 per arm). The randomization code will be maintained at the UW/KEMRI central office (in Nairobi) by the Principal Investigator, an external entity who will also be responsible for labeling of study drug. Treatment allocation (once assigned) will remain blinded to the participant, the study staff and the hospital clinicians during all data collection phases of the study. Resistance testing will occur after all participants are enrolled. We will randomly select 200 *E. coli* and 200 *S. pneumoniae* isolates from children from each of the enrollment, 90-day and 6-month follow up visits for antimicrobial susceptibility testing and other isolates will be stored for possible future determination of pathogen or commensal flora using standard culture and sensitivity or molecular identification. All isolates from Contact Cohort participants will be tested for antibiotic susceptibility.

Intervention

Caregivers of enrolled children will be provided a 5-day course of oral suspension formulation azithromycin **(Zithromax® from Pfizer)** (10 mg/kg on day 1, followed by 5mg/kg/day on days 2-5) or identically appearing and tasting placebo at discharge. Study staff will directly observe administration of the first dose and will provide information-motivation-behavior (IMB) theory based counseling on the importance of adhering to the intervention.[61] 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. Five day dosing results in detectable levels of azithromycin that persist beyond 72 hours following the final dose (Fig. 4).[62] The long half-life of azithromycin exceeds that of other antibiotics, such as amoxicillin (approximately 1 hour), contributing to the effectiveness of relatively short course dosing regimens.


Figure 4. Azithromycin concentration post dosing[1, 2]
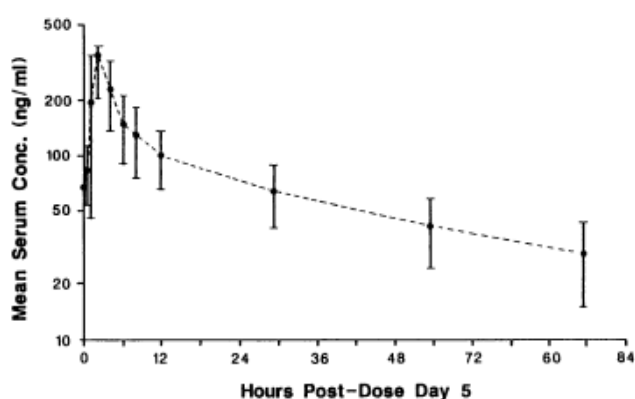
Follow-up Procedures

All enrolled children and primary caregivers will be scheduled to return to the health facility at 90 days and at 6 months following enrollment to collect clinical information and stool and nasopharyngeal samples. Anthropometric measurements will be obtained from all children at both follow up visits (height/length, weight, MUAC) and caregivers will be asked about any hospitalizations occurring since the last time the child was seen by study staff. A flowchart of follow-up and sample collection is shown in Figure 5. 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. Study staff will use a standardized questionnaire to ascertain history of recent

Figure 5. Data collection throughout follow-up
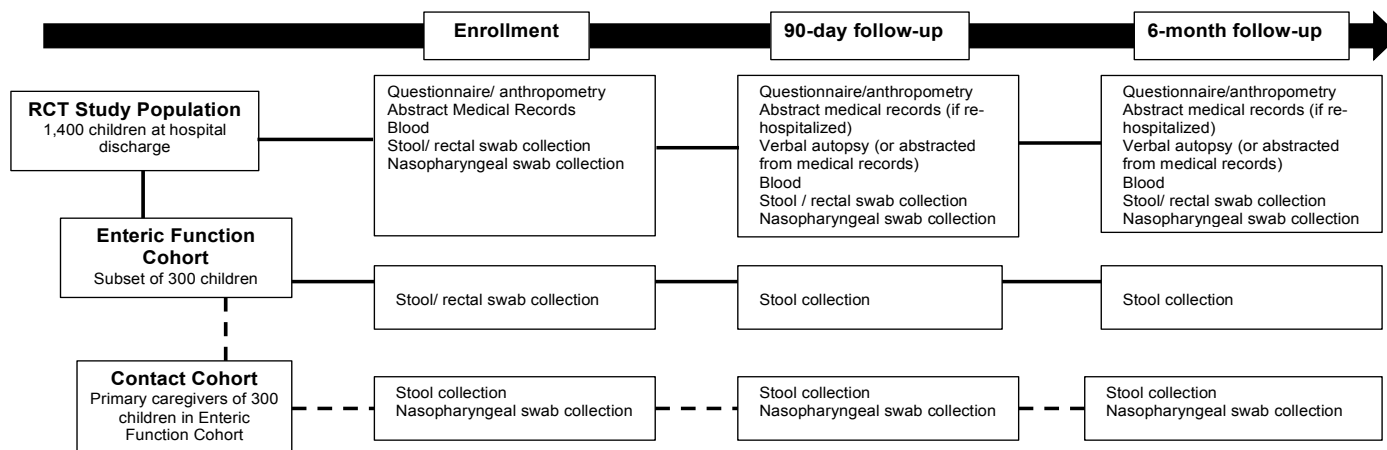
illness/morbidity, post-discharge medication use including antibiotic treatment, and current condition of the child (any hospitalizations, admission and discharge date of any hospitalization, alive or dead, date of death if applicable). If caregivers report a hospitalization, study staff will work with hospital staff to pull the hospital record of the re-admitted child and will abstract relevant information from the hospitalization, including use of antibiotics and other medications. In addition, study staff will work with hospital staff to monitor hospital admissions on a weekly basis to determine whether a previously enrolled child is re-admitted at the health facility and will follow-up on reported admissions. Study staff will capture and transcribe admission and discharge dates, date of death (if occurring in facility), diagnosis or causes of admission, medication administration, and length of stay from patient medical records. Caregivers will be encouraged, at enrollment and at each subsequent contact, to bring the child to the health facility where enrollment occurred for assessment and possible treatment of any medical conditions. If a child is admitted at a facility other than the designated study site, study staff will contact facility personnel at the relevant site to obtain information. If a caregiver or staff member reports that a child has died, study staff will review hospital records (if available) or conduct a standardized verbal autopsy at the household to ascertain the cause of death. The height, weight, and MUAC of the caregiver will be collected at the follow up visit as well.

Laboratory procedures and specimen collection and storage

Stool (rectal swabs), nasopharyngeal swabs and DBS will be collected as described above and undergo laboratory testing as described in Table 5. All biological samples will be collected by staff trained in biosafety and GCLP. Samples will be processed in Kenya when technology is available at one of the following laboratories: Kenya Medical Research Institute (KEMRI) (Wellcome Trust or Centre for Microbiology Research [CMR]) or at the University of Nairobi (Microbiology Department). Metagenomic analyses and/or analyses that require technology not available in Kenya will be performed at the University of Washington. If stool culture results report *Shigella* infection, the study staff will contact the child's caregiver and encourage the caregiver to bring the child back for treatment if the child is symptomatic.

Table 5. Laboratory testing of stool, nasopharyngeal swab and blood specimens collected from participants at enrollment, 90-days, and 6 months

Specimen Collected	Participants	Purpose	Tests Performed
Stool/ flocked rectal swabs	All enrolled children and adults in the Close Contact Cohort	Bacterial ID and storage for AST	Fresh or frozen stool samples/rectal swabs will be evaluated for enteric bacteria and antimicrobial susceptibility testing (AST) will be performed using standard microbiologic or molecular diagnostic protocols. Colonies of *Escherichia coli* (*E.coli*) and other enteric pathogens will be stored for future microbiologic or molecular testing.
	All enrolled children	Parasite detection	Fresh or frozen stool will be tested for parasites using microscopy or antigen test kits such as ImmunoCard STAT! *Cryptosporidium/Giardia* (Meridian Bioscience, Inc)*,* Xpect *Giardia*/ *Cryptosporidium* (Remel, Inc), Quik CheK or similar tests on stored stool/rectal swab samples. If available, molecular testing will be performed.
	Children enrolled in Enteric Function cohort, and adults in the Close Contact Cohort	Enteric inflammation	Commercially available enzyme-linked immunosorbent assays such as neopterin (NEO), alpha-anti-trypsin (AAT), myeloperoxidase (MPO), or similar tests, will be used to measure enteric inflammation on fresh or frozen stool samples..
	All enrolled children and adults in the Close Contact Cohort	Storage (eventual enteric infection detection and quantification)	Stool/ flocked swabs will be immediately placed in sample stabilizing reagents and stored at -80°C in the University of Washington/KEMRI storage facilities in Nairobi. Stored stool will be used for one or more of the following: enteric infection detection and quantification using multiplex real-time PCR methods, DNA extracted for metagenomic analysis to determine the composition of the microbial communities.
Nasopharyngeal Swabs	All enrolled children and adults in the Close Contact Cohort	Bacterial isolation, storage, and resistance testing	Pneumococcal colonies will be isolated using standard microbiologic or molecular diagnostic protocols and susceptibility testing performed using standard microbiologic or molecular techniques.
Blood	All enrolled children	Storage (eventual cytokine and inflammatory marker assessment), HIV, and Malaria testing	Pro-inflammatory cytokines (such as C-reactive protein, IFNγ, TNFα, IL-2, IL-17A, IL-17F, IL-22, IL-23 and IL-1β), anti-inflammatory cytokines (such as IL-4, IL-5, L-9, IL-6, IL-10, IL-13, IL-27, IL-37 and TGFβ), markers of intestinal damage (intestinal fatty acid binding protein) and microbial translocation (soluble CD14), and activity of the growth hormone axis (insulin-like growth factor [IGF-1])and insulin-like growth factor binding protein measured using enzyme-linked immunosorbent assays performed at the University of Washington. HIV testing will be performed per Kenyan National Guidelines and malaria microscopy performed using standard methods. Sickle-cell disease will be tested using Sickle SCAN (BioMedomics, Inc), HEMECHIP (Hemex Health, Inc) or with hemoglobin electrophoresis.

Data Storage

Data will be directly entered into a comprehensive and secure web-based clinical trials data management platform, such as RedCap. The database will be password-protected and web-based, The database will be hosted by University of Washington Institute of Translational Health Services (ITHS) and is Compliant with U.S. Food and Drug Administration (FDA) 21 CFR Part 11 and Good Clinical Practices (GCP). Each study staff member will be assigned a unique password that allows secure access to the centralized data. Rights to enter, access, and modify data will be restricted and managed by the Seattle-based data manager. Within the RedCap platform, all demographic, clinical and laboratory data for each patient are uniquely linked using a unique patient identifier (PID) number. The data are encrypted and stored in a restricted access server. The code linking the PID to individual identifying information will be kept in a separate secure location. The clinical staff at the sites will be able to access the identifying information linked to the PID only to match the laboratory results to individual children, as results become available, and to follow-up children and contacts. No identifying information other than the PID will be captured on the data collection forms and within the computer databases. All study staff are trained in data protection and privacy and are required to have certified CITI training in human subjects research (including data protection). All data and study files will be

stored in locked cabinets within locked study offices at each site. Study offices are accessible only to study staff. All databases are password protected. Only clinical files will contain patient identifiers and non-clinical study staff (including analysts) will receive only coded data. The link between identifiers and unique study ID numbers will also be kept in locked cabinets within the locked study offices and will be destroyed 3 years after study completion (in 2023).

Data verification and Quality assurance

A dedicated study data team will be responsible for all data entry, management and storage of study data. Each site will have a dedicated data clerk responsible for data entry and synchronization with the central server. The data team will be in close communication with the Seattle-based statistical team (under Dr. Richardson) for reporting, monitoring, and analysis. Entered data will be synchronized with the central database system on a daily basis and verified by the Seattle-based data management team. RedCap allows study staff at different locations to directly enter and review data online, with a complete auditing trail system tracks changes to data, and allows numerous real-time built-in data validation/quality checks with prompts during entry to ensure accuracy.

Data Analysis

The primary study endpoint is a combined outcome of mortality and hospital readmission, as re-hospitalization is highly associated with risk of subsequent poor outcome. Additional analyses will be conducted individually for these two outcomes. The secondary endpoints of cause-specific morbidity (diarrhea, acute respiratory infection, malnutrition, or malaria), pathogen carriage in stool or nasopharyngeal swabs and markers of enteric dysfunction will be used to better elucidate the mechanism by which azithromycin may effect the primary endpoints of mortality and re-hospitalization.

Aim 1. To compare rates of re-hospitalization and mortality in the 6 months following hospital discharge among Kenyan receiving 5-day azithromycin vs. placebo. Primary analyses will be intent-to-treat (ITT) based on randomization allocation to the 5-day course of azithromycin versus placebo. Cox proportional hazards regression analysis will be used to compare the rate of the mortality or first re-hospitalization, between the randomization groups. 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 ITT. Potential baseline confounders will be added stepwise in a multivariable model and maintained in the model if adjustment changes the hazard ratio by more than 10%. Mediating variables, such as post-discharge antibiotic use, will be considered in secondary analyses as time-varying covariates to elucidate possible mechanisms of effect. The rate of death or first re-hospitalization will be compared at 90-days and at 6 months post-randomization using Kaplan-Meier (K-M) survival analysis. 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.

Aim 2a. To evaluate possible mechanism(s) by which azithromycin may affect morbidity and mortality, by comparing reasons for re-hospitalization, prevalence of pathogen carriage, and markers of enteric dysfunction between the randomization arms. To evaluate the association between azithromycin and rate of cause-specific re-hospitalizations (hospitalization due to diarrhea, acute respiratory infection, malnutrition, or malaria) we will use Anderson-Gill proportional hazards modeling with previous re-hospitalizations included as time-dependent covariates in the model to capture the dependent structure of recurrence times. Because we will not have granularity in the time points other than 90-days and 6-months for assessment of pathogen carriage, we will compare the prevalence of pathogen carriage at 90-days and 6 months by randomization arm using generalized estimating equations (GEE) with a Poisson link, exchangeable correlation structure, and will adjust for baseline presence of a bacterial pathogen. To determine whether an observed association between the intervention and pathogen carriage wanes over time, we will test the hypothesis that the prevalence ratios comparing carriage in intervention arms are the same at the two follow-up time points using a chi-squared test. For the random subgroup of children in the **Enteric Function Cohort**, we will create a composite disease activity score ranging from 0 to 10 as previously described.[63] We will model the mean composite intestinal inflammation score by treatment group using linear regression. If the baseline comparison of randomization arms reveals an imbalance in characteristics between the treatment groups, we will additionally adjust for these variables, including post-discharge antibiotic use, in the regression models. Additionally, we will compare baseline differences between children randomized into the **Enteric Function Cohort** to those who were not, in order to evaluate representativeness of the random sample.

Aim 2b. To determine whether empiric administration of azithromycin at hospital discharge increases risk of antimicrobial resistance in commensal E. coli and pneumococcal isolates from treated children and their household contacts. Among children and adult household contacts in whom commensal *E. coli* and/or *S. pneumoniae* are isolated, we will compare the proportion of β-lactam or macrolide resistance, or both, between randomization arms and Contact Cohorts for each arm, at 90-days and 6 months using GEE with a

Poisson link and exchangeable correlation structure. A chi-squared test will be used to determine whether the association between intervention arm and antimicrobial resistance wanes over time. Because the likelihood of having a bacterial pathogen isolated may depend on baseline factors, including intervention arm, we will conduct secondary analyses utilizing propensity scores to 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.

Aim 3. To identify correlates and intermediate markers of post-discharge mortality and hospital-readmission among hospitalized Kenyan children. Enrollment hospital admission diagnosis, indicators of malnutrition, age, and randomization arm will be assessed in a multivariable Cox regression model to identify correlates of the primary endpoint of death and/or hospital-readmission 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.

Aim 4. To determine the cost-effectiveness of post-discharge administration of a 5-day course of azithromycin in settings of varying antibiotic use, re-hospitalization rates, and mortality rates. **Costs analysis**: We will assess the cost of all services and equipment necessary to implement the intervention (direct medical costs). The perspective will be the healthcare provider, i.e. Kenya's Ministry of Health. Using WHO guidelines and its ingredients approach, we will quantify the resources and associated unit costs required to deliver a 5-day course of azithromycin, organized in standard expenditure categories: personnel (salaries), supplies including drugs, equipment, services, space and overhead. We will also measure the costs incurred by severe child hospitalizations, the costs for the different types of personnel employed (e.g. nurses/doctors) and the time demanded from them for conducting the intervention.[64] When data are missing, they will be complemented by data extracted from the literature and other available sources. Full incremental costs will be derived, with estimation of the potential healthcare cost-savings realized in avoiding severe hospitalizations. Costs will be measured in local currency (Kenyan Shilling) and converted into US$. Our main metric will be cost per child treated. **Cost-effectiveness analysis (CEA)**: we will develop a CEA mathematical model, and estimate incremental costs and cost-effectiveness for implementation of the intervention. The model will include two components: costs (described immediately above) and health benefits. The study will provide clinical outcomes (mortality/morbidity) over a 6-month follow-up period. Subsequently, deaths averted, life-years saved and disability-adjusted life years (DALYs) averted by the intervention will be estimated. We will estimate: a) incremental costs and b) incremental cost-effectiveness of the intervention vs. status quo. *Incremental costs* are the net sum of the costs to implement the intervention compared with status quo, and the costs averted due to the decrease in severe child hospitalizations. The main metric will be cost per child treated. *Incremental cost-effectiveness* ratios (ICERs) will be estimated in cost per death averted, cost per life-year saved, and cost per DALY averted. We will use recent estimates for the disability weights for DALYs.[65, 66] Short-term (over study follow-up i.e. 6 months) and longer-term time horizons (extrapolated to 1, 5, and 10 years) will be used. DALYs and costs will be discounted at 3% per year, consistently with CEA guidelines (undiscounted results will also be presented). Sources of uncertainty in the results will be explored in univariate and probabilistic sensitivity analysis. [67, 68] Finally, we will compare our findings to CEA estimates for other health interventions in sub-Saharan Africa.[69, 70] Inclusion of the potential impact of antibiotic resistance: based on the results of Aim 2b, we will revisit and adjust our CEA estimates to account for the potentially observed increases in antibiotic resistance at the individual and the community level.

Data Safety and Monitoring Board Review

A Data Safety and Monitoring Board (DSMB) will be established at study initiation to monitor severe adverse events (SAEs) and to evaluate the statistical analysis plan and associated stopping rules for benefit, futility, or harm determined using O'Brien-Fleming stopping boundaries. The DSMB membership will include expertise in clinical trials, statistics, child mortality assessment, ethics, and pediatric care in resource limited settings. Severe adverse events (SAEs) will be monitored in real-time and will be summarized and reported to study investigators and relevant institutional review boards (IRBs) within 48 hours of occurrence. On a monthly basis, frequencies and descriptions of SAEs will be pooled and circulated to investigators and IRBs. When half of the person-time is accrued in the study, the DSMB will review an interim data analysis by arm to determine whether stopping boundaries have been crossed. Dr. Barbra Richardson will oversee preparation of interim analyses and presentation to the DSMB.

Study timeline

We anticipate that this study will take approximately 5 years to complete. In the first six months, we will apply for and obtain ethical approval from relevant institutions, develop and refine study tools, and finalize standard operating procedures. We will also conduct community sensitization and outreach to ensure the community is aware of the purpose of the project. Following IRB approval, field site preparation will take 3

months, including hiring and training of site staff. We will recruit and enroll participants and conduct follow-up over a 36-month period. Approximately 6-9 additional months will be needed for data verification and cleaning, ascertainment and transcription of any outstanding hospital and laboratory records, data analysis and manuscript preparation.

Table 8. Approximate study timeline

	Jan-May '15	Jun-Dec '15	Jan-May '16	Jun-Dec '16	Jan-May '17	Jun-Dec '17	Jan-May '18	Jun-Dec '18	Jan-May '19	Jun-Dec '19	Jan-May '20	Jun-Dec '20	Jan-May '21
IRB approvals	X	X											
CRF development	X	X											
Database development	X	X											
SOPs	X	X											
Patient enrollment			X	X	X	X	X	X	X	X			
DSMB Interim Analysis						X							
Data cleaning			X	X	X	X	X	X	X	X			
Data analysis										X	X	X	X
Manuscript prep and publications										X	X	X	X

Potential Challenges and Limitations

In order to ensure adequate power to detect a discernable clinically relevant difference between study groups in the primary outcome, we have combined hospital readmission with death. Preliminary studies suggest that sufficient numbers of children will reach this combined outcome. However, we have incorporated an interim analysis by the DSMB to review the accrued data and an adapted sample size could be considered if the combined event frequency is less than predicted. It is possible that since most children receive antibiotics during hospitalization, the benefit anticipated with the use of azithromycin based on previous trials of mass drug administration will not be observed. However, most hospitalized children are treated with penicillins, cephalosporins, gentamicin, or cotrimoxazole while in hospital and the broad spectrum of activity (including malaria prevention) and long half-life of azithromycin suggest that there may be additive treatment and/or prophylactic benefit. Interim analysis will allow us to determine whether children receiving specific agents during inpatient treatment are less likely to benefit and will allow us to adapt our study design, sample size, or approach if necessary. After discharge, it is difficult to ensure adherence with the full 5-day treatment course. We will attempt to improve adherence using the IMB model, as described, and will measure adherence using three different measures. In addition, the mortality benefit of azithromycin observed in Ethiopia was from a single dose and we will be able to directly observe the first dose.[10] While relying on caregiver report of mortality and morbidity may lead to bias due to outcome misclassification, this misclassification should not differ between randomization arms and therefore will be non-differential. Finally, resistance prevalence may be lower than predicted, limiting power to detect clinically relevant differences in resistance prevalence between the intervention arms. We will store all isolates in the event that a greater number of isolates are needed for antimicrobial resistance testing.

ETHICAL CONSIDERATIONS

Ethical approval
Study approvals will be obtained from the KEMRI SERU and the University of Washington IRB.

Risks to subjects
The study will involve care givers and children recruited from health facilities after discharge at hospitals in **Kisii and Homabay Counties**. This study carries a minimum risk to participants. Laboratory testing done for the study involves blood test that may involve bruising and possible infection at the site of needle entry. There is some risk that confidential information may be disclosed; however, all clinical and laboratory providers and other staff involved in the evaluation will be provided special training on confidentiality procedures and the importance of keeping personal information private. All study providers (nurses and

laboratory technicians) will be trained in confidentiality and privacy of information, consenting of patients, administration of the questionnaire, specifics of nasopharyngeal swab specimen collection, and appropriate HIV and Malaria testing prior to the start of the study. All study staff will be trained and receive certification in Good Clinical Practice standards.

Azithromycin arm: Azithromycin is a well-tolerated drug that is used frequently in pediatric patients. It is recommended for prophylaxis against pertussis in children as young as 1 month of age by the Centers for Disease Control and Prevention and is generally thought to be safe in young children.[71] Over 200 million doses of azithromycin have been distributed for treatment of trachoma, with many of these doses being provided to children as young as 6 months of age[72, 73]. Common side effects of azithromycin are largely gastrointestinal symptoms, including diarrhea, nausea, vomiting and abdominal pain. Azithromycin may cause a prolonged QT interval, with a possible increased risk of cardiac arrhythmia. As noted above, millions of doses of azithromycin have been distributed in trachoma endemic areas with significant *decreases* in overall childhood mortality. The short term (5 day) dosing of azithromycin is not expected to increase risk of arrhythmia in this population.

Placebo arm: Children randomized to the placebo arm may be at increased risk for persistent infection, recurrent infection, or new infection. The study is being conducted to determine if hospitalized children may benefit from azithromycin at discharge.

Adequacy of protection against risks
All study staff will be trained on proper infection prevention procedures and techniques for blood specimen collection and nasopharyngeal swabs to minimize any chances of bruising or infection. Participant files will be accessible only to investigators and will be stored in a locked office. All clinical personnel involved in the study will be trained in identifying and reporting of adverse events. Caregivers will be educated regarding symptoms that require urgent medical attention and counseled on the importance of contacting the study staff in the event of any change in a child's health. Any adverse events directly attributable to participation in the study will be managed by the appropriate clinic site, and if necessary, hospitalization. The costs of this care will be borne by the study. The clinical sites involved in this study include referral Hospital settings, all of which can provide emergency care if necessary. A Data Safety Monitoring Board (DSMB) will be instituted to monitor study progress and review safety data regularly to ensure continued benefit of the study to participants

Confidentiality
Data forms will contain no individually identifiable information. Databases will not include patient identifiers and will be encrypted and password protected. Study staff will keep signed consent forms and completed study questionnaires in a locked study cabinet. Each participant will be assigned a unique identification number at the time of enrollment. Case report forms, and laboratory samples will be identified with this number. The final study data will only have the participant's unique identification number with no link to their name or other personally identifying information. Once the study has been completed and data analyzed and disseminated, the IRB and bioethics committees will be informed of closure, and links and consent forms will be destroyed (within 3 years of study completion). All study staff involved will receive training and certification on standards of Good Clinical Practice before being allowed to work in this study.

Potential benefits of the proposed research to the subjects and others
Direct benefits: Direct benefits of participation in the study include individualized care and follow-up during the course of the study. In addition, transportation costs for return visits due to illness or scheduled follow up will be borne by the study. Transportation reimbursement is calculated based on the village of residence but averages about 400 Kenyan Shillings ($5) per visit. During screening for eligibility, if it is determined that a child did not receive HIV or malaria testing during admission, such tests will be conducted at the expense of the study. In addition, participants will receive diagnostic testing (bacterial culture of stool and nasopharyngeal samples) and some children will receive additional diagnostic testing (enteric function including intestinal inflammation and gut absorptive capacity from stool and urine samples) that may result in the identification of otherwise undiagnosed conditions. If participants have potential bacterial pathogens identified in stool or nasopharyngeal swabs, or if participants are found to have evidence of intestinal inflammation or malabsorption on stool or urine testing, these participants will be referred to hospital staff for management as appropriate. All children will benefit from experiencing additional exposure to the healthcare system during enrollment and follow-up, which may result in increased/improved identification and diagnosis of serious illness (via laboratory tests associated with the study, or referral to health facility for assessment and management as the result of an encounter with a study staff).

Indirect benefits: It is not known if the provision of antimicrobials to all children at hospital discharge can reduce morbidity and mortality and this study has the potential to dramatically impact national and international treatment guidelines for the management of hospitalized children in low-resource settings. Also by testing all children at baseline for resistance to macrolides and β-lactam resistance in this study we will have data on baseline rates of drug resistance. This information will be disseminated to hospital staff and we will conduct trainings on appropriate antibiotic use.

Importance of the knowledge to be gained
This study has the potential to benefit millions of children in low-resource settings who face high risk of morbidity and mortality after discharge from hospital. If beneficial, the provision of azithromycin to children at hospital discharge would be an inexpensive and practical intervention that could readily be delivered to reduce child morbidity and mortality in many settings. This study will also elucidate possible reasons why children recently discharged from hospitals are at a high risk of death in resource-limited settings. Finally, this study will provide important data on the risk of individual and household-level antimicrobial resistance from prophylactic antibiotic use which will be useful in guiding recommendations for such an intervention. The results of this trial will inform national and international treatment guidelines.

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