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STUDY PROTOCOL

# The CIPAZ study protocol: an open label randomised controlled trial of azithromycin versus ciprofloxacin for the treatment of children hospitalised with dysentery in Ho Chi Minh City, Vietnam [version 1; peer review: awaiting peer review]

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## Abstract

**Background:** Diarrhoeal disease remains a common cause of illness and death in children <5 years of age. Faecal-oral infection by *Shigella* spp. causing bacillary dysentery is a leading cause of moderate-to-severe diarrhoea, particularly in low and middle-income countries. In Southeast Asia, *S. sonnei* predominates and infections are frequently resistant to first-line treatment with the fluoroquinolone, ciprofloxacin. While resistance to all antimicrobials is increasing, there may be theoretical and clinical benefits to prioritizing treatment of bacillary dysentery with the azalide, azithromycin. In this study we aim to measure the efficacy of treatment with azithromycin compared with ciprofloxacin, the current standard of care, for the treatment of children with bacillary dysentery.

**Methods and analysis:** We will perform a multicentre, open-label, randomized controlled trial of two therapeutic options for the antimicrobial treatment of children hospitalised with dysentery. Children (6–60 months of age) presenting with symptoms and signs of dysentery at Children's Hospital 2 in Ho Chi Minh City will be

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Any reports and responses or comments on the article can be found at the end of the article.

randomised (1:1) to treatment with either oral ciprofloxacin (15mg/kg/twice daily for 3 days, standard-of-care) or oral azithromycin (10mg/kg/daily for 3 days). The primary endpoint will be the proportion of treatment failure (defined by clinical and microbiological parameters) by day 28 (+3 days) and will be compared between study arms by logistic regression modelling using treatment allocation as the main variable.

**Ethics and dissemination:** The study protocol (version 1.2 dated 27<sup>th</sup> December 2018) has been approved by the Oxford Tropical Research Ethics Committee (47-18) and the ethical review boards of Children's Hospital 2 (1341/NĐ2-CĐT). The study has also been approved by the Vietnamese Ministry of Health (5044/QĐ-BYT).

**Trial registration:** Clinicaltrials.gov: [NCT03854929](https://clinicaltrials.gov/ct2/show/study/NCT03854929) (February 26<sup>th</sup> 2019).

### Keywords

Shigella sonnei, bacterial dysentery, antimicrobial resistance, diarrhoea, Vietnam, ciprofloxacin, azithromycin



This article is included in the [Oxford University Clinical Research Unit \(OUCRU\) gateway](#).

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## Introduction

Diarrhoeal disease is a major childhood health issue and a leading cause of death in children <5 years of age<sup>1</sup>. Each year an estimated 1.73 billion episodes of diarrhoea occur worldwide<sup>2</sup>, and, while there have been reductions in the global burden of diarrhoea and diarrhoea-related deaths, the effect has been modest in Southeast Asia. In this region, data indicate that each child still experiences 2.4 episodes of diarrhoea every year, resulting in 427.4 million cases and 227,700 deaths annually<sup>2</sup>. Reasons for disparities in rates of reduction are thought to include differences in vaccination implementation, variation in breast-feeding practices and community-level case management practices<sup>1</sup>. Of note, there have been no major changes to international diarrhoea treatment algorithms over this time period<sup>3</sup>.

Faecal-oral infection by the Gram-negative bacilli *Shigella* spp. is a leading cause of moderate-to-severe diarrhoea in children, particularly in low and middle-income countries<sup>4,5</sup>. Shigellosis is particularly problematic in Southeast Asia where *S. sonnei* predominates and resistance to antimicrobials has reached a critical level<sup>6–8</sup>. Diarrhoea and dysentery (diarrhoea containing blood and/or mucus) due to infection by *Shigella* spp. are known to cause a significant burden of disease in Vietnam<sup>6,9,10</sup>, accounting for 49% of hospitalized cases of dysentery<sup>10</sup>. A key concern for the control and management of dysentery caused by *Shigella* spp. and other bacteria in Vietnam and elsewhere is the emergence of antimicrobial resistance, specifically against fluoroquinolones<sup>11–13</sup>.

Bacterial resistance to fluoroquinolones and 3<sup>rd</sup> generation cephalosporins has increased markedly over the last decade in Vietnam, in tandem with *S. sonnei* becoming the predominant *Shigella* species<sup>10</sup>. Most national and international guidelines advocate the antimicrobial treatment of all cases of dysentery in children, due to the high frequency of isolating *Shigella* sp. as the aetiological agent and the high rate of complications and death if not treated promptly<sup>14,15</sup>. In addition to preventing death and morbidity, effective treatment reduces shedding and transmission<sup>14,16</sup>. World Health Organization recommendations are to use the fluoroquinolone, ciprofloxacin (CIP), as 1<sup>st</sup>-line treatment and pivmecillinam, ceftriaxone or azithromycin, as alternative 2<sup>nd</sup>-line regimes according to local susceptibility data<sup>15–17</sup>. These recommendations were supported by a 2010 review of historical treatment failures, which estimated a 99% cure rate with these antimicrobials<sup>18</sup>. This high rate of success is contradicted by a previous study performed in our setting (Ho Chi Minh City, Vietnam) which suggested an 11% clinical failure rate when treating culture-confirmed *Shigella* dysentery cases with CIP<sup>19</sup>. Since these data were acquired, resistance rates have further increased over the last 10 years<sup>20</sup>. Currently, local resistance data suggests *Shigella* spp. non-susceptibility to CIP is ~60% with ~15% of patients failing to show a clinical response to treatment and trending upwards each year<sup>21</sup>.

## Rationale

With such high rates of resistance in Southeast Asia, there may be several direct and indirect benefits to using the 2<sup>nd</sup>-line

recommendation, azithromycin (AZI), in preference for the treatment of children with dysentery. In addition to its widespread availability and low cost, AZI has a large volume of distribution (30L/kg) and a long half-life (70hrs), which results in high tissue and intracellular concentrations at concentrations up to 100-fold those in serum<sup>22,23</sup>. Furthermore, incomplete absorption of AZI (low bioavailability; 17–37%) results in high concentrations of drug within colonic epithelial cells, which is likely to be particularly beneficial in colonic/diarrhoeal infection. Further theoretical indirect benefits to using AZI for the treatment of dysentery may include anti-inflammatory effects through IL-1 inhibition or CD<sub>4</sub> T-cell suppression<sup>24,25</sup>. These effects may result in a clinical treatment response even in the absence of direct *in vitro* microbicidal activity.

Only two previous randomised controlled trials have evaluated AZI for the treatment of dysentery. The first, performed in Dhaka in 1997, demonstrated that AZI was effective for treating *Shigella* dysentery in adult men<sup>26</sup>. Most cases were caused by epidemic *S. dysenteriae* ST1 strain and rates of multi-drug resistance (MDR) and nalidixic acid resistance were high. The second study, performed in Paraguay in 2003, demonstrated AZI was more effective than cefixime for bacterial eradication in children aged 6m – 5yrs, and suggested a trend for better clinical outcomes<sup>27</sup>. Bacterial isolates in this study were predominantly *S. flexneri* (~80%).

Antimicrobial resistance (AMR) is a well-established international emergency and children with diarrhoeal infection represent a significant proportion of the total global infectious disease burden. With the increasing rates of AMR observed in children presenting with dysentery in Vietnam and evidence of international transmission events<sup>28–30</sup>, new data supporting alternative treatment options, such as AZI, in particular for the new highly-antimicrobial resistant *S. sonnei* serotype, is urgently needed. While antimicrobial resistance to AZI is also now not infrequent<sup>31</sup>, we hypothesize that, due to the difference in pharmacokinetic/pharmacodynamic characteristics, bioavailability and theoretical indirect benefits, AZI may be superior treatment for children hospitalised with dysentery in Ho Chi Minh City compared to the current standard-of-care, CIP.

## Objectives and endpoints

The primary objective of our two-group superiority study is to measure the efficacy of azithromycin (AZI) compared with ciprofloxacin (CIP, standard of care) oral antimicrobials for the treatment of children hospitalised with dysentery in Ho Chi Minh City, Vietnam. The primary endpoint will be the proportion of treatment failures detected in each antimicrobial arm of the study, as defined by:

- **Clinical treatment failure** (fever  $\geq 38.0^{\circ}\text{C}$  or the persistence of signs or symptoms of the infection: vomiting, abdominal pain, +/-  $\geq 3$  loose stools +/- blood, mucus or both) after 120 hours of start of either treatment; **or**
- **Microbiological treatment failure** (positive PCR stool with original pathogen after completion of treatment course) after 72 hours of treatment.

Secondary objectives include:

1. Quantify differences in symptom duration between treatment groups, stratified by detection of *Shigella* spp. DNA in stool by PCR.
2. Assess the time to resolution of objective markers of infection and inflammation, including cessation of culture- and PCR-confirmed *Shigella* shedding, normalization of blood total white cell count, C-reactive protein and stool lipocalin concentrations.
3. Assess the rates of adverse events associated with exposure to the antimicrobial agents used.
4. Assess the effects of antimicrobial exposure on the host microbiome and resistome, including diversity and abundance of bacterial species in stool.

## Methods and analysis

### Study design

This study is designed as a single centre, open label, randomized controlled trial of two therapeutic options for the antimicrobial treatment of children hospitalised with dysentery. Eligible patients will be randomly assigned to treatment with either oral ciprofloxacin (15mg/kg/twice daily for 3 days, standard-of-care) or oral azithromycin (10mg/kg/daily for 3 days). Randomisation will be performed by using a set of computer-generated random numbers generated by the local CTU. Patients will be randomised 1:1 using a variable block design with blinding effected by use of identical sealed packs containing the allocated treatment generated by the central unblinded study pharmacist. Eligible participants will be assigned to the next available sequential treatment as recorded in the ward enrolment log.

### Patient recruitment

Study participants will be recruited from those children aged 6 to 60 months presenting to Children's Hospital 2, Ho Chi Minh City, Vietnam, with symptoms and signs of dysentery. Children's Hospital 2 is one of three major children's hospitals in the city, it has a 1,400-bed capacity, serving the local community and acts as tertiary referral center for children with severe infectious diseases and noncommunicable diseases in southern Vietnam. After identification, the admitting paediatric physician will approach the child's parents/guardians to discuss the study, to ask them to consider enrolment, and to explain the informed consent process. If eligible the attending study physician will obtain written informed consent.

### Eligibility criteria

Criteria for study inclusion are:

- Age 6 months to 60 months at time of presentation;
- Have signs/symptoms of dysentery, specifically passing stools containing mucus and/or blood with/without abdominal pain, tenesmus or fever ( $\geq 37.8^{\circ}\text{C}$ );
- Be within 72 hours of the onset of signs/symptoms;
- Have a parent/guardian present at admission willing to provide written informed consent.

Study exclusion criteria include:

- Those known to have specific medical/surgical conditions that may affect disease severity/presentation or response to treatment (e.g. affecting antimicrobial absorption), including: gastrointestinal abnormalities, including short bowel syndrome, chronic (inflammatory or irritable) bowel disease; inherited or acquired immune system deficiency rendering the patient immunocompromised, including chronic/long-term steroid treatment or other immunosuppressive treatment; patients with known congenital or acquired osteoarthritis, prolongation of the QT interval, congenital long QT syndrome;
- Presentation with severe infection requiring parenteral antimicrobial treatment, including shock jaundice, extensive gastrointestinal bleeding, convulsion, drowsiness or coma, reduced movements on stimulation, tachypnoea  $>60$  times per minute, grunting, chest retraction, poor sucking reflex;
- Known hypersensitivity to any of the trial drugs (ciprofloxacin or azithromycin);
- Coexisting infection requiring other or additional antimicrobials to be prescribed/administered.

### Interventions measured

The interventions measured will be ciprofloxacin 15mg/kg BW/ twice daily, with doses 12 hours apart against azithromycin 10mg/kg BW/once daily and administered by a study physician to ensure adherence.

**Ciprofloxacin.** CIP has a dual-ringed quinolone structure with the addition of fluorine at position 6 and a piperazinyl group at position 7. The bactericidal activity of CIP, which extends to Gram negative bacteria, is a result of inhibiting both the type II topoisomerase (DNA-gyrase) and type IV topoisomerase enzymes, which are required for bacterial DNA transcription, replication, repair and recombination. Inhibition of this mechanism results in rapid bacterial cell death.

Quinolone antimicrobials are well absorbed from the gastrointestinal system; CIP has an oral bioavailability of 70%. Peak concentrations of CIP are reached 1–3 hours after dose ingestion with a half-life of 4 hours. While food does not inhibit the absorption of quinolone agents, it may result in a delay in reaching peak concentrations. Enteral feeding may reduce the absorption of quinolone antimicrobials, whether given via the oral, nasogastric or jejunal route. CIP has a high volume of distribution ( $V_D$  231L) penetrating well into most tissues and the intracellular macrophage compartment, where concentrations are 2–100x plasma. CIP is eliminated through a combination of renal and non-renal routes, including intestinal secretion, which accounts for 10–15% of drug excretion. Other routes of elimination include hepatic metabolism to less active forms, which similarly accounts for 10–20% of CIP clearance.

CIP has a broad spectrum of activity, including the *Enterobacteriaceae*, Gram-negative cocci, intracellular atypical pneumonia pathogens and some Gram-positive organisms including *Mycobacteria* and *Staphylococci* species. Bacterial resistance

mechanisms include alterations of the target enzymes or permeability mechanisms. CIP resistance by *Shigella sonnei*, was first reported in 1993 and has been recently been characterised as occurring in a single MDR lineage (III) which is now dominant globally<sup>32</sup>. Genome sequencing has identified that the emergence of this globally dominant CIP-resistant strain has occurred as a single selection event likely in South East Asia, with resistance being acquired sequentially through accumulation of *gyrA* and *parC* chromosomal mutations within the DNA gyrase and topoisomerase IV genes, respectively.

**Azithromycin.** AZI is an azalide antimicrobial related to the macrolide erythromycin although with more favourable pharmacokinetic and side-effects profiles and a broader spectrum of activity. Structural differences with erythromycin include substitution of nitrogen for a methyl group within the lactone ring. This substitution enhances the stability of AZI in acidic conditions and thus improves the gastrointestinal absorption and bioavailability of the drug. The mechanism of action for AZI is similar to other macrolide antimicrobials; inhibition of the bacterial 50S ribosomal subunit inhibiting RNA-dependent protein synthesis.

The oral bioavailability of AZI is 36% and is further inhibited by food (50%), thus administration should be 1 hour before or 2 hours after meals. Absorption is also slowed by co-administration with magnesium or aluminium-containing antacids. AZI has a high volume of distribution (23–31L/kg) and is widely dispersed in body tissues including the lung and sputum with concentrations reaching 10–100x serum. Very high concentrations are found in macrophages and neutrophils, which is thought to be due to cellular uptake of the basic compound into acidic lysosomes by ionic trapping. AZI has a half-life of 2–4 days and antibacterial activity may persist for several days after completion of a course. Most elimination is of the non-metabolised drug via the gallbladder or trans-intestinal route into faeces.

AZI has more activity against Gram-negative bacteria in comparison to the other macrolides, erythromycin and clarithromycin, but is less active against various Gram-positive organisms including Streptococci and Staphylococci. This enhanced activity is thought to be due to enhanced ability to penetrate the Gram-negative cell wall. Resistance to AZI is by alteration of the target site (methylation of mRNA nucleotides or mutation of ribosomal components, e.g. *ermA/B/C*), by macrolide modification (by esterases or phosphotransferases e.g. *ereA/B* or *mphA/B/D*) or by efflux pump mechanisms (*mefA* or *msrA*)<sup>33</sup>. Although sporadically reported to-date, resistance by *Shigella* sp. to AZI is mostly through acquisition of a plasmid carrying *ermB* or *mphA* genes<sup>33–38</sup>.

### Sample size calculation

In this study, children aged 6 months to 60 months, presenting with symptoms of dysentery will be randomised to receive antimicrobial treatment with either AZI or CIP. The primary endpoint will be clinical or microbiological treatment failure. The sample size was based on the efficacy of CIP is 85%

based on non-clinical response (unpublished data; Duong et al. OUCRU Vietnam) and the efficacy of AZI is 95% based on non-clinical response (unpublished data; Duong et al. OUCRU Vietnam) with type I ( $\alpha$ ) errors = 0.05 and 80% of power. We need to recruit 140 patients per arm. To account for potential inadequacies in our assumptions and some loss to follow-up, sample size was increased by 30%. Thus, a total sample size of 364 participants, 182 in each arm, will be recruited. Parents of children meeting the study criteria will be approached daily until the participant number has been met. The ward will contain posters and flyers regarding the study and may be expanded to other sites if recruitment targets are not met.

### Standard-of-care procedures

Aside from the study specific procedures described, all patients admitted to participating wards and enrolled to the study will receive standard-of-care treatment according to national guidance<sup>39</sup>. Alterations to the management of each participant will be at the discretion of the treating physician; whilst in hospital, study participants may require additional procedures to be performed to optimise clinical management. In cases where the patient is failing to respond to the treatment allocated, rescue treatment with an alternative (AZI or third-generation cephalosporin) will be used. If antimicrobial susceptibility data becomes available, treatment will be altered as required.

### Data collection

After enrolment, data will be collected from participants during daily review, or if discharged before the final study visit on day 28, on days 3, 7 (+3 days allowable variation), and 28 (+3 days). Data sources will include the study participant and samples collected from them, the study participant's parent(s)/guardian(s) and the clinical care records, including but not limited to hospital case notes, imaging, laboratory results and nursing/observation record charts.

Comprehensive demographic data will be collected at the baseline/day 1 visit including complete previous medical and surgical history with participant birthing, breast-feeding and immunization record, and details of current and previous medication use including antimicrobials and visits to pharmacies/medicine sellers. Data collected from physical examinations performed will include assessment of hydration and nutritional status (weight, mean upper arm circumference, skin turgor, and mucus membrane moistness) and standard physical observations (heart rate, blood pressure, respiratory rate, oxygen saturations, and oral temperature measurement). Study visits will be performed twice-daily until day 5 with only physical observation and adverse event reporting data collected during the afternoon visit (Table 1; afternoon visits not shown).

**Laboratory methods.** Stool specimens will be collected in sterile containers as soon as possible on the day of hospital admission after informed consent has been obtained. After delivery to the laboratory, specimens will be investigated to determine a microbiological or parasitological cause for the dysentery

**Table 1. Visit schedule for the CIPAZ clinical trial.**

	Visit 1, day 0	Visit 3, day 1	Visit 5, day 3	Visit 7, day 4	Visit 9, day 5	Visit 11, day 7-10	Visit 12, day 28-31
<b>Informed consent</b>	X						
<b>Medical history and physical examination</b>	X	X	X	X	X	X	X
<b>Randomization</b>	X						
<b>Study intervention*</b>	X	X	X				
<b>Stool collection</b>	X	X	X	X <sup>†</sup>	X <sup>†</sup>	X <sup>†</sup>	X <sup>†</sup>
<b>Laboratory blood tests<sup>‡</sup></b>	X					X	
<b>Urine collection<sup>   </sup></b>	X						
<b>Adverse event assessment</b>	X	X	X	X	X	X	X

\* ciprofloxacin 15mg/kg BW/ twice daily, with doses 12 hours apart or azithromycin 10mg/kg BW/daily. <sup>†</sup> Only for hospitalised patients who produce stool; examination for ova, cysts and parasites will be performed on initial stool specimens only. <sup>‡</sup> Stool sample or rectal swab. <sup>§</sup> Full blood count and biochemistry, including urea, creatinine and electrolytes, liver function tests, random glucose, C-reactive protein (CRP). <sup>|||</sup> Sample for testing phenotypic antimicrobial activity.

episode. In addition, two aliquots of stool sample will be stored at -80°C as 10% suspensions in distilled PBS for batched viral identification and secondary analysis.

To examine stool for the presence of parasites, a fresh smear of a stool specimen will be prepared in phosphate buffered saline. 10µL of this solution will be examined at 400x magnification and examined for *E. histolytica* and *Giardia lamblia*. Further examination for *Cryptosporidium* cysts will be performed using Ziehl-Neelsen stain. This procedure will be performed on day of admission to the ward only.

**Culture methods.** All stool specimens will be examined for the presence of blood or mucus and cultured for the presence of bacterial pathogens. Fresh stool smears of each specimen will be examined by x400 microscopy for presence of red/white blood and pus cells, indicating an inflammatory colonic process. Stool cultures will be performed according to standard protocols; in brief, specimens will be cultured on MacConkey Agar (MC), Xylose-Lysine-Deoxycholate Agar (XLD), Selenite broth, and selective *Campylobacter* media. After overnight incubation at 37°C or ≥48 hours incubation at 42°C in a micro-aerophilic environment (for *Campylobacter* culture only), identification of potential pathogens will be performed using standard local protocols incorporating clinical mass-spectrometry identification approaches (Bruker MALDI-TOF Biotyper).

*Shigella* spp. causing dysentery will be identified through this method; primarily as non-lactose fermenting colonies growing on MC or XLD agar. Speciation will be performed by slide agglutination using polyvalent somatic (O) and monovalent serotype-specific antibodies (Denka Seiken, Japan), and confirmed by sequencing methods. Susceptibility testing of *Shigella* spp isolates will be performed using a disc diffusion

method and by minimum inhibitory concentration antimicrobial gradient diffusion, where necessary, on Mueller-Hinton agar (Oxoid). Assessment of extended-spectrum B-lactamase phenotype organisms will be performed using interpretative reading of the disc diffusion results and with the double-disc diffusion synergy test<sup>40</sup>. Further characterisation of resistance mechanisms will be performed using established in-house genotyping methods as appropriate, as previously described<sup>11,31</sup>.

All isolated pathogens will be stored for future testing and the isolated organism will be recorded in the study data. Pathogen identification and relevant susceptibility data will be reported to the clinical team as soon as it is available.

**Molecular detection methods.** To enhance our ability to identify putative pathogens causing dysenteric symptoms, additional non-culture-based diagnostics will be performed using nucleic acids (DNA or RNA) extracted from stool samples collected on the day of hospital admission. Total nucleic acids will be extracted from faecal specimens (homogenised and diluted 10% in PBS) either using the QIAamp viral RNA Mini kit (QIAGEN, Hilden, Germany) or a Roche MagNA pure 96 automated nucleic acid extraction machine (Roche). After extraction, an aliquot of RNA will be converted to complementary DNA (cDNA) by reverse transcription prior to storage at -80°C until further analysis. Bacterial (and viral) pathogen sequences will be detected in stool-extracted cDNA/DNA by multiplex real-time PCR using established in-house protocols<sup>41</sup>.

#### Data analysis plan

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised

into the CRF), clinical and office charts, laboratory and pharmacy records, and correspondence. CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name.

Analysis of the clinical trial data will be performed after dataset cleaning and not before the final enrolled participant has attended for their Day 3 visit. The primary analysis population consists of all participants enrolled and randomised to a treatment arm. Outcomes will be analysed according to randomised arm (intention-to-treat population). In addition, the primary endpoint will be assessed using the per-protocol population, which will include all participants completing the allocated full 3-day antimicrobial course. After data cleaning the only people with access to the dataset will be the head of the CTU at OUCRU and the trial statistician.

**Description of statistical methods.** The primary endpoint, the proportion of treatment failure (defined by clinical and microbiological parameters) by day 28 (+3 days) after enrolment will be compared between the study arm (either CIP or AZI) based on a logistic regression model with treatment arm as the main variable. The primary analysis will not adjust for any covariates, but in a second step we will also explore the effect of the following covariates on the treatment failure (in addition to the treatment arm): duration of diarrhoea prior to enrolment, age, and pathogen (norovirus, rotavirus, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., unknown). The secondary endpoints will be compared between the treatment arms based on a lognormal accelerated failure time regression model for time-to-event endpoints, logistic regression for rate of relapse within 7-days (+ 3 days) endpoint.

The primary endpoint, the proportion of treatment failure (defined by clinical and microbiological parameters) by day 28 (+3 days) after enrolment will be compared between study arms (either CIP or AZI) by logistic regression modelling using treatment allocation as the main variable. The primary analysis will not adjust for any covariates, but in a second step we will also explore the effect of the following additional covariates on treatment failure: duration of diarrhoea prior to enrolment, age, and pathogen identified (norovirus, rotavirus, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., unknown). The secondary endpoints will be compared between the treatment arms based on a lognormal accelerated failure time regression model for time-to-event endpoints and logistic regression for rate of relapse within the day 7 (+3 days) endpoint.

**Ethics and dissemination.** This study is sponsored by the University of Oxford and will be monitored by the Clinical Trials Unit at the Oxford University Clinical Research Unit, Vietnam. The principal investigator (SB) will ensure that this study is conducted in accordance with the principles of the

International Council on Harmonisation Guidelines for Good Clinical Practice and amendments<sup>42</sup>. This protocol and the relevant supporting documents have received approval by the Oxford Tropical Research Ethics Committee (47–18) and the ethical review boards of Children’s Hospital 2 (1341/NĐ2-CĐT). The study has also been approved by the Vietnamese Ministry of Health (5044/QĐ-BYT). The investigators will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents. All trial potential study participant’s parents/guardians will provide written informed consent in accordance with the Declaration of Helsinki<sup>43</sup>. SAEs and SARs will be notified to the OUCRU CTU immediately and within no more than 24 hours of the investigator becoming aware of the event. The trial will be audited annually by the OUCRU CTU audit team.

No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. The study monitor, other authorized representatives of the sponsor, representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records. The study participant’s contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations. Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Oxford University Clinical Research Unit, Vietnam (Ho Chi Minh City). This will not include the participant’s contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by OUCRU-Vietnam research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at OUCRU, Vietnam (Ho Chi Minh City).

### Study status

The trial has now received the necessary approvals and recruitment started in November 2019. We anticipate recruitment will continue for 18 months and we aim to complete data analysis by December 2021.

### Dissemination

We will disseminate the results from this study to local and regional policy makers to inform updates to current treatment guidelines in order to optimise the antimicrobial treatment for *Shigella* dysentery in the new era of widespread antimicrobial resistance. We would also aim to present these data at national (Vietnam) and international conferences and to report the results in open access journals, in keeping with the recommendations from the Funder. All people contributing to the protocol and downstream analysis will be eligible for inclusion as an author;

authorship will be determined by the Principal investigator; no professional writers will be sought. Public access to the full protocol, participant-level dataset, and statistical code can be requested from the Principal investigator.

## Data availability

### Underlying data

No data is associated with this article.

### Extended data

Zenodo: The CIPAZ study protocol: An open label randomised controlled trial of azithromycin versus ciprofloxacin for the treatment of children hospitalised with dysentery in Ho Chi Minh City, Vietnam, <http://doi.org/10.5281/zenodo.4004569><sup>44</sup>.

This project contains the following extended data:

- Full protocol
- Patient information sheet & informed consent form

## Reporting guidelines

Repository name: SPIRIT checklist for ‘The CIPAZ study protocol: An open label randomised controlled trial of azithromycin versus ciprofloxacin for the treatment of children hospitalised with dysentery in Ho Chi Minh City, Vietnam’, <http://doi.org/10.5281/zenodo.4004569><sup>44</sup>.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

## References

1. Black RE, Cousens S, Johnson HL, *et al.*: **Global, regional, and national causes of child mortality in 2008: a systematic analysis.** *Lancet.* 2010; **375**(9730): 1969–87. [published Online First: 2010/05/15]. [PubMed Abstract](#) | [Publisher Full Text](#)
2. Walker CLF, Rudan I, Liu L, *et al.*: **Global burden of childhood pneumonia and diarrhoea.** *Lancet.* 2013; **381**(9875): 1405–16. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. WHO/UNICEF: **Diarrhoea: why children are still dying and what can be done.** Geneva/New York: World Health Organisation/United Nations International Children’s Emergency Fund. 2009. [Reference Source](#)
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): 209–22. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
5. 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–e75. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
6. von Seidlein L, Kim DR, Ali M, *et al.*: **A multicentre study of Shigella diarrhoea in six Asian countries: disease burden, clinical manifestations, and microbiology.** *PLoS Med.* 2006; **3**(9): e353. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Livio S, Strockbine NA, Panchalingam S, *et al.*: **Shigella isolates from the global enteric multicenter study inform vaccine development.** *Clin Infect Dis.* 2014; **59**(7): 933–41. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. Bardhan P, Faruque AS, Naheed A, *et al.*: **Decrease in shigellosis-related deaths without Shigella spp.-specific interventions, Asia.** *Emerg Infect Dis.* 2010; **16**(11): 1718–23. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Anders KL, Thompson CN, Thuy NT, *et al.*: **The epidemiology and aetiology of diarrhoeal disease in infancy in southern Vietnam: a birth cohort study.** *Int J Infect Dis.* 2015; **35**: 3–10. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Thompson CN, Phan MV, Hoang NV, *et al.*: **A prospective multi-center observational study of children hospitalized with diarrhea in Ho Chi Minh City, Vietnam.** *Am J Trop Med Hyg.* 2015; **92**(5): 1045–52. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
11. Thompson CN, Thieu NT, Vinh PV, *et al.*: **Clinical implications of reduced susceptibility to fluoroquinolones in paediatric Shigella sonnei and Shigella flexneri infections.** *J Antimicrob Chemother.* 2016; **71**(3): 807–15. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
12. Thompson CN, Duy PT, Baker S: **The Rising Dominance of Shigella sonnei: An Intercontinental Shift in the Etiology of Bacillary Dysentery.** *PLoS Negl Trop Dis.* 2015; **9**(6): e0003708. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
13. Chang Z, Zhang J, Ran L, *et al.*: **The changing epidemiology of bacillary dysentery and characteristics of antimicrobial resistance of Shigella isolated in China from 2004-2014.** *BMC Infect Dis.* 2016; **16**(1): 685. [published Online First: 2016/11/20]. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
14. World Health Organisation: **The Management of Bloody Diarrhoea in Young Children.** In: Programme for Control of Diarrhoeal Disease, ed. Geneva: WHO 1994. [Reference Source](#)
15. World Health Organisation: **The Treatment of Diarrhoea: a manual for physicians and other senior health workers.** In: Department of Child and Adolescent Health and Development, ed. 4 ed. Geneva: WHO 2005. [Reference Source](#)
16. World Health Organisation: **Guidelines for the control of shigellosis, including epidemics due to Shigella dysenteriae, type 1.** In: WHO Department of Child and Adolescent Health and Development, ed. Geneva: WHO 2005. [Reference Source](#)
17. World Health Organisation: **Antibiotics in the management of shigellosis.** *Wkly Epidemiol Rec.* 2004; **79**(39): 349–56. [PubMed Abstract](#)
18. Traa BS, Walker CL, Munos M, *et al.*: **Antibiotics for the treatment of dysentery in children.** *Int J Epidemiol.* 2010; **39** Suppl 1(Suppl 1): i70–4. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
19. Vinh H, Anh VT, Anh ND, *et al.*: **A multi-center randomized trial to assess the efficacy of gatifloxacin versus ciprofloxacin for the treatment of shigellosis in Vietnamese children.** *PLoS Negl Trop Dis.* 2011; **5**(8): e1264. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Holt KE, Thieu Nga TV, Thanh DP, *et al.*: **Tracking the establishment of local endemic populations of an emergent enteric pathogen.** *Proc Natl Acad Sci U S A.* 2013; **110**(43): 17522–7. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Duong VT, Tuyen HT, Van Minh P, *et al.*: **No Clinical Benefit of Empirical Antimicrobial Therapy for Pediatric Diarrhea in a High-Usage, High-Resistance Setting.** *Clin Infect Dis.* 2018; **66**(4): 504–11. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
22. Parnham MJ, Erakovic Haber V, Giamarellos-Bourboulis EJ, *et al.*: **Azithromycin: mechanisms of action and their relevance for clinical applications.** *Pharmacol Ther.* 2014; **143**(2): 225–45. [PubMed Abstract](#) | [Publisher Full Text](#)
23. Gladue RP, Bright GM, Isaacson RE, *et al.*: **In vitro and in vivo uptake of azithromycin (CP-62,993) by phagocytic cells: possible mechanism of delivery and release at sites of infection.** *Antimicrob Agents Chemother.* 1989; **33**(3): 277–82. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
24. Gualdoni GA, Lingscheid T, Schmetterer KG, *et al.*: **Azithromycin inhibits IL-1 secretion and non-canonical inflammasome activation.** *Sci Rep.* 2015; **5**: 12016. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

25. Ratzinger F, Haslacher H, Poepl W, *et al.*: **Azithromycin suppresses CD4(+) T-cell activation by direct modulation of mTOR activity.** *Sci Rep.* 2014; **4**: 7438. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
26. Khan WA, Seas C, Dhar U, *et al.*: **Treatment of shigellosis: V. Comparison of azithromycin and ciprofloxacin. A double-blind, randomized, controlled trial.** *Ann Intern Med.* 1997; **126**(9): 697–703. [PubMed Abstract](#) | [Publisher Full Text](#)
27. Basualdo W, Arbo A: **Randomized comparison of azithromycin versus cefixime for treatment of shigellosis in children.** *Pediatr Infect Dis J.* 2003; **22**(4): 374–7. [PubMed Abstract](#)
28. Kim JS, Kim JJ, Kim SJ, *et al.*: **Outbreak of Ciprofloxacin-Resistant *Shigella sonnei* Associated with Travel to Vietnam, Republic of Korea.** *Emerg Infect Dis.* 2015; **21**(7): 1247–50. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
29. Baker KS, Dallman TJ, Ashton PM, *et al.*: **Intercontinental dissemination of azithromycin-resistant shigellosis through sexual transmission: a cross-sectional study.** *Lancet Infect Dis.* 2015; **15**(8): 913–21. [PubMed Abstract](#) | [Publisher Full Text](#)
30. Chung The H, Rabaa MA, Pham Thanh D, *et al.*: **South Asia as a Reservoir for the Global Spread of Ciprofloxacin-Resistant *Shigella sonnei*: A Cross-Sectional Study.** *PLoS Med.* 2016; **13**(8): e1002055. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. Darton TC, Tuyen HT, The HC, *et al.*: **Azithromycin Resistance in *Shigella* spp. in Southeast Asia.** *Antimicrob Agents Chemother.* 2018; **62**(4): e01748–17. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
32. The HC, Thanh DP, Holt KE, *et al.*: **The genomic signatures of *Shigella* evolution, adaptation and geographical spread.** *Nat Rev Microbiol.* 2016; **14**(4): 235–50. [PubMed Abstract](#) | [Publisher Full Text](#)
33. Gomes C, Martinez-Puchol S, Palma N, *et al.*: **Macrolide resistance mechanisms in *Enterobacteriaceae*: Focus on azithromycin.** *Crit Rev Microbiol.* 2017; **43**(1): 1–30. [PubMed Abstract](#) | [Publisher Full Text](#)
34. Zhang C, Zhang R, Yu Q, *et al.*: **Decreased Susceptibility to Azithromycin Among Clinical *Shigella* Isolates from China.** *Microb Drug Resist.* 2017; **23**(5): 596–601. [PubMed Abstract](#) | [Publisher Full Text](#)
35. Murray K, Reddy V, Kornblum JS, *et al.*: **Increasing Antibiotic Resistance in *Shigella* spp. from Infected New York City Residents, New York, USA.** *Emerg Infect Dis.* 2017; **23**(2): 332–35. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
36. Nuesch-Inderbinen M, Heini N, Zurfluh K, *et al.*: ***Shigella* Antimicrobial Drug Resistance Mechanisms, 2004–2014.** *Emerg Infect Dis.* 2016; **22**(6): 1083–5. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
37. Poramathikul K, Bodhidatta L, Chiek S, *et al.*: **Multidrug-Resistant *Shigella* Infections in Patients with Diarrhea, Cambodia, 2014–2015.** *Emerg Infect Dis.* 2016; **22**(9): 1640–3. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Thanh Duy P, Thi Nguyen TN, Vu Thuy D, *et al.*: **Commensal *Escherichia coli* are a reservoir for the transfer of XDR plasmids into epidemic fluoroquinolone-resistant *Shigella sonnei*.** *Nat Microbiol.* 2020; **5**(2): 256–64. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
39. **Management of Infectious Diarrhoeal Diseases in Children.** Ministry of Health, Vietnam. 2009.
40. Thomson KS, Sanders CC: **Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the double-disk and three-dimensional tests.** *Antimicrob Agents Chemother.* 1992; **36**(9): 1877–82. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
41. Dung TT, Phat VV, Nga TV, *et al.*: **The validation and utility of a quantitative one-step multiplex RT real-time PCR targeting rotavirus A and norovirus.** *J Virol Methods.* 2013; **187**(1): 138–43. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
42. **ICH harmonized tripartite guideline: Guideline for Good Clinical Practice.** *Journal of postgraduate medicine.* 2001; **47**(1): 45–50. [Reference Source](#)
43. World Medical Association: **World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects.** *JAMA.* 2013; **310**(20): 2191–4. [PubMed Abstract](#) | [Publisher Full Text](#)
44. Baker S: **The CIPAZ study protocol: An open label randomised controlled trial of azithromycin versus ciprofloxacin for the treatment of children hospitalised with dysentery in Ho Chi Minh City, Vietnam (Version 2.0).** *Zenodo.* 2020. <http://www.doi.org/10.5281/zenodo.4004569>