Antibiotics for Children with Severe Diarrhea (ABCD) Trial: Kenya Field Site

Overall Study Coordination:
World Health Organization
Maternal, Newborn, Child, & Adolescent Health
Research and Development Team
Geneva, Switzerland

Kenya Protocol Chairs:
Judd Walson, MD, MPH
University of Washington, Department of Global Health
Seattle, Washington, USA

Benson Singa, MBChB, MPH
Kenya Medical Research Institute
Nairobi, Kenya

Version: 25January2019

Table of Contents

ABBREVIATIONS & ACRONYMS

AMR	Antimicrobial resistance
DOT	Direct observed therapy
E. coli	*Escherichia coli*
EPEC	enteropathogenic *E. coli*
ERC	Ethical Review Committee
ETEC	enterotoxigenic *E. coli*
GEMS	Global Enterics Multicenter Study
GCLP	Good Clinical and Laboratory Practice
HCW	Health care worker
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
IMNCI	Integrated Management of Neonatal and Childhood Illnesses
ITT	Intent-to-treat
IV	Intravenous
KEMRI	Kenya Medical Research Institute
LAZ	Length-for-age z-score
MCA	Maternal, Newborn, Child and Adolescent Health
MCH	Maternal Child Health
MOH	Ministry of Health
MSD	Moderate to severe diarrhea
MUAC	Mid-upper arm circumference
NASCOP	National AIDS & STI Control Programme
PID	Patient Identifier
PI	Principal Investigator
ORS	Oral rehydration salts
OND	Office of New Drugs
SAE	Serious Adverse Event
SAM	Severe-acute malnutrition
SD	Standard deviation
SERU	Scientific and Ethics Review Unit
TAG	Technical Advisory Group
UW	University of Washington
WHO	World Health Organization
WLZ	Weight-for-length z-score

STUDY INVESTIGATORS

KENYA SITE

Judd Walson, MD, MPH Associate Professor, Departments of Global Health, Medicine, Pediatrics, and Epidemiology (Adjunct) University of Washington Box 359931 325 Ninth Avenue Seattle, Washington, USA Tel: +1-206-543-4278 walson@u.washington.edu	Benson Singa, MBChB, MPH Kenya Medical Research Institute Centre for Clinical Research Off Mbagathi Way, Box 20778, 00202, Nairobi, Kenya Tel**:** +254-725 234844 singabo2008@gmail.com bsinga@kemri.org
Patricia Pavlinac, PhD, MS Assistant Professor Department of Global Health University of Washington Box 359931 325 Ninth Avenue, Seattle WA 98104 Tel: +1-206-685-8464 ppav@u.washington.edu	Christine McGrath, PhD, MPH Assistant Professor Department of Global Health University of Washington Visiting Scientist, Kenya Medical Research Institute, Centre for Clinical Research PO Box 2651, Nairobi, Kenya 00202 Tel: +254 717 689 194 mcgrathc@u.washington.edu
Hannah Atlas Study Coordinator, Department of Global Health University of Washington Box 359931 325 Ninth Avenue, Seattle WA 98104 Tel: +1-206-685-8464 hatlas@uw.edu	Churchil Nyabinda, CO Study Coordinator, Kenya Medical Research Institute Centre for Clinical Research Off Mbagathi Way, Box 20778, 00202, Nairobi, Kenya Tel: +254 724 438 179 cnyabinda@gmail.com
Liru Meshack Wekesa, MBChB MS Department of Pediatrics Homa Bay County Referral Hospital Kenya Ministry of Health Tel: +254-724-073-773 lirumeshack@gmail.com	

104 **OTHER CLINICAL SITES**

Site	Study site institution	Institution (Contractual)	Site Principal Investigators	Alternate Contact
Tanzania	Muhimbili University of Health and Allied Sciences	BOSTON CHILDREN'S HOSPITAL	Dr. Karim Manji	Dr. Chris Duggan
Mali	Centre National di Appui à la Lutte contre la Maladie (CNAM)	UNIVERSITY OF MARYLAND	Dr. Samba Sow	Dr. Karen Kotloff
Malawi	Queen Elizabeth Central Hospital, Blantyre	UNIVERSITY OF LIVERPOOL	Dr. Queen Dube	Dr. Naor Ben-Zeev
Bangladesh	International Centre for Diarrhoeal Disease Research, Bangladesh (ICCDDR, B)	ICDDR, B (INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH)	Dr. Tahmeed Ahmed	Dr. Md. Jobayer Chisti
India	Center for Public Health Kinetics	CENTER FOR PUBLIC HEALTH KINETICS	Dr. Sunil Sawazal	Dr. Usha Dhingra
Pakistan	Aga Khan University	AGA KHAN UNIVERSITY	Dr. Farah Qamar	Dr. Tahir Yousafzai

105

ABSTRACT

Diarrhea remains a leading cause of death in children, and the majority of diarrhea deaths occur in children under 2 years of age living in sub-Saharan Africa and Asia. Three of the four leading infectious causes of diarrhea are potentially treatable with antimicrobial agents and antimicrobials have demonstrated a mortality benefit in certain high-risk children. The purpose of this double-blind placebo-controlled clinical trial is to determine the efficacy of an antibiotic in reducing risk of death and malnutrition in children less than two years of age with diarrhea and dehydration or malnutrition. A total of eleven thousand five hundred children will be enrolled from 7 study sites (Kenya, Mali, Malawi, Tanzania, Bangladesh, India, and Pakistan) and randomized to a three-day course of azithromycin or placebo. Children will be followed to determine risk of death and linear growth faltering (a marker of chronic malnutrition) in the 180-days after study enrollment. Whole stool and rectal swabs will be collected from all children at enrollment. Rectal swabs will be collected from all children at 90-days and whole stool or rectal swabs from a subset of children and from another child or caregiver in their household at 90 and 180-days. Nasopharyngeal swabs will be collected from a subset of children and another child in their household at 90 and 180-days. Infections identified from stool and nasopharyngeal swabs will be used to evaluate the impact of the antibiotics on enteric and nasopharyngeal pathogens and antimicrobial resistance. The Kenya Medical Research Institute/University of Washington (KEMRI/UW) team will collaborate with investigators at all field sites for data analysis, but is primarily responsible for research conducted at the Kenya sites under guidance from the World Health Organization (WHO) study coordinators.

LAY SUMMARY

In some parts of Africa and Asia, children under 2 years of age who have had diarrhea may continue to have health problems and grow more slowly than children without diarrhea. The usual treatment of diarrhea includes oral rehydration solution (ORS), zinc, and feeding advice. Medicines to fight the germs that cause diarrhea (called antibiotics) are only recommended for children with suspected cholera or when there is blood in the child's stool. Some experts think that there may be other types of diarrhea that could benefit from an antibiotic. The purpose of this study is to find out if children treated with the antibiotic azithromycin for three days will recover from their diarrhea faster and grow better. We plan to enroll 11,500 children from 7 different countries in this study and approximately 2,000 children in Kenya.

DEFINITION OF TERMS

Diarrhea	Diagnosed as diarrhea per caregiver perception AND 3 or more unusually loose stools within 24 hours
High risk diarrhea	Diarrhea associated with high risk of death. For study purposes: diarrhea with severe dehydration OR wasting OR stunting
Moderate malnutrition/wasting	Mid upper arm circumference (MUAC) <125 mm (but >115 mm) OR weight-for-length z-score (WLZ) >-3 SD and ≤-2 SD
Severe Acute Malnutrition	MUAC < 115 mm OR WLZ < -3 SD
Moderate-to-severe diarrhea	Diarrhea AND at least one of the following: sunken eyes, loss of skin turgor, intravenous hydration administration or prescribed, dysentery, or admission to hospital with diarrhea or dysentery (GEMS)
Stunting	Length-for-age z-score (LAZ) <-2 SD
Severe stunting	Length-for-age z-score (LAZ) <-3 SD

BACKGROUND & LITERATURE REVIEW

Despite dramatic reduction in childhood mortality during the past three decades, an estimated 6.3 million children died in 2013 before reaching their 5th birthday [1]. The reduction in mortality has been especially impressive for diarrheal disease. In 1980, an estimated 4.6 million under-five children were dying every year because of diarrhea [2] while in 2013, the number of deaths due to diarrhea in this age group was reduced to 0.6 million per year [3]. This very significant reduction was partly due to the worldwide diarrhea control strategy implemented in the 1980s, which includes the treatment/prevention of dehydration with oral rehydration salts (ORS) and/or intravenous (IV) fluids, administration of zinc, and feeding advice.

While there have been substantial reductions in diarrhea-associated morbidity and mortality, there continues to be over 600,000 deaths/year in under-five children attributed to diarrhea [3]. Data from a recent large multi-center observational study (the Global Enterics Multicenter Study [GEMS]) suggests that only one-third of diarrhea-associated deaths occur in the first week following diagnosis. When follow-up visits were conducted 50-90 days after the health care encounter, the timing of deaths among children with diarrhea was as follows: 34% occurred on days 0–7 after enrollment, 33% on days 8–21 and 33% after day 21 [4]. The majority of children (70%) who die from diarrhea received ORS prior to death [5]. In addition, diarrhea episodes considered as moderate-to-severe had an 8.5 fold higher risk of death during the 50-90 day follow-up period compared to their matched controls in spite of appropriate treatment of the episode, including ORS, zinc, and feeding advice [4]. In the Nyanza province of Kenya, 3.5% of children with moderate-to-severe diarrhea died in the subsequent 50-90 day period compared to 0.5% of controls. The risk of death was significantly associated with degree of chronic malnutrition [measured as height/length-for-age Z-score (LAZ)] at the diarrhea episode. Although 26% of deaths in cases occurred during the enrollment encounter at the health care facility and 19% during a subsequent medical contact, 55% occurred at home or outside of a medical facility and most (88%) deaths occurred in children under 24 months [4]. These data suggest that current estimates, which capture predominantly immediate deaths, may dramatically underestimate diarrhea-associated mortality. These data also suggest that a proportion of deaths due to diarrhea, especially in young children and in children with moderate-to-severe diarrhea, may not be avoided by using the current treatment recommendations, ORS, zinc and continued feeding. Additional treatment may be necessary to reduce diarrhea-associated mortality in these children.

WHO currently recommends the use of antibiotics only for the treatment of dysentery (as a proxy for suspected shigellosis) and cholera [6,7]. Recent studies have shown that dysentery is a poor proxy for treatable bacteria suggesting that current treatment guidelines may be missing the opportunity to appropriately provide antibiotics to a group of young children at particularly high risk of diarrhea-associated mortality [8,9]. Recent data also suggests that multiple bacterial pathogens (typical enteropathogenic *E.coli* (tEPEC) and ST-enterotoxigenc E.coli [ETEC], *Campylobacter*, and *Shigella*) are important in the etiology of moderate-to-severe diarrhea and these pathogens are significantly associated with death following a diarrhea episode [4,8]. Expanding the use of antibiotics for a subset of children with diarrhea associated with high risk of subsequent mortality (such as children under 2 years of age and those with malnutrition) may be an important intervention to reduce the currently unaddressed deaths occurring after a diarrhea episode.

Data suggest that up to 60% of all children with diarrhea currently receive antibiotics, despite WHO guidelines restricting antibiotics to a small subset of children with dysentery or suspected cholera [10]. This may be due, in part, to the lack of high-quality, data-driven evidence supporting guidelines for appropriate antibiotic indications. Data from clinical trials of antibiotics in acute respiratory illness suggest that when clear, evidence-based guidelines are provided for the use of antibiotics

in high-risk children, antibiotic prescriptions in health facilities decrease and resistance among isolated pathogens is reduced [11,12]. By targeting the indication of antibiotics for a small subgroup of severely ill children at high risk of death from a diarrheal episode, overall facility-based antibiotic use for diarrhea, and associated resistance, may be reduced because treatment will be limited to those deemed high-risk.

Although expanded antibiotic treatment of acute diarrhea episodes in very young children with severe disease may offer the opportunity to significantly reduce diarrhea-associated deaths in the 90 days following presentation for acute diarrhea and may also improve growth, an increase in antibiotic resistance may also occur. Antibiotic resistance reduces the effectiveness of antibiotics, which is of particular concern in resource-limited settings where antibiotic options are limited. In evaluating the cost and benefit associated with antibiotic use in children with diarrhea, policy-makers need information specific to their settings on antimicrobial resistance to the antibiotics of interest and other commonly used antibiotics.

Optimizing antibiotic treatment of acute diarrhea episodes in very young children with severe disease may offer the opportunity to significantly reduce diarrhea associated deaths in the 90 days following presentation for acute diarrhea and may also improve growth. Antibiotics may have benefit from direct antimicrobial effects on pathogens or from other incompletely understood mechanisms including improved nutrition, alterations in immune tolerance, or improved enteric function. Alternatively, antibiotics use may lead to antibiotic resistance in enteric and nasopharyngeal bacteria resulting in clinical failure. We propose to evaluate and compare in a randomized, double-blind, two-arm, placebo-controlled trial, the efficacy of an antibiotic (azithromycin) delivered to young children (2-23 months of age) at high risk of diarrhea-associated mortality with the aim of reducing mortality and linear growth faltering in the subsequent 3 months. Azithromycin is a broad spectrum macrolide antibiotic, with anti-inflammatory properties, indicated for gram positive and gram negative bacterial infections in children such as pneumonia and for traveler's diarrhea [13]. Azithromycin is being trialed because in some settings, such as in South Asia, fluoroquinolone resistance is high which may mean that ciprofloxacin may no longer be an efficacious antibiotic choice for children with diarrhea [14,15]. Azithromycin is also the recommended first-line agent from *Campylobacter* and in some settings, diarrehgeanic *E.colis* such as EAEC and ETEC [13]. Azithromycin has demonstrated safety in young children. A review of studies having used azithromycin in young children concluded that there are no safety or tolerance concerns in the use of azithromycin for the treatment of acute bacterial infections [16].

To assess the potential for increasing antibiotic resistance in children randomized to the antibiotic arms, we will collect stool and nasopharyngeal samples from a sub-set of children, and their child household contacts, for resistance determination in commensal gut and nasopharyngeal bacteria and will follow this subset of children for 6 months **(**AMR sub-study). These aims will inform future diarrhea case management guidelines by empirically determining the potential benefits and harms associated with targeted antibiotic use in children with severe diarrhea.

KENYA STUDY SITE

The Nyanza region of Kenya, the location of the KEMRI-UW study sites, covers a population of 1.9 million people living within the two counties (50% of which are under age 15) and a total area of 5780 km [17]. Cases of moderate-to-severe diarrhea (MSD) from the GEMS Kenya site in the Demographic Surveillance System (DSS) area of the nearby Kisumu County, had a case-fatality rate of 3.5% [4]. Eight district, 12 sub-district, and four faith-based hospitals serve Homa Bay and Migori County and are the referral centers for dispensaries and health centers. Enteric pathogens potentially treatable with an antibiotic are common (ETEC [4.4%], EPEC [6.3%], *Shigella* [5.2%]) at three district hospitals in the region (Homa Bay, Migori, and Kisii) [17]. Over 25% of children

presenting with acute diarrhea have at least one bacterial pathogen that may be treatable with an antibiotic (ETEC, EPEC, *Shigella*, *Campylobacter*, and EAEC), an expected prevalence that is likely underestimated due to culture-based methods used for bacteria isolates other than for detection of diarrheagenic *E. coli* [18]. The HIV prevalence among children presenting with acute diarrhea is 5.2% and 11.3% of HIV-uninfected children are HIV-exposed [17].

OBJECTIVES

PRIMARY OBJECTIVES

1. To compare all-cause mortality rates in the 180 days following an episode of high risk diarrhea without dysentery among children 2 to 23 months of age in low resource settings randomized to receive a 3-day course of azithromycin or placebo.
2. To compare the average change in linear growth (∆LAZ) in the 90 days following an episode of high risk diarrhea without dysentery among children 2 to 23 months of age in low resource settings randomized to receive a 3-day course of azithromycin or placebo.

SECONDARY OBJECTIVES

1. To compare changes in markers of acute malnutrition (∆MUAC and ∆WLZ and ∆weight) in the 90 days following an episode of high-risk diarrhea without dysentery among children 2 to 23 months of age in low resource settings randomized to receive a 3-day course of azithromycin or placebo.
2. To evaluate (as determined by verbal and social autopsy-VSA) cause-specific mortality rates across randomization arms in the 90 days following an episode of high risk diarrhea without dysentery among children 2 to 23 months of age in low resource settings randomized to receive a 3-day course of azithromycin or placebo.
3. To compare the proportion of children hospitalized in the 90 days following enrolment for an episode of high risk diarrhoea without dysentery among children 2 to 23 months of age living in low resource settings who are randomized to receive a 3-day course of azithromycin or placebo.
4. To compare the proportion of children hospitalized or died in the 90 days following enrolment for an episode of high risk diarrhoea without dysentery among children 2 to 23 months of age living in low resource settings who are randomized to receive a 3-day course of azithromycin or placebo.
5. To compare the proportion of children hospitalized or died in the 10 days following enrolment for an episode of high risk diarrhoea without dysentery among children 2 to 23 months of age living in low resource settings who are randomized to receive a 3-day course of azithromycin or placebo.
6. To compare the proportion of strains of *E.coli,* isolated from stool samples, resistant to selected antibiotics at enrolment among the study population. Since children entering later into the trial are coming from the same communities, they can serve as a comparator group indicative of the community level of resistance.
7. To compare the proportion of strains of *Streptococcus pneumonia (S. pneumoniae),* isolated from nasopharyngeal swabs, resistant to selected antibiotics at DAY 90 and DAY 180 in a randomly selected sub-sample of children enrolled in the study and their child household contacts (children 6-59 months of age, who have slept in the same household as the enrolled child for 5 of the last 7 nights, has the same primary caregiver as the study child, and whose primary caregiver provides informed consent).

METHODS

STUDY SITES

The study will be conducted in health facilities in 7 countries in South Asia (Bangladesh, India and Pakistan) and Sub-Saharan Africa (Kenya, Malawi, Mali, and Tanzania). The countries have been selected based on study site characteristics as well as the strengths and experience of the country teams in conducting large intervention trials. Within each country, 2-10 individual health facilities will be the sites of patient enrollment.

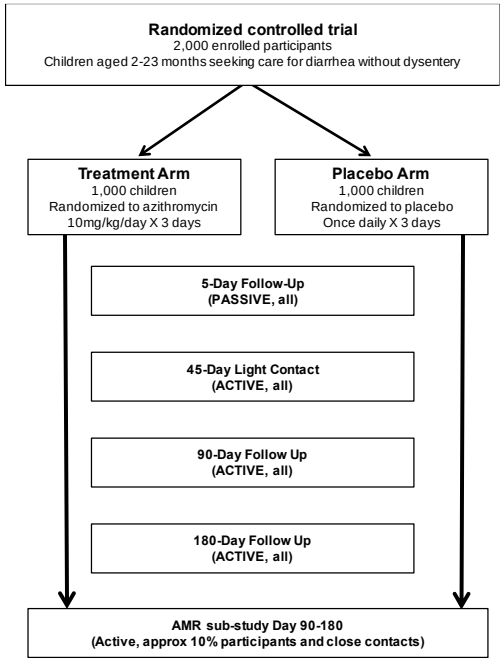
The KEMRI/UW team will operate as part of the larger global study and will be responsible for all operation in field sites within Kenya. The Kenya field sites will include six to eight hospitals, depending on enrollment, in Homa Bay and Migori counties in the Nyanza region of Kenya. The eight study sites include Awendo, Rongo, Isibania, Mbita, Rachuonyo, Ndhiwa, Kendu Bay and Migori hospitals. The Kenya government, through the Ministry of Health (MOH), is the main provider of health services in the study area. The various health facilities are graded from Level 1 to 5, depending on the services offered, and lower level facilities refer to higher-level facilities depending on availability of resources and/or illness severity. Level 1 facilities offer community-level health education and promotion services provided by community health workers. Level 2 Dispensaries and Level 3 Health Centre's largely provide outpatient services and limited health care and laboratory services. Level 4 sub-county and Level 5 county hospitals offer inpatient and outpatient services, and limited laboratory and radiological services.

Private and faith-based health facilities complement the services offered by the government clinics at a minimal cost. These facilities generally offer inpatient, outpatient, and laboratory services as well as routine immunizations. These facilities collaborate with the department of public health in disease surveillance and research activities within the study area.

STUDY DESIGN

We propose a randomized, multi-country, multi-site, double-blind, placebo-controlled clinical trial with an embedded sub-study focused on antimicrobial resistance (AMR). The KEMRI/UW team will be responsible for the portion of the clinical trial administered in Kenya. Figure 1 represents the study design used in each of the study countries. Enrollment based on study tier includes:

- Overall WHO study: 11,500 children (5,750 per treatment arm) across 7 countries
- Kenya: 2,000 children (1,000 per treatment arm) across all field sites
- Overall WHO AMR sub-study: 1,150 children (575 per treatment arm) across 7 countries
- Kenya AMR sub-study: **259** children across all field sites



In Kenya, 2,000 children will be enrolled and randomized to one of two treatment arms *(See section 5.2 Randomization for more information).* All study participants will be followed for 180 days with the final clinical outcome assessment at 180-days of follow-up.

ELIGIBILITY CRITERIA

The age group (2-23 months) corresponds to a standard age grouping as defined in Integrated Management of Neonatal and Childhood Illnesses (IMNCI), and is used globally to define treatment recommendations. Moreover, diarrhea-associated mortality has been demonstrated to be highest in children less than 2 years of age, particularly among malnourished children. Children aged 2-23 months presenting to health facilities for diarrhea will be screened for enrollment. For the purpose of this trial, we are seeking to enroll young children at high risk of mortality in the 180 days following an acute diarrhea episode. This includes children with relatively severe diarrhea. Since there is no standard definition used in clinical practice for assessment of severity of all diarrhea in these settings, we propose using signs and symptoms that are usually agreed upon as criteria and objective signs associated with increased risk of death and are also used and understood by health care workers. These signs include those of "some" or "severe" dehydration as per the 2013 WHO Pocket Book for Hospital Care for Children which are established to select the group of children with most severe diarrhea. In addition, children with evidence of malnutrition (stunting and wasting) are at high risk of diarrhea-associated death (associated with as many as 11% of deaths).

Per current WHO guidelines, children presenting with any of the following signs or symptoms should receive antibiotic therapy and cannot ethically be randomized to the placebo arm:

- Dysentery,
- Suspected cholera,
- Severe malnutrition, and/or
- Other signs of infections requiring antibiotics

As a result, children presenting with a history of bloody stool (per caregiver report or healthcare worker [HCW] observation), children in a location with a declared cholera outbreak and presenting with presumed cholera as defined by WHO guidelines (child is 2 years or older and has severe dehydration), children with Severe Acute Malnutrition (SAM), and/or children with signs of associated infections, will be excluded. Associated infections will be suspected if a child has signs of pneumonia/severe pneumonia (cough or difficulty breathing with any one of the following signs: general danger signs as per WHO guidelines, chest in-drawing, or fast breathing), severe febrile illness (or temperature ≥ 38.0°C with either danger signs or stiff neck), mastoiditis or acute ear infection (ear problem with any of the following signs: tender swelling behind the ear, pus draining from the ear and discharge reported for less than 14 days, or ear pain). Children with hypothermia, defined as temperature <34.5°C, will be excluded because of suspected sepsis.

Therefore, the inclusion/exclusion criteria are as follows:

Inclusion criteria

- Children aged 2 – 23 months, presenting to a designated health care facility at a participating study site *WITH*
- Diarrhea per caregiver perception *AND* at least 3 unusually loose or watery stools in the previous 24 hours,
- Diarrhea for <14 days prior to screening *AND* at least one of the following criteria at presentation:
    A. Signs of some or severe dehydration as per IMCI *OR*
    B. Moderate wasting defined as MUAC <125 mm (but >115 mm) or WLZ >-3 SD and ≤-2 SD after rehydration during stabilization period *OR*
    C. Severely stunted (LAZ <-3 SD) *AND*

- Parent or guardian (caregiver) able to provide consent on child's behalf, according to local standards,
- Parent or guardian (caregiver) willing to allow household visits on DAY 2 and DAY 3 and willing to return to facility on DAY 90 and DAY 180

Exclusion criteria

- Dysentery (gross blood in stool as reported by parent or observed by HCW),
- Suspected cholera (determined according to WHO guidelines or by clinical suspicion),
- Previously or currently enrolled in the ABCD study,
- Concurrently enrolled in another interventional clinical trial,
- Sibling or other child in the household enrolled as a case and who is currently taking study medication,
- Signs of associated infections (pneumonia, severe febrile illness, sepsis, meningitis, abscess, mastoiditis or acute ear infection), requiring alternate antibiotic treatment,
- Documented antibiotic use in the 14 days prior to screening (not including standard use of prophylactic antibiotics, i.e., cotrimoxazole use in HIV-exposed children),
- Documented use of metronidazole in the 14 days prior to screening,
- Known allergy to antibiotic azithromycin,
- Severe acute malnutrition (SAM) defined as WLZ <−3 SD or MUAC <115 mm or the presence of edema of both feet,
- Known contraindication to macrolide antibiotics,
- Living too far from the enrollment health center to ensure adequate Directly Observed Therapy (DOT) on DAY 2 and DAY 3.

INTERVENTION

3-day course of azithromycin (brand name Throza DPS Universal Corporation, Nairobi, Kenya) vs. 3-day course of placebo.

FOLLOW-UP DURATION

All children in the main study will be followed for 180 days. At the day 180 follow-up visit, a subset of children enrolled in the AMR sub-study will also provide stool and nasopharyngeal samples.

SAMPLE SIZE

We expect to enroll approximately 13 children per week from all Kenya enrollment facilities combined to reach a target enrollment of 2,000 children within the 30-month recruitment period with an additional 6 month follow up. Across the entire study, we anticipate enrolling 140 children per week from all country sites to reach a target enrollment of 11,500 children within the study period. At the WHO coordination level, after one year of enrollment, the WHO Technical Advisory Group (TAG) will review the enrollment rates and the events rate at each country site to determine more precisely the total number of children that each site will have to enroll. Primary objectives are powered for the larger overall study (not for each country-site individually).

PRIMARY OBJECTIVES

1. *To compare rates of all-cause mortality between the control group and the intervention groups from enrollment to 180 day.*

The 60-day mortality in children under five years of age with moderate to severe diarrhea in the GEMS study averaged 2.0% across all sites. Excluding the two low mortality sites in Bangladesh and West Bengal (India), this mortality rate was 2.8%. Furthermore, 88% of the deaths occurring in the GEMS study were among children less than two years of age. The sites included in this

study are expected to have higher rates of mortality and the children included in this trial are anticipated to be at higher risk of mortality than observed in GEMS.

For the purposes of sample size determination, we have conservatively estimated that the 90-day mortality in the control (placebo) group will be 2.7%. Assuming this baseline mortality, a relative risk in the intervention group of 0.65 (35% reduction in mortality in the intervention group), 90% power, 95% confidence, assumed loss to follow-up 10%, and 1:1 ratio in the numbers of participants in the control and intervention group, the required sample size would be 5,696 per group or 11,392 in total. Since there is an initial plan to conduct one interim analysis for safety (under Fleming bounds, assuming equally spaced analyses and maintaining total alpha=0.05 and beta=0.1, and two-sided symmetric bounds), the sample size will be inflated by a factor of 1.009 [19]. Hence, the total planned sample size will be 5,750 per group and 11,500 in total.

The estimated overall mortality in the control group is a summary function of earlier data from the GEMS study as well as the assumed proportions of children with various risk factors in the sample and respective sub-group specific mortality rates. The 60-day mortality in children under five years of age with moderate to severe diarrhea or dysentery in the GEMS study averaged 2.0% across all sites. Excluding the two low mortality sites in Bangladesh and West Bengal (India), this mortality rate was 2.8%. The mortality estimates for children with various degrees of stunting or wasting come from GEMS 1a study (which covers children with less severe diarrhea), supported by meta-analyses of some 10 child cohorts, collected between 1977 and 1997 [20,21].

As the study is implemented in 7 countries, the Kenya site will need to enroll approximately 2,000 children (about 1,000 per study group). During a 30-month enrollment period, approximately 13 children per country site will need to be enrolled per week. However, 12 months after study initiation, the Steering Committee and DSMB of the study will evaluate the rate of enrollment and the rate of events at each study site, and calculate or adjust the total number of children to be enrolled at each site.

2. To compare ∆LAZ between the control group and the intervention group from enrollment to 90 days.

We have estimated that we will have 80% power to detect at least a 0.04 difference in mean ∆LAZ between study groups using a two-sided t-test to compare the difference in mean change in LAZ between two groups with α=0.05 and standard deviation (SD) of 0.7 in both groups. This is comparable to results observed in GEMS. As the SD of the difference in ∆LAZ has been shown to vary, the table below outlines the minimum detectable difference in ∆LAZ with varying SD. We will also have adequate power for detecting a difference in ∆LAZ between each intervention group using linear regression models, adjusting for enrollment LAZ. Sample size and minimum detectable difference in ∆LAZ with varying power and standard deviation are shown in Table 2.

Table 1. Objective 2 sample size to detect difference in ∆LAZ between control and treatment groups

Scenario	Minimum detectable difference in mean *∆LAZ*	Standard deviation (equal SD per group)	Sample size (per group) at 90% power	Sample size (per group) at 80% power
A	0.06	0.7	2862	2138
		0.6	2103	1571
		0.5	1461	1092
B	0.05	0.7	4120	3078
		0.6	3028	2262

		0.5	2103	1571
C	0.04	0.7	6437	4809
		0.6	4730	3533
		0.5	3285	2454

We have estimated that we will have 90% power to detect at least a 0.04 difference in mean ΔLAZ between study groups (5,750 children per group) using a two-sided t-test to compare the difference in mean ΔLAZ between two groups with α=0.05 and SD of 0.6 in both groups. Assuming 50% of children will be less than 12 months of age at enrollment (2,875 infants per group), we will have 90% power to detect at least a 0.06 difference in mean ΔLAZ between study groups comprised of infants and separately, between study groups comprised of toddlers (12-23 months). As a point of reference, combined across all sites in GEMS, MSD cases lost 0.04 more in LAZ, on average, than controls among infants (p<0.001) and among toddlers, MSD cases lost 0.10 more in LAZ than controls (p<0.001).

SECONDARY OBJECTIVES

1. *To compare changes in markers of acute malnutrition (ΔMUAC and ΔWLZ and Δweight)* ***between*** *the control and intervention from enrollment to 90 days.*

The study is powered for the primary objectives analyzed at the level of the larger study. With 5,750 participants per arm, we will have 80% power to detect a ΔMUAC and WLZ effect of 0.04.

2. *To evaluate (as determined by verbal autopsy) cause-specific mortality rates across randomization arms in the 90 days.*

The study is powered for the primary objectives analyzed at the level of the larger study. Assuming the placebo study group experiences a 90-day cause-specific mortality of 1% and 5,750 participants per arm, we will have 80% power to detect a relative difference in mortality of 30%.

3. *To compare the proportion of children hospitalized in the 90 days following enrolment for an episode of high risk diarrhoea without dysentery among children 2 to 23 months of age living in low resource settings who are randomized to receive a 3-day course of azithromycin or placebo.*

4. *To compare the proportion of children hospitalized or dying in the 90 days following enrolment for an episode of high risk diarrhoea without dysentery among children 2 to 23 months of age living in low resource settings who are randomized to receive a 3-day course of azithromycin or placebo.*

5. *To compare the proportion of children hospitalized or dying in the initial 10 days following enrolment for an episode of high risk diarrhoea without dysentery among children 2 to 23 months of age living in low resource settings who are randomized to receive a 3-day course of azithromycin or placebo.*

6. *To compare the proportion of strains of E.coli isolated from stool samples, resistant to selected antibiotics at enrolment among the study population. Since children entering later into the trial are coming from the same communities, they can serve as a comparator group indicative of the community level of resistance.*

All enrolled children in the AMR sub-study (575 children per arm) will be eligible to have a child household contact enrolled. Assuming the same loss-to-follow-up rate of 10% of the index child, and assuming 10% will not have an eligible child household contact, we expect 460 child household contacts to provide rectal and nasopharyngeal swab samples at 90 days per index-child treatment arm and assuming 90% of those same children are available at 180 days for sample collection, 414 will be available at 180 days. Using the same carriage estimate of 65%, we estimate that 269 *E. coli* isolates will be available from child household contact for antibiotic resistance testing, per index-child treatment arm at the 90-day time point. As a secondary objective of the study, we will be powered to only detect relatively large differences in prevalence, such a prevalence of 35% in the child household contacts of antibiotic-treated children compared to 15% in contacts of placebo-treated children at 90-day of follow-up.

7. *To compare the proportion of strains of E. coli (from stool samples) and Streptococcus pneumonia (S. pneumoniae), isolated from nasopharyngeal swabs, resistant to selected antibiotics at DAY 90 and DAY 180 in a randomly selected sub-sample of children enrolled in the study and their siblings or child household contacts (children 6-59 months of age, who have slept in the same household as the enrolled child for 5 of the last 7 nights, whose has the same primary caregiver as the study child, and whose primary caregiver provides informed consent).*

All enrolled children in the AMR sub-study (575 children per arm) will also be eligible to have a child household contact enrolled. Assuming the same loss-to-follow-up rate of 10% of the index child, and assuming 10% will not have an eligible child household contact, we expect 460 child household contacts to provide stool samples and nasopharyngeal swab samples at 90 days per index-child treatment arm and assuming 90% of those same children are available at 180 days for sample collection, 414 will be available at 180 days. Using the same carriage estimate of 65%, we estimate that 299 each of *E. coli* and *S. pneumoniae* isolates will be available from child household contacts for antibiotic resistance testing, per index-child treatment arm at the 90 day time point, and 269 each of *E. coli* and *S. pneumoniae* isolates will be available from child household contacts for antibiotic resistance testing, per index-child treatment arm at the 180 day time point. As a secondary objective of the study, we will be powered to only detect relatively large differences, in prevalence, such a prevalence of 35% in the contacts of antibiotic-treated children compared to 15% in contacts of placebo-treated children at 90 days of follow-up.

RECRUITMENT

Three permanent study staff (a clinical officer, study nurse, follow-up counselor) will be placed at each of the selected hospitals at which enrollment is occurring. Study staff will work closely with hospital staff to determine when there is a potentially eligible child either on the ward or in the outpatient unit. The study clinician/nurse will also participate in the daily morning health talk to sensitize caregivers about the study and actively identify potentially eligible participants. To further enrich enrollment, a mobile team focused on community sensitization, directly observed therapy (DOT), and participant follow-up will be deployed in the community. Each enrollment center will be assigned a mobile team member who will travel throughout the community via motorbike.

Screening

All children presenting to the pediatric (under 5 years) outpatient clinics and admitted to the inpatient ward will be pre-screened based on age between 2 and 24 months, presentation with diarrhea, and living within 10 km from any ABCD enrollment facilities. After a potentially eligible child has been identified based on pre-screening criteria, the study clinician/nurse will consent the caregiver to screen the child for potential participation in the study. The parent or guardian (primary

caregiver) must sign written informed consent (or provide a witnessed thumbprint if not literate) prior to screening. After obtaining consent, the study clinician/nurse will screen the child for eligibility based on the above-mentioned inclusion and exclusion criteria using a standardized screening form. Data will be collected on the child's current and recent medical history, care seeking behaviors, and additional information on the family background. The height/length, weight, and mid-upper arm circumference (MUAC) of all enrolled children and their primary caregiver will be also ascertained during screening. Maternal/caregiver anthropometric measures (weight, height, MUAC) will be collected to assess the relationship between maternal/caregiver nutritional status and infant nutritional status and diarrhea morbidity. Maternal BMI has been shown to be associated with diarrhea morbidity in breastfeeding infants born to HIV-infected mothers and will serve as a proxy for household nutritional status [22]. If the child presents with diarrhea and "no signs" of dehydration, screening will be completed at once. If the child has evidence of dehydration, the child will be rehydrated and re-assessed after hydration has been successfully completed.

Study staff will explain to the primary caregiver that the child and caregiver must be willing to be visited at the home (or at a pre-determined nearby location) for directly observed therapy (DOT) for the next two days, be willing to return to the health facility 90 and 180 days after enrollment and be willing to provide a stool specimen and 2 rectal swabs from the child at enrollment, 2 rectal swabs at the day 90 and 180 visits, and possibly another whole stool specimen and nasopharyngeal swab at 90 and 180 days (for a subset of randomly selected children for AMR).

Stabilization period

If the child has signs of "some" or "severe" dehydration, or if the child requires urgent care or the screening physician is unsure of the diagnosis, the child will be kept under observation. During this "stabilization" period, oral and/or IV rehydration will be conducted and treatment of all urgent conditions will be performed using standard treatment in accordance with the WHO Pocket Book of Hospital Care for Children, 2013. If rehydration of the child is successfully completed within 24 hours, as well as urgent care provided and diagnosis confirmed, screening will be continued (anthropometric measurements, physical examination, etc.) and the child eventually enrolled if eligible. However, if the child is not stabilized within 24 hours or requires additional treatment, the child will not be screened further. The child will be eligible again if stabilized at a later stage (after 24 hrs), but in this case the screening process will be started all over again.

Consent

After the child has been found to be eligible to be enrolled in the study, the accompanying primary caregiver will undergo informed consent in the language of the respondent's choosing (English, Kiswahili, Kuria, or Luo). During consent, the purpose of the study and study procedures will be explained to the caregiver, including administration of study drug, follow-up visits, and collection of stool and nasopharyngeal samples (see attached Informed Consent Form). The parent or guardian (primary caregiver) must sign written informed consent (or provide a witnessed thumbprint if not literate) prior to enrollment.

ENROLLMENT

Following consent, each participant will be assigned a unique study Patient Identification Number (PID). A card detailing the PID and the contact information for the medical personnel responsible for enrollment will be given to the primary caregiver of enrolled children. If laboratory results (including HIV and malaria status) are not available from the hospital record, study staff will work with the facility partner to perform HIV testing as indicated by Kenya NASCOP Guidelines. According to Kenya National AIDS & STI Control Programme (NASCOP) Guidelines, HIV testing should be performed in all children presenting to a healthcare facility. Any child newly diagnosed

with HIV will be referred to the HIV Care Clinic at the respective study site for follow-up care and treatment. The study staff will interview the primary caregiver of the child to collect information on socio-demographic characteristics, breastfeeding and vaccination history, and HIV status of the primary caregiver. A stool sample (if available) and 2 rectal swabs will be collected from all enrolled children as described in the sample collection section.

The study clinician/nurse will collect detailed contact information from the caregivers using a standardized patient locator form, including drawing a map of the participant's home location with the help of the caregiver and using Google Maps satellite to confirm the drawn map. Study staff will also ascertain mobile phone information of the caregiver for follow-up visit reminders and home tracing. If a caregiver does not have a personal mobile phone we will obtain the mobile phone information of a household member or friend of the caregivers' choosing. The patient locator form and associated log will be the only link between the PID and the participant's name, and will be stored in a locked file cabinet at each site accessible by only the study clinician and follow-up staff for the purpose of patient tracing.

AMR Sub-study

Once enrolled in the ABCD Trial, up to 259 children will be randomly selected to participate in the antimicrobial resistance (AMR) sub-study. Random assignment into the AMR sub-study will be determined by the WHO Central Coordinating Office and will be part of the intervention randomization code, stratified by intervention arm. Children enrolled in the AMR sub-study will also have a child household contact enrolled in the contact study. (Secondary Objective 4). The caregiver will be instructed to bring the eligible contact back to the facility with the index child at the 90-day visit. Eligible child household contacts are children between 6-59 months, who have slept in the same household as the enrolled child for 5 of the last 7 nights, and under the same caretaker who provides consent. If there are multiple child household contacts who meet eligibility criteria, then the child closest in age to the index child will be included in the contact study. Index children randomized into the AMR sub-study will be enrolled with or without an eligible child household contact (e.g. those without an eligible child household contact will still be enrolled in the AMR sub-study). Although it is determined at study enrollment whether or not the index child will participate in the AMR sub-study (including screening of a child household contact), the child household contact will not be enrolled in the study until the 90-day visit, and samples for the child household contact will be collected at the 90 and 180-day time points. The child household contact will be the same child at 90 and 180 days in order to maintain the ability to evaluate within-individual change in resistance.

STANDARD MANAGEMENT AND INTERVENTION

Both randomization arms will receive standard of care for diarrheal disease, including zinc, rehydration, and nutritional counseling following WHO and Kenya MOH guidelines. Children with some or severe dehydration will be rehydrated and stabilized prior to completion of screening. In children with acute diarrhea, standard of care will include:

- Standardized assessment of hydration status,
- Rehydration and maintenance fluids with IV or ORS as indicated,
- Oral zinc therapy for 10-14 days (10 mg for infants <6 months old; 20 mg for ≥6 months),
- Recommendation for increased fluid intake and continued feeding at home, and
- Instructions on when to seek follow-up care.

As part of standard case management, caregivers will be advised to seek care immediately if their child is unable to drink or to breastfeed, develops fever, starts passing blood in the stool with continued diarrhea or becomes sicker. In addition, as per WHO guidelines, the caregiver will be

advised to bring their child back to the health facility for a follow-up at DAY 5 if the child's clinical status is not improving.

Active tracing and follow up of children at home will not be performed on DAY 5. Caregivers will be counseled at enrollment that children with continuing diarrhea at DAY 5 should return to an appropriate health facility for further treatment.

Study Drug Intervention

Based on the PID (enrollment number), the packet with the same number label containing the PID labels and AMR randomization for the child will be opened. Additionally, the study staff will select the study medication bottle corresponding to the assigned PID. These bottles will be such that they cannot be contaminated with any external substance and oral syringes for study administration are "baby friendly" for ease of administering the dose. The details will be described in the manual of Standard Operating Procedures (SOP).

The study medication bottle will contain either a 3-day course of azithromycin or placebo and will be given as 1 dose per day (10 mg/kg). The first dose of the study drugs will be given at the health facility by a trained study worker. At this time, the caregiver will be trained on how to administer the study drug at home. On DAY 2 and DAY 3, a study health worker will visit the home of all enrolled children, to provide the subsequent doses of the study drugs or to observe the caregiver giving it.

Randomization and blinding

Randomization (1:1) will be done in random sized blocks (with varying block sizes of 4, 6 and 8). All drug and placebo will be procured by WHO MCA Department where a separate randomization list for each of the sites will be generated. This list will be converted into unique sequential serial numbers for each enrolled child at each country site. The list provided to the site will include only the serial numbers with the first letter depicting the respective country site. Each individual child's supply of study drugs will be provided to each site and labeled with this serial number. At the country sites, enrolled children will receive the drugs contained in the packet with the randomization number identical to the enrollment number. The sites will not have the randomization code.

To ensure blinding, the number of bottles and doses for each of the two groups will be identical. The drug and control bottles will be similar in all aspects including the content, color and taste. Treatment allocation (once assigned) will remain blinded to the participant, site Principal Investigator (PI), site staff and hospital clinicians during all data collection phases of the trial.

Study Drug Administration

First dose of study medications

Following consent, collection of enrollment information, stool and 2 rectal swab sample collection, and acute management, the study clinician/nurse will identify the pre-labeled intervention packet corresponding to the PID. The study clinician/nurse will explain the regimen and plan for direct observation, directly observed administration of the first dose, and will provide counseling on the importance of adhering to the intervention. At enrollment, the study staff enrolling the child will administer the first dose of the medication assigned to the child according to her/his enrollment number. In addition to administering the medication the study staff will educate and demonstrate to the primary caregiver, the method of administration and the quantity to be administered from the bottle using the measuring system. This training will help the caregiver to administer doses 2

and 3 in the case the study community health worker cannot visit the household. In addition, the study staff will provide the caregiver user-friendly instructions in the local language and pictorially, both for quantity and administration of the assigned drugs to be taken home. In case the child is admitted in the facility, study staff will administer all the doses of study medication while the child is admitted. When the child is sent home the education previously imparted will be reinforced.

If a child is enrolled in the afternoon, the child will still receive the second daily dose in the morning of the following day. After administration of the first dose of the medications, the child will be observed for 30 minutes. If vomiting occurs during these 30 minutes, a second dose of the medications will be administered only once. If this dose is vomited, a second repeat dose will NOT be given.

A new measuring and dispensing device will be used for each dose and disposed of after use.
At this point, the caregiver will be asked to return to the health facility if any of the following signs appear in the next 10 days:

-Anaphylactic reaction, Quincke oedema (angioedema), convulsions, cutaneous rash, arthralgia, urticaria, and severe colitis (*See section 6 on Serious Adverse Events*).

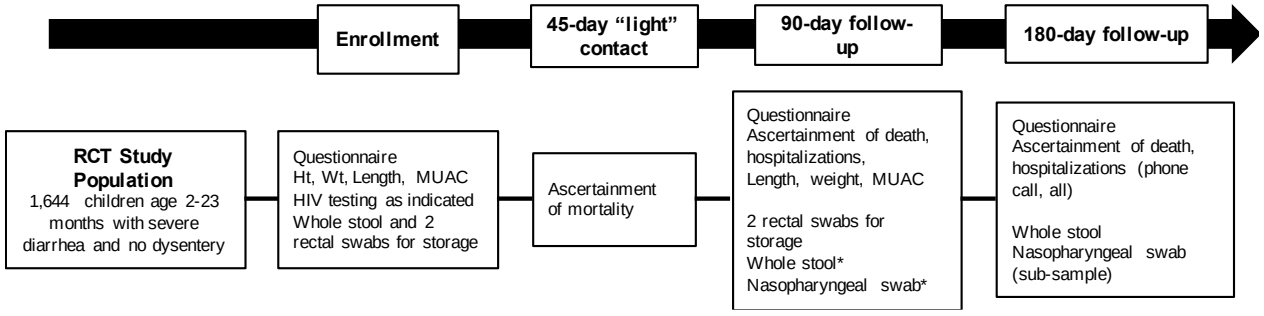
Other doses of study drugs

When the child is sent home (day 1 for outpatient or short admissions and days 2/3 if the child stays in the hospital longer) the study staff send the caregiver home with the medication bottles with the measuring device and reinforce the message concerning administration of the subsequent doses. At the end of each day at the study sites, the study community health worker team will work the rest of the facility study staff to determine the visitation schedule for participants enrolled earlier that day and walk through the patient locator form with the study clinician/nurse.

On DAY 2 and DAY 3, the mobile team will visit the household to administer the medication to the child in her/his presence or to give the study drugs if the guardian prefers. At these visits the study staff will fill in the dosing information on the PID card in consultation with the caregiver. The study staff will instruct the caregiver on how to prepare an additional dose in case child vomits within 30 minutes of taking the dose. On the last dosing day, the study staff will collect the bottles for the completed medications and enter this information on the PID card. A user-friendly PID card will be given to the caregiver, which will have details by day for the expected dosing of the child, contact information, and dates of scheduled follow-up visits. The study staff and caregiver will complete this card. The study staff will also record compliance information as well as any vomiting events in the data collection forms.

FOLLOW UP

Figure 2: Follow-up activities and timeline



Day 45 Follow up

Study staff will contact participants, using a method of "light contact" at DAY 45 (range DAY 40 to DAY 50). The preferred method of contact for the 45-day follow-up will be mobile phone. A standardized script and brief questionnaire will be used to ascertain vital status, hospitalizations, and to remind the caregiver of the 90-day follow-up visit. No other outcome determination will be performed at this contact.

Day 90 Follow up

At the DAY 90 follow up (range DAY 80 to DAY 100), a standardized assessment will be performed by study staff to ascertain vital status, hospitalizations, and health of the enrolled child, as well as her/his anthropometric measurements (weight, length, and MUAC). Participants will be contacted by phone up to three times within the DAY 80 to DAY 100 period to confirm an appointment for this DAY 90 follow up visit at the health facility. Anthropometric measurements will be performed by trained study staff according to SOPs. All participants will be reimbursed transportation costs at enrollment and follow-up visits. In the event that a participant does not return for their scheduled study visit, study staff will attempt to contact the primary caregiver via mobile phone; if no mobile number is provided, or if the participant cannot be reached by mobile phone, study staff will trace the child to the household and bring the caregiver and child to the enrollment center.

Two rectal swabs will be collected from all index children at the 90-day visit. For index children enrolled in the AMR sub-study, up to **259** children, a whole stool (or rectal swab) and a nasopharyngeal swab will also be collected at the 90-day follow up visit. Additionally, if there is an eligible child household contact available (children 6-59 months of age, who have slept in the same household as the enrolled child for 5 of the last 7 nights, whose has the same primary caregiver as the study child, and whose primary caregiver provides informed consent), the caregiver will undergo informed consent for the contact child's enrollment in the AMR sub-study. Whole stool and nasopharyngeal swab samples will be collected from the enrolled child household contact at this point.

If a child is reported to have died at the DAY 45 contact or at the DAY 90 visit, or at any other point during the study follow up period, study staff will review the participant's hospital record (if available) and conduct a standardized verbal autopsy interview at the household to ascertain the cause of death. The verbal autopsy effort will include capturing all relevant hospital/facility-based information on a child that dies in a facility. Upon learning about the death, the bereaved family will be contacted as soon as possible after the culturally appropriate mourning period has ended (between two to four weeks) and not to exceed one year. The verbal autopsy questionnaire will primarily be completed by the primary caregiver of the child who was with the child in the period leading to death. The head of the household will always be invited to the verbal autopsy interview as a courtesy, regardless of whether s/he was involved in caring for the child during the illness leading to death. Written informed consent will be sought from each respondent using the KEMRI-approved Verbal Autopsy Consent Form. As with all data collected in the ABCD trial, only anonymized data will be used for data analysis and reporting of results. The data will be safeguarded using procedures for data storage and security for the ABCD trial.

Day 180 Follow up

All participants will return to the clinic for a DAY 180 visit. The anthropometry (weight, length, and MUAC), vital status, and overall health status (including any hospital admissions since the DAY 90 visit) will be obtained from all the index child.

The AMR subset of children and the child household contacts who provided a whole stool / rectal swab sample at the DAY 90 visit will also return to the clinic for a Day 180 visit. At this DAY 180 visit, a whole stool sample or rectal swab will be collected will be taken as well as a nasopharyngeal swab from both the index and the contact child.

Day 90 and 180 Follow up

All participants will be reimbursed transportation costs at enrollment and follow-up visits (Ksh 200 for enrollment and Ksh 400 for each scheduled follow-up visit per child). In the event that a participant does not return for their scheduled study visit, study staff will attempt to make contact with the primary caregiver via mobile phone; if no mobile number is provided, or if the participant cannot be reached by mobile phone, study staff will trace the child to the household and bring the caregiver and child to the enrollment center. If participants are unwilling to return to the health facility, then vital status and other information available by phone interview will be collected from the caregiver en lieu of the in-person visit.

Outcome Measures

- **Mortality** and time of death to be ascertained at the DAY 45 contact, DAY 90, and DAY 180 visit, or at any other time point during the 180-day study period.
- **Hospitalization** (dates and categorized causes) between days 0 and 180
- **Anthropometric measurements:** length or height, weight and MUAC will be measured at enrollment, the DAY 90 visit (DAY 80 to DAY 100), and the DAY 180 visit (DAY 170-200).
- **Antimicrobial resistance:** prevalence of resistance to **commonly used antibiotics** in isolated *E. coli* (stool sample) at enrollment and DAY 90 (on all children), and *S. pneumococcus* (nasopharyngeal swabs) at DAY 90 and at DAY 180 (on the AMR subset) will be measured. Isolates will be frozen in -80 freezers for future genotypic resistance testing.

SPECIMENS

Collection

Sample collection time points and populations are described in Table 2. All children will provide a stool sample at enrollment and rectal swabs at both enrollment and the 90 day visit. For children enrolled in the AMR sub-study and their child contact, whole stool and nasopharyngeal swabs will also be collected at the 90-day and the 180 day visit. At the 90-day and 180-day visit if the index child or contact has not passed stool within 2-4 hours of the start of the clinic visit, and the primary caregiver gives permission to he procedures, one of the two rectal swabs collected will be used for the AMR sub study in place of the whole stool sample. No nasopharyngeal swabs will be collected from enrolled children at baseline in the chance the sample collection process deters caregivers from returning for follow-up visits.

Table 2. Specimen collection time points and populations

	Baseline			90 day visit			180 day visit	
	Rectal swab	Whole stool	Naso swab	Rectal swab	Whole stool	Naso swab	Whole stool	Naso swab
All ABCD participants	✓	✓		✓				
AMR ABCD participants					✓	✓	✓	✓
Child household of AMR					✓	✓	✓	✓

Processing

Samples will be processed for bacterial culture, phenotypic antibiotic resistance testing, and molecular pathogen detection in Kenya 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 and molecular analyses that require technology not available in Kenya will be performed at the University of Washington, University of British Columbia-Vancouver, University of Virginia (TaqMan), Kilimanjaro Christian Medical Center (TaqMan), or Malawi-Liverpool-Wellcome Trust Clinical Research Programme using samples coded with patient ID. Metagenomic and molecular analysis may be performed outside of Kenya because samples across the 7 country sites will be analyzed at a single laboratory to avoid inter-laboratory variability and because metagenomic technology is not currently available in Kenya. Sample processes are outlined in Table 3.

Storage

A small amount of stool and rectal swabs will be placed without media in a cryovial and immediately frozen at -80°C for future molecular identification detection, analysis of enteric pathogens, their antimicrobial resistance genes, and microbiome characterization.

The stored samples are planned to be analyzed in batches, within four years after all the sample collection for the trial is completed and with up-to-date methods and at sites that have capacity to do those analyses. Any remaining samples will be destroyed at the end of the above-defined storage period. During the storage, only the local PIs and researchers designated by them will have access to the samples.

The study participants or their caregivers can at any point request the discontinuation of storage and destruction of samples collected from them, by contacting the Principal Investigator.

Table 3. Overview of sample requirements and estimated quantities

Population	Sample	Time Points	Immediate Processing	Eventual processing
All enrolled children	Flocked rectal swab and whole stool	Baseline, 90-day	Placed in -80°C	Molecular determination of enteropathogens (TaqMan) and metagenomic analysis of microbiota composition and markers of enteric dysfunction
Enrolled children randomly chosen for the AMR sub-study	Whole stool or flocked rectal swab if whole stool unavailable	Baseline, 90-day, 180-day	Placed in Cary-Blair transport media	*E.coli,* and other common bacteria isolation, AST, and isolate storage
	Flocked naso-pharyngeal swab	90-day, 180-day	Placed in skim milk, tryptone, glucose, and glycerin transport media	*S. pneumoniae* and other common bacteria isolation, AST, and isolate storage
Child household contacts of enrolled children randomly selected for	Whole stool or flocked rectal swab if whole stool unavailable	90-day, 180-day	Placed in Cary-Blair transport media	*E.coli* and other common bacteria isolation, AST, and isolate storage
	Flocked naso-pharyngeal swab	90-day, 180-day	Placed in skim milk, tryptone, glucose, and	*S. pneumoniae* and other common bacteria isolation, AST, and isolate storage

the AMR sub-study.			glycerin transport media	

E. coli

Children enrolled in the AMR sub-study (and child household contacts) will have a small amount of stool sample or the additional rectal swab immediately placed in Cary-Blair transport media and shipped to microbiology facilities (maintaining temperatures between 2-8°C) within 48 hours of collection at each of the three time-points (baseline, 90-day and 180-day). Upon receipt at the laboratory, specimens will be immediately plated on selective media, incubated at 35°C to 37°C for 24 hours, and examined for colonies consistent with *E. coli.* Antibiotic resistance testing will be performed on distinct *E. coli* isolates and all isolates will be frozen in -80°C freezers.

S. pneumoniae

After collection, the nasopharyngeal swab will be immediately placed in skim milk, tryptone, glucose, and glycerin (STGG) transport medium, the sample split into the main and back-up sample, and either frozen or processed immediately. The swab will be plated onto selective media and incubated at 35-37°C in 5% CO_2 overnight and examined for colonies consistent with *S. pneumoniae* [23]. Antibiotic resistance resting will be performed on distinct *S. pneumoniae* colonies and all isolates will be frozen in -80°C freezers.

Resistance Testing

Phenotypic Resistance Testing

E. coli and *S. pneumoniae* isolates will be restored and antibiotic susceptibility testing performed in batches using the Beckman Coulter MicroScan, *auto*SCAN-4 (an automated analytic system). In situations when a desired antibiotic or antibiotic combination is not available from the MicroScan System, an inert non-porous plastic carrier strips, impregnated with a predefined stable antimicrobial gradient of selected antibiotics (so called E-test, each strip containing on antibiotic) will be used. With these tests, minimum inhibitory concentrations (MICs) for *E. coli* and *S. pneumoniae* will be determined for commonly used antibiotics. The *E. coli* and *S. pneumoniae* isolates will be maintained in -80°C freezers for eventual genotypic characterization.

Genotypic Resistance Testing

A subset of *E. coli* and *S. pneumoniae* isolates will be sent to a central laboratory (University of Washington, University of British Columbia Vancouver or Malawi-Liverpool-Wellcome Trust Clinical Research Programme) for molecular characterization. DNA will be extracted from the isolate and one of two methods used for genotypic resistance testing: PCR of specific transmissible resistance genes (macrolide genes such as mefA and ermB) and (fluoroquinolone resistance genes such as qnrA, qnrB, qnrS) or whole genome sequencing which will include all presence/absence and abundance of all resistance genes (some of which may not yet be characterized).

SERIOUS ADVERSE EVENTS

Serious Adverse Events (SAE) will be recorded throughout the study period. At enrollment, caregivers will be told to come back to the facility if the enrolled child presents with any of the following signs: Anaphylactic reaction, Quincke oedema, convulsions, cutaneous rash, arthralgia, urticaria, and severe colitis. All signs will be considered a SAE. On DAY 2 and DAY 3 visit for the DOT, the study staff will record any reported SAE.

In case there is a suspected allergic reaction caused by the study drug or other SAE, the participant will not get any further doses of the study drug. In case of a suspected allergic reaction, the study data manager at WHO will break the randomization code for that participant, to know which product the participant was receiving. The data manager will provide this information to clinicians taking care of the participant, so that they can take in into account in the medical management and also counsel the participant's caregivers about any need to avoid certain drugs in future.

TIMELINE

We anticipate that this study will take approximately 4 years to complete (Table 4). In the first six months, we will apply for and obtain ethical approval from relevant institutions, develop and refine study tools, and finalize SOPs. We will also conduct community sensitization and outreach to ensure the community is aware of the purpose of the trial. Following receipt of IRB approvals, 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 4. Approximate study timeline

	Jan-May 2016	Jun-Dec 2016	Jan-May 2017	Jun-Dec 2017	Jan-May 2018	Jun-Dec 2018	Jan-May 2019	Jun-Dec 2019	Jan-May 2020	Jun-Dec 2020	Jan-May 2021
IRB approvals											
CRF development											
Database development											
SOPs											
Patient enrollment											
DSMB Interim Analysis											
Data cleaning											
Data analysis											
Manuscript prep and publications											

DATA ANALYSIS

The analysis plan for the primary objectives is outlined below. The primary objectives will be analyzed at the larger (overall) study level and will include all 11,500 participants from 7 countries and not by individual site. Primary analyses will be intent-to-treat (ITT) based on randomization allocation to the 3-day course of azithromycin or placebo.

The primary end-points in this trial are death and change in LAZ:

- ***Deaths*** will be ascertained by study staff either through community health workers, hospital staff, or determined at follow-up visits. Date of death will be determined by verbal autopsy or medical/hospital record in those cases where deaths occurred in a facility.
- ***Length*** will be assessed using a length board at enrollment and at the day 90 follow-up visit by study staff trained in anthropometry. Length will be measured twice at each visit, a third time if the two repeated measures vary by more than 10%, and median length will be used for analysis. LAZ will be calculated using the WHO ANTHRO software. Implausible length

values will be considered on a case-by-case basis at each site, and implausible lengths set to missing.

- ***Loss to follow-up*** will be determined at 180 days after enrolment and defined as non-attendance at the 90-day follow-up visit and inability to be contacted after three subsequent attempts by mobile phone and/or one home visit. All analyses will utilize a complete case analysis other than in time-to-event analyses where children who are lost to follow-up will be censored at the date of last contact with study staff. All hypothesis testing will utilize an alpha of 0.05 to determine statistical significance.

Primary Objectives

1. *To compare all-cause mortality rates in the 180 days following an episode of high-risk diarrhea without dysentery among children 2 to 23 months of age in low resource settings randomized to receive a 3-day course of azithromycin or placebo.*

Period prevalence of death will be compared between randomly assigned treatment groups using relative risk regression. Mortality will be defined as a binary variable and will be defined as any event of death from time of randomization to end of day 180. Time to death will not be considered. Censored individuals will be retained in the denominator. For the primary ITT analysis, the following model will be used:

$$\boldsymbol{E(Y|randomisation\ arm) = e^{(\beta_0+\beta_1 X_{azm})}}$$

where x_{azm} is an indicator variable specifying randomized to the azithromycin group (x_{azm}=1) or not (x_{azm}=0). The risk ratio describing the risk of death in children randomized to azithromycin compared to placebo will be determined by e^{β_1}. The statistical significance of this comparison will be determined by Wald test. For all Cox regression models, the assumption of proportional hazards will be checked using graphical methods including a ln(-ln(S(t))) plot for the treatment group and assessing the parallelism of the three lines and by plotting Schoenfeld residuals over time. Kaplan-Meier (K-M) plots for time to death among the two randomization arms will also be prepared and two-way equivalence of survival functions (azithromycin vs. placebo) will be tested using a log-rank test. A log-rank test will be used as opposed to a Wilcoxon test because the former puts relatively more weight on differences in the survival function at larger values of time (i.e. deaths that occur later in follow-up [late deaths]).

If the baseline assessment of randomization reveals an imbalance in characteristics between randomization arms, we will evaluate these characteristics as potential confounders in a sub-analysis secondary to the ITT analysis. The following baseline characteristics will be evaluated for balance between randomization arms (median age, stunting prevalence, wasting prevalence, prevalence of “some” and “severe” dehydration, site [Bangladesh, India, Kenya, Malawi, Mali, Pakistan, Tanzania], hospital admission, and median duration of diarrhea). Potential baseline confounders will be added stepwise in the relative risk model with randomization arm as follows:

$$\boldsymbol{e^{(\beta_0+\beta_1 X_{azm}+\beta_i X_i)}}$$

Where *i* are the baseline characteristic(s) included in the model. These will be maintained in the model if their inclusion in the model changes the hazard ratio by more than 10%. Also, in a sub-analysis, separate analysis for each site (Bangladesh, India Kenya, Malawi, Mali, Pakistan, Tanzania) will be presented to describe differences in treatment effects between sites (no formal hypothesis tests within each site stratum will be performed).

In a per-protocol analysis, also secondary to the ITT analysis, relative risk regression will be fit as described above among the subset of children with documented completion of the full course of treatment (direct observation of 3 daily doses and) with at-risk-time beginning on date of observed third dose.

2. *To compare changes in linear growth (∆LAZ) in the 90 days following an episode of high-risk diarrhea without dysentery among children 2 to 23 months of age in low resource settings randomized to receive a 3 day course of azithromycin or placebo.*

Linear regression models will be used to compare mean ∆LAZ across treatment groups. ∆LAZ will be operationalized as the difference in LAZ between the 90-day follow-up visit and LAZ at baseline. For the primary ITT analysis, the following model will be used:

$$E(Y|randomisation\ arm) = \beta_0 + \beta X_{azm}$$

where Y=mean ∆LAZ and x_{azm} is an indicator variable of being randomized to the azithromycin group (x_{azm}=1) or not (x_{azm}=0). The mean difference in ∆LAZ among children randomized to azithromycin vs. placebo will be determined by (β_1). The statistical significance of this comparison will be determined by independent t-tests. Scatter plots of the residuals will be used to determine whether the assumption of approximate linearity, implicit in linear regression, is valid.

If the baseline assessment of randomization reveals an imbalance in characteristics between the randomization arms, we will evaluate these characteristics as potential confounders in a sub-analysis secondary to the ITT analysis as described in the mortality time-to-event analyses. Potential baseline confounders will be added stepwise in the linear regression model with randomization arms and maintained in the model if inclusion in the model changes the effect estimates (difference in means) by more than 10%.

In a per-protocol analysis, also secondary to the ITT analysis, a linear regression model will be fit as described above among the subset of children with documented completion of the full course of treatment (direct observation of 3 daily doses).

Secondary Objectives

1. *To compare changes in markers of acute malnutrition (∆MUAC and ∆W**L**Z and ∆weight) in the 90 days following an episode of high risk diarrhea without dysentery among children 2 to 23 months of age in low resource settings randomized to receive a 3-day course of azithromycin or placebo.*

Linear regression (and associated t-test) will be used to compare mean ∆MUAC, ∆WLZ, and weight across treatment groups (as described for primary objective 2).

2. *To evaluate cause-specific mortality rates across randomization arms in in the 90 days following an episode of high risk diarrhea without dysentery among children 2 to 23 months of age in low resource settings randomized to receive a 3-day course of azithromycin or placebo.*

We will conduct Cox regression and K-M survival analyses (as described for primary objective 1) for time to cause-specific mortality (diarrhea, pneumonia, malnutrition, other) as separate endpoints to understand intervention effects on specific causes of death.

3. *To compare the proportion of children hospitalized in the 90 days following enrolment for an episode of high risk diarrhoea without dysentery among children 2 to 23 months of age living in low resource settings who are randomized to receive a 3-day course of azithromycin or placebo.*

4. *To compare the proportion of children hospitalized or dying in the 90 days following enrolment for an episode of high risk diarrhoea without dysentery among children 2 to 23 months of age living in low resource settings who are randomized to receive a 3-day course of azithromycin or placebo.*

5. *To compare the proportion of children hospitalized or dying in the initial 10 days following enrolment for an episode of high risk diarrhoea without dysentery among children 2 to 23 months of age living in low resource settings who are randomized to receive a 3-day course of azithromycin or placebo.*

6. *To compare the presence of strains of E.coli resistant to selected antibiotics at enrolment between children in the different treatment arms entering the trial over time. Since children enter later into the trial are coming from the same communities, they can serve as a comparator group indicative of community level of resistance.*

Average prevalence of resistance to selected antibiotics (with Wilson binomial 95% confidence intervals) will be calculated on all samples collected in Year 1 of the study, in Year 2 of the study and Year 3 of the study and compared using chi-squared test to determine the secular trend of evolution of antibiotic resistance at each site.

7. *To compare the presence of strains of E. coli, isolated from stools, and S. pneumoniae isolated from nasopharyngeal swabs, resistant, β-lactamase and quinolone at DAY 90 and DAY 180 in a randomly selected sub-sample of children enrolled in the study and other children under age 5 years living in the same household.*

Prevalence of antibiotic resistance as a function of treatment group and time will be modelled using generalized estimating equations (GEE), with a Poisson link and exchangeable correlation structure for each antibiotic and each isolate, to account for repeated measurements within individuals. The GEE model will include three time-points (baseline, DAY 90 and DAY 180) for antibiotic resistance data from *E. coli* isolates and two time-points (DAY 90 and DAY 180 for *S. pneumoniae* isolates). Pairwise comparisons by treatment groups (azithromycin vs. placebo) at DAY 90 and DAY 180 and between baseline and DAY 180 (among the *E. coli* isolates) will be conducted using Wald tests. A Wald test will also be used to test the hypothesis that the magnitude of change in prevalence, between treatment groups, differs at 90 days and 180 days (effect modification by time). Because multiple hypothesis tests will be performed (4 Wald tests per each of 8 antibiotics per each of 2 isolates types =56 tests) we will report p-values adjusted for multiple comparisons using a Bonferroni correction or a similar method.

For the child household contacts, the same analytical methods will be used. However, no *E. coli* data from baseline will be collected therefore the *E. coli* GEE antibiotic resistance models will contain only two time points (DAY 90 and DAY 180).

RISK MITIGATION

Azithromycin is approved for infants >6 months of age and has been widely used without significant safety concerns in neonates and infants for a wide-range of bacterial infections. Safety and pharmacokinetics have been evaluated in newborn infants with gestational ages as low as 24 weeks [16,24]. While several case reports of pyloric stenosis have been reported in infants receiving azithromycin, the FDA OND Maternal Health Team concluded in 2010 that available data do not demonstrate an association between azithromycin use and increased risk of pyloric stenosis.[25] However, to monitor possible incidence of this adverse reaction, all study participants will be queried about hospitalizations (for abdominal surgery, for diarrhea and for some other defined causes) at the day 45 and day 90 follow-up visits.

Large-scale use of antibiotics raises concern for the possibility that antimicrobial resistance will be induced and ultimately could negatively impact clinical outcomes in the communities in which resistance emerges. Several studies have examined the emergence of antibiotic-resistance among pharyngeal pneumococcal isolates following mass treatment with azithromycin [26,27]. Multiple studies have documented relatively rapid emergence of macrolide resistance following mass drug administration with azithromycin [26,27] although rates of resistance decline quickly after discontinuation of treatment [28,29].

Current efforts to reduce antimicrobial use are failing and new strategies and approaches to change prescribing practices and patient demand factors are urgently required. Given the widespread and inappropriate use of antibiotics, efforts to identify and focus use in a sub-population of children with well-defined and highly specific acute diarrhea episodes could reduce overall use. This dynamic has been observed for other infectious diseases [11,12]. The ABCD Trial results, if robust, might, paradoxically, lead to reduced use of antibiotics and lessen the ecologic pressure pushing resistance. Current efforts to reduce antimicrobial use are failing and new strategies and approaches to change prescribing practices and patient demand factors are urgently required. To further limit antibiotic use, the ABCD trial enrolment criteria have been made as strict as possible only including children whom a guardian brings to the health facility and who has at least moderate dehydration, moderate wasting, or severe stunting.

STUDY LIMITATIONS

The mortality estimates used to determine the sample size required to achieve adequate power are based on a single large multi-center observational study (GEMS) that enrolled participants between 2001 and 2007. Although the estimates used are considered conservative based on other available data and preference was given to the selection of sites thought to experience high diarrhea associated mortality, it is conceivable that there will be fewer mortality events observed in this trial due to overall declining trends in mortality, which would have implications for study power. Because of risk of randomizing children to a placebo group, children for whom there are indications for antibiotic use, such as those with dysentery, severe acute malnutrition, and/or other comorbidities are excluded yet could be the children at highest risk for diarrhea-associated morbidity and mortality. Finally, a limitation of the study is the lack of frequent follow-up visits to evaluate morbidity (such as length of diarrhea). The follow-up visits are intentionally infrequent so as to best replicate a real-world setting where there is minimal active follow-up after contact with a health facility, however this limits the study's ability to determine the impact of the antibiotics on diarrhea associated morbidity other than morbidities that do not lead to hospitalization or death.

STUDY MANAGEMENT AND OVERSIGHT

Coordination

The WHO coordinators in the Department of MCA will be responsible for the oversight of this trial, including harmonization of methods and procedures across study sites. MCA is responsible for developing technical guidelines, including management of infections in children less than five years of age. MCA has the operational advantages of building on existing WHO facilities at the International, Regional and Country level. This includes a communications network and a managerial group with experience in successful handling of projects of a similar size and complexity. MCA also has a large network of collaborators in academic and other institutions around the world, which have expertise in epidemiology, clinical, microbiological, radiological and diagnostic areas of childhood illnesses including diarrheal disease. The WHO coordinating team will provide technical support in the development of site protocols, site-specific study instruments and SOPs. The WHO coordinating team will also oversee randomization and will be responsible for procuring the antibiotics and placebo, arranging for their adequate packaging into full-treatment courses and delivering the packaged treatments to the study sites. Specifically, the WHO team will arrange the purchase of study investigational products from manufacturers Universal Corporation (Nairobi, Kenya) and Madibios Laboratories (Mumbai, India) who will guarantee quality manufacturing and production practices. Products will be imported into Kenya by appointed clearing/logistics agent and will be received and stored centrally in a locked room at the KEMRI-UW Office in Nairobi where daily temperature monitoring will be maintained. Batches of study investigational product will be shipped to the study sites which will be stored at the sites in locked refrigerators with daily temperature monitoring. Strict investigational product accountability procedures will be in place so that each bottle is tracked and accounted for.

The WHO coordinating team will develop guidelines for monitoring and evaluation of the progress of the trial and will be responsible for internal and external monitoring and oversight of quality assurance procedures. WHO study coordinators and others identified by them will ensure that at least two structured monitoring visits are conducted to each site every year. The monitoring visits will have as their primary aim quality control and the improvement of study implementation. The monitors will make direct observations of all relevant study procedures and data management activities. The content of monitoring will vary in response to the stage of study implementation. Monitoring will start with a review of the sites' readiness to begin implementation. After implementation has started, it will shift its focus to the adequacy of procedures for recruitment (including informed consent and adherence to enrollment criteria), treatment delivery and follow-up. More intensive monitoring visits are planned during the first six months of study implementation than in the later part of the study. This process is intended to ensure early problem-identification and prompt resolution at the individual site level and the identification of any variability of procedures across sites that might require rectification.

Local Governance

A PI from each country site will be the leader of the local research team and will be responsible for the execution of the agreed protocol and data integrity. The PI will be responsible for local financial management as well as for obtaining the necessary local ethical approvals. Drs. Judd Walson, and Benson Singa are the identified PIs for the Kenya country site. They will be supported by the UW team to manage finances and operations in the hospital sites within Kenya.

Ethical Review

The study protocol and associated documents (consent forms, CRFs, etc.) will be submitted to the IRB at the WHO and at each participating institution for ethical approval.

The study will be conducted according to GCLP, the Declaration of Helsinki, IRB and local rules and regulations of Kenya. Submission of the protocol and any protocol amendments to regulatory agencies will occur in accordance with local regulatory requirements. Ethical Review board at the

UW and KEMRI Scientific and Ethical Review Unit (KEMRI-SERU) are the IRBs of record for the Kenyan Site. The protocol will be submitted to KEMRI-SERU in addition to UW for review and approval. After approval by KEMRI-SERU and UW, it will be submitted to the Expert Committee on Clinical Trials (ECCT) of the Pharmacy and Poisons Board (PPB) at the Kenya MOH for review and approval before the protocol is implemented.

Trial Advisory Group

The Trial Advisory Group (TAG) will provide advice to the WHO coordinating team. This small group of leading experts will provide strategic guidance and assist with independent advice in the case of difficult issues in the conduct of the trial. The Technical Steering Committee (TSC) will be comprised of the co-PIs from each study site and include members from the WHO coordinating team and the Bill & Melinda Gates Foundation. The initial tasks for TSC will include assisting in the finalization of the site-specific protocols and study instruments. The TAG and TSC will review the progress in the implementation of the study, the planning and review of the data analyses and presentation of the study results. The WHO will facilitate meetings of the TAG at least once per year, whereas the TSG will meet at least monthly, normally electronically.

Data Safety and Monitoring Board

A Data Safety and Monitoring Board (DSMB) has been established at study initiation to monitor SAEs and to approve the statistical analysis plan and associated stopping rules for benefit, futility, or harm determined using O'Brien-Fleming stopping boundaries. This initial meeting will be held before patient accruals are initiated. The DSMB includes five members with expertise in clinical trials, statistics, child mortality assessment, ethics, and pediatric care in resource limited settings, and it is represented by both study regions. The DSMB will include at least 5 individuals and will include at least one member from each study region. The DSMB will meet electronically or in person at least every 6 months and an in-person meeting of the DSMB will occur at least one time per year. SAEs related to study participation will be monitored in real-time and will be summarized and reported to study investigators, WHO and relevant IRBs within 48 hours of occurrence. On a monthly basis, frequencies and descriptions of SAEs will be pooled by the data management team at the WHO and circulated to investigators, DSMB 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.

Quality assurance

Field supervisors will be responsible for assuring the training of the field staff is of high quality and rigorous. They will schedule the testing and retraining as required at their individual site. Assessment of individual study personnel's abilities to use the standardized enrollment criteria and conduct the anthropometric measurements consistently across the study population are key responsibilities. They also will be accountable for the site-specific approaches to minimizing loss-to-follow-up. These quality assurance approaches will be reinforced by the WHO coordinating team during the early site visits.

Data cleaning quality assurance will be performed using consistency and range checks both at the study sites and at the WHO data coordination Centre as described in the data management section. Data quality checks will also be applied on a quarterly basis at the WHO and feedback will be provided to the PIs and site study managers.

PIs will provide brief monthly progress reports during the entire study period and will participate in regular telephone conferences with WHO staff. The monthly progress reports will include the number children assessed, number of children recruited, home visits due to be conducted, actual

visits conducted, child hospitalizations, deaths and verbal autopsies conducted. The templates of the monthly progress report will be developed by WHO Data Coordination Centre with inputs from the sites. The trial will be registered by the WHO as a clinical trial in one or more Primary Registries in the WHO Registry Network.

Data management

The WHO coordinating team will ensure harmonization of data collection and data management processes across sites. All sites will collect information on a core set of variables with standard definitions. The WHO will provide a set of range and consistency checks that must be applied to these variables, although the exact procedures used to carry out these checks will be left to the individual sites. As a general principle, there should be maximum flexibility for each of the sites since they have been selected on the basis of their capabilities to carry out large trials.

Each site will be responsible for data entry and initial cleaning of the data, including running range and consistency checks as well as periodic reviews of distributions and identification of outliers. In Kenya, all data will be collected on paper forms and entered into the database within 48 hours of collection. The database is password-protected and all site staff responsible for entering data will have unique usernames and passwords that are controlled by the data manager in Nairobi and will only have rights to enter data. The Nairobi-based data manager evaluates data daily and generates reports on a weekly basis to identify any missing or erroneous data. On a monthly basis, the Seattle-based data manager will crosscheck the data being entered by generating a detailed monthly report. Changes to existing data will be tracked using an electronic auditing system and adverse events will be recorded in the database.

Individual sites will be required to provide data on the core set of variables in SQL readable format monthly to a central data repository established for the trial at the WHO. The WHO team will run another set of range and consistency checks including checking of consistency of data quality across sites quarterly. Any inconsistencies or queries will be notified to the study site, which will be expected to check and address the list of queries and resubmit data. Cleaned data from all sites will be pooled and stored in a SQL database at the WHO. Data analysis workshops will be held in Geneva following completion and unblinding of the trial data.

The WHO Coordination group will maintain close communication with both the PIs and the Bill & Melinda Gates Foundation assigned Program Officer. Monthly check in calls to review progress and challenges will take place between the WHO and BMGF. In addition, BMGF will be invited to attend all meetings of the Trial Advisory Group.

REFERENCES

1. Liu J, Kabir F, Manneh J, et al. Development and assessment of molecular diagnostic tests for 15 enteropathogens causing childhood diarrhoea: a multicentre study. *Lancet Infect Dis.* 2014;14(8):716-724.
2. Snyder JD, Merson MH. The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. *Bull World Health Organ.* 1982;60(4):605-613.
3. Collaborators. GMaCoD. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2015;385(9963):117-171.
4. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet.* 2013;382(9888):209-222.

5. Rahman AE, Moinuddin M, Molla M, et al. Childhood diarrhoeal deaths in seven low- and middle-income countries. *Bull World Health Organ.* 2014;92(9):664-671.
6. WHO. *Guidelines for treatment of Shigellosis.* WHO-Geneva2005.
7. WHO. *The treatment of diarrhoea: A manual for physicians and senior health workers.* WHO-Geneva2005.
8. Pernica JM, Steenhoff AP, Welch H, et al. Correlation of Clinical Outcomes With Multiplex Molecular Testing of Stool From Children Admitted to Hospital With Gastroenteritis in Botswana. *J Pediatric Infect Dis Soc.* 2015.
9. Pavlinac PB, Denno DM, John-Stewart GC, et al. Failure of Syndrome-Based Diarrhea Management Guidelines to Detect Shigella Infections in Kenyan Children. *J Pediatric Infect Dis Soc.* 2015.
10. Platts-Mills JA, Babji S, Bodhidatta L, et al. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). *Lancet Glob Health.* 2015;3(9):e564-575.
11. Gouws E, Bryce J, Habicht JP, et al. Improving antimicrobial use among health workers in first-level facilities: results from the multi-country evaluation of the Integrated Management of Childhood Illness strategy. *Bull World Health Organ.* 2004;82(7):509-515.
12. Qazi SA, Rehman GN, Khan MA. Standard management of acute respiratory infections in a children's hospital in Pakistan: impact on antibiotic use and case fatality. *Bull World Health Organ.* 1996;74(5):501-507.
13. O'Ryan GM, Ashkenazi-Hoffnung L, O'Ryan-Soriano MA, Ashkenazi S. Management of acute infectious diarrhea for children living in resource-limited settings. *Expert Rev Anti Infect Ther.* 2014;12(5):621-632.
14. Khan WA, Saha D, Ahmed S, Salam MA, Bennish ML. Efficacy of Ciprofloxacin for Treatment of Cholera Associated with Diminished Susceptibility to Ciprofloxacin to Vibrio cholerae O1. *PLoS One.* 2015;10(8):e0134921.
15. Bhattacharya D, Bhattacharya H, Sayi DS, et al. Changing patterns and widening of antibiotic resistance in Shigella spp. over a decade (2000-2011), Andaman Islands, India. *Epidemiol Infect.* 2015;143(3):470-477.
16. Goldman JA, Kearns GL. *Fluoroquinolone Use in Paediatrics: Focus on Safety and Place in Therapy.* 2011.
17. Pavlinac PB, John-Stewart GC, Naulikha JM, et al. High-risk enteric pathogens associated with HIV infection and HIV exposure in Kenyan children with acute diarrhoea. *Aids.* 2014;28(15):2287-2296.
18. Liu J, Platts-Mills JA, Juma J, et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. *Lancet.* 2016;388(10051):1291-1301.
19. Jennison C, Turnbull B. *Group Sequential Methods with Applications to Clinical Trials.* Chapman and Hall/CRC; 1999.
20. Black R, Allen L, Bhutta Z, et al. Maternal and child undernutrition: global and regional exposures and health consequences. *The Lancet.* 2008;371(9608):243-260.
21. Olofin I, McDonald C, Ezzati M, et al. Associations of Suboptimal Growth with All-Cause and Cause-Specific Mortality in Children under Five Years: A pooled Analysis of Ten Prospective Studies. *PLoS ONE.* 2013;8(5).
22. Fawzy A, Arpadi S, Kankasa C, et al. Early weaning increases diarrhea morbidity and mortality among uninfected children born to HIV-infected mothers in Zambia. *J Infect Dis.* 2011;203(9):1222-1230.
23. Satzke C, Turner P, Virolainen-Julkunen A, et al. Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. *Vaccine.* 2013;32(1):165-179.

24. Chaudhari S, Suryawanshi P, Ambardekar S, Chinchwadkar M, Kinare A. Safety profile of ciprofloxacin used for neonatal septicemia. *Indian Pediatr.* 2004;41(12):1246-1251.
25. Treadway G, Pontani D. Paediatric safety of azithromycin: worldwide experience. *J Antimicrob Chemother.* 1996;37 Suppl C:143-149.
26. Skalet AH, Cevallos V, Ayele B, et al. Antibiotic selection pressure and macrolide resistance in nasopharyngeal Streptococcus pneumoniae: a cluster-randomized clinical trial. *PLoS Med.* 2010;7(12):e1000377.
27. Seidman JC, Coles CL, Silbergeld EK, et al. Increased carriage of macrolide-resistant fecal E. coli following mass distribution of azithromycin for trachoma control. *Int J Epidemiol.* 2014;43(4):1105-1113.
28. Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I. Quinolone and Macrolide Resistance in Campylobactor jejuni and C. Coli: Resistance Mechanisms and Trends in Human Isolates. *Emerging Infectious Diseases.* 2001;7(1).
29. Haug S, Lakew T, Habtemariam G, et al. The decline of pneumococcal resistance after cessation of mass antibiotic distributions for trachoma. *Clin Infect Dis.* 2010;51(5):571-574.