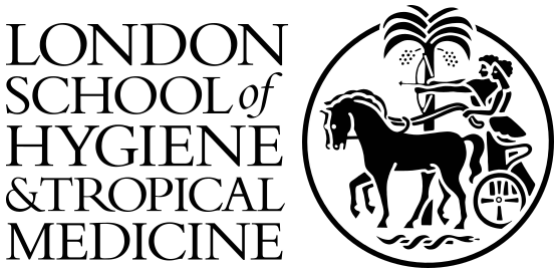

Azithromycin mass drug administration for reducing child mortality in Malawi

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I, John Daniel Hart, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis

Abstract

Background: Child mortality has decreased considerably in recent years but more than one in ten children still die before their fifth birthday in several African countries. The MORDOR study investigated whether azithromycin mass drug administration (MDA) reduces child mortality in three countries in Africa. The study described within this thesis was conducted at the MORDOR-Malawi site and aims to provide additional detail on potential mechanisms of effect of the intervention; macrolide resistance; and cost-effectiveness.

Methods: The study involved cluster randomisation of communities in Mangochi District, Malawi, to biannual azithromycin or placebo MDA. Household visits were conducted to update the census and perform verbal autopsies (VAs) to assess causes of death. Indicators related to healthcare access; malaria risk; and water, sanitation and hygiene were measured. Nasopharyngeal samples were collected to assess macrolide resistance. Cost data were collected for one complete round of fieldwork.

Results: The study included 334 clusters. The mortality rate ratio in azithromycin-treated compared to placebo-treated communities was 0.91 (95%CI: 0.79–1.05); *P*=0.20. There was evidence for an effect of the intervention in infants aged 1-5 months: 0.70 (95%CI: 0.50-0.99); *P*=0.04; but not in older age groups. The VA analyses suggested possible effects on pneumonia, HIV/AIDS and diarrhoea mortality. The intervention was highly cost-effective according to the WHO's willingness-to-pay thresholds, costing $898 per death averted. At the 12-month and 24-month follow-up rounds, macrolide resistance in *Streptococcus pneumoniae* was higher in the azithromycin group compared to placebo.

Conclusion: The mortality findings at the MORDOR-Malawi site could be explained by the broad spectrum of activity of azithromycin against gut and respiratory organisms, including non-vaccine pneumococcal serotypes and other aetiological causes of pneumonia, sepsis and meningitis. Azithromycin MDA is a feasible short-term intervention to reduce child mortality, whilst longer term sustainable health system improvements are pursued. Vigilance of antibiotic resistance is required.

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Abbreviations

ARR	Annual rate of reduction
MDGs	Millennium Development Goals
SDGs	Sustainable Development Goals
WHO	World Health Organisation
EPI	Expanded Programme on Immunisation
HDSS	Health and demographic surveillance systems
ICD-10	International Statistical Classification of Diseases Tenth Revision
NDVI	Normalized Difference Vegetation Index

Chapter 1: Introduction

Global burden of child mortality

Child mortality remains high in many low-income settings, despite considerable progress in recent years. Improving child health and reducing child mortality have been key global health priorities of the last two decades. The United Nations (UN) Millennium Development Goals (MDGs) included a target to reduce the under-5 mortality rate (U5MR) by two-thirds from 1990 levels by 2015.[1] The MDGs have been superseded by the Sustainable Development Goals (SDGs), which include a further target to reduce U5MR in all countries to below 25 per 1,000 live births by 2030.[2]

The MDG target for child mortality was not attained globally, with U5MR decreasing by 54% from 93 to 43 deaths per 1,000 live births and only 62 of 195 countries achieving the target, including 12 low income and 12 lower-middle income countries[3]. However, significant reductions in child mortality have been achieved, with estimates of U5MR in sub-Saharan Africa falling from 180 in 1990 to 86 in 2015, at an overall annual rate of reduction (ARR) of 3.1%.[4] Many African countries at least tripled their rate of progress but, for comparison, an ARR of 4.4% was required for the whole period between 1990 and 2015 for a country to attain the MDG target – at the current rate of decline, it is estimated that the target would be met globally in 2026 (Figure 1.1).[4]

The ARR and how it has changed are useful for assessing progress and countries' potential to attain future targets. The ARR for sub-Saharan Africa increased from 1.9% between 1990 and 2000 to 4.0% between 2000 and 2009 but decreased again in the last decade to 3.4.[3] To attain the SDG target for U5MR by 2030, the ARR would have to increase substantially, especially in sub-Saharan Africa. Figure 1.2 shows country U5MRs, highlighting continuing high child mortality in sub-Saharan Africa, including the 5 countries where U5MR remains greater than 100 per 1,000 live births.

Figure 1.1: Annual rate of reduction (ARR) in under-five mortality in 2000-2015 compared to 1990-2000[4]

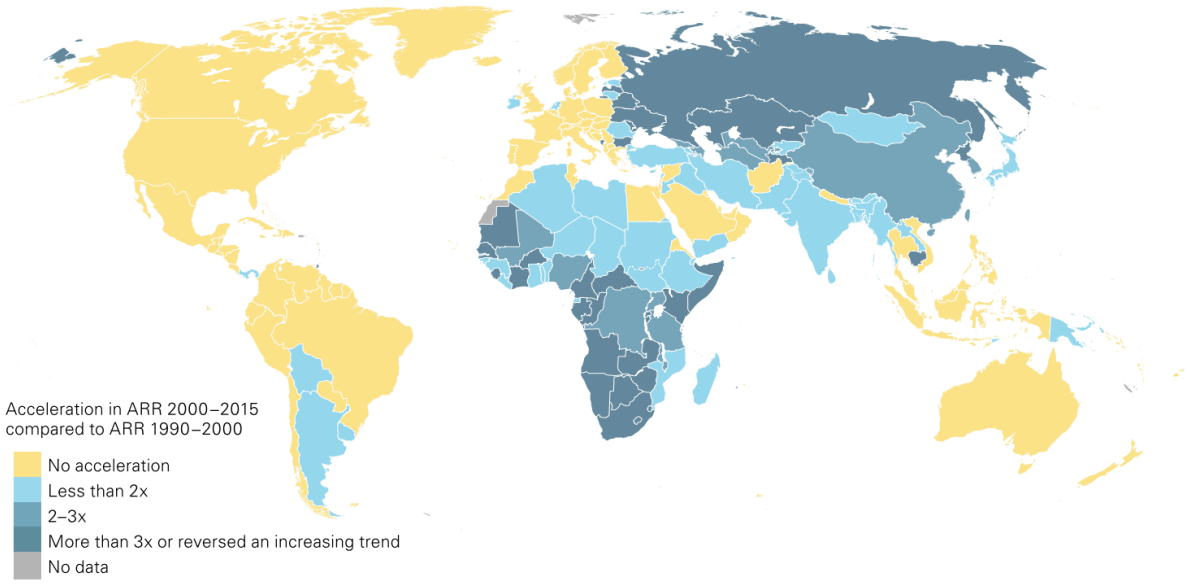
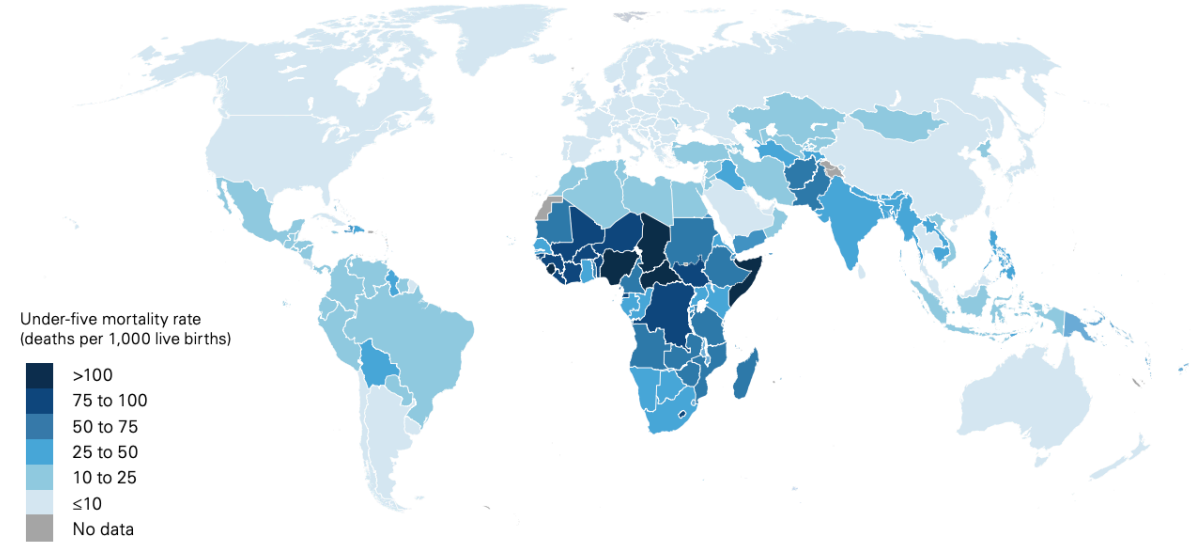


Figure 1.2: Under-five mortality rate (deaths per 1000 live births) by country, 2019[3]



Between 1990 and 2016, under-5 mortality fell from 93 to 41 deaths per 1,000 live births, infant mortality from 65 to 31, and neonatal mortality from 37 to 19.[5] The proportion of under-5 deaths that occur in the neonatal period increased to approximately 46% in 2016 from under 40% in 1990 as neonatal mortality reductions have lagged behind reductions in mortality in older children in all MDG regions. The main neonatal causes of death are preterm birth complications, intrapartum-related events, sepsis or meningitis, congenital abnormalities and pneumonia[5]. Management of these conditions generally requires significant resources and improved health services that facilitate, for example, detection of foetal complications during labour and newborn resuscitation.[6] To highlight the point, for one condition for which an affordable intervention was available – maternal vaccination to prevent neonatal tetanus – an ARR of 9.5% between 2000 and 2010 was achieved, compared to ARRs closer to 2% for the main causes of neonatal mortality.[7]

Amongst the major causes of post-neonatal mortality to have reduced significantly between 2000 and 2017, pneumonia mortality in 1-59 month old children decreased from 11.1 to 4.7 per 1,000 live births, a reduction from 1.45 million to 653,000 deaths per year. Over the same period, diarrhoea mortality in 1-59 month old children decreased from 8.9 to 3.0 per 1,000 live births, a reduction from 1.16 million to 424,000 deaths per year. Malaria mortality decreased from 5.0 to 1.9 per 1,000 live births, from 656,000 to 263,000 deaths.[8]

Child mortality interventions

The main interventions and programs that have enabled significant reductions in child mortality include immunization, use of insecticide treated bed nets, micronutrient supplementation and improved access to and quality of health care, which are discussed in more detail below. However, as reflected by the downturn in the ARR in the last decade, further increasing the rate of reduction of child mortality is likely to be particularly challenging for two reasons. Firstly, as child mortality decreases, the proportion of deaths due to each aetiological cause changes and the proportion of deaths due to unknown causes increases[9,10]. Research to describe the changing causality of child deaths will become more important for optimal implementation of available interventions as well as directing development of new interventions, hence major investment in studies such as the Child Health and Mortality Prevention Surveillance (CHAMPS) global health network.[11] Secondly, the ARR produced by

an intervention will increase with initial roll-out then tend towards zero as that intervention reaches full coverage. Therefore, after an initial "easy" reduction in mortality from the very high levels still seen in many countries in the late twentieth century, maintaining high rates of reduction will require either continued addition of new interventions or sustained socio-economic development with associated improvement in health and health care provision.

The programs with maximum potential to reduce mortality from a public health perspective are generally mass interventions to whole communities. The most important example is immunization, which due to increasing global coverage, and an increasing arsenal of vaccines, now prevents many millions of deaths.[12] Targeting mass coverage of an intervention to whole populations at risk as opposed to testing, diagnosing and treating individuals with a particular condition has several potential benefits: reducing cost per person treated; maximising population coverage; preventing or treating cases early in the clinical course of disease; and reducing community prevalence of pathogens and disease. Cost effectiveness is likely to be further enhanced where different MDA and vaccination programs can be combined, as is the case with the World Health Organisation's (WHO) Expanded Programme on Immunisation (EPI) schedule.[13]

Some interventions may reduce child mortality via multiple mechanisms, for example, breast feeding, WASH interventions and zinc supplementation all reduce both diarrhoea and pneumonia mortality.[14] Although not a medical intervention, the benefits of breast feeding are multiple, including immunological, social and economic. Educational and promotional interventions may be particularly cost-effective in increasing rates of breast feeding and reducing child mortality.[15] Exclusive breast feeding for 6 months with continued breast feeding until 24 months may reduce all-cause mortality 5-fold in those aged 6-11 months and 2-fold in those aged 12-23 months.[16] The optimum mix of cost-effective interventions for reducing avoidable child mortality will depend on local context and may include disease-specific as well as general health, hygiene and nutritional interventions.

Undernutrition, including foetal growth restriction, stunting, wasting and micronutrient deficiency, is believed to be a factor in 45% of child deaths.[17] Whilst less commonly the primary cause of death, nutritional deficiencies reduce the ability to mount an effective immune response against infectious challenges. Many poorer countries rely on a narrow range of energy-dense, micronutrient-poor foods, which can result in micronutrient

deficiency. The four most common micronutrient deficiencies are iron, vitamin A, iodine and zinc, with hundreds of millions of people affected by each of these in low-income settings.[18] Micronutrient supplementation, including large scale fortification of flour, oil, salt and other foods, is an important strategy for improving children's nutritional status.[19] Multiple micronutrient supplementation during pregnancy and breastfeeding is also recommended for maternal and child health benefits.[20]

Multiple factors have contributed to reducing pneumonia mortality, including improved case management, pneumonia-related vaccines, improved nutrition and reduced exposure to indoor smoke from burning solid fuels. The use of *Haemophilus influenzae* type b (Hib) vaccine and pneumococcal conjugate vaccine (PCV), in particular, have reduced the prevalence and changed the aetiology of pneumonia in resource limited settings: viral pneumonia and multiple-pathogen pneumonia are now more common and the leading bacterial causes are *Staphylococcus aureus* and non-type b *H. influenzae*.[21]

Highly effective interventions for reducing diarrhoea mortality include improvements to water, sanitation and hygiene, mass supplementation with zinc, and rotavirus vaccine.[22–25] Interventions to reduce malaria mortality in addition to improved case management include intermittent preventive treatment, indoor residual spraying, intermittent spraying, and, most cost-effective, the use of long-lasting insecticide treated bed nets.[26–28]

There are several examples of mass drug administration for reducing child mortality. Intermittent preventive treatment of infants and children through routine immunization services, and seasonal malaria chemoprevention with sulfadoxine-pyrimethamine plus amodiaquine, are proven interventions for reducing child mortality.[29–31] In HIV-infected children, co-trimoxazole has been shown to reduce mortality and morbidity both before the commencement of anti-retroviral therapy and subsequently.[32–34] Interestingly, there is widespread resistance to cotrimoxazole in Africa, which does not appear to impact the effectiveness of the cotrimoxazole intervention, the reasons for which are not clear.[35]

Precision public health and cost-effectiveness of child mortality interventions

Precision public health refers to the use of data to more efficiently guide interventions for maximum public health benefit. This requires robust surveillance data, sophisticated analyses to understand the geographical distribution of disease and the capacity to act on such

information.[36,37] The use of datasets and precise analyses is normal in all sectors in high income settings, particularly guiding public health interventions. Whilst much improvement in data gathering and analysis capacity is required in many low income settings, progress is being made towards greater precision in public health interventions, including in the understanding of child mortality.[38] Five by five kilometer grid square mapping of child mortality in sub-Saharan Africa has used available data, albeit relatively sparse for many countries, to produce more detailed predictions of child mortality.[39]

The extent to which child mortality varies even within countries is considerable, as shown in relatively small geographical areas for many countries by Health and demographic surveillance systems (HDSS) data.[40–48] Understanding spatial variation in child mortality may be used to identify areas of increased risk, allowing policymakers to target these with relevant public health interventions, and researchers to develop hypotheses regarding the reasons for the geographical pattern. Examples of the use of geostatistical tools to guide public health interventions include analyses of childhood growth failure, and diphtheria–pertussis–tetanus vaccine coverage and dropout, both across Africa.[49,50]

The cost-effectiveness of public health programs is closely related to precision public health, where the ultimate aim is to improve efficiency. Cost-effectiveness considerations are important for programs to maximise benefit with frequently scarce resources. However, the direct benefits of many interventions can be difficult to define, for example nutritional interventions may improve child health in multiple ways and reduce mortality from several specific causes. Well conducted trials are one method of isolating the effect of an intervention and enabling cost-effectiveness analyses. More complex analyses to estimate inefficiencies in a health system and the potential benefits of reallocation of resources to more cost-effective interventions can also be conducted.[51]

For childhood pneumonia, cost-effectiveness analyses suggest nutritional interventions, community case management and vaccination are particularly cost-effective, whilst improving facility-based care and introducing cleaner cooking methods may be less so.[52] For reducing diarrhoea mortality, rotavirus vaccine is a particularly cost-effective intervention, whereas the evidence is less clear for the cost-effectiveness of attempts to change hygiene practices.[53,54] For interventions related to general improvements in living standards, such as installation of water and sanitation facilities, improvements in several morbidity and

mortality indicators may be difficult to isolate. Furthermore, such interventions, related to socioeconomic development, are likely to have multiple non-health as well as health benefits that may be considered in broader cost-effectiveness analyses.

Measuring child mortality and causes of death

Attempts to estimate mortality rates and causes of death globally are based on available surveys, censuses and vital registration data. Models for estimating global burden of disease vary considerably, for example the Institute for Health Metrics and Evaluation's (IHME) estimate of global U5MR differed by 800,000 - more than 10% - from the WHO's Child Health Epidemiology Reference Group's estimate for the same year.[55] There is a current focus towards achieving more accurate estimates of global mortality and morbidity, in order to improve health policy at a local level and the targeting of interventions to reduce mortality. This is illustrated in the Bill and Melinda Gates Foundation's 20-year pledge to support improved surveillance for child morbidity and mortality: the Child Health and Mortality Prevention Surveillance Network (CHAMPS).[56] CHAMPS aims to use Minimally Invasive Tissue Sampling (MITS) to improve the understanding of deaths from unknown causes. The research will take place at selected sites and require significant resources. More widespread data on cause of death in areas lacking healthcare professionals, diagnostic and research facilities and vital statistics registration, may be achievable using verbal autopsy (VA). VA would not generally be considered comparable to medical certification of cause of death by a health professional but it may offer a practical, feasible and cheap method of collecting cause of death data.[57,58] There is likely to be continued need for mortality estimates from methods such as VA for rural areas of developing countries in the coming decades despite recent global improvements in access to health care.[59]

Systematic interviews for determining cause of death have been used since the 1950s and 1960s in Keneba, The Gambia, and Khanna and Narangwal, India.[57,60] Multiple different VA questionnaires have been used, making reliability and comparability historically difficult. The WHO produced its first VA tool in 2007, aiming to standardize VA across regions and countries[61]. Physician review of the interviews was required, with coding according to International Statistical Classification of Diseases Tenth Revision (ICD-10) classification of disease, a labour intensive and costly process.[62] WHO working groups updated the

questionnaire in 2012, recommending routine use of freely available InterVA software for grading the interviews, after several studies showed diagnosis from the algorithm was non-inferior to physician review.[63,64] There is no consensus as to the superiority of diagnosis using computerized algorithms or physician review of VAs but with at least comparable results, the feasibility of automated methods is an advantage in many settings.[65]

The two most widely used programs for automated coding of VA after 2012 have been InterVA, produced by the Umea Centre for Global Health Research; and SmartVA using the Tariff method designed by IHME.[66,67] Neither program was clearly better than the other in comparative studies, which led to the WHO releasing an updated questionnaire in 2014 that incorporated questions to be used with both methods and also excluded questions relating to conditions that had previously been poorly diagnosed by VA.[68,69]

InterVA software uses input of dichotomous questions and estimated probabilities related to diseases and symptoms, informed by expert panel discussions and data from the field.[70] SmartVA, using the Tariff method, applies a scoring system reflecting the importance and uniqueness of each symptom to each cause of death in a large dataset with hospital diagnosis as the gold standard cause of death.[71,72] It is an additive algorithm where the scores for each cause of death are summed and the summed scores used to assign most likely causes of death.

Azithromycin and child health

Azithromycin is an azalide antibiotic, a newer sub-class of macrolide antibiotics with a 15-membered lactone ring as opposed to the 14-membered macrolides such as erythromycin, clarithromycin and roxithromycin. It has a similar mechanism of action to the older macrolides, inhibiting protein synthesis through binding the 50S subunit of the bacterial ribosome, but is considered to have improved safety and tolerability.[73,74] It has a broad spectrum of activity, including against a range of respiratory and gastrointestinal pathogens, as well as antimalarial properties.

Azithromycin is an effective treatment for ocular *Chlamydia trachomatis* infection, the causal organism of trachoma, the leading infectious cause of blindness globally.[75,76] It forms a core part of the SAFE strategy for elimination of trachoma (Surgery for entropion/trichiasis; Antibiotics for infectious trachoma; Facial cleanliness to reduce transmission; and

Environmental improvements, such as control of disease-spreading flies and access to clean water).[77] Following widespread rollout of azithromycin MDA for trachoma control, sub-studies within trachoma trials indicated that azithromycin may reduce all-cause mortality in children.[78,79] The MORDOR trial (see below) was the first study designed to assess the effect of azithromycin MDA on child mortality.[80]

Azithromycin is an effective treatment for community-acquired pneumonia, including the major aetiological causes, *S. pneumoniae* and *Haemophilus influenzae*.[81] Single dose azithromycin has been shown to reduce carriage of *S. pneumoniae* and decrease incidence of pneumonia.[82,83] When given as MDA, a single round of azithromycin has also been reported to decrease carriage of *S. pneumoniae* for 1-3 months after treatment, with recolonisation mostly complete after 6 months.[84,85] Rollout of *H. influenzae* type b (Hib) vaccine and pneumococcal conjugate vaccine (PCV) in much of sub-Saharan Africa is contributing to significantly reduced pneumonia morbidity and mortality.[86] However, the activity of azithromycin against these organisms, including non-vaccine pneumococcal serotypes and other aetiological causes of pneumonia, could augment the reductions in child mortality. In Tanzania, a 38% decrease in risk of childhood pneumonia in the month immediately following azithromycin MDA has been reported.[87]

Rotavirus in the leading overall cause of death from diarrhoea globally but more than 250,000 children still die each year from the main bacterial causes, namely enteropathogenic and enterotoxigenic *Escherichia coli*, *Shigella spp*., *Campylobacter spp*., *Salmonella spp*. and *Vibrio cholerae*[88]. Azithromycin has good efficacy in treating diarrhoea caused by all of these pathogens and is a recommended empirical treatment for diarrhoea.[89–92] Following azithromycin MDA, a 40% reduction in diarrhoea episodes was reported in The Gambia in the following 28 days.[93] A study in Nepal reported a reduction in the prevalence of diarrhoea in children from 32% to 11% in the 10 days following azithromycin MDA.[94]

In addition to its antibacterial effects, azithromycin has good efficacy against *Plasmodium falciparum* and *P. vivax* when used as either mono- or combination-therapy for malaria prophylaxis or treatment.[95–97] Azithromycin MDA has been shown to decrease malaria symptoms and malaria parasitaemia.[93,98–100] Further to its broad anti-microbial effects, azithromycin also exhibits advantageous pharmacokinetic and pharmacodynamic properties compared to older macrolides that could prove beneficial for its use in the context of MDA.

In addition to the time-dependent antibacterial effects shared with other macrolides, azithromycin displays concentration-dependent bactericidal effects and a post antibiotic effect, sustaining suppression of bacterial growth even after azithromycin concentration has decreased.[101–103]

Azithromycin accumulates extensively in cells, including tissue fibroblasts, macrophages and polymorphonuclear leucocytes, attaining intracellular concentrations up to several hundred-fold higher than in extracellular fluid.[104–106] The high concentrations in phagocytic cells are believed to produce a targeted delivery mechanism for the drug to sites of infection, via the body's normal immune response, with release during the process of phagocytosis.[106,107] Azithromycin has a particularly long terminal half-life of 68 hours, that combined with phagocyte delivery to sites of infection, may maintain effective tissue concentrations for several days.[108,109] This sustained therapeutic window and the post antibiotic effect, may enhance the potential for single dose azithromycin MDA to have beneficial effects on child health and mortality.

In addition to delivery of the drug to sites of infection, two further mechanisms may augment the beneficial effects of azithromycin. Firstly, even at sub-inhibitory concentrations, azithromycin has an impact on several bacterial virulence factors, including adhesion to epithelial cells, toxin production and susceptibility to the host immune response.[110,111] Secondly, immunomodulation has been proposed as the reason both the 14- and 15-membered macrolides show benefit in the treatment of chronic lung diseases such as diffuse pan-bronchiolitis and cystic fibrosis separately to any anti-microbial effects.[112] The effect appears to be through modulation of polymorphonuclear leucocyte response and the pro-inflammatory cytokines IL-1, IL-6 and IL-8.[113] Chronic immunostimulation, such as caused by environmental enteropathy, has been shown to be a contributing factor in malnutrition and growth retardation.[114,115] As malnutrition is a major contributor to under-five morbidity and mortality, it is feasible that reduced inflammation and the metabolic requirements for the acute phase response, could improve child health and nutritional status, thereby contributing to a decrease in mortality.

The MORDOR study

Following the evidence from trachoma studies that azithromycin MDA may have broader health benefits, a trial was designed specifically to assess the effect of azithromycin on child mortality. The Mortality Reduction after Oral Azithromycin (MORDOR) study was a multicentre cluster randomized trial involving biannual azithromycin MDA to children aged 1-59 months. The London School of Hygiene and Tropical Medicine managed the trial in Malawi; Johns Hopkins University in Tanzania; and the University of California, San Francisco (UCSF) in Niger (ClinicalTrials.gov identifiers for mortality and morbidity components, respectively: NCT02047981, NCT02048007). These three countries were selected for the study as they have varying rates of child mortality. Malawi was chosen as the country with intermediate child mortality; Niger for high mortality; and Tanzania for low mortality. The U5MR in Malawi was 64 per 1,000 live births in 2015, having decreased from 242 deaths per 1,000 live births in 1990 at an ARR of 5.3%.[4] The respective rates in Niger and Tanzania at the start of the study in 2015 were 96 and 49 deaths per 1,000 live births.

The southern lakeside district of Mangochi was chosen as the MORDOR-Malawi study site. Mangochi is one of the poorer districts in Malawi with high birth rates, low literacy and little formal employment.[116] At the last HDSS in 2010, 35.3% of women and 17.8% of men in Mangochi had received no education, the highest figures for Malawi.[116] The majority of the population belong to the Yao tribe and the two main sources of sustenance and income are farming maize and fishing on Lake Malawi.

The randomisation unit for MORDOR-Malawi was the population covered by a Health Surveillance Assistant (HSA). HSAs receive three months of basic clinical and community health training and are involved with any health-related activities in their communities. They commonly work some days of the week in their local health centre. The total population of each HSA's catchment village, or group of villages, is approximately one thousand people. For the MORDOR study, communities covered by each HSA, known as HSA areas, were randomised to receive 4 biannual distributions of either azithromycin or placebo syrup to all children aged 1-59 months. Follow-up census was conducted biannually until 6 months after the final treatment. In addition to the main mortality analysis, a subset of 30 communities were randomly selected for more intensive follow up and assessment of morbidity indicators, including biannual collection of anthropometric indices on all children and collection of

samples from a random selection of 40 children annually, including: blood spots, malaria slides and haemoglobin measurement, nasopharyngeal (NP) and conjunctival swabs and stool samples.

Rationale for this work

This study reports on supplementary analyses of the data gathered by the MORDOR-Malawi study and is intended to inform the main MORDOR mortality results. It aims to explore factors associated with the effects of azithromycin MDA on child mortality, heterogeneity in the effect of the intervention, the effect on malaria parasitaemia, macrolide resistance, and cost-effectiveness. Malawi, as the site with intermediate child mortality, compared to Niger and Tanzania, may be particularly useful for understanding the effects of the intervention. Indeed, if mortality varies over a smaller scale in Mangochi District, understanding that heterogeneity and any associated heterogeneity in the effect of azithromycin, could help inform future plans for azithromycin MDA as an intervention for reducing child mortality.

Malaria parasitaemia was estimated to be 20% in Malawi in 2009 so community distribution of a drug with some antimalarial activity in such a high prevalence setting may reduce malaria morbidity and mortality.[117] Given the previous studies reporting reductions in malaria morbidity following azithromycin MDA, assessment of malaria parasitaemia, as well as malaria mortality, in the setting of biannual azithromycin MDA targeted to children only, will be important for understanding the effect of the intervention and how this may differ depending on the background malaria prevalence.

Interventions involving mass drug administration with antibiotics raise concerns regarding the development of antibiotic resistance and potentially reduced efficacy of the intervention or other negative health effects. Macrolide resistance has generally been reported to increase following whole community MDA for trachoma elimination.[118–120] There is, however, little evidence for the effect on macrolide resistance following MDA targeted only to children. Understanding the development of macrolide resistance in this setting, as well as its effect on the efficacy of the intervention, will be important considerations for future rollout of the intervention. It is notable that treatment with co-trimoxazole is given to particular at-risk populations, such as children with HIV, even in settings where there is known resistance to

the drug, as it maintains a beneficial effect.[35] Therefore there are multiple considerations regarding the effectiveness of the intervention for reducing mortality; the levels of resistance identified and the potential consequences of that resistance; as well as potential alterations in the community microbiome and prevalence of pathogenic organisms.

Further to assessing the effectiveness of the intervention, an assessment of cost-effectiveness will be conducted as this will be a key consideration regarding rollout of the intervention.

Study hypotheses

1. Azithromycin MDA reduces child mortality from the major causes, including pneumonia, diarrhoea and malaria, as identified by verbal autopsy.
2. The effect of azithromycin MDA on mortality in children aged 1-59 months exhibits spatial heterogeneity associated with: access to healthcare; malaria risk factors; and water, sanitation and hygiene indicators.
    a. Azithromycin MDA reduces the prevalence of malaria parasitaemia.
3. Azithromycin MDA increases macrolide resistance in nasopharyngeal *S. pneumoniae*.
4. Azithromycin MDA is a cost-effective intervention for reducing child mortality.

Chapter 2: Methods

Study design

The methodology and initial findings of the multi-centred MORDOR trial are reported elsewhere.[80] The PhD study described herein was nested within the work carried out at the MOROR-Malawi study site, the field work of which was coordinated by the author.

Implementation of study activities at the MORDOR-Malawi site of Mangochi District first involved analysis of population estimates from the 2008 government census. Urban centres were excluded from the study, as were any villages with a total population estimate greater than 4,000 from the government census.[121] A pre-baseline census of rural communities with a population <4,000 from the government census was then conducted during October-December 2014. Finally, communities with child population >600 on the pre-baseline census were excluded and the remaining 334 communities were eligible for inclusion in the study.

Fifteen communities per arm (30 in total) were randomly selected from the overall study sample of 334 communities to be part of the morbidity monitoring study. Selecting 40 children per community, that is 600 children per arm, would provide 90% power to detect a reduction in malaria parasitaemia from 20% to 10% in the treated group compared to placebo. It would also provide 80% power to detect a 20% increase in carriage of macrolide resistant pneumococci in the treated group, assuming alpha of 0.05, pneumococcal carriage in 40% of children and an ICC of 0.11.[119] The random selection of morbidity communities was conducted by the team at UCSF from the list of study communities provided.

Study communities were randomised to receive either azithromycin or placebo and all children present in the communities were offered biannual MDA with the allocated study drug for a total of four distributions. Study census was conducted biannually to: update the status of each child; update the cohort (with entry and exit due to migration and age); and identify children for treatment. To ensure study drug masking, ten different letters to label azithromycin and placebo drug bottles were produced for use in the HSA areas included in the mortality study, and six further drug letters for the 30 morbidity study communities. The allocation of drug letters was also facilitated by the team at UCSF, in order to maintain masking of all study personnel involved in the MORDOR-Malawi study.

Training

Recruitment and training for enumerators to conduct census work started in Monkey Bay zone in October 2014. HSAs identified candidates from their villages who had a secondary school leaver's certificate. Candidates were invited to a training day, where the study was explained and their ability to learn on touch-screen devices assessed. The best candidates, selected by data input and aptitude tests, underwent two further days of training and assessment. This training involved data collection and editing as well as an introduction to research ethics and the rationale for the study. Discussion and roleplay were used to prepare enumerators for recording multiple eventualities, including different types of family structure and unusual situations at the household.

The pre-baseline census, primarily implemented to produce accurate village populations, also allowed the census enumerators to gain experience, and enabled troubleshooting of the data collection application (app). The app was custom-made by an independent contractor for use on Android devices and required many updates and bug fixes, particularly in the early stages. Regular staff meetings were held during this period for enumerators to share experiences, discuss challenges, and learn from each other the best strategies for the work. Immediately prior to the baseline visit, enumerators received refresher training as well as learning the additional skills required to find and update household lists and record the treatment administered to each individual. Twenty-four enumerators were selected for the first round of fieldwork in Monkey Bay zone, and additional enumerators were recruited and trained in subsequent zones. Before the second round of fieldwork, a core team of ten enumerators was selected to become permanent staff who would work with 14 enumerators recruited locally in each zone for each round of fieldwork.

The HSAs attended one day of training prior to the study commencing, plus an additional refresher day before each biannual round of fieldwork. HSAs were trained on aspects of receiving, storing and returning study drug; suspending the powder to make azithromycin or placebo syrup; and administering the drug using disposable syringes, as shown in Figure 2.1. HSAs were also taught to determine drug dose using height-dosing sticks for those aged 1 year and over, and by weighing infants under 1 year of age. Training also included the study

rationale, research ethics, and the expected roles of enumerators, HSAs and village volunteers.

A total of ten nurses, registered with the Nurses and Midwives Council of Malawi, were recruited to collect samples and data for the assessment of morbidity. They received training on the clinical procedures as well as sample labelling, transport and storage. Three of the study nurses were also trained to perform verbal autopsy interviews with family members of children who died in the study. This training was of one day duration and included practical aspects as well as ethical considerations and discussion of local customs and sensitivities.

Figure 2.1 Photographs of mass drug administration to a (A) baby; and (B) child during the MORDOR-Malawi fieldwork

A

B

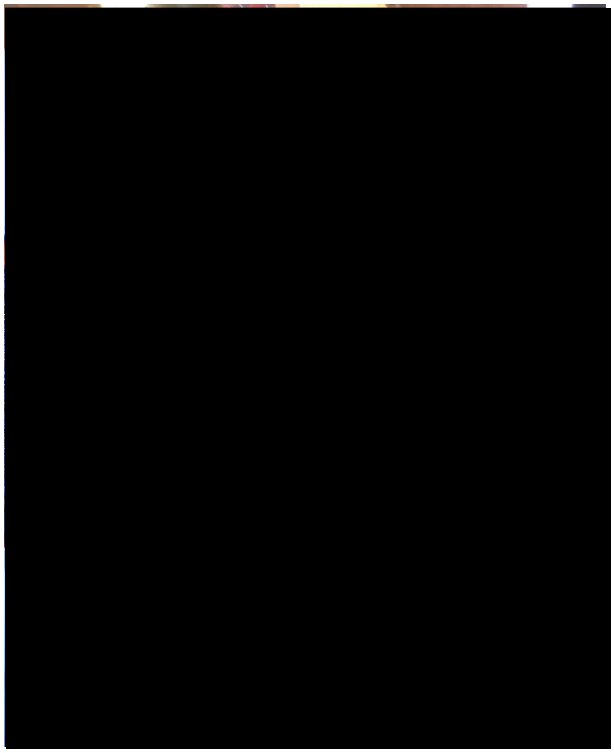
Fieldwork

Fieldwork for this study was coordinated in collaboration with the District Health Office in Mangochi District. An allowance was provided by the study for the HSAs to administer the MDA. Mangochi District is divided into five administrative zones: Monkey Bay, Chilipa, Makanjira, Namwera and Mangochi. Fieldwork was conducted by zone for logistical reasons, particularly to organise the work with the HSAs. Several study and hire vehicles were used to provide transport for enumerators allocated to work in villages distant from their home (and on occasion for HSAs who were not residing in their communities).

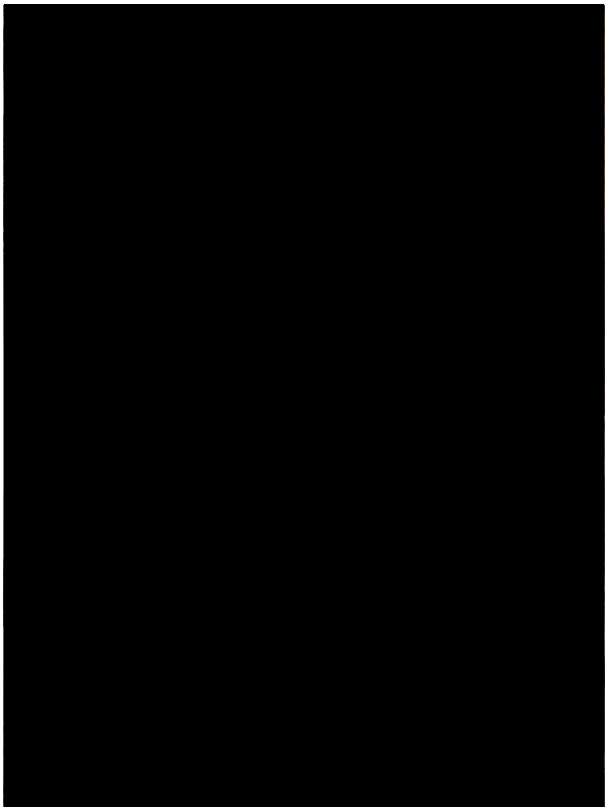
One enumerator worked in each HSA area in a team of three with the HSA and a village volunteer (an unpaid village representative selected by the community who the HSA may call upon for support). When a team completed their area, the enumerator moved to a new HSA area and continued the process. The team updated the census for each household and distributed study drug to all children aged 1-59 months in the MORDOR mortality communities. In morbidity communities, the census was first updated to enable random selection for morbidity sample collection, which was conducted at a second visit, and followed by a third visit for the MDA. The final two visits were separated to remove the potential for sample contamination with study drug if sample collection and MDA were to occur on the same day. Children under one year of age were weighed and those weighing ≥3.8kg were administered a volume of study drug equivalent to 20mg/kg of azithromycin. Children weighing <3.8kg were not treated due to the risk of infantile hypertrophic pyloric stenosis[122]. Children aged 1 year and over were given a dose according to their height (see detail below and Figure 2.2). Distribution of study drug took place at 4 census rounds: baseline and follow-up at 6, 12 and 18 months. A final census update was undertaken 24 months post-baseline.

Figure 2.2: Photographs of (A) estimating dose of study drug using the height-dose stick; and B) weighing an infant to calculate dose based on weight

A



B



Dose calibration

Children enrolled in the study were to be offered 20mg/kg of azithromycin syrup at a concentration of 40mg/ml, or the equivalent volume of placebo syrup. Due to the number of children in this study, weighing each child to administer an exact dose of 20mg/kg azithromycin was not a feasible option. It was therefore decided to estimate dose based on height, an approach that can save a significant amount of time, especially when a height-dosing stick is used. A height-dosing stick is a stick with bands on it that each child stands against to estimate dose, as shown in Figure 2.2. The International Trachoma Initiative (ITI) recommend a height-dose stick that is calibrated to deliver 20-40mg of azithromycin to children, which would significantly overdose children for this study.[123] As this study aimed to determine the effect of azithromycin at a dose of 20mg/kg on child health, data were collected during the pre-baseline census to optimise a height-dose stick for the Malawi setting.

Height and weight were measured for 794 children in Monkey Bay zone, Mangochi District. The dose of azithromycin (mg/kg) that would be offered to each individual if they received whole integer volumes of study drug (2-10ml) was then calculated. Cut-offs were determined that would minimise the number of individuals receiving <15mg or >30mg, shown in Table 2.1. Having optimised the height-dosing stick, only two children (0.3%) fell outside that range.

Anthropometry data collected for 1,421 children across all zones of Mangochi District at the baseline visit were used to validate the height stick. Based on these data, if the height-dosing sticks were used exactly as designed, six children (0.4%) would not have received 15-30mg/kg (two children <15mg and four children >30mg). The mean dose offered would have been 21.9mg/kg. Following this validation, the height-dosing sticks were kept the same for subsequent treatment rounds.

Table 2.1: Height-based dosing schedule for azithromycin optimised and validated using data from Mangochi District

Height (cm)	Dose (ml)	Dose (mg)
53.5-61.4	3	120
61.5-69.4	4	160
69.5-78.4	5	200
78.5-86.4	6	240
86.5-95.4	7	280
95.5-103.4	8	320
103.5-112.4	9	360
112.5+	10	400

Data collection

Mortality data were collected using biannual census updates. Census data recorded included the head of each household; any children under five years of age, their date of birth (or age if date of birth unknown); and their mother or guardian. Pregnant women were also recorded as a means of maximising follow-up for all births at the following census visit. To be recorded as present, a child had to have slept at the household the night before. If this was not the case, the child was recorded as temporarily absent if the family expected the child to return to the household within one month; and permanently absent if they were expected to have moved away for longer than one month. The location of each household was recorded at each census visit. In addition to the main purpose of enabling geospatial analyses, having GPS coordinates for each household visit also provided supervisors with an additional data quality check. At the 24-month visit, location data were also collected for all health facilities in the study area.

Following each census round (approximately three months duration), data identifying deaths of study participants were compiled by cluster, and household visits were organised by the VA interviewers. The three study nurses trained on VA methodology conducted the interviews using the WHO 2014 questionnaire. This was administered using ODK software on Android devices (Google Nexus 7 tablets) and data were uploaded to a secure server at LSHTM. Prior to VA interviews in a cluster, one of the study nurses contacted the HSA whose area it was. HSAs had been briefed on the VA process during their training and were asked to discuss this

with the families of the deceased children and request a suitable day for the visit. At least three attempts were made to visit each family that agreed to the VA interview. When visiting to conduct the interview, the nurses took a small token of appreciation and condolence, as is culturally appropriate when visiting a bereaved family. This was usually a bar of soap plus some sugar or maize flour. The interviewer would generally sit at the front of the house and request non-family members remain at a distance to maintain privacy, as crowds, particularly of children, commonly follow study teams during visits.

Thirty HSA areas were randomly selected from across the study district for the collection of data to assess child morbidity. In order to ensure the pace of sample collection fieldwork matched that of the census fieldwork, facilitating travel arrangements and other logistical considerations, the randomisation was stratified so that six HSA areas were selected from each of the five administrative zones of Mangochi District. This was also expected to be beneficial in ensuring sample collection from geographically diverse areas. To ensure adequate drug supply, the randomisation was also restricted, using the pre-baseline census data, so that the total number of children to be treated with each of the six morbidity drug letters (three corresponding to azithromycin and three to placebo) each round was not expected to be greater than 1,250 children.

Samples were collected at the baseline, 12-month and 24-month follow-up visits from 40 randomly selected children in each morbidity HSA area. The random selection was made for each area by the MORDOR-Malawi study coordinator after the census had been completed for that cluster. Height or length, and weight, measurements were taken from all children aged 1-59 months at the baseline visit and on the same cohort at each biannual follow-up visit, plus new children who entered the study census.

During sample collection visits, a station was set up at a central location in a community, usually outside and under a large tree for shade. Tables were set up for registration of children, taking anthropometry measurements, and collection of samples. The HSAs informed community members in advance and requested that parents bring their children on the day of the visit. Children presenting to the registration station were first identified on a sample collection app on the tablet devices. The app indicated whether each child had been randomly selected for both sample collection and anthropometry measurements or only anthropometry measurements. Anthropometry was conducted first. The length of children

under one year of age was measured supine using infant length boards (Seca 417). Height was measured for those aged 1 year and older using a Leicester portable height measure. Children were weighed using digital flat scales (Seca 877). Weight for small children unable to stand on the scale themselves was measured by asking the carer to first stand on the scale alone, taring the scale, then asking the carer to stand on the scale whilst carrying the child. All anthropometry measurements were taken in triplicate and the middle value was used in the analyses.

Following anthropometry, there was no further testing for the majority of children. The 40 children in each community randomly selected for sample collection then proceeded to a table for finger prick blood testing. This included Hb measurement using a Hemocue 201 device (Ängelholm, Sweden); collection of blood spots on filter paper; and thick and thin blood films on a single glass microscope slide. Unique numbers and QR codes on pre-printed stickers were attached to the filter papers and slides and scanned under both the appropriate test and the appropriate child using the data collection app.

Children then proceeded to a separate table where the nurses would take a nasopharyngeal swab using a COPAN FLOQSwab^{TM}. Sample tubes were also labelled with a unique number and QR code, and scanned using the data collection app (multiple Android tablets were used at the different stations). Samples were stored in skim milk tryptone glucose glycerine (STGG) media and placed on ice in the field. On the team's return to Mangochi town each evening, samples were frozen at -80°C at Mangochi District Hospital. Finally, children proceeded to a table where their accompanying parent or guardian was provided with a stool collection kit and instructions on how to collect the stool sample. Some samples were returned to the field team the same day and a member of the study team returned to the community the following day to collect additional samples. The guardians of children selected for sampling were given a sack of maize flour as a token of appreciation for their time.

At the 24-month follow-up visit, additional data were collected in the morbidity communities. A survey of water, sanitation and hygiene indicators was designed and added to the data collection app to be administered to one third of households in the morbidity HSA areas. Data collected included time taken to collect water; use of improved water sources; and use of any latrine or improved latrine. Fieldworkers also requested to photograph the vaccination

records in the health cards of all children in the morbidity HSA areas. The vaccination data were subsequently compiled to produce cluster-level vaccination coverage data.

Cost data were collected to assess cost of the MDA and calculate cost-effectiveness. Budgeting for the study was organized on a zonal basis and the study employed a finance officer to assist with administering the budget; distributing per diems and allowances to study staff, HSAs and village volunteers; purchasing fuel for vehicles; and collecting receipts and accounting for funds spent. All costs for the 12-month follow-up visit were recorded in order to analyse financial costs for the MDA overall at the study site, as well as disaggregated by zone.

In addition to the data collected directly by the study, data on mortality and various other health indicators were requested from the District Health Office. Data routinely collected by the HSAs, as a part of their work for the Ministry of Health, include mortality data disaggregated to <5 years and ≥5 years, as well as community access to water sources, latrines, healthcare and other health indicators.

Data processing

As GPS coordinates were collected for most households at each census visit, the following process was followed to derive a single point location for each household. First, the median latitude and median longitude for each household from all visits were calculated, then the latitude and longitude for each visit were subtracted from the medians. Any measurements that differed by more than 0.01 degrees (approximately 108m) were excluded. The mean was then taken of the remaining measurements to give the final household coordinates. HSA areas were then mapped, and additional cleaning was conducted to remove coordinates of households clearly outlying from their communities.

In addition to the GPS coordinates collected during the study, maps of the road network in Mangochi District and of Lake Malawi and Lake Malombe were provided by the National Statistics Office in Zomba, Malawi. These were used to calculate the distance from each household to the nearest road and to the nearest large water body using ArcGIS software. Distances were similarly derived for the distance from each household to the nearest health facility locations, as collected in the study.

Environmental data were also obtained from publicly available sources. Land surface temperature data were downloaded from the WorldClim Global Climate Data website with a resolution of 30 arc seconds (approximately 1km^2).[124] Normalized Difference Vegetation Index (NDVI) data at the same resolution were downloaded from the United States Geological Survey; data are derived from the Moderate Resolution Imaging Spectroradiometer (MODIS) instrument that operates from NASA spacecraft.[125] Values were extracted from raster maps of mean annual temperature and mean NDVI for the month of March (end of the wet season in Malawi) to households in the study area using ArcGIS software.

District Health Office data for the year from May 2014 to April 2015 were provided for this study. Data were linked to the study HSA areas using both the HSA names and village names. The HSAs frequently change communities, village names can have different spellings, and villages also split to create new villages not infrequently. Consequently, data were matched for 298 of the 334 study clusters.

Chapter 3: Effect of azithromycin mass drug administration on mortality and causes of death

Introduction

This chapter investigates the effect of azithromycin MDA on all-cause and cause-specific mortality at the MORDOR-Malawi site. With few published studies available on the effect of this intervention on child mortality, particularly cause-specific mortality, this study may provide important insights into the mechanism of effect of azithromycin on child mortality and guide future research on the intervention. Whilst the study was not powered to detect effects on cause-specific mortality, available evidence is presented for the purposes of hypothesis generation.

Official recording of cause of death is rarely conducted in Mangochi District, similar to many low-income settings, due to limited medical personnel and capacity for completing medical certificates of cause of death. Many deaths occur in the community unknown to the health system. For these reasons, verbal autopsy (VA) interviews were conducted by nurses at the Malawi site for deaths that were identified from the census, in lieu of more formal data on cause of death. The VA methods used have been validated to predict population level causes of death with relative accuracy.[69] The bulk of the methodology and results for this chapter – relating to cause specific mortality – have been published in a peer-reviewed journal (see below).[126] The published manuscript is supplemented in this chapter with analyses of mortality overall in the study and validation of the study findings using government data collected by the HSAs as a comparator.

Methods

As detailed in Chapter 2, the MORDOR-Malawi study site included clusters solely for assessment of mortality plus additional morbidity clusters in which clinical samples and anthropometry measurements were also taken. The morbidity clusters received three visits during each round of field work (as opposed to one visit for the mortality clusters): a census update visit, sample collection visit, and treatment visit. Prior to finalization of the datasets

and data analysis, and following discussion with other MORDOR investigators, it was decided that in order to increase the power of the analyses of cause-specific mortality (this chapter), and heterogeneity of effect of the intervention (Chapter 4), to use the combined dataset of morbidity and mortality clusters. The combined dataset was first analysed by age group using a Poisson regression model with random effects for randomisation unit.

The VA interviews, collected using the WHO 2014 VA questionnaire, were analysed using both the SmartVA and InterVA algorithms. The InterVA program can use the WHO 2014 questionnaire directly as input. To run the SmartVA program, the questionnaire was mapped to the required input format, as detailed in the published manuscript included in this chapter.[126]

The mortality data obtained from the Malawian health system's routine population counts, as reported quarterly by the HSAs to the DHO were summarized at the zonal level to use as a verification of mortality data derived from the biannual censuses for this study.

Mortality rates were calculated by zone for both this study and the DHO data for the year prior to commencing the study. Difference between treatment arms for the complete dataset for this study, as well as the mortality and morbidity communities separately, was tested using Poisson regression with random effects for clustering at the level of the MORDOR randomization unit.

Results

Three hundred and four clusters in Malawi were included in the three-country MORDOR mortality analysis. These were combined with the 30 morbidity clusters for the analyses of mortality at the Malawi site alone. The baseline characteristics of the mortality and morbidity communities separately, as well as the final combined dataset, are presented in Table 3.1. Morbidity and mortality communities were similar in terms of number of children, age and sex distribution.

Mortality was 9% lower in azithromycin-treated communities compared to placebo-treated communities (rate ratio 0.91 [95% CI: 0.79–1.05]; P = 0.20). In the placebo arm, mortality rate was highest in the 1-5 month age group (21.1 deaths per 1,000 person-years [95% CI: 17.2-25.8]) and lowest in the 24-59 month age group (6.2 deaths per 1,000 person-years [95% CI:

5.5-7.0]), as shown in Table 3.2. Mortality in 1-5 month old children was lower in the azithromycin-treated group compared to placebo: rate ratio 0.70 (95% CI: 0.50-0.99); *P* = 0.04. There was no evidence for an effect of the intervention in the older age groups.

Mortality rate by zone in azithromycin and placebo clusters, compared to official mortality statistics from Mangochi District Health Office, is shown in Table 3.3. Overall, the DHO data suggest mortality was higher in azithromycin clusters compared to placebo clusters in the year prior to this study commencing (2014-2015). Mortality measurements were lowest in Monkey Bay from both the DHO data and in this study. Mortality was highest in placebo communities in Chilipa and Namwera in both data sets.

Table 3.1: Baseline characteristics of the combined dataset and mortality and morbidity clusters by treatment allocation

	Combined dataset		Mortality clusters		Morbidity clusters	
	Placebo	Azithromycin	Placebo	Azithromycin	Placebo	Azithromycin
Number of clusters	167	167	152	152	15	15
Number of children	42,825	43,105	38,578	38,631	4,247	4,474
Number of children per cluster (mean±SD)	256±121	258±117	254±121	254±119	283±122	298±94
Male sex (%)	50.0	50.0	50.0	50.2	50.1	49.1
Age (%)						
1-5 months	7.0	7.2	7.1	7.1	6.9	8.3
6-11 months	11.7	11.4	11.7	11.4	11.6	11.7
12-23 months	20.5	20.6	20.5	20.7	20.7	20.1
24-59 months	60.8	60.8	60.8	60.9	60.8	59.9

Table 3.2: Mortality rate in azithromycin- and placebo-treated clusters by age group

	Deaths/ person-years	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**
1-5 months				
Placebo	93/4,414	21.1 (17.2-25.8)	1	
Azithro	64/4,354	14.7 (11.5-18.8)	0.70 (0.50-0.99)	0.04
6-11 months				
Placebo	109/6,809	16.0 (13.3-19.3)	1	
Azithro	104/6,689	15.5 (12.8-18.8)	0.96 (0.72-1.29)	0.80
12-23 months				
Placebo	164/14,297	11.5 (9.8-13.4)	1	
Azithro	149/14,178	10.5 (9.0-12.3)	0.92 (0.73-1.18)	0.53
24-59 months				
Placebo	256/41,416	6.2 (5.5-7.0)	1	
Azithro	248/41,617	6.0 (5.3-6.7)	0.96 (0.79-1.17)	0.70

Table 3.3: Mortality rate in 1-59 month old children by zone in azithromycin- and placebo-treated clusters compared to official under-five mortality data from Mangochi District Health Office (DHO) in the year preceding the study

	Mortality rate in this study		Mortality rate from DHO data	
	Placebo clusters (deaths/1,000 person-years)	Azithromycin clusters (deaths/1,000 person-years)	Placebo clusters (deaths/1,000 person-years)	Azithromycin clusters (deaths/1,000 person-years)
Monkey Bay	7.0	6.7	7.5	6.4
Chilipa	10.9	10.3	12.3	17.9
Makanjira	8.8	9.3	10.3	10.1
Namwera	10.6	7.7	14.4	13.8
Mangochi	8.1	8.6	11.1	15.9
Total	9.3	8.4	11.6	13.7

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SECTION A – Student Details

Student ID Number	248702	**Title**	Dr
First Name(s)	John Daniel		
Surname/Family Name	Hart		
Thesis Title	Azithromycin mass drug administration for reducing child mortality in Malawi		
Primary Supervisor	Robin Bailey		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	American Journal of Tropical Medicine and Hygiene		
When was the work published?	March 2020		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I coordinated data collection, conducted all data analysis and wrote the manuscript

SECTION E

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Date	14/06/2021

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Effect of Mass Treatment with Azithromycin on Causes of Death in Children in Malawi: Secondary Analysis from the MORDOR Trial

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Abstract. Recent evidence indicates mass drug administration with azithromycin may reduce child mortality. This study uses verbal autopsy (VA) to investigate the causes of individual deaths during the Macrolides Oraux pour Réduire les Décès avec un Oeil sur la Résistance (MORDOR) trial in Malawi. Cluster randomization was performed as part of MORDOR. Biannual household visits were conducted to distribute azithromycin or placebo to children aged 1–59 months and update the census to identify deaths for VA. MORDOR was not powered to investigate mortality effects at individual sites, but the available evidence is presented here for hypothesis generation regarding the mechanism through which azithromycin may reduce child mortality. Automated VA analysis was performed to infer the likely cause of death using two major analysis programs, InterVA and SmartVA. A total of 334 communities were randomized to azithromycin or placebo, with more than 130,000 person-years of follow-up. During the study, there were 1,184 deaths, of which 1,131 were followed up with VA. Mortality was 9% lower in azithromycin-treated communities than in placebo communities (rate ratio 0.91 [95% CI: 0.79–1.05]; $P = 0.20$). The intention-to-treat analysis by cause using InterVA suggested fewer HIV/AIDS deaths in azithromycin-treated communities (rate ratio 0.70 [95% CI: 0.50–0.97]; $P = 0.03$) and fewer pneumonia deaths (rate ratio 0.82 [95% CI: 0.60–1.12]; $P = 0.22$). The use of the SmartVA algorithm suggested fewer diarrhea deaths (rate ratio 0.71 [95% CI: 0.51–1.00]; $P = 0.05$) and fewer pneumonia deaths (rate ratio 0.58 [95% CI: 0.33–1.00]; $P = 0.05$). Although this study is not able to provide strong evidence, the data suggest that the mortality reduction during MORDOR in Malawi may have been due to effects on pneumonia and diarrhea or HIV/AIDS mortality.

INTRODUCTION

Mass drug administration (MDA) with azithromycin is widely used by trachoma control programs as part of efforts to eliminate trachoma, the leading infectious cause of blindness globally.[1] During interventions principally aimed at eliminating trachoma, evidence emerged of reductions in a number of infectious diseases following azithromycin MDA, including diarrhea,[2] pneumonia,[3] and malaria.[4–6] A study from Ethiopia reported a large reduction in child mortality following azithromycin MDA.[7] Expert opinion suggested that a reduction in mortality was likely but to a lesser extent than estimated in this single available trial.[8] MORDOR was a large multicenter trial that recently tested the hypothesis that biannual azithromycin MDA to children aged 1–59 months would reduce childhood mortality, reporting a 14% overall reduction in mortality in those assigned to azithromycin versus placebo.[9] There is limited literature related to the mechanism through which azithromycin MDA may reduce child mortality. As a broad-spectrum antibiotic with weak antimalarial activity that has shown reductions in morbidity from pneumonia, diarrhea, and malaria, an effect on mortality from any of these major causes is feasible. In addition, azithromycin may reduce opportunistic infections in HIV-positive individuals, including *Mycobacterium avium* complex, and investigation is underway regarding its effect on HIV-associated chronic lung disease.[10,11]

Ideally, the underlying cause of death would be determined from medical certificates of cause of death completed by physicians with access to adequate investigative resources. However, at the MORDOR-Malawi study site, as in low-income settings in much of sub-Saharan Africa, there was limited access to physicians who would be able to complete death certificates. In such contexts, verbal autopsy (VA)—a structured interview with relatives of the deceased to ascertain cause of death—is increasingly used to infer likely causes of death. Development of standard electronic questionnaires on mobile devices and automated analysis programs have improved the feasibility of VA and comparability of results, compared with the previous requirement for physician review of the interviews.[12] Prediction of cause-specific mortality fractions in a population using VA may be approximately 60–80% accurate, although lower for individual-level diagnosis.[13,14] In settings where more accurate determination of cause of death is not possible, VA may be used to produce probable cause of death data with the caveat of it being an imperfect tool.[15] The two most commonly used VA analysis programs are InterVA[16] and SmartVA.[17] InterVA uses a probabilistic model based on the relationship between indicators and causes, as captured through expert panel discussions.[18] SmartVA, or the Tariff method, is based on the symptom–cause information from the Population Health Metrics Research Consortium (PHMRC) study that included more than 12,000 VA interviews performed on deaths with a gold standard diagnosis derived from stringent diagnostic criteria.[19,20]

The MORDOR trial was designed to assess overall mortality over three country sites and cause of death has not previously been compared between treatment arms for this study. Although azithromycin MDA has been shown to reduce morbidity due to the leading causes of child mortality outlined previously, there is no evidence to date for a cause-specific mortality effect of azithromycin MDA. As interest and evidence increases regarding the mortality benefit of azithromycin, an understanding of the mechanism for that benefit remains unclear. This study used VA methods to assess causes of death in children enrolled in the MORDOR trial in Malawi.

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METHODS

Trial design and participants. The methods for the main MORDOR trial in Niger, Tanzania, and Malawi are described elsewhere.[9] Briefly, MORDOR assessed the effects of biannual single-dose azithromycin MDA compared with placebo on mortality in children aged 1–59 months. The trial was cluster randomized and in Malawi used the catchment area of a Health Surveillance Assistant (HSA) as a cluster. The study area covered the whole of Mangochi District and took place between March 2015 and June 2017, including a total of four treatment rounds and five census visits. Children were included if they were aged between 1 and 59 months at the start of any inter-census period. Biannual house-to-house census was conducted to identify all deaths as well as new births and migrations into or out of the study area.

The MORDOR trial included 304 clusters randomly selected from a pool of 334 clusters meeting the inclusion criteria identified from a pre-baseline census. The remaining 30 clusters, randomly located throughout the study area, were used for assessment of morbidity outcomes. This study, assessing cause-specific mortality, included deaths that occurred in all 334 communities, in the interests of presenting all available data.

Interventions. Azithromycin was administered at a dose of 20 mg/kg to all children available in study communities. Additional mop-up visits were conducted to increase the number of children treated. Children able to stand received an approximate dose based on their height, and small children were weighed. Placebo bottles and suspension were identical in appearance to azithromycin. Distribution of drug was performed by HSAs and MORDOR fieldworkers conducting house-to-house visits. Guardians were asked to inform the HSA of any adverse events that occurred within 7 days of receiving the study drug and HSAs were trained to inform the study team.

Outcomes. The primary prespecified outcome was cause of death, inferred using VA methods, for deaths in children aged 1–59 months at the prior census. A secondary prespecified outcome was seasonality of deaths. Both the InterVA and SmartVA automated analysis algorithms are endorsed by the WHO and performance overall is not greatly dissimilar between the methods.[14] The sensitivity and specificity for diagnosis of specific causes does, however, vary considerably between the two algorithms, including for the four major causes of child mortality in this setting: malaria, pneumonia, diarrhea, and HIV/AIDS, as identified by Murray et al.[14] and shown in Supplemental Table 7 for reference and discussion in the following paragraphs. This study used the WHO 2014 VA questionnaire with analysis using both the InterVA and SmartVA algorithms to provide a comprehensive and transparent assessment of the available data.

Data collection. Census data collection used custom-made software on Google Android devices. Deaths were identified from census updates and were followed up with VA interviews. Three nurses were trained to conduct VA interviews and aimed to complete these within 12 months of the death as recall is expected to diminish after this time.[21] The WHO 2014 VA questionnaire was installed on Android devices using ODK Collect software. Data were uploaded to a secure server at the London School of Hygiene and Tropical Medicine.

Sample size. The MORDOR trial was designed to assess the effect of azithromycin on overall mortality in children aged 1–59 months with 84% power to detect a 15% effect over 2 years. The study was not designed to assess cause-specific mortality, which would require a significantly larger trial size.

Randomization and blinding. The study drug was labeled with 16 letters by the manufacturer (Pfizer Inc., New York, NY), with half corresponding to azithromycin and half to placebo. Communities, being the villages under a single HSA, were randomly assigned to a drug letter by the study statistician in San Francisco using the statistical package R (R Foundation for Statistical Computing, Vienna, Austria). All staff and participants in Malawi were blind to the treatment code until all data collection and cleaning was complete.

Statistical methods. The automated analysis programs used to produce inferred cause of death data, InterVA version 4 (InterVA version 4, University of Umeå, Umeå, Sweden) and SmartVA-Analyze (SmartVA-Analyze, Institute for Health Metrics and Evaluation, University of Washington, Seattle, WA), have thresholds for identifying the cause of death and if these are not met, the outcome is listed as "indeterminate" or "undetermined."[16,17] The SmartVA analysis software redistributes unknown causes of death according to the certainty of the algorithm in predicting different causes of death and Global Burden of Disease patterns for the country.[22] As the SmartVA redistribution reflects the inherent ability of the program to predict each cause, redistribution may provide more accurate estimation of cause-specific mortality rates than the SmartVA output without redistribution; where possible, output with and without redistribution is presented. Output with redistribution is presented without CIs as this is produced by the SmartVA software at the population level rather than subsequently calculated from individual-level cause of death data.

Statistical analysis was performed using Stata version 15.1. Primary analyses were by intention-to-treat (ITT); to investigate if any cause-specific effects on mortality were due to individual or community-level effects of azithromycin, secondary per protocol (PP) analyses were performed that included only individuals who received treatment as indicated at the previous visit. Cause-specific mortality rates were calculated, and Poisson regression models produced, for the four main causes of mortality in the study area (pneumonia, diarrhea, HIV/AIDS, and malaria), with treatment allocation as the main predictor variable and random effects for clustering at the level of the MORDOR randomization unit. Point estimates with CIs from the models are presented as the best available estimates of the effect of azithromycin on cause-specific mortality in this population.

As an independent analysis to the VA algorithm outputs, the proportion of individuals with open response terms and positive responses to VA questions related to the major causes of child mortality were also assessed, specifically the open response terms "malaria," "pneumonia," and "diarrhea" and VA items "maternal test positive for HIV," "frequent loose/liquid stool continuing until death," "very severe cough," and "severe fever". A two-tailed test of proportions was used and *P* values are presented. Maternal HIV status was selected to estimate HIV exposure as very few children had a known HIV status, whereas mothers were more likely to have been tested for HIV during antenatal visits.

Study visits took place around the beginning and end of the dry season in Malawi, which occurs approximately between May and October. The baseline, 12-month, and 24-month visits took place between April and June 2015, 2016, and 2017, respectively; and the 6-month and 18-month visits between September and December 2015 and 2016. For the analysis of seasonality, dry season deaths were defined as those occurring between the baseline and 6-month visits and between the 12-month and 18-month visits, and wet season deaths as those occurring between the 6-month and 12-month visits and between the 18-month and 24-month visits. Univariate Poisson regression models were produced to assess the effects of treatment allocation during both the wet and dry seasons on each of the main causes of child mortality.

RESULTS

In the 334 randomization units in the MORDOR trial in Malawi, there were 133,772 person-years of follow-up for children aged 1–59 months at the prior census. Person-years of follow-up and mortality rates were similar between males and females at each round, shown in Table 1. Over the course of the study, there were 1,184 deaths and VAs were completed for 1,131 of these. Median delay from death to VA was 5 months (range 1–26 months), with 87% of VAs completed within 12 months of death.

Mortality was 9% lower in azithromycin-treated communities compared with those allocated to placebo (rate ratio 0.91 [95% CI: 0.79–1.05]; $P = 0.20$). The top 10 causes of death using the InterVA algorithm are shown in Table 2, and using the SmartVA algorithm in Table 3. Malaria was the leading cause of death overall, followed by HIV/AIDS, using both algorithms without redistribution of undetermined cases, and diarrhea, pneumonia, and either acute abdomen (InterVA) or other digestive diseases (SmartVA) made up the top five causes. The top 10 causes including redistribution of undetermined cases for SmartVA are also shown in Table 3. Pneumonia, malaria, diarrhea, and HIV/AIDS accounted for 83% and 78% of the inferred causes of death using InterVA and SmartVA with redistribution, respectively.

TABLE 1

Person-years enrolled in the study and number of deaths by follow-up period and gender

	Total person-years	Number of deaths (followed up with verbal autopsy)	Rate per 1,000 person-years (95% CI)
0–6 months follow-up			
Female	13,371	124 (118)	9.27 (7.78–11.06)
Male	13,398	135 (126)	10.08 (8.51–11.93)
Total	26,768	259 (244)	9.68 (8.57–10.93)
6–12 months follow-up			
Female	19,095	174 (168)	9.11 (7.85–10.57)
Male	19,031	181 (175)	9.51 (8.22–11.00)
Total	38,126	355 (343)	9.31 (8.39–10.33)
12–18 months follow-up			
Female	15,547	124 (123)	7.98 (6.69–9.51)
Male	15,356	117 (108)	7.62 (6.36–9.13)
Total	30,903	241 (231)	7.80 (6.87–8.85)
18–24 months follow-up			
Female	19,100	162 (154)	8.48 (7.27–9.89)
Male	18,875	167 (159)	8.85 (7.60–10.30)
Total	37,975	329 (313)	8.66 (7.78–9.65)
Grand total	133,772	1,184 (1,131)	8.85 (8.36–9.37)

The ITT analyses of cause-specific mortality rates are shown using InterVA in Table 4 and SmartVA in Table 5. The analysis using InterVA showed 30% lower HIV/AIDS mortality in azithromycin-treated communities (rate ratio 0.70 [95% CI: 0.50–0.97]; $P = 0.03$), but this trend was not evident using SmartVA (rate ratio 0.99 [95% CI: 0.71–1.38]; $P = 0.95$) without redistribution and rate ratio 0.92 with redistribution. The effect estimate for pneumonia mortality was lower in azithromycin communities using both InterVA (rate ratio 0.82 [95% CI: 0.60–1.12]; $P = 0.22$) and SmartVA (rate ratio 0.58 [95% CI: 0.33–1.00]; $P = 0.05$) without redistribution and rate ratio 0.87 with redistribution.

Inferred malaria mortality was similar in azithromycin communities compared with placebo using InterVA (rate ratio 0.95 [95% CI: 0.78–1.16]; $P = 0.64$) and SmartVA (rate ratio 0.93 [95% CI: 0.76–1.14]; $P = 0.49$) without redistribution and rate ratio 0.94 with redistribution. The effect estimate for diarrhea mortality was similar in azithromycin communities compared with placebo when analyzed using InterVA (rate ratio 0.95 [95% CI: 0.61–1.49]; $P = 0.84$) but was lower in azithromycin communities when analyzed using SmartVA (rate ratio 0.71 [95% CI: 0.51–1.00]; $P = 0.05$) without redistribution and rate ratio 0.72 with redistribution. The analyses of cause-specific mortality rates PP are shown in Supplemental Table 1 using InterVA, and Supplemental Table 2 using SmartVA. Cause-specific rate ratios PP were similar to those by ITT; within 10% for all causes.

Cause-specific mortality rates for the leading causes of child mortality are shown by follow-up period in Figure 1. Pneumonia mortality rates were lower in azithromycin than placebo communities at three of the four follow-up visits when analyzed using InterVA and at all follow-up visits using SmartVA. HIV/AIDS mortality appears generally lower in azithromycin communities using InterVA and diarrhea mortality generally lower using SmartVA; no such patterns are evident for malaria mortality.

The comparison of VA response terms is shown in Table 6. In the ITT analysis, the open response term "pneumonia" was more frequently reported in VAs conducted in placebo communities than azithromycin: 8.2% versus 5.1%, respectively; $P = 0.03$. The open response term "diarrhea" was also more frequently reported in placebo than azithromycin communities: 21.9% versus 16.9%, respectively; $P = 0.03$. The VA item "frequent loose/liquid stool continuing until death" was more frequently endorsed in placebo than azithromycin communities: 37.2% versus 30.1%, respectively; $P = 0.01$. The open response term "malaria" was similar between placebo and azithromycin arms: 45.7% versus 49.1%, respectively; $P = 0.26$. "Maternal test positive for HIV," "very severe cough," and "severe fever" were all similar between treatment arms. The PP analyses showed similar trends to the ITT results.

Assessment of seasonality by ITT using InterVA and SmartVA did not identify clear trends for differences in cause-specific mortality between treatment arms; these analyses are included as Supplemental Data. Analysis using the InterVA algorithm suggested an effect of azithromycin on pneumonia mortality during the wet season but not the dry (wet season rate ratio 0.64 [95% CI: 0.41–1.00]; $P = 0.05$) and dry season rate ratio 1.02 [95% CI: 0.65–1.61]; $P = 0.93$) (Supplemental Table 3). Analysis using SmartVA, however, predicted approximately two-thirds fewer pneumonia deaths overall and the same seasonal trend was not evident (wet season rate

TABLE 2
Deaths in placebo- and azithromycin-treated clusters due to the top 10 causes using InterVA

InterVA output cause	Number of deaths in placebo arm (%)	Number of deaths in azithromycin arm (%)	Number of deaths in both arms (%)
Malaria	256 (42.9)	245 (45.9)	501 (44.3)
HIV/AIDS-related death	103 (17.3)	71 (13.3)	174 (15.4)
Acute respiratory infection, including pneumonia	94 (15.7)	77 (14.4)	171 (15.1)
Diarrheal diseases	48 (8.0)	45 (8.4)	93 (8.2)
Indeterminate	19 (3.2)	15 (2.8)	34 (3.0)
Acute abdomen	16 (2.7)	15 (2.8)	31 (2.7)
Meningitis and encephalitis	10 (1.7)	16 (3.0)	26 (2.3)
Severe malnutrition	12 (2.0)	9 (1.7)	21 (1.9)
Accidental exposure to smoke fire and flame	7 (1.2)	6 (1.1)	13 (1.2)
Epilepsy	2 (0.3)	9 (1.7)	11 (1.0)
Other	30 (5.0)	26 (4.9)	56 (4.9)
Total	597 (100)	534 (100)	1,131 (100)

ratio 0.71 [95% CI: 0.32–1.61]; $P = 0.42$ and dry season rate ratio 0.48 [95% CI: 0.23–1.02]; $P = 0.06$) (Supplemental Table 4). Analysis using InterVA suggested lower HIV/AIDS mortality in azithromycin communities during both the wet and dry seasons, whereas the SmartVA analysis suggested lower diarrhea mortality in azithromycin communities during both seasons. The PP analyses using InterVA and SmartVA show generally similar results to the ITT analysis and are included for completeness as Supplemental Tables 5 and 6, respectively.

DISCUSSION

This study investigated the effect of azithromycin MDA on each of the major causes of child mortality in Mangochi District, Malawi, during the MORDOR trial. Verbal autopsy was used to infer the likely causes of death. The relative effects of azithromycin on cause-specific mortality during the wet and dry seasons was also assessed. The effect estimates produced in this study are mostly compatible with there being zero effect but are the only estimates available to date for the effect of azithromycin MDA on cause-specific child mortality.

The effect estimates using the InterVA analysis algorithm suggest lower pneumonia and HIV/AIDS mortality in azithromycin-treated than in placebo communities. Using SmartVA, the results suggest lower pneumonia and diarrhea mortality in azithromycin communities. The analysis of VA responses indicates that the terms "pneumonia" and "diarrhea" in the open narrative and "frequent loose/liquid stool continuing until death" were less commonly reported following deaths in azithromycin-treated than placebo communities. The relatively low sensitivity of InterVA for predicting both HIV/AIDS and diarrhea reported by Murray et al.,[14,19] and shown in Supplemental Table 7, indicates the algorithm may underestimate mortality from these causes. Compared with SmartVA, InterVA did predict fewer diarrhea deaths, although more HIV/AIDS deaths in this study. The relatively higher sensitivity and lower specificity of InterVA for identifying pneumonia may lead to an overestimate of pneumonia mortality. SmartVA, on the other hand, has a particularly low sensitivity for pneumonia, which would contribute to the lower proportions of pneumonia reported in this study using the SmartVA algorithm, especially before redistribution of unknown causes.

The differences in sensitivity and specificity of the two algorithms reflect the fact that VA is a blunt diagnostic tool, less suited to individual-level diagnosis compared with population-level prediction of cause-specific mortality fractions. Nonetheless, primary analyses by the two algorithms are not necessarily contradictory, both suggesting lower pneumonia mortality in azithromycin communities and InterVA suggesting lower HIV/AIDS mortality, whereas SmartVA suggests lower diarrhea mortality. These diagnoses are particularly difficult to distinguish retrospectively in the absence of clinical investigation; diarrhea may often be the immediate cause of death when the underlying cause is HIV/AIDS. The direct analysis of VA responses would suggest lower pneumonia and diarrhea in the azithromycin group; the similar maternal HIV positivity between groups, estimating HIV exposure, does not

TABLE 3
Deaths in placebo and azithromycin-treated clusters due to the top 10 causes using SmartVA

SmartVA output cause	Number of deaths in placebo arm (%)	Number of deaths in azithromycin arm (%)	Number of deaths in both arms (%)	Percentage of deaths in placebo arm after redistribution	Percentage of deaths in azithromycin arm after redistribution	Percentage of deaths in both arms after redistribution
Malaria	198 (33.2)	184 (34.5)	382 (33.8)	39.1	40.9	40.0
Undetermined	133 (22.3)	131 (24.5)	264 (23.3)	0.0	0.0	0.0
AIDS	71 (11.9)	70 (13.1)	141 (12.5)	12.8	13.2	13.0
Diarrhea/dysentery	79 (13.2)	56 (10.5)	135 (11.9)	16.1	13.0	14.6
Other digestive diseases	44 (7.4)	50 (9.4)	94 (8.3)	7.6	9.5	8.5
Pneumonia	35 (5.9)	20 (3.8)	55 (4.9)	10.3	10.0	10.1
Fires	8 (1.3)	6 (1.1)	14 (1.2)	1.5	1.3	1.4
Childhood cardiovascular diseases	5 (0.8)	4 (0.8)	9 (0.8)	1.1	1.1	1.1
Meningitis	5 (0.8)	3 (0.6)	8 (0.7)	2.0	2.5	2.2
Other infectious diseases	6 (1.0)	1 (0.2)	7 (0.6)	1.8	1.3	1.6
Other	13 (2.2)	9 (1.7)	22 (2.0)	7.7	7.2	7.5
Total	597 (100)	534 (100)	1,131 (100)	100	100	100

TABLE 4
Cause-specific mortality by intention-to-treat for the four main inferred causes of death in the study area using InterVA

	Deaths/person-years	Rate per 1,000 person-years (95% CI)	Rate ratio* (95% CI)	*P*-value
Pneumonia				
Placebo	94/66,935	1.40 (1.15–1.72)	1	
Azithromycin	77/66,837	1.15 (0.92–1.44)	0.82 (0.60–1.12)	0.22
Malaria				
Placebo	256/66,935	3.82 (3.38–4.32)	1	
Azithromycin	245/66,837	3.67 (3.23–4.15)	0.95 (0.78–1.16)	0.64
HIV/AIDS				
Placebo	103/66,935	1.54 (1.27–1.87)	1	
Azithromycin	71/66,837	1.06 (0.84–1.34)	0.70 (0.50–0.97)	0.03
Diarrhea				
Placebo	48/66,935	0.72 (0.54–0.95)	1	
Azithromycin	45/66,837	0.67 (0.50–0.90)	0.95 (0.61–1.49)	0.84

* From random-effects Poisson model adjusting for clustering at the level of the randomization unit.

provide evidence for whether or not the diarrhea is likely to be HIV-related. The PP analyses did not show any greater effect than the ITT analyses for pneumonia, diarrhea, or HIV/AIDS, providing no evidence that these were solely individual-level effects in those treated, as opposed to community-level effects.

An effect of azithromycin on respiratory and gastrointestinal infections, including on a background of HIV exposure or infection, leading to a reduction in the rate of pneumonia and diarrhea as immediate causes of death, is certainly plausible. Azithromycin is an effective treatment for community-acquired pneumonia, including the major etiological causes, *Streptococcus pneumoniae* and *Haemophilus influenzae*.[23] Single-dose azithromycin has been shown to reduce oropharyngeal carriage of *S. pneumoniae* and decrease incidence of pneumonia.[24,25]

Azithromycin has efficacy against the main bacterial causes of fatal childhood diarrhea, namely, enteropathogenic and enterotoxigenic *Escherichia coli*, *Shigella* spp., *Campylobacter* spp., *Salmonella* spp., and *Vibrio cholerae*.[26] Furthermore, azithromycin has anti-inflammatory properties that, combined with a reduction in gut pathogens, may reduce chronic immunostimulation caused by environmental enteric dysfunction and consequently improve nutritional status.[27,28] Evidence from the MORDOR Niger study site indicates there may be changes to the gut microbiome following azithromycin MDA, although improvements in anthropometry measurements, as an assessment of nutritional status, have not previously been associated with azithromycin MDA.[29–31]

The REALITY trial identified a 27% reduction in mortality over 24 weeks in adults and children aged 5 years and older when commencing antiretroviral therapy (ART) plus an enhanced antimicrobial prophylactic regimen of trimethoprim–sulfamethoxazole, isoniazid–pyridoxine, fluconazole, albendazole, and a 5-day course of azithromycin, compared with commencing ART plus standard prophylaxis of trimethoprim–sulfamethoxazole only.[32] The multiple dimensions to the enhanced regimen make it difficult to identify the specific intervention causing the reduction in mortality, but further analysis by Post et al.[33] indicated mortality reductions from cryptococcosis and unknown causes, and not from severe bacterial infections, potentially azithromycin-responsive infections or tuberculosis.

REALITY investigated mortality in adults and older children during ART commencement, so it is not directly comparable with child mortality in the MORDOR trial; antimicrobial benefits and prophylactic effects of azithromycin against chronic disease-causing pathogens such as *M. avium* complex and *Pneumocystis jirovecii* could play a role in reducing HIV/AIDS mortality in low-resource settings where HIV is underdiagnosed.[34] Prevalence of HIV infection in Malawi is approximately 9.2% in adults aged 15–49 years.[35] HIV transmission is known to be a problem in fishing communities in Mangochi District because of practices associated with the market chain.[36] Among HIV-infected children aged 0–14 years nationally, approximately 61% were estimated to be receiving ART in 2018.[35] In the setting of significant levels of

TABLE 5
Cause-specific mortality by intention-to-treat for the four main causes of death in the study area using SmartVA

	Number of cases/person-years	Rate per 1,000 person-years (95% CI)	Rate ratio* (95% CI)	*P*-value	Rate per 1,000 person-years after redistribution	Rate ratio after redistribution
Pneumonia						
Placebo	35/66,935	0.52 (0.38–0.73)	1		0.92	1
Azithromycin	20/66,837	0.30 (0.19–0.46)	0.58 (0.33–1.00)	0.05	0.80	0.87
Malaria						
Placebo	198/66,935	2.96 (2.57–3.40)	1		3.49	1
Azithromycin	184/66,837	2.75 (2.38–3.18)	0.93 (0.76–1.14)	0.49	3.27	0.94
HIV/AIDS						
Placebo	71/66,935	1.06 (0.84–1.34)	1		1.14	1
Azithromycin	70/66,837	1.05 (0.83–1.32)	0.99 (0.71–1.38)	0.95	1.05	0.92
Diarrhea						
Placebo	79/66,935	1.18 (0.95–1.47)	1		1.44	1
Azithromycin	56/66,837	0.84 (0.64–1.09)	0.71 (0.51–1.00)	0.05	1.04	0.72

* From random-effects Poisson model adjusting for clustering at the level of the randomization unit.

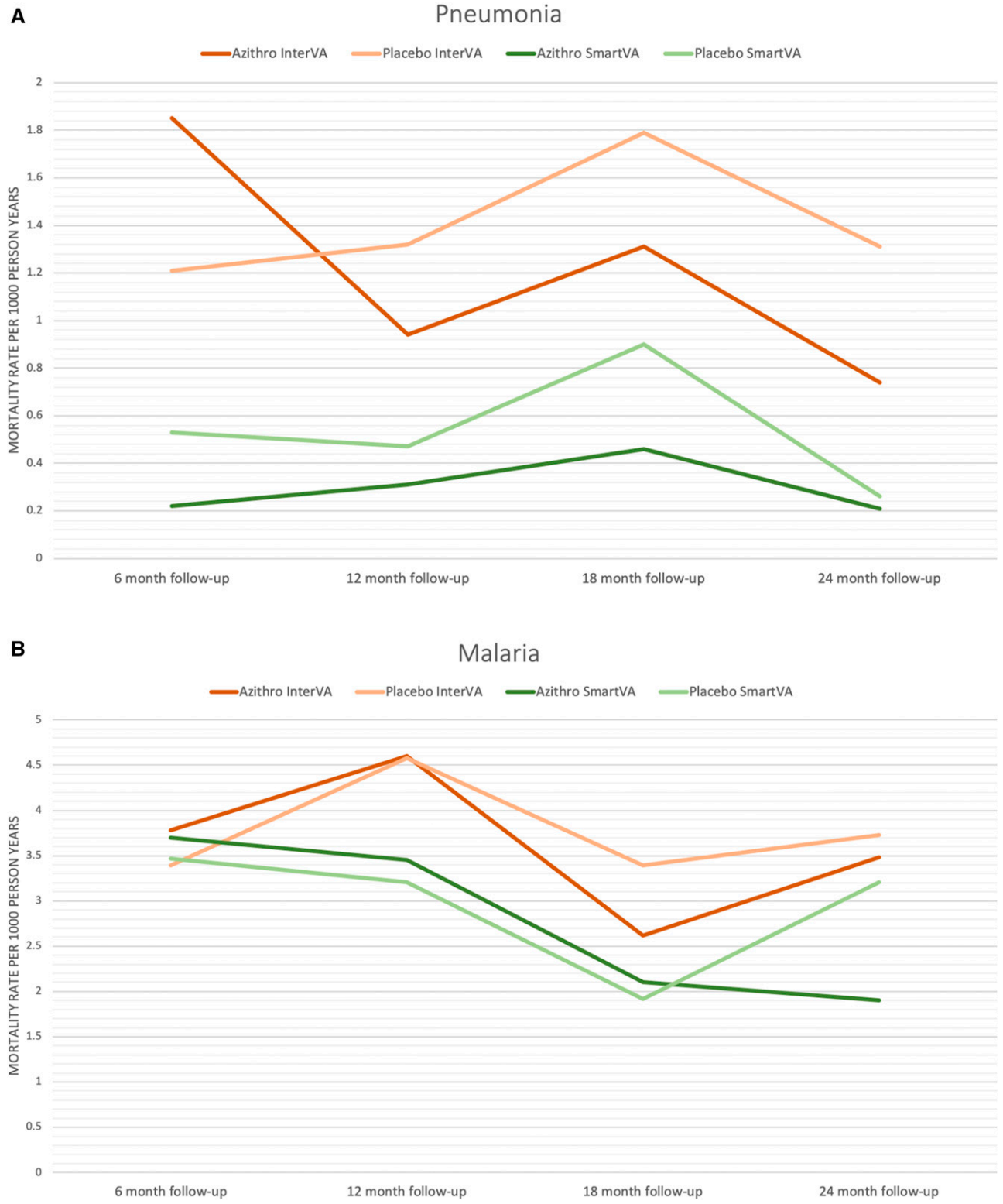
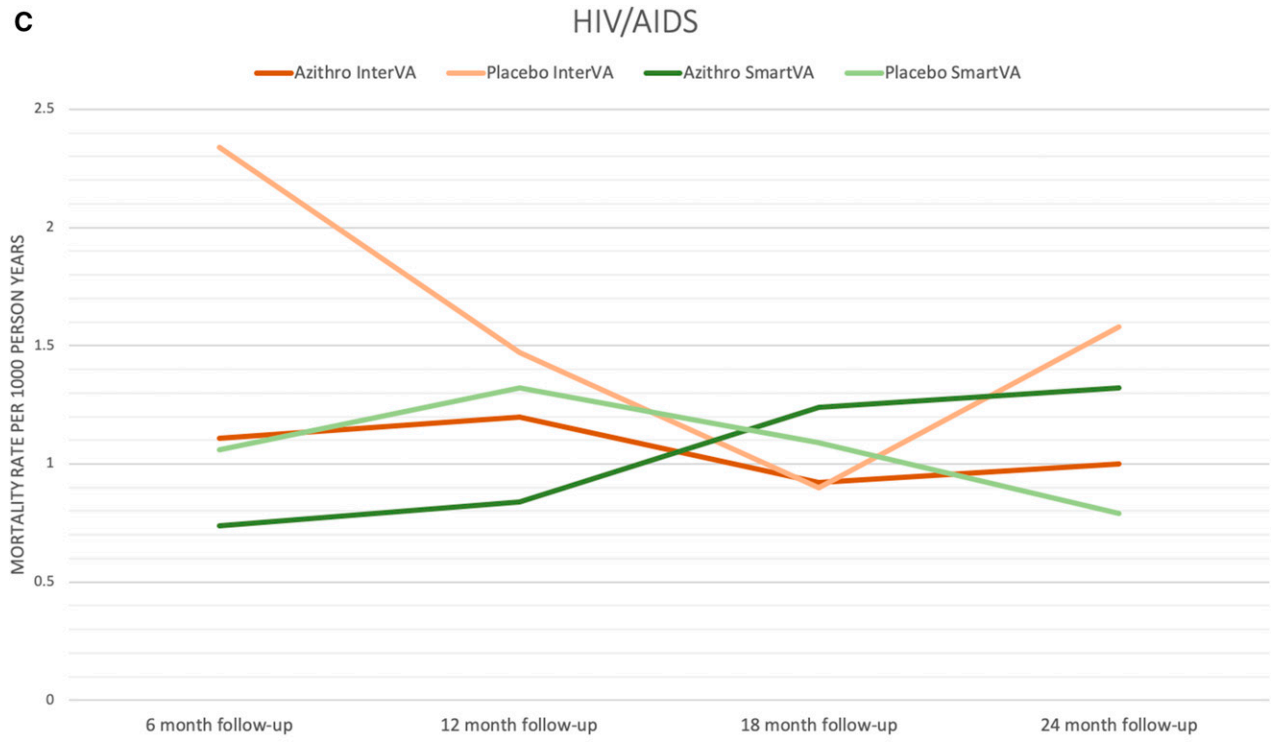


FIGURE 1. Cause-specific mortality rates by intention-to-treat for the leading causes of child mortality over the four follow-up periods of the study.

undiagnosed HIV/AIDS, an antibacterial agent could prevent or delay serious infections.

In this study, malaria was the most common cause predicted by both VA analysis algorithms but there was no evidence for an effect of azithromycin MDA on malaria mortality. Azithromycin is a weak antimalarial agent, and previous studies have indicated there may be reductions in malaria morbidity following azithromycin MDA.[4–6,37] Recent assessment from the MORDOR trial in Niger indicated reduced malaria parasitemia in azithromycin-treated communities.[38] However, morbidity assessments as part of the MORDOR trial in Tanzania indicated no difference in fever



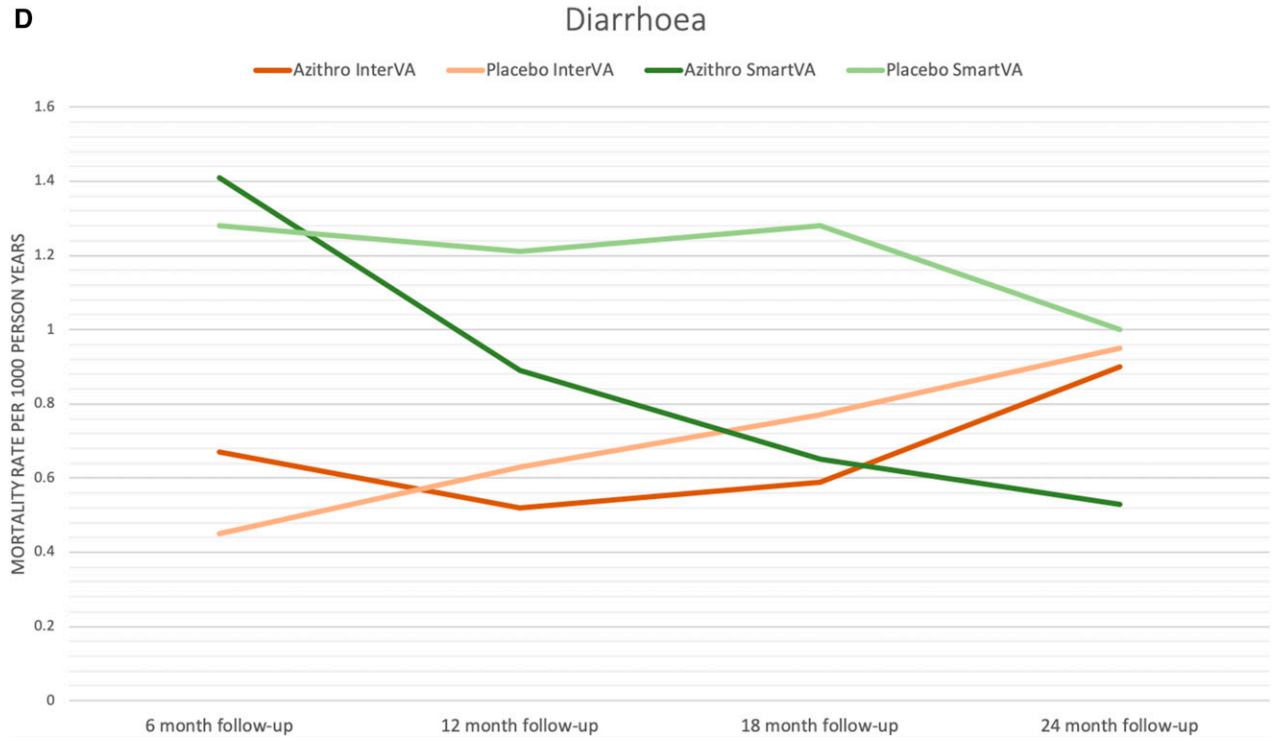


FIGURE 1. Continued.

or anemia between azithromycin and placebo arms.[39] A study comparing seasonal malaria chemoprevention (SMC) to SMC plus azithromycin in Burkina Faso and Mali, which have lower year-round malaria transmission than Malawi, reported no difference in mortality between groups, although reductions were evident in morbidity due to gastrointestinal and respiratory infections and nonmalarial febrile illness with the addition of azithromycin.[40]

A significant limitation of this study is that MORDOR was powered to detect a difference in overall mortality at three study sites and consequently the sample size is low for this study, first because it is limited to one of the three MORDOR

TABLE 6

Comparison of verbal autopsy open response terms and question endorsements related to the major causes of child mortality between azithromycin- and placebo-treated communities

	Intention-to-treat analysis			Per protocol analysis		
VA item	Total deaths	Number of interviews in which item endorsed (%)	*P*-value*	Total deaths	Number of interviews in which item endorsed (%)	*P*-value*
Open response term "malaria"						
Placebo	597	273 (45.7)		461	220 (47.7)	
Azithromycin	534	262 (49.1)	0.26	431	217 (50.3)	0.44
Open response term "pneumonia"						
Placebo	597	49 (8.2)		461	40 (8.7)	
Azithromycin	534	27 (5.1)	0.03	431	22 (5.1)	0.04
Open response term "diarrhea"						
Placebo	597	131 (21.9)		461	96 (20.8)	
Azithromycin	534	90 (16.9)	0.03	431	75 (17.4)	0.19
Maternal test positive for HIV						
Placebo	597	45 (7.5)		461	35 (7.6)	
Azithromycin	534	41 (7.7)	0.93	431	37 (8.6)	0.59
Frequent loose/liquid stool continuing until death						
Placebo	597	222 (37.2)		461	170 (36.9)	
Azithromycin	534	161 (30.1)	0.01	431	133 (30.9)	0.06
Very severe cough						
Placebo	597	56 (9.4)		461	44 (9.5)	
Azithromycin	534	48 (9.0)	0.82	431	38 (8.8)	0.70
Severe fever						
Placebo	597	282 (47.2)		461	225 (48.8)	
Azithromycin	534	261 (48.9)	0.58	431	202 (46.9)	0.56

* From test of proportions.

sites and second because it is assessing cause-specific mortality rather than all-cause mortality. The study is underpowered to provide strong conclusions regarding cause-specific mortality rates, and the small effect estimates may represent type II error. Relatively small effect sizes for the major causes of child mortality may be clinically important and this study provides first estimates for how azithromycin may reduce child mortality for hypothesis generation and further investigation.

Verbal autopsy is recognized as the only feasible method for determining the cause of death in the absence of a clinical diagnosis in low-resource settings.[41] Automated analysis of VA interviews improves the affordability and reproducibility of VA compared with physician review; however, the accuracy of individual-level diagnoses should not be assumed to be as accurate as medical certification of cause of death.[12] Using the two main VA questionnaires, there is variation between the prediction of the main causes of death, so for the purposes of this investigation of the potential mechanisms of action of azithromycin, output from both algorithms was presented. The use of medical records and medical certificates of cause of death for in-facility deaths was investigated before the study but not pursued as, paradoxically, deaths that occurred in or on the way to a health facility were among the hardest to identify and categorize as there was no requirement for HSAs to report them, and health center data were generally absent or impossible to link to the community census. Any medical records retained by the family were reviewed by the VA team, and there are specific VA questions concerning diagnoses from the health system that are used in the algorithms for predicting cause of death.

In addition, VA, despite being used increasingly to estimate cause of death patterns where more sophisticated methods are not practicable, is a relatively imprecise instrument that relies on details of the final illness recalled by family members. Accuracy of recall for VA has been estimated to decrease by approximately 0.6% per month following the death, and it is recommended that, where possible, VA should be completed within 12 months.[21,42] Verbal autopsy completion for this study took place as soon as possible following identification of the death from the census, and after the customary 1-month mourning period, however, many visits were required to trace family members for some children as households in the study area move quite frequently, especially after a child's death. The median delay of 5 months in this study and with 87% of VAs completed within 12 months is within the normally accepted range and is not expected to have significantly affected the accuracy of predicted diagnoses.

Finally, this study used the WHO 2014 VA questionnaire, which was designed to facilitate the use of both SmartVA and InterVA for assigning cause of death.[43] The 2014 instrument includes all questions required for InterVA, although not the exact wording for all questions from the PHMRC questionnaire used by SmartVA. A further updated questionnaire, released in 2016 after this study started, is fully compatible with both algorithms. Most questions required as input for SmartVA are similar to those in the 2014 questionnaire, although the performance of SmartVA may be slightly reduced compared with having input from the WHO 2016 questionnaire or PHMRC questionnaire. The benefits of having analysis from these two main algorithms for a wider discussion of the implications of the data were deemed to outweigh the potential for a slight decrease in performance. There is a more recently developed statistical tool for producing cause of death data from VA interviews (InSilicoVA), which aimed to improve on the other methods by sharing uncertainty between cause of death assigned for specific individuals and the population distribution of causes of death.[44] Adding a third analysis method with associated increased complexity was not attempted for this study.

Although this study is not able to provide strong evidence on the causes of death in the MORDOR trial, the data have been presented fully to enable generation of hypotheses regarding mechanisms of effect of azithromycin on child mortality. The

data suggest the mortality reduction in the MORDOR trial in Malawi may have been due to effects on pneumonia and diarrhea or HIV/AIDS mortality. Larger studies will be required to clearly define the effects of azithromycin MDA on cause-specific child mortality.

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Discussion

The overall result of this study was 9% lower mortality in azithromycin communities in the MORDOR mortality and morbidity assessment clusters combined. The reported effect in the MORDOR-Malawi mortality clusters alone was 6%.[80] The greater effect estimate in the combined dataset may be either due to chance or that the additional sample collection visit somehow led to a greater effect, potential mechanisms for which are difficult to envisage. Morbidity clusters were excluded from the main (three country) MORDOR analysis due to the increased potential for unblinding, for example from identification of higher levels of macrolide resistance in samples from azithromycin communities.[80] In addition, the increased contact with study personnel could potentially lead to a reduction in mortality at the study site.

At the Malawi site, all lab staff were masked to the community from which samples were collected and all samples were labeled in the same say so looked identical. Hence any attempt to identify patterns in characteristics of the samples prior to completion of the study would not have resulted in unmasking the treatment allocation. Census and treatment activities were similar in the morbidity communities but separated by the additional visit for sample collection. The slightly different protocols may not have been appropriate for the MORDOR study, where the emphasis was on a large simple trial design and the minimum visits were made to the communities to reduce the risk of biasing the result or unblinding the treatment allocation. However, due to a relatively low sample size for more detailed investigation of mortality effects at the Malawi site, the decision to include the morbidity clusters to maximise the data available for additional analyses was, on balance, deemed most appropriate for this study. This was decided prior to any exploration of mortality effects.

The point estimate for a mortality reduction of 30% in children aged 1-5 months in the azithromycin-treated arm compared to placebo using the combined dataset for this study corresponds to effect estimates of approximately 25-30% at each of the three country sites in the primary MORDOR mortality analysis.[80] It may be expected that in younger age groups with higher child mortality, including from infectious causes, a greater proportion of deaths may be preventable with azithromycin.

The mortality comparison with DHO data provides a useful validation of the robustness of the study methods. The DHO estimates are generally higher than the study estimates, which would be expected given the DHO figures are for all under-5 deaths, as opposed to deaths in 1-59 month old children in the MORDOR study. Given the estimate that close to 50% of under-5 mortality occurred in the neonatal period at the time of the study, the study may in fact have identified more deaths in the post-neonatal period than the official estimates.[5] It is possible that for historical cultural reasons related to high mortality, deaths might be under-notified to official authorities and recorded in the DHO figures. The rigorous house-to-house census activities in this study would be expected to identify the majority of deaths. Any missed deaths may have been more likely to occur in temporary residents or more mobile families due to the study criteria requiring identification of the individual on the census before and after the death. Overall, the mortality distribution by zone between the DHO data and this study is relatively consistent, with lowest mortality in Monkey Bay and highest in Chilipa and Namwera.

A limitation of this work is the use of VA, as it is a blunt diagnostic tool compared to physician diagnosis. However, VA is now accepted as a useful method to infer most likely cause of death in settings where a clinical diagnosis is not possible. VA methods are increasingly used beyond research as part of countries' civil registration and vital statistics systems, especially now that diagnostic algorithms can provide more consistent diagnoses more cheaply than physician review of the interviews.[127]

Key findings from this work, including the published paper on cause of death are, firstly, that the DHO data validate the study methods and, secondly, that azithromycin MDA may have reduced pneumonia and diarrhea or HIV/AIDS mortality in Malawi. As discussed above, an effect of the intervention on mortality from these causes could be explained by azithromycin MDA treating or preventing invasive bacterial disease.

Chapter 4: Heterogeneity of effect of azithromycin mass drug administration on child mortality

Introduction

A key question with regard to wider implementation of azithromycin MDA for reducing child mortality is in what way the effect may vary temporally and spatially. Understanding how the effects of the intervention are influenced by geographical factors and disease epidemiology are likely to be important considerations for optimization of the intervention strategy. Variation in effect might enable targeting of the intervention to different areas and at different times of the year in order to maximise benefit and minimise potential negative effects such as the development of antibiotic resistance. This chapter investigates heterogeneity in the effect of azithromycin MDA on child mortality through describing spatial and temporal patterns in the effect of the intervention, as well as associations between the mortality effects and distance and climatic variables. These assessments are supplemented with malaria prevalence data collected in this study.

As other interventions to reduce child mortality are likely to influence the effect of azithromycin on child mortality, in order to set the background for the intervention, such interventions in Mangochi District are described. As a first step in exploring heterogeneity in the effect of the intervention, mortality rates between the five health zones were explored. The zones comprise distinct geographical areas with diverse socio-cultural, tribal and religious backgrounds. Selected variables related to specific diseases as well as water, sanitation and hygiene indicators, and access to healthcare, that could feasibly be related to a mortality effect of azithromycin, were also analysed.

Accessibility of health services is multidimensional with factors such as social support and caregiver autonomy increasingly understood to play a role.[128–130] However, studies in Africa have shown that proximity to health facilities is an important determinant of child mortality.[131–135] Investigation of straight line distance between dwellings and health facilities in The Gambia showed a nearly three-fold greater risk of all-cause mortality with distances greater than 5 km compared to less than 2 km.[136] In rural Kenya, high mortality has been shown to occur in clusters and remain so over several years, associated with several

geospatial indicators.[137] Higher infant mortality was associated with close proximity to streams and greater distance from public transport roads; and higher mortality in children aged 1-4 years was associated with living in areas of sparse population. These results would be consistent with a mortality effect related to either access to health care or exposure and transmission of infectious diseases, both of which could feasibly influence the effect of azithromycin MDA on mortality.

Higher malaria prevalence has been reported near large lakes in Africa, leading to concern with the building of dams.[138] Although malaria-carrying mosquitoes tend to breed in smaller stagnant pools of water, these can include almost any small man-made and natural pools, which may be more common near large water bodies. Mosquitoes may travel up to a few kilometres but their prevalence falls sharply even 500 metres from a breeding site.[139] A study along the shores of Lake Victoria showed mosquito prevalence was associated with proximity to breeding sites, particularly during the dry season.[140] However, this may not always translate to higher malaria prevalence as where there are significant levels of nuisance mosquitoes, inhabitants may be more likely to use mosquito nets.[141] This study provides the opportunity to investigate the effect of proximity to Lake Malawi on all-cause and malaria mortality and how this may influence the effect of azithromycin MDA.

Child mortality and its major aetiological causes show seasonal variation.[142–144] This has been shown to be similar through different age groups, likely related to different climatic conditions affecting particular disease processes or pathogens in a constant manner.[145] In addition, in locations where scarcity of food occurs, this usually follows an annual cycle, which may exacerbate seasonal peaks in mortality. The micro-climate may also play a role in parts of countries like Malawi, where rainfall, temperature and altitude vary considerably over short distances and may also affect mortality.[146,147] Malaria transmission is well documented to be associated with altitude and land surface temperature, as well as rainfall and NDVI, a graphical indicator of vegetation cover[148,149]. Pneumonia is commonly associated with the cooler drier months, as reported in a South African study of pneumonia in infants, with a four-fold higher incidence of pneumonia compared to the summer months.[150] Conversely, bacterial causes of diarrhoea are generally more common in the hotter, wetter months.[151] The relative impact of azithromycin on mortality from these causes, if known, could prove a useful consideration regarding timing of the intervention.

A significant impact on child mortality may be possible through interventions in water, sanitation and hygiene. One study of interventions supported by community health workers in South Africa suggested hand washing with soap may be the most cost-effective intervention for reducing child mortality, above case treatment of pneumonia and diarrhoea, and supplementary feeding.[152] An analysis using data from 59 countries suggested that just under 10% of the reduction in child mortality seen between 1990 and 2015 may be accounted for by sanitation improvements.[153]

Whilst mortality effects are the main focus of this work, the blood samples collected to assess malaria prevalence may provide useful additional evidence. The greater number of cases of malaria for each malaria death may enable identification of patterns that would not be possible from the investigation of mortality alone. The limited number of pathogens causing malaria may also facilitate more accurate assessment of disease prevalence compared to analysis of pathogens related to other causes of child mortality. Malaria is the leading cause of child mortality in Malawi, and as single dose azithromycin MDA has been associated with improvements in malaria morbidity indicators, assessment of malaria morbidity is an important component of this work.[99,154]

Finally, it was decided to make use of the secondary dataset provided by the District Health Office in Mangochi to provide data on various health indicators for the majority of MORDOR clusters, as opposed to just the six morbidity clusters per zone in which health indicator data were collected for this study. The District Health Office data included water, sanitation and hygiene indicators; insecticide treated bed net usage; community outreach (health visits); and the number of children per community being followed up for malnutrition.

Data analysis methods

The mortality database for this study was compiled using data from the four biannual census rounds conducted between March 2015 and June 2017. If a child was present at the start and end of any follow-up period, the total time of the intercensal period was used as the person-time contributed to the study. If a child's status was not known at the follow-up census, or the child was reported to have moved, half of the intercensal period was used for the person-time. Half of the follow-up time was used because the main outcome (death) was likely to be known and reported to the census enumerator by a family member or neighbour at the

follow-up visit if the child died before the family moved to live elsewhere and, on average, we would expect children to have moved away halfway through the follow-up period.

Information on current interventions to reduce mortality in the study area were gathered through discussions with health staff in Mangochi District. Background mortality at the study site was estimated by zone in placebo clusters using a Poisson regression model with random effects for clustering at the level of the randomization unit. Variables related to specific diseases or assessing specific domains, such as access to healthcare, or water, sanitation and hygiene, were grouped to produce separate models. All statistical tests were two-sided with a 5% significance level. A Poisson model on individual level mortality counts, with random effects to account for clustering at the level of the HSA area, was used to explore associations between mortality and geospatial and environmental predictors. This was conducted for all-cause mortality for all variable groupings and for specific causes where an association was considered feasible, for example between diarrhoea and water, sanitation and hygiene indicators.

Water, sanitation and hygiene and other data from the District Health Office were summarised to produce community level means and zonal level means. To test associations with all-cause and cause-specific mortality, variables were generally dichotomized rather than categorized into multiple groups due to the relatively small sample size and number of deaths at the MORDOR-Malawi site. Dichotomization took place around the median unless there were strong reasons for this not to be the case. For example, with distance to Lake Malawi expected to be a risk factor for malaria, the median distance of 9km would not make logical sense as the distance to dichotomize the data as this is well beyond the flight distance of mosquitoes. As the prevalence of mosquitoes falls sharply even 500m from breeding sites, and these are likely to occur close to the lake, the data were dichotomized around a distance of 1km from the lake.

Composite scores were created from the dichotomized variables, grouping those related to access to healthcare; malaria; and water, sanitation and hygiene. For access to healthcare, the composite score included 1 or 0 for households less than or greater than 2,500m from a health facility, respectively; 1 or 0 for households less than or greater than 1,000m from the nearest road, respectively; and 1 or 0 for communities with or without established outreach clinics, respectively. A higher score indicated better health care access. For malaria risk, the

composite score included 1 or 0 for less than or greater than 25% of the community using insecticide treated bed nets, respectively; 1 or 0 for households less than or greater than 1km from the lake, respectively; and 1 or 0 for temperature greater than or less than 24 degrees Celsius, respectively. A higher score indicated greater malaria risk. NDVI was not included in the composite score as these geographical indicators are likely to be associated with each other and also both reflect altitude in the Mangochi setting, where higher land is cooler and more forested. NDVI was included in the univariate analyses. The composite score for water, sanitation and hygiene risk included 1 or 0 for less than or greater than 50% of the community using an improved water source, respectively; 1 or 0 for less than or greater than 5% of the community using improved latrines, respectively; and 1 or 0 for less than or greater than 5% of the community having handwashing facilities at the household. A higher score indicated greater risk from disease associated with poor water, sanitation and hygiene. A summary of the composite scores is shown in Table 4.1.

Table 4.1: Construction of composite scores for access to healthcare, malaria risk and risk from water, sanitation and hygiene

Variable grouping	**Criteria to score 1 point**			**Score guide**
Access to healthcare	Household <2,500m from a health facility	Household <1,000m from the nearest road	Established outreach clinic in community	Score 0 to 3; Higher score indicates better access to healthcare
Malaria	<25% of the community using insecticide treated bed nets	<1km from Lake Malawi or lake Malombe	Temperature >24 degrees Celsius	Score 0 to 3; Higher score indicates greater malaria risk
Water, sanitation and hygiene	<50% of the community using an improved water source	<5% of community using improved latrines	<5% of community using handwashing facilities	Score 0 to 3; higher score indicates greater risk from disease associated with poor water, sanitation and hygiene

ArcGIS version 10 software was used to create variables for the distance from each household to the nearest lake (Lake Malawi or Lake Malombe), nearest road and nearest health facility. For all distance measurements, data were projected to Universal Transverse Mercator (UTM) zone 36S, which corresponds to Malawi's location. For raster data, such as land surface temperature, cell values were extracted to households. QGIS software was used to produce maps displaying mortality rate in azithromycin and placebo communities separately. Inverse distance weighting was used with settings to minimize the change in rate displayed with distance from each community. As measurements were only available for clusters, with no data points at geographical locations in between clusters, it was decided this approach, which maps a relatively constant rate around each community with increasing distance until the rate is influenced by that of a neighbouring community, would be the best approach. As each cluster only provided data for azithromycin or placebo clusters, these were mapped separately. A map was then created of the difference in mortality rate between placebo and azithromycin clusters for the study site by subtracting mortality rate in azithromycin clusters extrapolated to the study site from mortality rate in placebo clusters extrapolated to the study site.

Results

Geographical variation

Mortality in children aged 1-59 months in placebo clusters varied between 6.99 (5.34-9.15) deaths per 1,000 person-years in Monkey Bay zone and 10.91 (9.21-12.93) in Chilipa zone. This would correspond to total post-neonatal mortality of approximately 35-55 deaths per 1,000 live births, which is broadly similar to Malawi's estimated post-neonatal mortality of 42 in 2015.[4] Mortality in placebo clusters was higher in Namwera (rate ratio: 1.52 (95% CI: 1.07-2.17), P = 0.020) and Chilipa (rate ratio: 1.55 (95% CI: 1.07-2.26, P = 0.022) compared to Monkey Bay. The mortality rate in both the placebo and azithromycin arms by zone is shown in Figure 4.1. Mortality was lower in the azithromycin arm compared to placebo in Namwera: rate ratio 0.73 (95% CI: 0.54-0.97), P = 0.031. There was no evidence for a mortality difference between treatment arms in the other four zones. Mortality rate is displayed geographically for placebo and azithromycin clusters, extrapolated over the study site, in Figure 4.2 and

Figure 4.3, respectively. The difference between treatment arms, representing the effect of the intervention, is shown in Figure 4.4. Mortality varied considerably in all zones across the study area, in both azithromycin and placebo clusters. The difference in mortality between treatment arms, representing the effect of the intervention geographically, also varied considerably.

Key interventions that may have reduced mortality in the study setting are listed in Table 4.2. Cause-specific mortality (as identified using both InterVA and SmartVA) by zone showed stochastic variation due to relatively low numbers of deaths from each cause but suggested there may be an effect of azithromycin on pneumonia, malaria and HIV mortality in Namwera zone using InterVA (data presented in Appendix 2) and an effect on pneumonia, malaria and diarrhoea mortality using SmartVA in the same zone (Appendix 3). This would appear to reflect the analyses in Chapter 3 that suggested an effect of azithromycin MDA on mortality from these four causes and that the bulk of the effect at the Malawi site was in Namwera zone.

Characteristics of the five zones of Mangochi District, collected from both the morbidity communities and extracted from DHO data, are summarized in Table 4.3. From data collected in this study, PCV coverage was estimated to be highest in Mangochi and Namwera zones (approximately 75%) and lowest in Monkey Bay and Chilipa zones (approximately 65%). Any education of the household head was lowest in Namwera and Mangochi (under 20%) and highest in Monkey Bay (60%). The proportion with household wash stations was over 30% in Mangochi and Monkey Bay but approximately 10% or lower in Chilipa, Makanjira and Namwera. Weight-for height, weight-for-age and height-for-age z scores of study participants were not greatly dissimilar by zone at baseline. Malaria parasitaemia was highest in Namwera at baseline (47%) and lowest in Monkey Bay and Mangochi (approximately 20%). Religion, reflecting the ethnic background of the people, varied considerably from 82.3% Christian (mostly Chewa tribe) in Monkey Bay to 3.7% Christian in Namwera (mostly Yao).

Analysis of the DHO data to study clusters suggested >90% of households in all zones had a latrine although the highest proportion with an improved latrine was only 18.5% in Namwera zone. The proportion of households with handwashing facilities was highest in Namwera (41.9%) and lowest in Chilipa (5.7%).

Table 4.2: Government programs to reduce child mortality in Mangochi District, Malawi during the study

Intervention	**Year introduced**
PCV-13 vaccine*	2011
Rotavirus vaccine*	2012
Integrated management of childhood illness	2000
Insecticide treated bed nets	2012
Water, sanitation and hygiene	Ongoing education and information programs
Vitamin A supplementation	2000
Prevention of mother-to-child transmission of HIV	Expansion from 2003
Health surveillance assistants	1960s; expansion 2002-2008

* In addition to earlier introduction of *H. influenzae* type b, measles, diphtheria-tetanus-pertussis, hepatitis B, polio and BCG vaccinations

Figure 4.1: Mortality rate in azithromycin- and placebo-treated clusters in the five administrative zones of Mangochi District

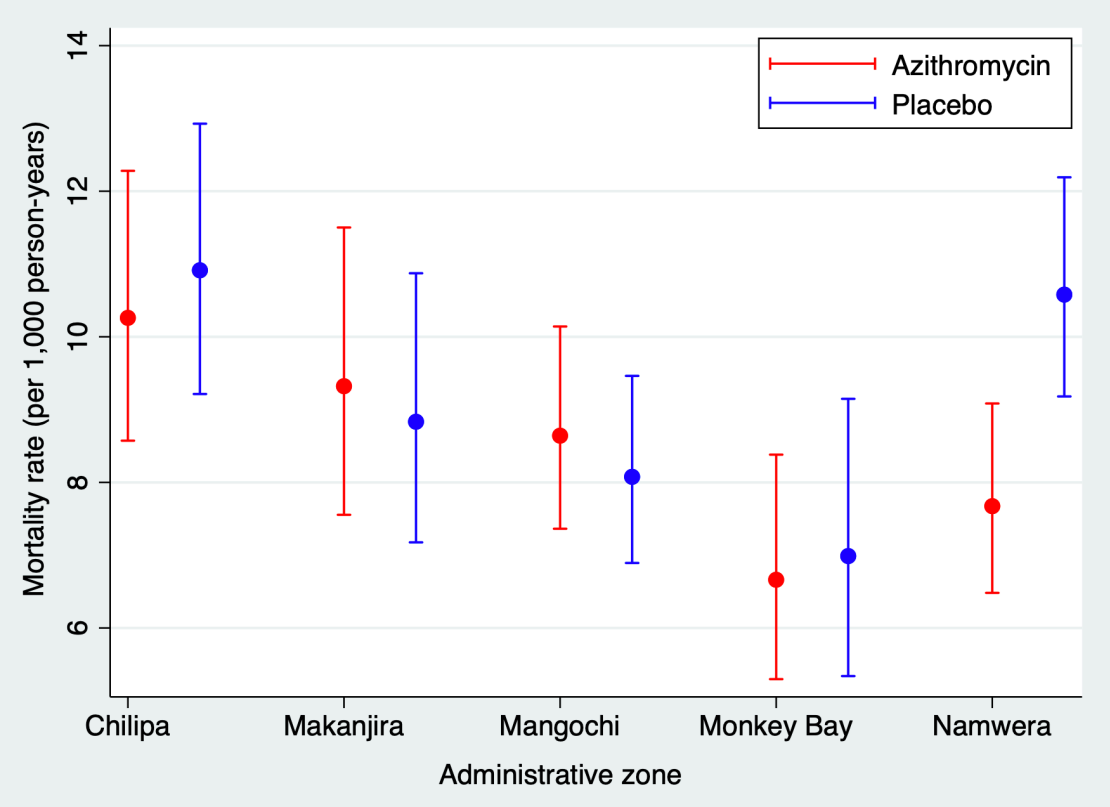

Figure 4.2: Mortality rate in placebo clusters extrapolated to Mangochi District

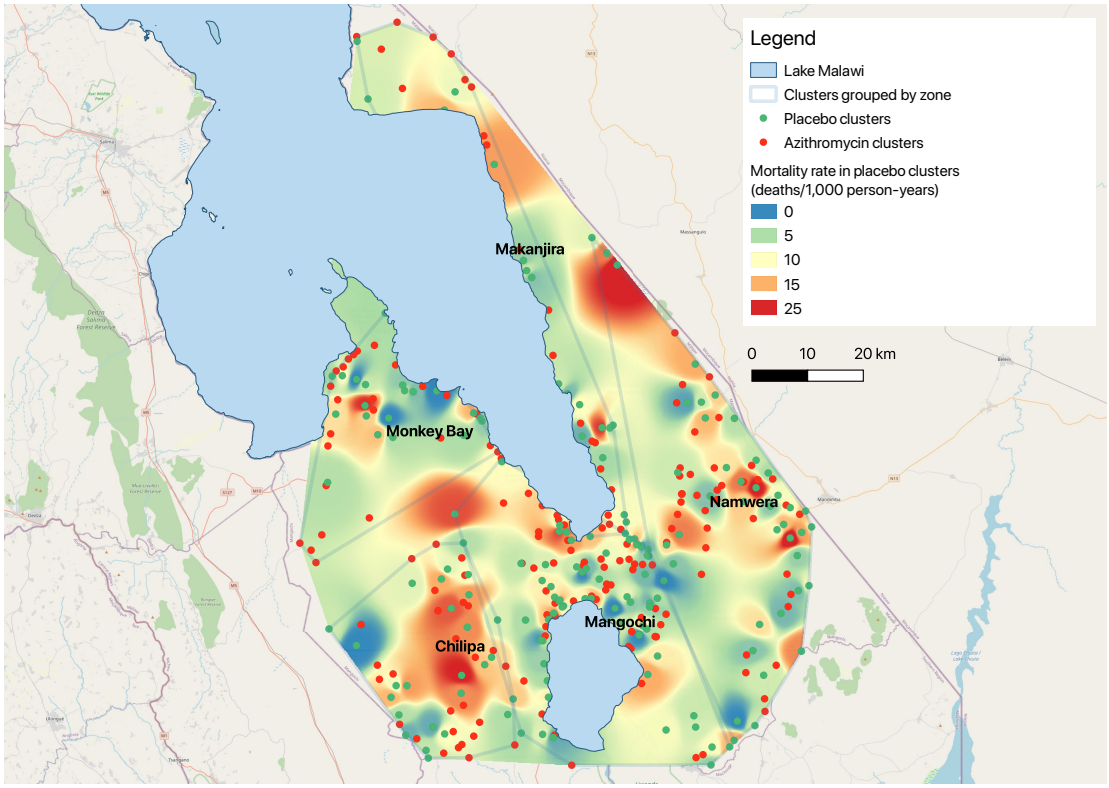

Figure 4.3: Mortality rate in azithromycin clusters extrapolated to Mangochi District

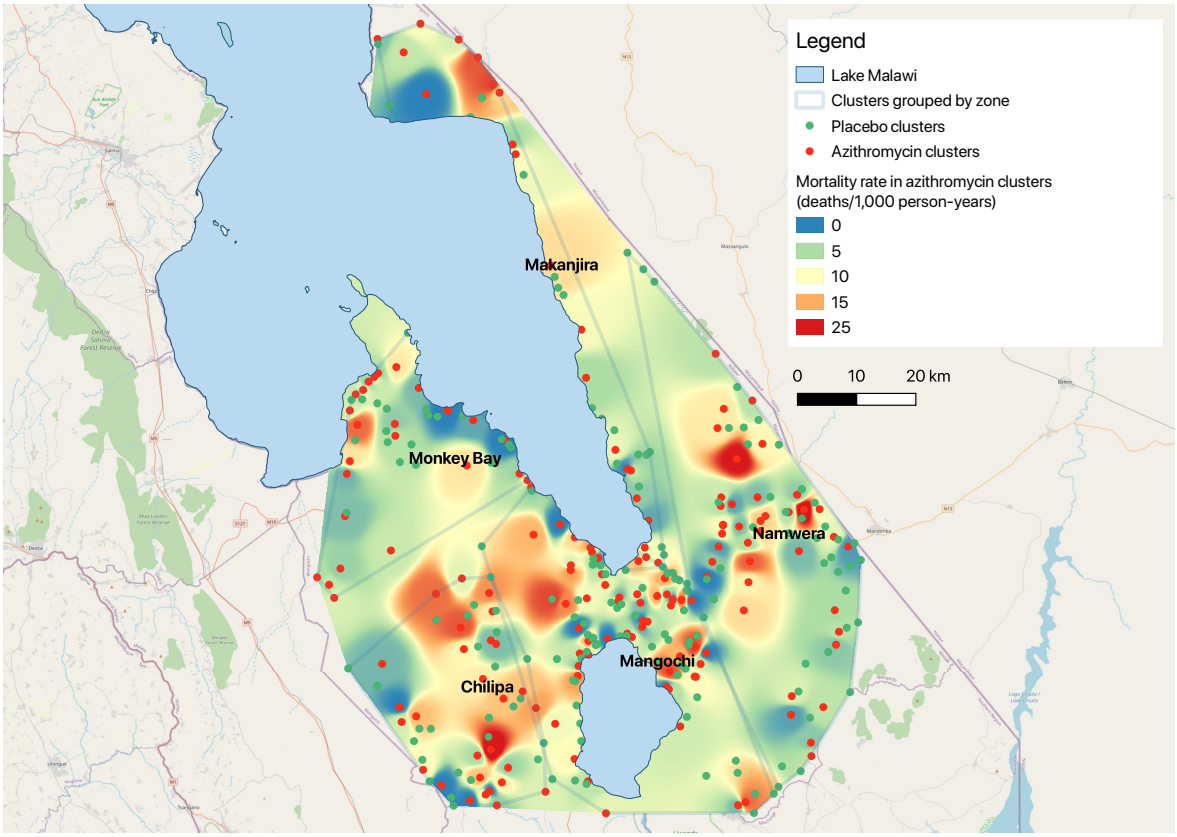

Figure 4.4: Mortality rate difference between azithromycin and placebo clusters

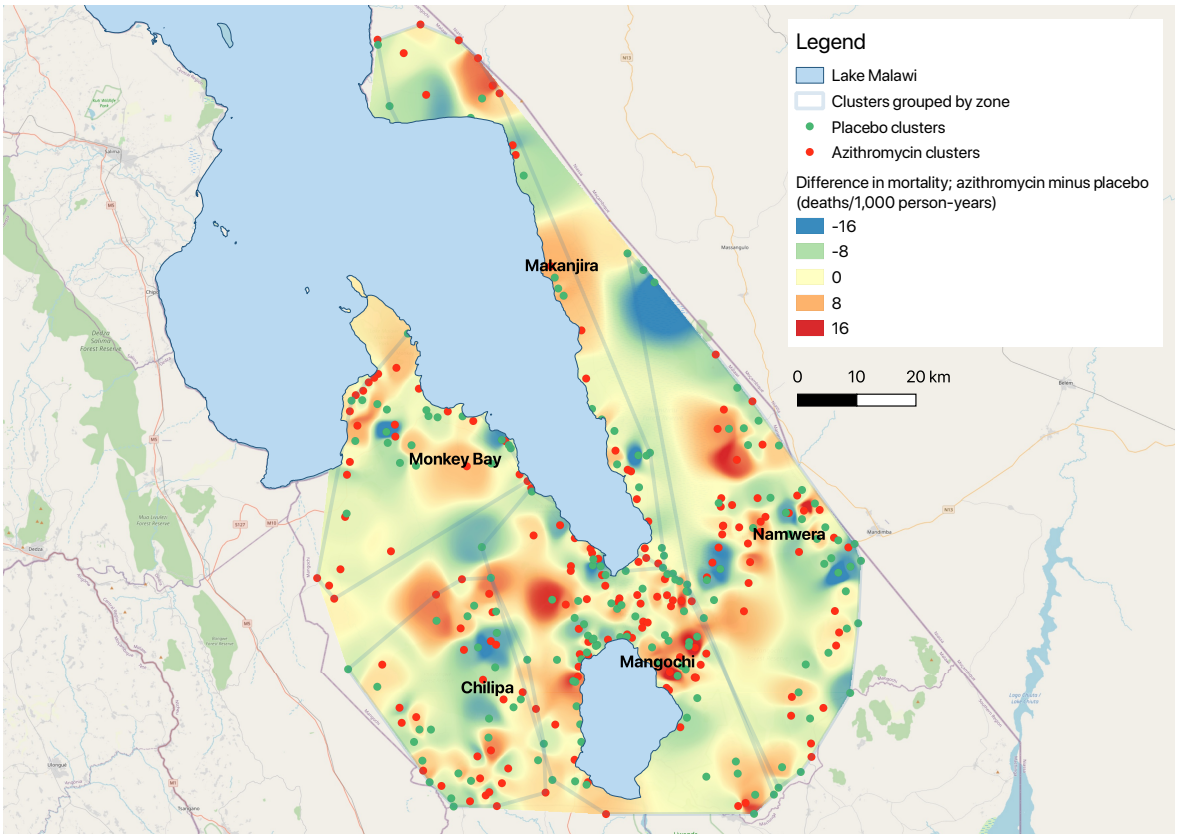

Table 4.3: Characteristics of the five health zones in Mangochi District

Zone	Monkey Bay	Chilipa	Makanjira	Namwera	Mangochi	Total
Study data from morbidity communities						
PCV coverage	65.9%	65.0%	-	74.9%	76.3%	70.5%
Any adult education	60.1%	42.9%	32.6%	19.4%	17.3%	34.1%
Household bicycle	36.7%	44.4%	44.2%	45.5%	36.0%	41.6%
Household wash station	32.3%	9.4%	7.9%	10.6%	37.2%	18.9%
Households with any latrine	51.0%	68.5%	60.5%	59.0%	52.2%	58.7%
Water collection time (minutes)	27	29	22	19	16	23
Improved water source (borehole; protected spring; protected hand dug well with or without pump)	83.0%	78.3%	76.7%	90.6%	95.9%	84.9%
Protestant (overall 61% Muslim, 38% Protestant, 1% other)	82.3%	41.9%	27.1%	3.7%	40.6%	37.8%
whz < -2 at baseline	1.3%	3.4%	1.1%	4.5%	5.0%	3.4%
waz < -2 at baseline	12.7%	16.2%	13.1%	11.9%	14.9%	14.4%
haz < -2 at baseline	35.7%	38.4%	42.9%	43.1%	35.7%	38.0%
Malaria parasite positive at baseline	19.0%	33.5%	29.9%	47.0%	21.6%	29.7%
Data from District Health Office						
Access to improved water source within 500m	67.6%	51.3%	68.7%	71.7%	66.9%	66.0%
Households with improved latrine	7.1%	5.3%	11.7%	18.5%	6.7%	10.6%
Households with any latrine	90.7%	90.2%	91.8%	92.6%	90.8%	91.3%
Household handwashing facilities	13.6%	5.7%	11.6%	41.9%	15.5%	20.3%
Children malnourished	2.5%	3.5%	4.4%	8.6%	4.4%	5.1%
Households with insecticide treated nets	46.0%	45.8%	36.8%	50.7%	41.0%	44.3%
Communities with outreach clinic	34.2%	45.6%	36.1%	35.5%	20.8%	33.1%

Access to healthcare

The associations between indicators of access to healthcare and the effect of azithromycin are shown in Table 4.4. The effect estimates by distance to health facility were similar in the azithromycin and placebo arms. The effect estimates by whether the community received health outreach visits suggested that there may be an effect of the intervention only in communities that did not receive such visits. There was a similar trend suggesting the effect occurred only in communities close to the road, convincing reasons for which are difficult to envisage. There was no clear association evident between the composite score for access to healthcare and the effect estimate in azithromycin and placebo arms, as would be expected given the lack of clear associations with the individual indicators (Table 4.5).

Table 4.4: Association between variables related to healthcare access and effect of azithromycin (distance to heath facility, distance to road, and presence of outreach clinic)

	Deaths/ person-years	Rate per 1000 person-years (95% CI)	Rate Ratio (95% CI)	*P*-value
Distance to health facility				
0-2,499 metres				
Placebo	248/29,482	8.4 (7.4-9.5)	1	
Azithro	255/33,471	7.6 (6.7-8.6)	0.92 (0.75-1.12)	0.40
2,500-19,400 metres				
Placebo	343/34,383	10.0 (9.0-11.1)	1	
Azithro	277/29,738	9.3 (8.3-10.5)	0.92 (0.76-1.11)	0.39
Outreach in community				
No				
Placebo	355/37,614	9.4 (8.5-10.5)	1	
Azithro	347/43,447	8.0 (7.2-8.9)	0.85 (0.71-1.02)	0.08
Yes				
Placebo	213/22,753	9.4 (8.2-10.7)	1	
Azithro	166/17,810	9.3 (8.0-10.9)	0.99 (0.76-1.28)	0.94
Distance to road				
0-999 metres				
Placebo	334/36,908	9.0 (8.1-10.1)	1	
Azithro	316/40,558	7.8 (7.0-8.7)	0.86 (0.71-1.03)	0.10
1000-20,200 metres				
Placebo	257/26,957	9.5 (8.4-10.8)	1	
Azithro	216/22,652	9.5 (8.3-10.9)	1.01 (0.81-1.25)	0.95

Data on distance to health facility and distance to road available for all clusters; presence of outreach services only available for clusters linked to DHO data (298 of 334 clusters)

Table 4.5: Mortality rate in azithromycin- and placebo-treated clusters by a composite score for access to healthcare (higher score indicates better access to healthcare)

	Deaths/ person-years	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value
Composite score for access to healthcare				
0				
Placebo	82/8,260	9.9 (8.0-12.3)	1	
Azithro	50/6,063	8.2 (6.3-10.9)	0.82 (0.56-1.22)	0.33
1				
Placebo	231/23,291	9.9 (8.7-11.3)	1	
Azithro	238/25,021	9.5 (8.4-10.8)	0.95 (0.77-1.18)	0.65
2				
Placebo	163/18,927	8.6 (7.4-10.0)	1	
Azithro	154/20,913	7.4 (6.3-8.6)	0.87 (0.68-1.12)	0.28
3				
Placebo	64/7,131	9.0 (7.0-11.5)	1	
Azithro	42/5,959	7.0 (5.2-9.5)	0.76 (0.49-1.19)	0.23

Data available for clusters linked to DHO data (298 of 334 clusters)
Note higher score indicates better access to healthcare

Malaria risk factors

Associations between indicators of malaria risk and the effect of azithromycin on all-cause mortality, as well as malaria mortality, as identified by the VA algorithms, are shown in Table 4.6. There was evidence for an effect of azithromycin compared to placebo on all-cause mortality in communities where the proportion of households using ITNs was lower than 25%: rate ratio = 0.79 (0.63-0.99), P = 0.039. There was no evidence for an effect of azithromycin in communities where >25% of households used ITNs: rate ratio = 0.99 (0.79-1.24), P = 0.93. Interestingly, similar patterns were not evident for malaria mortality as identified from both the InterVA and SmartVA algorithms.

The mortality rate ratio in azithromycin- compared placebo-treated individuals in households <1,000 metres from Lake Malawi or Lake Malombe was 0.77 (0.55-1.07), P = 0.12. The respective rate ratio in households ≥1,000 metres from the lakes was 0.95 (0.81-1.11), P = 0.51. Similar patterns were evident, with a greater effect estimate close to the lakes, for malaria mortality, as identified from both VA algorithms.

The rate ratios in azithromycin- compared to placebo-treated individuals in areas with NDVI <0.60, and temperature <24 degrees, respectively, were: 0.81 (0.65-1.03), P = 0.085; and 0.85 (0.70-1.04), P = 0.11. The effect estimates were much smaller for NDVI ≥0.60 and temperature >24 degrees. A similar pattern was evident, with a greater effect estimate in areas with NDVI <0.60, for malaria mortality, as identified from both VA algorithms. The same pattern in malaria mortality was not evident by temperature.

The estimate for the effect of azithromycin on all-cause mortality increased with an increase in the composite score for malaria risk, and the patterns were not dissimilar for the effect on malaria mortality inferred from the two VA algorithms (Table 4.7).

Table 4.6: Association between variables related to malaria (insecticide treated bed net (ITN) usage, distance to Lake Malawi, NDVI and temperature) and effect of azithromycin on all-cause and malaria mortality (identified from verbal autopsy using InterVA and SmartVA)

		Overall mortality				Malaria mortality using InterVA				Malaria mortality using SmartVA			
	Person-years at risk	Number of deaths	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value	Number of deaths	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value	Number of deaths	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value
Proportion of households using ITNs													
0.0-24.9%													
Placebo	22,057	212	9.6 (8.4-11.0)	1		75	3.4 (2.7-4.3)	1		64	2.9 (2.3-3.7)	1	
Azithro	29,429	224	7.6 (6.7-8.7)	0.79 (0.63-0.99)	0.039	96	3.3 (2.7-4.0)	0.96 (0.69-1.32)	0.79	76	2.6 (2.1-3.2)	0.89 (0.63-1.26)	0.52
25.0-100.0%													
Placebo	31,074	282	9.1 (8.1-10.2)	1		124	4.0 (3.3-4.8)	1		94	3.0 (2.5-3.7)	1	
Azithro	22,992	204	8.9 (7.7-10.2)	0.99 (0.79-1.24)	0.93	85	3.7 (3.0-4.6)	0.93 (0.69-1.26)	0.64	65	2.8 (2.2-3.6)	0.94 (0.68-1.31)	0.71
Distance to Lake Malawi													
0-999 metres													
Placebo	11,777	99	8.4 (6.9-10.2)	1		44	3.7 (2.8-5.0)	1		36	3.1 (2.2-4.2)	1	
Azithro	13,621	92	6.8 (5.5-8.3)	0.77 (0.55-1.07)	0.12	33	2.4 (1.7-3.4)	0.63 (0.39-1.02)	0.060	30	2.2 (1.5-3.2)	0.72 (0.44-1.19)	0.20
1,000-40,000 metres													
Placebo	52,087	492	9.4 (8.6-10.3)	1		201	3.9 (3.4-4.4)	1		152	2.9 (2.5-3.4)	1	
Azithro	49,589	440	8.9 (8.1-9.7)	0.95 (0.81-1.11)	0.51	195	3.9 (3.4-4.5)	1.02 (0.83-1.27)	0.84	141	2.8 (2.4-3.4)	0.98 (0.77-1.24)	0.84
NDVI													
<0.60													
Placebo	22,070	200	9.1 (7.9-10.4)	1		92	4.2 (3.4-5.1)	1		67	3.0 (2.4-3.9)	1	
Azithro	22,058	162	7.3 (6.3-8.6)	0.81 (0.65-1.03)	0.085	63	2.9 (2.2-3.7)	0.67 (0.48-0.94)	0.022	54	2.4 (1.9-3.2)	0.81 (0.56-1.16)	0.25
≥0.60													
Placebo	41,794	391	9.4 (8.5-10.3)	1		153	3.7 (3.1-4.3)	1		121	2.9 (2.4-3.5)	1	
Azithro	41,151	370	9.0 (8.1-10.0)	0.96 (0.81-1.13)	0.63	165	4.0 (3.4-4.7)	1.10 (0.87-1.39)	0.43	117	2.8 (2.4-3.4)	0.98 (0.76-1.28)	0.91
Temperature													
19.0-23.9 degrees													
Placebo	32,083	332	10.3 (9.3-11.5)	1		145	4.5 (3.8-5.3)	1		98	3.1 (2.5-3.7)	1	
Azithro	31,186	273	8.8 (7.8-9.9)	0.85 (0.70-1.04)	0.11	126	4.0 (3.4-4.8)	0.90 (0.69-1.16)	0.41	95	3.0 (2.5-3.7)	1.00 (0.74-1.34)	0.99
24.0-24.9 degrees													
Placebo	31,782	259	8.1 (7.2-9.2)	1		100	3.1 (2.6-3.8)	1		90	2.8 (2.3-3.5)	1	
Azithro	32,024	259	8.1 (7.2-9.1)	0.98 (0.80-1.21)	0.88	102	3.2 (2.6-3.9)	1.00 (0.75-1.35)	0.98	76	2.4 (1.9-3.0)	0.84 (0.61-1.15)	0.28

Distance to Lake Malawi and data on NDVI and temperature available for all clusters; data on use of ITNs available for clusters linked to DHO data (298 of 334 clusters)

Table 4.7: All-cause mortality and malaria mortality (as identified from verbal autopsy using InterVA and SmartVA) in azithromycin- and placebo-treated clusters by a composite score for malaria risk

		Overall mortality				Malaria mortality using InterVA				Malaria mortality using SmartVA			
	Person-years at risk	Number of deaths	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value	Number of deaths	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value	Number of deaths	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value
Composite score													
0													
Placebo	16,712	158	9.4 (8.1-11.0)	1		75	4.5 (3.6-5.6)	1		51	3.0 (2.3-4.0)	1	
Azithro	10,904	108	9.9 (8.2-12.0)	1.08 (0.80-1.45)	0.61	48	4.4 (3.3-5.8)	0.99 (0.68-1.45)	0.96	36	3.3 (2.4-4.6)	1.09 (0.70-1.69)	0.70
1													
Placebo	17,950	169	9.4 (8.1-10.9)	1		64	3.6 (2.8-4.6)	1		48	2.7 (2.0-3.5)	1	
Azithro	19,454	166	8.5 (7.3-9.9)	0.91 (0.71-1.17)	0.47	69	3.5 (2.8-4.5)	1.00 (0.70-1.42)	0.99	58	3.0 (2.3-3.9)	1.12 (0.76-1.65)	0.58
2													
Placebo	13,042	115	8.8 (7.3-10.6)	1		41	3.1 (2.3-4.3)	1		43	3.3 (2.4-4.4)	1	
Azithro	11,398	85	7.5 (6.0-9.2)	0.84 (0.61-1.16)	0.29	32	2.8 (2.0-4.0)	0.89 (0.56-1.44)	0.65	24	2.1 (1.4-3.1)	0.64 (0.39-1.07)	0.088
3													
Placebo	2,856	28	9.8 (6.8-14.2)	1		11	3.9 (2.1-7.0)	1		8	2.8 (1.4-5.6)	1	
Azithro	7,752	48	6.2 (4.7-8.2)	0.62 (0.37-1.05)	0.076	20	2.6 (1.7-4.0)	0.67 (0.31-1.42)	0.29	16	2.1 (1.3-3.4)	0.74 (0.31-1.75)	0.49

Data available for clusters linked to DHO data (298 of 334 clusters)
Note higher score indicates greater risk of malaria

Water, sanitation and hygiene indicators

The rate ratio for the effect of azithromycin compared to placebo on all-cause mortality in communities where <50% of households had access to improved water sources was 0.75 (0.58-0.97), *P* = 0.028 (Table 4.8). In communities where ≥50% of households had access to improved water sources, there was no such evidence for an effect of the intervention: rate ratio 1.06 (0.85-1.32), P = 0.60. The rate ratios in the azithromycin compared to placebo groups in communities where <5% of households had access to improved latrines and those where ≥5% had improved latrines, respectively, were 0.83 (0.69-0.99), P = 0.043; and 1.00 (0.77-1.30), P = 0.98. There was no such pattern in effect estimates by whether communities had more than or less than 5% of households with handwashing facilities.

The analyses by diarrhoea mortality show more stochastic variation due to relatively low numbers of inferred diarrhoea mortality from the VA algorithms, as shown in Table 4.8.

The rate ratios for the effect of azithromycin on all-cause and diarrhoea mortality by a composite score for WASH risk are shown in Table 4.9. The trend is of an increasing effect of the intervention on all-cause mortality with increasing risk from poor WASH indicators. The same pattern is evident for diarrhoea mortality inferred using the InterVA algorithm; the pattern is less clear for diarrhoea mortality inferred using the SmartVA algorithm.

Table 4.8: Association between variables related to water, sanitation and hygiene (access to improved water source, improved latrines and handwashing facilities) and effect of azithromycin on all-cause mortality and diarrhoea mortality (as identified from verbal autopsy using InterVA and SmartVA)

		Overall mortality				Diarrhoea mortality using InterVA				Diarrhoea mortality using SmartVA			
	Person-years at risk	Number of deaths	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value	Number of deaths	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value	Number of deaths	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value
Proportion of community with access to improved water source													
0.0-49.9%													
Placebo	22,658	229	10.1 (8.9-11.5)	1		16	0.7 (0.4-1.2)	1		26	1.1 (0.8-1.7)	1	
Azithro	19,789	150	7.6 (6.5-8.9)	0.75 (0.58-0.97	0.028	15	0.8 (0.5-1.3)	1.08 (0.48-2.42)	0.84	12	0.6 (0.3-1.1)	0.53 (0.26-1.07)	0.076
50.0-100.0%													
Placebo	26,401	230	8.7 (7.7-9.9)	1		22	0.8 (0.5-1.3)	1		28	1.1 (0.7-1.5)	1	
Azithro	31,935	299	9.4 (8.4-10.5)	1.06 (0.85-1.32)	0.60	20	0.6 (0.4-1.0)	0.77 (0.39-1.54)	0.46	33	1.0 (0.7-1.5)	0.97 (0.57-1.64)	0.90
Proportion of community with improved latrines													
0.0-4.9%													
Placebo	38,086	361	9.5 (8.5-10.5)	1		36	0.9 (0.7-1.3)	1		49	1.3 (1.0-1.7)	1	
Azithro	41,575	330	7.9 (7.1-8.8)	0.83 (0.69-0.99)	0.043	28	0.7 (0.5-1.0)	0.72 (0.42-1.23)	0.23	37	0.9 (0.6-1.2)	0.68 (0.44-1.07)	0.097
5.0-100.0%													
Placebo	20,147	184	9.1 (7.9-10.6)	1		6	0.3 (0.1-0.7)	1		18	0.9 (0.6-1.4)	1	
Azithro	18,943	170	9.0 (7.7-10.4)	1.00 (0.77-1.30)	0.98	11	0.6 (0.3-1.0)	1.99 (0.70-5.64)	0.19	10	0.5 (0.3-1.0)	0.59 (0.27-1.32)	0.20
Proportion of community with handwashing facilities													
0.0-4.9%													
Placebo	32,429	290	8.9 (8.0-10.0)	1		23	0.7 (0.5-1.1)	1		35	1.1 (0.8-1.5)	1	
Azithro	35,334	283	8.0 (7.1-9.0)	0.88 (0.72-1.08)	0.22	21	0.6 (0.4-0.9)	0.83 (0.43-1.60)	0.58	29	0.8 (0.6-1.2)	0.75 (0.45-1.26)	0.27
5.0-100.0%													
Placebo	25,212	262	10.4 (9.2-11.7)	1		20	0.8 (0.5-1.2)	1		34	1.3 (1.0-1.9)	1	
Azithro	22,263	191	8.6 (7.4-9.9)	0.84 (0.66-1.06)	0.14	15	0.7 (0.4-1.1)	0.90 (0.42-1.92)	0.78	17	0.8 (0.5-1.2)	0.57 (0.31-1.05)	0.069

Data available for clusters linked to DHO data (298 of 334 clusters)

Table 4.9: All-cause mortality and diarrhoea mortality (as identified from verbal autopsy using InterVA and SmartVA) in azithromycin- and placebo-treated clusters by a composite score for water, sanitation and hygiene risk

		Overall mortality				Diarrhoea mortality using InterVA				Diarrhoea mortality using SmartVA			
	Person-years at risk	**Number of deaths**	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**	**Number of deaths**	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**	**Number of deaths**	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**
Composite score for WASH risk													
0													
Placebo	3,878	21	5.4 (3.5-8.3)	1		0	0 (0)	1		1	0.3 (0.0-1.8)	1	
Azithro	5,100	44	8.6 (6.4-11.6)	1.60 (0.87-2.93)	0.13	0	0 (0)	1.00 (0)	1.00	1	0.2 (0.0-1.4)	0.76 (0.05-12.16	0.85
1													
Placebo	15,412	168	10.9 (9.4-12.7)	1		14	0.9 (0.5-1.5)	1		19	1.2 (0.8-1.9)	1	
Azithro	13,163	133	10.1 (8.5-12.0)	0.91 (0.68-1.21)	0.52	11	0.8 (0.4-1.7)	0.94 (0.39-2.30)	0.90	16	1.20.7-2.0)	0.99 (0.51-1.92)	0.97
2													
Placebo	18,589	175	9.4 (8.1-10.9)	1		16	0.9 (0.5-1.4)	1		22	1.2 (0.8-1.8)	1	
Azithro	17,829	149	8.4 (7.1-8.8)	0.88 (0.67-1.16)	0.37	11	0.6 (0.3-1.1)	0.75 (0.32-1.77)	0.51	15	0.8 (0.5-1.4)	0.71 (0.37-1.37)	0.31
3													
Placebo	9,564	83	8.7 (7.0-10.8)	1		8	0.8 (0.4-1.7)	1		8	0.8 (0.4-1.7)	1	
Azithro	11,973	85	7.1 (5.7-8.8)	0.80 (0.56-1.15)	0.23	7	0.6 (0.3-1.2)	0.66 (0.21-2.00)	0.46	8	0.7 (0.3-1.3)	0.80 (0.30-2.13)	0.65

Data available for clusters linked to DHO data (298 of 334 clusters)
Note higher score indicates poorer WASH indicators

Nutritional status

In placebo communities, all-cause mortality was more than twice as high in infants aged 1-11 months with a weight-for-age z score <-2 compared to those with a z score ≥-2: 35.7 (95% CI: 26.3-48.4) vs 15.3 (95% CI: 12.6-18.7), shown in Table 4.10. The rate ratio for the effect of azithromycin compared to placebo on mortality in infants with a weight-for-age z score <-2 was 0.71 (95% CI: 0.43-1.16), *P* = 0.17; and in infants with a weight-for-age z score ≥-2 the rate ratio was 0.86 (95% CI: 0.64-1.18), *P* = 0.35.

Table 4.10: Mortality by weight-for-age z score in infants 1-11 months of age

	Deaths/ person-years	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**
Z score <-2				
Placebo	41/1,149	35.7 (26.3-48.4)	1	
Azithro	27/1,046	25.8 (17.7-37.6)	0.71 (0.43-1.16)	0.17
Z score ≥-2				
Placebo	97/6,328	15.3 (12.6-18.7)	1	
Azithro	82/6,170	13.3 (10.7-16.5)	0.86 (0.64-1.18)	0.35

Malaria morbidity

The analysis of malaria morbidity in this study has been published in a peer reviewed journal and is included here.

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SECTION A – Student Details

Student ID Number	248702	**Title**	Dr
First Name(s)	John Daniel		
Surname/Family Name	Hart		
Thesis Title	Azithromycin mass drug administration for reducing child mortality in Malawi		
Primary Supervisor	Robin Bailey		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	American Journal of Tropical Medicine and Hygiene		
When was the work published?	March 2020		
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SECTION E

Student Signature	John Hart
Date	14/06/2021

Supervisor Signature	Robin Bailey
Date	14/06/2021

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Effects of Biannual Azithromycin Mass Drug Administration on Malaria in Malawian Children: A Cluster-Randomized Trial

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Abstract. Reductions in malaria morbidity have been reported following azithromycin mass drug administration (MDA) for trachoma. The recent Macrolides Oraux pour Reduire les Deces avec un Oeil sur la Resistance (MORDOR) trial reported a reduction in child mortality following biannual azithromycin MDA. Here, we investigate the effects of azithromycin MDA on malaria at the MORDOR-Malawi study site. A cluster-randomized double-blind placebo-controlled trial, with 15 clusters per arm, was conducted. House-to-house census was updated biannually, and azithromycin or placebo syrup was distributed to children aged 1–59 months for a total of four biannual distributions. At baseline, 12-month, and 24-month follow-up visits, a random sample of 1,200 children was assessed for malaria with thick and thin blood smears and hemoglobin measurement. In the community-level analysis, there was no difference in the prevalence of parasitemia (1.0% lower in azithromycin-treated communities; 95% CI: −8.2 to 6.1), gametocytemia (0.7% lower in azithromycin-treated communities; 95% CI: −2.8 to 1.5), or anemia (1.7% lower in azithromycin-treated communities; 95% CI: −8.1 to 4.6) between placebo and azithromycin communities. Further interrogation of the data at the individual level, both per-protocol (including only those who received treatment 6 months previously) and by intention-to-treat, did not identify differences in parasitemia between treatment arms. In contrast to several previous reports, this study did not show an effect of azithromycin MDA on malaria parasitemia at the community or individual levels.

INTRODUCTION

The Macrolides Oraux pour Reduire les Deces avec un Oeil sur la Resistance (MORDOR) trial, conducted in Niger, Malawi, and Tanzania, demonstrated a reduction in child mortality following biannual mass drug administration (MDA) with azithromycin.[1] The mechanism through which such a reduction in child mortality may occur is not clear. Azithromycin is a broad spectrum antibiotic with a relatively long half-life which is used in the treatment of pneumonia and diarrhea but also displays antimalarial activity.[2,3] Field trials of the effects of azithromycin MDA on malaria infection and symptoms have previously reported reductions in malariometric indices; and recent results from the Niger MORDOR site indicate an association between azithromycin MDA and lower parasitemia.[3–7] It is feasible that the mortality benefit seen with azithromycin MDA may be due, at least in part, to a decrease in malaria prevalence or severity.

This study reports malaria parasitemia, parasite density, and gametocytemia data from the MORDOR Malawi study site, aiming to improve our understanding of the effects of azithromycin MDA on malaria infection. Samples were collected from children in villages representative of the MORDOR trial and used the same biannual census updates and cluster-randomized trial structure to make the results as representative as possible of the wider study area and to assess community-level effects of any outcomes. The hypothesis for this study was that azithromycin MDA would reduce the community prevalence of malaria compared with placebo.

METHODS

Trial design. The randomization unit for the MORDOR trial in Malawi was defined as the catchment area of a health surveillance assistant (HSA), approximately 1,000 total population. Communities with a population < 200 or > 2,000 on a pre-baseline census were excluded. Thirty communities were randomly selected from the pool of communities for the MORDOR trial for follow-up as part of this malaria prevalence study. The randomization was stratified to produce six communities in each of the five geographical zones of Mangochi district for geographical generalizability and for logistical reasons regarding fieldwork. Biannual census updates were performed, and communities received study drug in the same treatment rounds as the MORDOR trial.

Participants. All children aged 1–59 months and weighing ≥ 3.8 kg were eligible for treatment at each of four biannual mass distributions. At the baseline, 12-month, and 24-month follow-up visits, guardians of a randomly selected sample of 40 children per community were asked to provide written informed consent for finger-prick blood samples. The procedures and study were explained by trained local nursing staff who subsequently collected thick and thin blood smears if consent was obtained. Illiterate guardians provided a thumb print to acknowledge consent.

Interventions. Azithromycin was administered at a dose of 20 mg/kg. Children old enough to stand received an approximate dose estimated from their height, and younger children were weighed. The placebo bottles and suspension appeared identical to azithromycin. Distribution of drug took place after sample collection was complete and was performed by the HSAs and field-workers conducting house-to-house visits. Guardians were asked to inform the HSA of any adverse events that occurred within 7 days of receiving study drug. Health surveillance assistants subsequently informed the study team.

Outcomes. The primary prespecified outcome was prevalence of malaria parasites on thick blood smears in children aged 1–59 months. Prespecified secondary outcomes included parasite density, gametocyte prevalence and density,

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hemoglobin concentration, and presence of anemia (Hb < 11 g/dL). Primary analyses were by intention-to-treat, and per-protocol analyses were secondary.

Sample collection. Sample collection took place during the baseline visit (May–July 2015) and at 12-month and 24-month visits (April–June 2016 and 2017, respectively), approximately 6 months after the second and fourth treatment rounds. Sample collection involved selected children receiving a finger stick and thick and thin blood smears collected on a single slide (hemoglobin measurement was performed using a Hemocue 201 device (Ängelholm, Sweden)). Slides were labeled with a random number and barcode and scanned using the data collection app to link to census data. The thin smear was fixed with absolute methanol and the slide stained with 8% Giemsa. Parasite density was assessed from the thick smear by two independent slide readers at MORDOR trial laboratories in Mangochi and Blantyre. Parasite density was estimated as parasites per microliter by assessing up to 100 high-power fields and assuming that 500 high-power fields contain the equivalent of one microliter of blood. If after two reads there was a discrepancy of greater than 20%, a third reading was taken as the final read. Thin slides were used to assess malaria species.

Sample size. Fifteen communities per arm, with 40 children sampled from each community (600 children per arm), provided 90% power to detect a reduction of malaria parasitemia from 20% to half that value at each study phase.

Randomization and blinding. Study drug was labeled with six letters by the manufacturer (Pfizer Inc., New York, NY), with three letters corresponding to azithromycin and three to placebo. Communities were randomly assigned to one of the six drug letters by the study statistician using the statistical package R (R Foundation for Statistical Computing, Vienna, Austria). All field and laboratory staff and participants in Malawi were blinded to the treatment code until after all data collection was complete.

Statistical methods. The main analysis was by intention-to-treat at the community level, in keeping with the cluster-randomized design of the MORDOR trial. Further analyses by both intention-to-treat and per-protocol at the individual level were used to further explore the data.

Community-level prevalence of parasitemia, anemia, and gametocytemia by treatment arm were assessed for the 12-month and 24-month visits using mixed-effects linear regression models including fixed effects for baseline prevalence and study phase and a random effect for community.

Individual-level parasitemia, parasite density, hemoglobin, and gametocytemia by treatment arm were assessed for the 12-month and 24-month visits using mixed-effects logistic or linear regression models, as appropriate, including fixed effects for age, baseline prevalence, and study phase and nested random effects for individuals within communities. Individual-level analyses were performed by both intention-to-treat and per-protocol, including only those who received study drug as indicated at the previous phase. Intra-class correlation coefficients (ICCs) were derived from the regression models at the level of the randomization unit.

The hemoglobin levels in individuals between the treatment arms were split by the presence of parasitemia and compared using Student's *t*-test. Association between the presence of parasitemia and the hemoglobin level was assessed at the individual level using Student's *t*-test.

Ethical approval. Ethical approval for morbidity assessments alongside the MORDOR trial was obtained from the College of Medicine, University of Malawi; the London School of Hygiene and Tropical Medicine; and the UCSF Committee on Human Research. Written consent was obtained from the guardians of participants. There were no incentives for participation.

RESULTS

Demographic details of sampled children in the 30 study communities are shown in Table 1. Age and gender distributions of sampled children were similar between the azithromycin- and placebo-treated communities. Study drug was distributed in all 30 communities at each round, with 76.6% of eligible children treated in azithromycin communities over all phases and 73.5% in placebo communities. The trial flow is shown in Figure 1, including the number of malaria blood films taken. No serious adverse events attributable to study drug were reported.

Over 98% of malaria species detected were *Plasmodium falciparum*, and the remainder were *Plasmodium malariae*. Analyses included both malaria species. The community-level prevalence of malaria parasitemia, anemia, and gametocytemia was similar between placebo and azithromycin groups at baseline (unadjusted data shown in Table 2). Malaria parasitemia did not change significantly between treatment groups at the 12- and 24-month follow-up rounds: 1.0% lower in azithromycin-treated communities after adjusting for baseline parasitemia and follow-up phase (95% CI: –8.2 to 6.1%, $P = 0.78$; ICC = 0.51). The prevalence of anemia, defined as Hb < 11 g/dL, was not significantly different between treatment groups at the 12- and 24-month follow-up visits: 1.7% lower in azithromycin-treated communities (95% CI: –8.1 to 4.6%, $P = 0.59$; ICC = 0.09). The prevalence of gametocytemia also remained similar between treatment groups at the follow-up rounds: 0.7% lower in azithromycin-treated communities (95% CI: –2.8 to 1.5%, $P = 0.53$; ICC < 0.01).

Individual-level unadjusted data are shown by intention-to-treat in Table 3 and per-protocol in Table 4. The prevalence of parasitemia, parasite density, and hemoglobin was similar at baseline. By intention-to-treat, parasitemia was not significantly different between treatment groups at the 12- and 24-month follow-up visits: the odds ratio for individuals in azithromycin- compared with placebo-treated communities after adjusting for age, baseline parasitemia, and follow-up phase was 0.89 (95% CI: 0.53 to 1.50, $P = 0.67$; ICC = 0.06).

TABLE 1

Characteristics of children sampled in the study communities at the start of each follow-up period

	Placebo *N* (%)	Azithromycin *N* (%)
Age distribution (months)		
1–11	259 (47.9)	282 (52.1)
12–23	385 (51.2)	367 (48.8)
24–35	362 (50.2)	359 (49.8)
36–47	351 (51.1)	336 (48.9)
48–59	324 (50.2)	321 (49.8)
Gender		
Female	878 (50.3)	866 (49.7)
Male	841 (50.4)	828 (49.6)

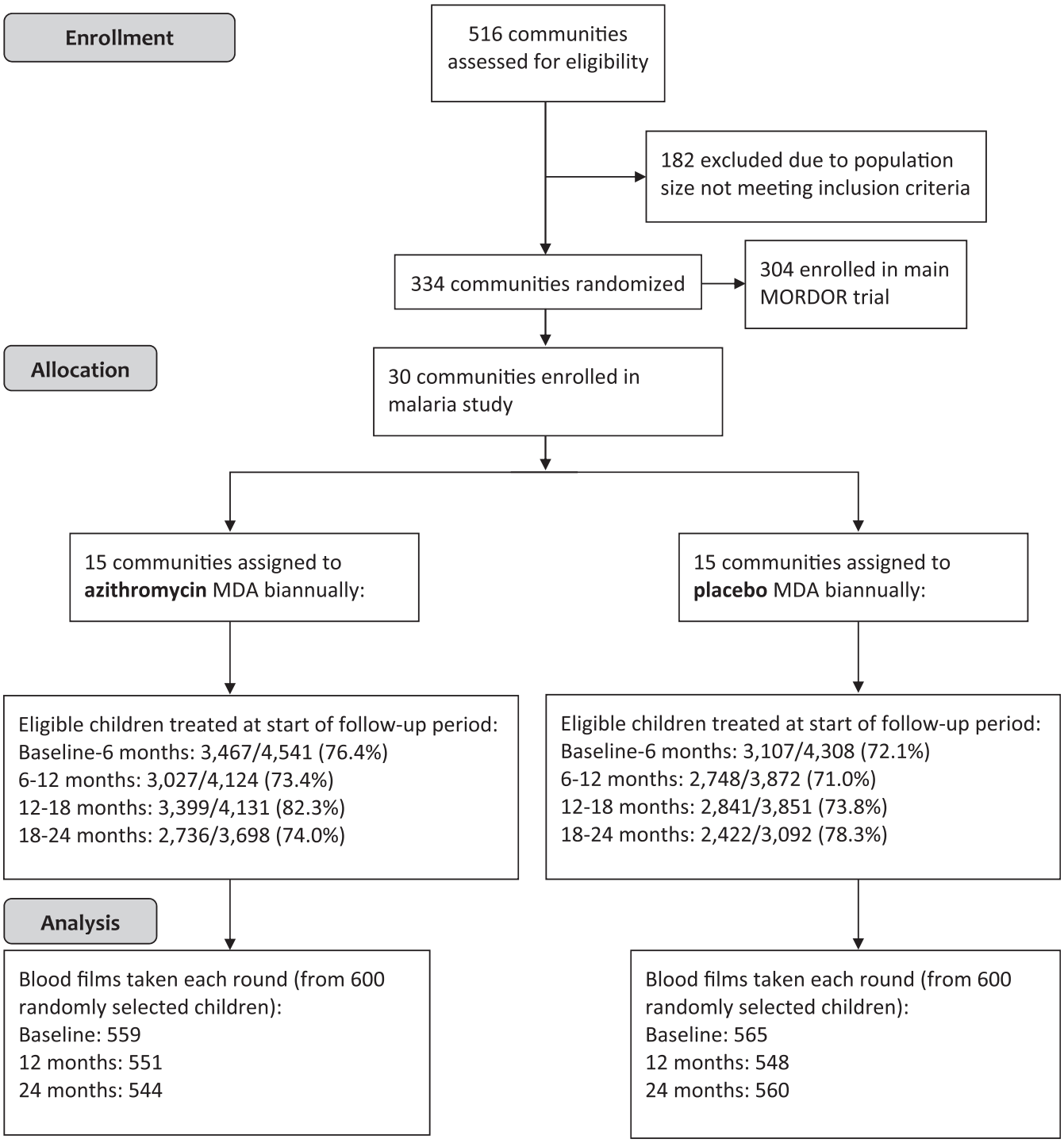

FIGURE 1. Trial flow. Communities were randomly selected from the same pool as the main MORDOR mortality study. Individuals could join the cohort at each of the biannual follow-up censuses.

Parasite density in parasitemic individuals was similar between treatment groups at the 12- and 24-month follow-up visits: 23 parasites/μL lower in individuals in azithromycin-treated communities after adjusting for age, baseline parasitemia, and follow-up phase (95% CI: –67 to 22 parasites/μL, $P = 0.32$; ICC = 0.02). Hemoglobin was also similar between treatment groups at 12- and 24-month follow-up visits: 0.08 g/dL lower in individuals in azithromycin-treated communities after adjusting for age, baseline community prevalence of anemia, and follow-up phase (95% CI: –0.36 to 0.19, $P = 0.56$; ICC = 0.05).

In the per-protocol analysis, the odds ratio for parasitemia in azithromycin- compared with placebo-treated individuals at 12- and 24-month visits after adjusting for age, baseline parasitemia, and follow-up phase was 0.71 (95% CI: 0.43 to 1.16, $P = 0.17$; ICC = 0.06). Parasite density in parasitemic individuals was similar between treatment groups at 12- and 24-month visits after adjusting for age, baseline parasitemia,

TABLE 2
Community level prevalence of malaria parasitemia, anemia (Hb < 11 g/dL), and gametocytemia in the 30 study communities by treatment arm (unadjusted)

	Mean prevalence of parasitemia (95% CI)		Mean prevalence of anemia (95% CI)		Mean prevalence of gametocytemia (95% CI)	
Study phase	Placebo	Azithromycin	Placebo	Azithromycin	Placebo	Azithromycin
Baseline	29.2% (18.8–39.6%)	31.8% (21.8–41.8%)	58.1% (50.8–65.4%)	57.2% (50.1–64.2%)	6.2% (3.4–9.0%)	8.0% (4.3–11.7%)
12 months	34.8% (25.9–43.8%)	37.3% (27.7–47.0%)	59.2% (52.5–65.9%)	56.4% (49.7–63.2%)	5.0% (1.8–8.0%)	4.7% (2.3–7.1%)
24 months	29.2% (21.6–36.9%)	27.8% (18.6–37.0%)	51.7% (41.9–61.4%)	50.2% (43.5–56.8%)	3.5% (1.6–5.4%)	3.3% (0.6–6.1%)

and follow-up phase: 19 parasites/μL lower in azithromycin-treated individuals (95% CI: −45 to 8, $P = 0.17$; ICC = 0.03). Hemoglobin levels were also similar between treatment groups at the 12- and 24-month follow-up visits after adjusting for age, baseline community prevalence of anemia, and follow-up phase: 0.03 g/dL lower in azithromycin-treated individuals (95% CI: −0.32 to 0.25, $P = 0.81$; ICC = 0.04), shown in Table 4. There were no significant differences in gametocyte prevalence or gametocyte density in gametocyte-positive individuals when analyzed by intention-to-treat or per-protocol, shown in Supplemental Tables 1 and 2.

In the individual-level per-protocol and intention-to-treat analyses, there was a positive association between the outcomes (parasitemia and hemoglobin) and both age and baseline community parasitemia or hemoglobin. The effect of treatment did not vary with age when an interaction term between the treatment arm and age was included in the models.

Hemoglobin levels were approximately 1 g/dL lower in parasitemic individuals at all study phases and in azithromycin and placebo groups, shown in Supplemental Table 3.

DISCUSSION

Several morbidity sub-studies were nested within the MORDOR trial to investigate mechanisms through which azithromycin MDA may reduce mortality. This research was not carried out in the MORDOR mortality study communities to reduce the risk of interventions impacting the primary MORDOR outcome of mortality. Morbidity study communities were randomly selected from the same pool as the main MORDOR trial, designed so that results would be representative of the mortality study. This malaria study, nested within MORDOR, did not identify a reduction in malaria parasitemia, gametocytemia, or hemoglobin in azithromycin-treated compared with placebo-treated communities. Secondary analyses at the individual level, by intention-to-treat and per-protocol, including only those who received study drug at the previous phase, approximately 6 months earlier, also did not identify significant differences in the prevalence of parasitemia or parasite density in parasitemic individuals.

Previous published reports of the effect of azithromycin MDA on malaria have suggested a reduction in malaria over 6-month duration.[4,5,7] The mechanism through which a reduction in parasitemia over this time period may occur is unclear. Azithromycin displays delayed activity against the malaria parasite, preventing the progeny of antibiotic-treated parasites from fully maturing, and has a long terminal half-life of approximately 68 hours. These are properties that could feasibly contribute to a period of antimalarial activity of days to weeks but not months.[8] The studies showing longer term effects were in Niger and the Gambia, in areas with lower background levels of parasitemia, which may be more amenable to reductions in transmission that could last longer than individual-level prophylaxis and treatment. This study assessed malaria gametocytemia as an effect of azithromycin on the sexual stages of the parasite which could explain the longer term community-level reductions in parasitemia as previously reported. Reductions in gametocytemia were not identified, which is consistent with previous evidence.[9]

In the context of the MORDOR trial showing a reduction in child mortality, this study does not provide evidence for an effect of azithromycin MDA on malaria mortality at the Malawi site.[1] The best estimates for the odds ratio for the presence of parasitemia in individuals in azithromycin communities compared with placebo ones were 0.89 by intention-to-treat and 0.71 per-protocol, and it is possible that this study of limited size with follow-up restricted to 6 months post-MDA represents type II error. However, while the main MORDOR trial suggested increased child survival benefit in those younger than 6 months, in this study, age and parasitemia were positively associated (i.e., lower parasitemia in infants), further suggesting that the child survival benefits in MORDOR were not best explained through effects on malaria. Further investigation of the effects of azithromycin MDA on malaria severity and mortality, as well as cause-specific mortality for all the major causes of child mortality, is required to improve our understanding of whether the reported mortality reductions may be due to specific anti-pathogen effects or to other mechanisms, such as the immunomodulatory and anti-inflammatory effects of azithromycin.[10,11]

The prevalence of malaria parasitemia in this study was approximately 30% in children aged 1–59 months. Malawi is hyperendemic for malaria with 95% of the population susceptible to infection, and large-scale surveys before and after this study reported malaria parasitemia prevalence of 33% and 24%, respectively.[12,13] Malaria was also the commonest inferred cause of death from verbal autopsy in the MORDOR trial in Malawi.[14] Parasitemic children had significantly lower mean hemoglobin levels at all follow-up rounds, consistent with known effects of malaria as a cause of anemia in children.[15–17] This analysis provides some validation of the integrity of the data. In addition, the ICCs were low for all analyses, suggesting considerable heterogeneity in malaria infection and hemoglobin levels by community, consistent with usual epidemiological patterns of malaria and similar to the MORDOR Niger site data.[7]

One limitation of this study is that treatment took place at the beginning and end of each dry season for logistical reasons. Mathematical modeling of the effect of azithromycin MDA on malaria in areas of highly seasonal transmission suggests there may be most benefit from treatment during the low-transmission season when treatment of established infection may produce more sustained benefit because of the lower risk of reinfection.[18] At the community level, this may also reduce

TABLE 3

Individual level parasitemia, parasite density in parasite-positive individuals, and hemoglobin analyzed by intention-to-treat (unadjusted)

	Prevalence of parasitemia				Parasite density (parasites/μL)				Hemoglobin (g/dL)			
	Placebo		Azithromycin		Placebo		Azithromycin		Placebo		Azithromycin	
Study phase	*N**	Mean (95% CI)	*N**	Mean (95% CI)	*N*	Mean (95% CI)	*N*	Mean (95% CI)	*N**	Mean (95% CI)	*N**	Mean (95% CI)
Baseline	565	28.5% (24.8–32.2%)	559	30.9% (27.1–34.8%)	161	352 (277–426)	173	368 (305–431)	564	10.5 (10.4–10.6)	559	10.6 (10.4–10.7)
12 months	548	34.5% (30.5–38.5%)	551	37.0% (33.0–41.1%)	189	195 (161–228)	204	172 (142–203)	547	10.5 (10.4–10.6)	549	10.5 (10.4–10.6)
24 months	560	29.3% (25.5–33.1%)	544	27.0% (23.3–30.8%)	164	214 (175–254)	147	200 (156–244)	558	10.8 (10.7–10.9)	544	10.8 (10.7–11.0)

* Different values between parasitemia and hemoglobin as a very small proportion of individuals did not have all tests.

TABLE 4

Individual level parasitemia, parasite density in parasite-positive individuals, and hemoglobin analyzed per-protocol, including only those who received treatment at the previous phase (unadjusted)

	Prevalence of parasitemia				Parasite density (parasites/μL)				Hemoglobin (g/dL)			
	Placebo		Azithromycin		Placebo		Azithromycin		Placebo		Azithromycin	
Study phase	*N**	Mean (95% CI)	*N**	Mean (95% CI)	*N*	Mean (95% CI)	*N*	Mean (95% CI)	*N**	Mean (95% CI)	*N**	Mean (95% CI)
12 months	391	36.3% (31.5–41.1%)	404	36.4% (31.7–41.1%)	142	191 (153–228)	147	180 (141–220)	390	10.5 (10.3–10.7)	402	10.5 (10.4–10.7)
24 months	385	31.4% (26.8–36.1%)	367	23.7% (19.3–28.1%)	121	220 (171–268)	87	182 (139–224)	385	10.8 (10.7–11.0)	367	10.9 (10.8–11.1)

* Different values between parasitemia and hemoglobin as a very small proportion of individuals did not have all tests.

the reservoir of infectious individuals at the beginning of the following high-transmission period. In Malawi, where despite some seasonality, malaria prevalence remains high year-round, the optimal time to treat, if indeed there is any benefit from the intervention, is unclear.

Monitoring for infection took place during the baseline, 12-month, and 24-month visits, early in the dry season, and approximately 6 months after the previous treatment round for the latter two visits. This seasonality and timing of sample collection may not provide a complete understanding of the effect of the biannual azithromycin MDA on malaria prevalence, which may vary with time lapsed after administration. Finally, while carefully designed to be representative of the MORDOR trial site in Malawi, the data may not be representative of other countries with different malaria prevalence and healthcare interventions, although some data are available from other MORDOR sites.[7] Any effect of azithromycin MDA on malaria may be impacted by other malaria interventions, such as seasonal malaria chemoprophylaxis (SMC), which was not taking place at the MORDOR-Malawi site. A recent study indicated no additional child mortality benefit when adding azithromycin MDA to SMC in Mali and Burkina Faso.[19]

In conclusion, a cluster-randomized placebo-controlled study of the effects of azithromycin MDA on malaria parasitemia was unable to detect a difference in malaria parasitemia at the community or individual levels, providing no evidence that any child survival benefit was mediated by effects on malaria mortality. Further investigation is required to understand the effect of azithromycin MDA on malaria morbidity and mortality and, indeed, to elucidate the mechanisms by which azithromycin MDA may reduce child mortality.

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Discussion

This study investigated heterogeneity in the effect of azithromycin on child mortality at the MORDOR-Malawi study site. Unadjusted effect estimates are presented, without adjustment for multiple testing, in the interests of displaying all available data. This is an established approach for post-hoc investigation of epidemiological datasets.[155] It is accepted that this approach cannot produce strong evidence for an effect but can facilitate hypothesis generation for further investigation.

The background information on child mortality interventions presented in Table 4.1 identifies a number of key interventions that would be expected to reduce child mortality in the study setting. In particular, recent expansion of the vaccination schedule to include PCV-13 and rotavirus, and insecticide treated bed net rollout, would be expected to significantly reduce mortality from causes that might be amenable to azithromycin MDA. It is likely, therefore, that the estimated effect of azithromycin at the time of the study may be lower than if the study were conducted prior to the introduction of the other interventions or, indeed, in settings without similar interventions.

The effect of the intervention on child mortality was greatest in Namwera. However, mapping mortality rate across Mangochi District in azithromycin and placebo communities identified considerable variation over smaller geographical areas than the administrative zones. No clear pattern was evident visually from the mapping of mortality difference between azithromycin and placebo clusters, perhaps suggesting that any true heterogeneity in effect of the intervention may be related to more granular details of specific variables than can be visualized at the district level.

The analysis of development indicators, measured only in the sample of morbidity communities, suggested Namwera scores relatively poorly in some, such as any adult education and presence of household wash stations. However, Namwera scored relatively better than other zones in some indicators, particularly those collected by the HSAs, including improved water source, access to improved latrine, and handwashing facilities. The proportion of households with handwashing facilities/wash stations differed considerably between the study estimate from a sample of six morbidity clusters, and the DHO data (close to four-fold difference in Namwera). This suggests that either the study data were not

representative of the zone or that the definition/interpretation of the indicator was different between study fieldworkers and HSAs as there was no standardisation between these personnel. The lack of a clear pattern at the zonal level that would explain the geographical heterogeneity of effect of the intervention necessitates more detailed analysis of the data to understand potential reasons for the mortality patterns and to hypothesise which populations may benefit most from the intervention.

Key findings from the individual level mortality analysis were the suggestion of a greater effect of the intervention in populations without outreach health services in their community; those living within 1km of a road; those in communities where <25% of households used ITNs; those living within 1km of a large water body; those in areas with NDVI <0.60 or temperature <24 degrees; those in communities where <50% of households had access to improved water sources; and those in communities where <5% of households had access to improved latrines. In addition, this study showed a greater effect of the intervention in infants identified to be underweight by weight-for-age z score.

For variables likely to contribute to a particular cause of death, analysis was also conducted of the effect of the intervention on cause-specific mortality. Interestingly, the effect estimate of the intervention on cause specific mortality by these variables was often not greater than the effect estimate on all-cause mortality, as would be expected if the difference in effect were due to that specific cause alone. For example, the point estimate of effect in communities with lower uptake of ITN usage was greater for all-cause mortality than for malaria mortality. Use of ITNs has been reported to reduce all-cause child mortality by significant amounts, for example a reduction in under-five all-cause mortality of 23%-50% in Malawi, and of 44% in 1-59 month old children in Kenya.[156,157] It is possible that several of the variables identified in this study as associated with an effect of the intervention may be associated indirectly as well as directly with the intervention. Many of the assessed variables are also indicators of socioeconomic status and some of the associations could relate to either poverty or cultural attitudes regarding modern medicine and healthcare.

Evidence for the greater effect in underweight children could be associated with a protective effect against bacterial infection in this particularly vulnerable group. Indeed, antibiotics are indicated as part of the management of acute malnutrition due to the high prevalence of concurrent infection and associated high mortality in these children.[158] The analysis of causes

of death using the InterVA algorithm (Chapter 3) suggested an effect of azithromycin on pneumonia and HIV/AIDS mortality; and using the SmartVA algorithm, an effect on pneumonia and diarrhoea. It is entirely feasibly that children with these conditions, particularly HIV/AIDS and diarrhoea (which may not be well differentiated by the algorithms), would be more likely to be underweight and to also benefit from azithromycin MDA.

Analysis of the VA algorithms reveals that in the child SmartVA algorithm, the VA item "becoming very thin during the final illness" only contributes "tariffs" to the diagnosis of diarrhoea, pneumonia and "other defined causes". "Other defined causes" includes malnutrition, which SmartVA does not diagnose separately due to poor ability to predict this cause from the gold standard dataset used to produce the algorithm. The VA item "becoming very thin during the final illness" does not contribute to the diagnosis of HIV/AIDS using the SmartVA algorithm, which may be related to the fact the gold standard dataset used to produce the algorithm only included 20 confirmed child HIV/AIDS cases.[159] The InterVA algorithm uses physician-derived conditional probabilities describing the typical likelihood of each indicator for deaths from each cause, and "noticeable weight loss" has a highly ranked conditional probability that most likely contributes to the diagnosis of HIV/AIDS in children. Given these factors in the design of the VA algorithms, an effect of the intervention in underweight children would contribute to pneumonia and diarrhoea predictions using the SmartVA algorithm, and HIV/AIDS using InterVA, i.e., the causes that the algorithms predicted the intervention had the greatest impact on.

One key indicator measured in the study communities was malaria parasitaemia – close to half the children were parasitaemic at baseline in Namwera zone, compared to one third or fewer in the other zones. The detailed analysis of malaria morbidity presented and discussed in the peer-reviewed publication that is included in this chapter did not provide strong evidence for an effect of the intervention on malaria infection.[160] The odds ratios for a sustained reduction in malaria parasitaemia over 6 months duration were 0.89 (95% CI: 0.53 to 1.50, $P = 0.67$) by intention-to-treat and 0.71 (95% CI: 0.43 to 1.16, $P = 0.17$) per-protocol. As previously suggested, the study is underpowered to identify effect sizes of this magnitude and these could represent type II error. Further, it is likely that in areas with higher malaria parasitaemia in children, pregnancy-associated malaria is also more common.[161] This often silent and therefore untreated condition is a major cause of low birth weight and associated

morbidity and mortality in infants. It is entirely feasible that azithromycin would have a greater impact on child mortality in higher prevalence malaria areas independent of any direct effect on malaria, by treating or preventing the common bacterial causes of child mortality, that particularly affect the underweight child.

This study investigated whether temperature and NDVI (vegetation cover) could be useful predictors of mortality and the effect of azithromycin MDA. Malaria transmission is well known to increase at higher temperatures and research has indicated the optimum temperature for malaria transmission may be 26-29 degrees Celsius.[162] Mechanisms behind the association with temperature may relate both to more active mosquitoes at higher temperatures, as well as a lower extrinsic incubation period, i.e. a more rapid progression of the malaria life cycle in the mosquito host.[163] Higher NDVI reflects greater rainfall and potential malaria vector breeding sites, and is generally associated with higher malaria transmission, although higher NDVI has also been associated with lower malaria risk, perhaps explained by very heavy rainfall leading to "flashing" of breeding sites, preventing mosquito larvae from maturing.[149] A study in Malawi, using NDVI as a proxy measurement of vegetation greenness and rainfall, identified an association with malaria prevalence only in Dedza region, with villages in Mangochi being far more homogeneous.[164] The research also identified an association between altitude (a proxy for temperature) and malaria in Dedza only. This may reflect difficulty identifying associations between some climate variables and malaria in a relatively homogeneous area and on a relatively fine scale.

In this study, data were dichotomised at NDVI of 0.6 reflecting the transition between high density green leafy plant cover above this value, compared to lower density vegetation or less healthy plants when NDVI <0.6.[165] The point estimates of effect reported in this study were of a greater effect of the intervention at both lower NDVI and lower temperatures. If these findings are supported in additional studies, this could be due to a greater effect of the intervention in relatively lower prevalence malaria settings, as discussed previously, where the treatment of established infections may produce more sustained benefit because of the lower risk of reinfection. Modelling predicts that the effect of azithromycin MDA on malaria may be greatest during the lower transmission period for this reason.[166]

One limitation of this work, as discussed in Chapter 3, is the sample size is not adequate to produce strong evidence for variation in the effect estimate by the additional variables

assessed. However, the aim of this work is hypothesis generation given the absence of any other studies being available on heterogeneity of effect of azithromycin MDA on all-cause mortality and specific causes of death.

Secondly, the geographical mapping of mortality by treatment arm is challenging to interpret compared to if it were possible to have measurements for both arms of the study at the same location. In addition, the colour representing the mortality rate (and rate difference) around a remote cluster will be displayed over a greater area of the map before it is influenced by the rate in neighbouring clusters, compared to around more densely clustered communities. Finally, the data cannot provide evidence as to whether a specific community is a "hotspot" or "coldspot" for the effect of azithromycin on mortality. Given the nature of the study, these challenges were expected, and the maps produced provide a visual overview of the effect of the intervention and how this varies considerably within all zones over small geographical areas, as may be expected given the relatively small population (and number of deaths) per cluster.

This study was not powered to provide strong evidence for associations between the indicators assessed and the effect of azithromycin MDA on child mortality. However, many of these analyses have not been conducted previously and the associations identified, particularly related to access to healthcare, malaria risk, WASH indicators and poverty, may prove useful to guide future research. Other analyses add weight to the results of other published studies, such as the apparent greater effect of the intervention in younger children; underweight children; and on pneumonia and diarrhoea or HIV/AIDS mortality. Given the evidence now available for an effect of azithromycin on child mortality, the results presented here may guide future research regarding factors associated with heterogeneity of effect of the intervention that will be useful for countries planning to implement the intervention.

Chapter 5: Cost-effectiveness

Introduction

A key consideration for azithromycin MDA to be implemented more widely is whether the intervention proves cost-effective compared to other child health interventions that could produce similar reductions in child mortality. A potential cost-reducing benefit of MDA programs is that no immediate assessment or diagnosis is required prior to drug delivery, although assessments of disease prevalence may be required. This benefit must be weighed against the prevalence of the condition being targeted and the benefit produced by the drug – as prevalence decreases, it will become relatively more cost-effective to spend resources identifying cases with disease or restricted populations at risk. In the case of azithromycin MDA for reducing child mortality, it is feasible that in areas with a higher burden of child mortality, particularly higher infectious mortality, that the intervention could have the greatest effect and hence greatest cost-effectiveness. Factors related to the mechanism of effect of azithromycin MDA on child mortality, for example the potential for treatment of subclinical infections and reducing community-level pathogen carriage (i.e. producing a herd protective effect), may greatly influence the effectiveness of the intervention and mean that targeting individuals at risk could considerably decrease the overall benefit. The investigation of cost-effectiveness of the intervention in this study has been published in a peer-reviewed journal and is included below.

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Primary Supervisor	Robin Bailey		

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Cost-Effectiveness of Mass Treatment with Azithromycin for Reducing Child Mortality in Malawi: Secondary Analysis from the MORDOR Trial

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Abstract. The recent Macrolides Oraux pour Réduire les Décès avec un Oeil sur la Résistance (MORDOR) trial reported a reduction in child mortality following biannual azithromycin mass drug administration (MDA). Here, we investigate the financial costs and cost-effectiveness from the health provider perspective of azithromycin MDA at the MORDOR-Malawi study site. During MORDOR, a cluster-randomized trial involving biannual azithromycin MDA or placebo to children aged 1–59 months, fieldwork-related costs were collected, including personnel, transport, consumables, overheads, training, and supervision. Mortality rates in azithromycin- and placebo-treated clusters were calculated overall and for the five health zones of Mangochi district. These were used to estimate the number needed to treat to avert one death and the costs per death and disability-adjusted life year (DALY) averted. The cost per dose of MDA was $0.74 overall, varying between $0.63 and $0.94 in the five zones. Overall, the number needed to treat to avert one death was 1,213 children; the cost per death averted was $898.47, and the cost per DALY averted was $9.98. In the three zones where mortality was lower in azithromycin-treated clusters, the number needed to treat to avert one death, cost per death averted, and cost per DALY averted, respectively, were as follows: 3,070, $2,899.24, and $32.31 in Monkey Bay zone; 1,530, $1,214.42, and $13.49 in Chilipa zone; and 344, $217.98, and $2.42 in Namwera zone. This study is a preliminary cost-effectiveness analysis that indicates azithromycin MDA for reducing child mortality has the potential to be highly cost-effective in some settings in Malawi, but the reasons for geographical variation in effectiveness require further investigation.

INTRODUCTION

Azithromycin mass drug administration (MDA) is a key part of the global campaign to eliminate blinding trachoma.[1] Research alongside trachoma programs has indicated a beneficial effect of azithromycin MDA on child morbidity indicators,[2–6] and increasing evidence suggests this may include a reduction in child mortality.[7,8]

Despite accelerating reductions in child mortality over the past two decades, progress is not uniform, with more than one in 10 children still dying before their fifth birthday in six countries, five of them in sub-Saharan Africa.[9] At current annual rates of reduction in child mortality, many countries will not meet the Sustainable Development Goals for reduction in child mortality by 2030, with the 2015 Millennium Development Goal for child mortality not likely to be met globally before 2026.[9] Socioeconomic progress and improvements in health systems are key to long-term progress in reducing child mortality. However, in the short term, practical, cost-effective interventions to reduce child mortality are of particular interest.

The MORDOR trial, investigating the effect of biannual azithromycin MDA to children aged 1–59 months on child mortality in Malawi, Niger, and Tanzania, reported 14% lower mortality in azithromycin- compared to placebo-treated communities.[8] The effect was not uniform, with mortality reduction of 6% in Malawi, 18% in Niger, and 3% in Tanzania. In addition, the effect differed significantly by age-group. Such heterogeneity raises important questions about who should be targeted and where, if wider country campaigns of azithromycin MDA to reduce child mortality are to be considered. Cost-effectiveness will play a key role in such policy decisions. As cost data were not collected uniformly at the three MORDOR study sites, this study reports child mortality in different geographical areas of the MORDOR trial in Malawi and the associated financial costs and cost-effectiveness of the intervention from the health provider perspective.

METHODS

MORDOR trial. The MORDOR trial in Malawi was conducted in Mangochi district, one of the poorer districts in Malawi, with associated high birth rates, low literacy, and little formal employment.[10] Mangochi district is shown on the map (Figure 2). The MORDOR trial methodology has been reported elsewhere.[8] Briefly, MORDOR used a cluster-randomized, placebo-controlled trial design to assess the effects of biannual single-dose azithromycin MDA on mortality in children aged 1–59 months.

Clusters were defined as the catchment areas of health surveillance assistants (HSAs), which have approximately a total population of 1,000. The study included house-to-house visits for a total of four treatment rounds and five census visits between March 2015 and June 2017. The study cohort was updated biannually, as each census identified deaths as well as new births and migrations into or out of the study area. The MORDOR trial included 334 clusters that were randomly assigned to either the main mortality study (304 clusters) or for the assessment of morbidity outcomes (30 clusters). All clusters met the same inclusion criteria and were identified from a pre-baseline census. The cost-effectiveness analyses include all 334 clusters. Mangochi district is divided into five zones for health administration purposes. MORDOR fieldwork and the collection of cost data were organized by the five distinct geographical zones (Mangochi, Namwera, Chilipa, Monkey Bay, and Makanjira).

Intervention. Treatment was administered as 20 mg/kg azithromycin syrup or an equivalent volume of placebo. Young children were weighed to determine dose, and those old enough to stand were measured and given an approximate

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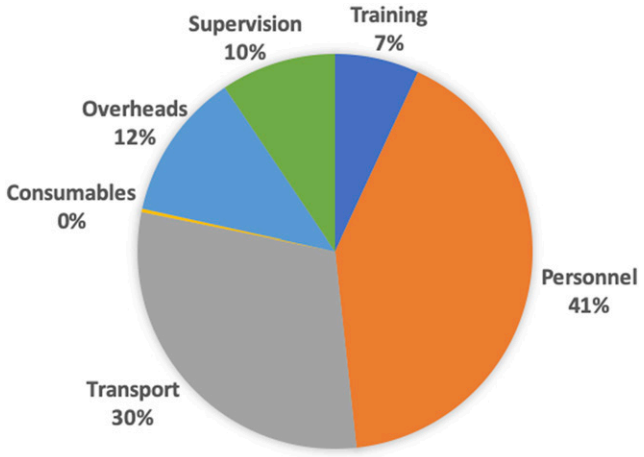

FIGURE 1. Breakdown of mass drug administration costs by broad category. This figure appears in color at www.ajtmh.org.

dose based on their height. Mass drug administration was performed by HSAs in their own communities with assistance from village volunteers and MORDOR field-workers, who also recorded the data using tablet devices. Adverse event reporting was encouraged for events occurring within 7 days of the MDA via the HSA to the study team.

Randomization and blinding. The study drug manufacturer (Pfizer Inc., New York, NY) labeled medicine bottles with eight letters corresponding to azithromycin and eight to placebo to reduce risks of unblinding. Study clusters were randomly assigned to a drug letter by the study statistician using the statistical package R (the R Foundation for Statistical Computing, Vienna, Austria). All field and laboratory staff, supervisors, data managers, and participants were blind to the treatment code until after all fieldwork was complete.

Outcomes. The primary prespecified outcome for this study was the cost-effectiveness of azithromycin MDA at the MORDOR-Malawi study site. The secondary outcome was a comparison of the differences in cost-effectiveness of azithromycin MDA by health zone in Mangochi district. The study was designed to assess cost-effectiveness from the perspective of service providers and not from a societal perspective.

Cost data collection. Cost data were collected at the 12-month follow-up visit and included personnel, transport, consumables, overheads, training, and supervision. Details of sources of cost data are provided in Table 1. Efforts were made to exclude costs related solely to research, such as the electronic capture of census and treatment data. All daily fieldwork costs were recorded and cross-checked with budgets and funds released by the MORDOR trial finance officer in coordination with the District Environmental Health Officer. Costs were collected in Malawian Kwacha (MWK) and converted to U.S. dollars ($) at the exchange rate of MWK700 = $1.00, the commonly used exchange rate in Malawi in 2016, where U.S. dollars are often used for major purchases. The value of the Malawian kwacha fluctuates significantly on the international market, and the exact exchange rate fluctuated above and below the value used for this study during the 12-month follow-up visit for MORDOR fieldwork between March and June 2016. All costs presented in this study are in 2016 USD. There were no missing cost data as costs were proactively captured during the fieldwork from budgets and receipts.

Personnel costs included per diems for HSAs, village volunteers, drivers, MORDOR field-workers, and supervisors. Salary costs were not included for the main analysis, although HSA salaries were included in a subsequent sensitivity analysis. Training costs were included for HSAs to conduct the MDA; separate training costs for field-workers specifically on the use of tablet devices were excluded. Morbidity assessment costs for field-workers collecting samples and laboratory staff working on research activities were excluded.

Vehicle depreciation was calculated daily for two MORDOR vehicles by dividing the capital cost of the new vehicles by their 10-year life expectancy multiplied by 345, an assumed number of working days per year. Fuel costs were taken from the fuel receipts used specifically for the fieldwork in each zone. Additional transport costs, including bicycle and boat taxis, were included as required.

Azithromycin and placebo suspension were donated by Pfizer, so no costs were included for study drug in the main analysis, although the value of azithromycin was included in a sensitivity analysis. Overheads included rental costs for office and storage space in two locations and associated security, utilities, and upkeep costs. Supervision costs were mainly related to the MORDOR trial supervisors; zonal environmental health officer's (EHO's) costs were included to attend trainings.

Cost-effectiveness calculations. Total costs were summed for each of the five zones of Mangochi district. The cost per treatment in each zone and overall was calculated using the total number of treatments distributed at the 12-month follow-up visit. Mortality rates per 1,000 person-years were calculated for azithromycin- and placebo-treated clusters using the total deaths and person-years of follow-up over the full 2 years and four intercensal periods of the MORDOR trial. The number needed to treat to avert one death was calculated from the rate difference between azithromycin and placebo clusters. The cost per death averted was then calculated by zone and overall using the number needed to treat and cost per treatment. Cost per disability-adjusted life year (DALY) averted was also calculated from years of life lost in the study using the mean age of mortality of children in the study area and WHO standard life tables with no age weighting or time discounting.[11,12]

A lifetime time horizon was used for this study to capture all effects associated with a round of azithromycin MDA, as opposed to assuming, in effect, that for lives saved during the intervention, those individuals would die instantly at the end of any shorter follow-up period. The evidence available to date regarding the effect of azithromycin MDA on child mortality does not indicate any reduction in effect with subsequent rounds of treatment. Indeed, the aggregate efficacy of azithromycin as compared with placebo tended to increase with each progressive round of treatment in the MORDOR trial.[8] In addition, most of the protective effect of azithromycin MDA occurred in the 3 months after distribution, so this study assumed that each biannual MDA has an equal and independent effect on mortality.[13]

Cost-effectiveness of the intervention was compared with the WHO willingness-to-pay thresholds, specifically the estimate that an intervention costing less than three times the national annual GDP per capita per DALY avoided, may be

TABLE 1
Sources of cost data

Costs	How data were collected/costs estimated*
Training	
Environmental health officers' (EHOs) phone credit, fuel, and allowance	Distributed to EHOs to attend training and make fieldwork supervisory visits (between $34.29 and $142.86 for each of the five zones depending on size, accessibility, and number of EHOs)
Health surveillance assistants' (HSAs) allowance	As provided to each HSA ($17.86 each)
HSAs' transport	Provided to HSAs traveling long distances to attend training ($2.86 per HSA)
Refreshments	Actual expenditure for training (between $34.29 and $100.00)
Hall hire (health center or teacher training hall)	Actual expenditure (between $8.57 and $10.00 per day)
Personnel	
HSAs' per diem	Paid as agreed with the district ddministration ($3.57 per day for 5 days for a total of $17.86 per HSA); for the sensitivity analysis, each HSA salary was included for the allocated 1 week of work ($14.29 per HSA)
Volunteers' per diem	Paid as agreed with the district ddministration ($2.14 per day for 5 days for a total of $10.71 per volunteer in each community)
MORDOR field-workers' pay/allowances	Local enumerators staying at home paid $2.86 per day for 5 days for a total of $14.29 per community. Enumerators staying away from home paid $5.00 per day including per diem.
Field-worker accommodation	Actual expenditure for field-workers not able to return home at night (rooms costing between $2.86 and $6.43 per night)
Transport	
Vehicle depreciation	2 Toyota LandCruiser vehicles costing $60,000 each, with life expectancy of 10 years, assuming 345 working days per year ($17.39 per day per vehicle)
Fuel	Actual expenditure for fieldwork (between $1,721.56 and $2,752.24 per zone)
Boat fare	Actual expenditure for fieldwork (between $0.00 and $28.57 per zone, required to get to some remote communities without road access)
Bicycle fare	Actual expenditure for fieldwork ($1.43 per day for 3 days for one HSA to reach remote community)
Consumables	
Study drug	No cost for donated drug; for the sensitivity analysis, cost of azithromycin was taken from the WHO International Product Price Guide for 2015 ($0.94 per 30 mL bottle of syrup, with three doses per bottle, as allocated for fieldwork)
Waste bags	Actual expenditure for fieldwork ($28.57 per zone)
Overheads	
Office and storage space rent	Rental cost for two offices and storage space during the fieldwork ($1,128.57/month)
Office security and upkeep	Security and upkeep costs for rented premises during the fieldwork ($102.86/month)
Office utilities	Utilities for rented premises during the fieldwork ($205.71/month)
Supervision	
MORDOR staff per diems, accommodation, and lunch allowances	2–3 staff plus drivers ($14.29 per diem when staying away from Mangochi town; rooms costing between $4.29 and $8.57 per night; and lunch allowance of $3.57 provided every day)

* All costs were measured during the 12-month follow-up round of MORDOR activities.

considered cost-effective, whereas one costing less than the national annual GDP per capita may be considered highly cost-effective.[14] The WHO willingness-to-pay criteria applied to Malawi in 2016, with GDP per capita of $316, indicate that a cost-effective intervention would cost less than $948 per DALY averted and a highly cost-effective intervention would cost less than $316 per DALY averted.[15]

Sensitivity analyses were conducted: firstly, adding the value of azithromycin using the median price estimated for the buyer using the WHO International Product Price Guide and, secondly, adding both the value of azithromycin and the salaried time of HSAs.[16]

Ethical approval. Approval for the MORDOR trial was obtained from the College of Medicine, University of Malawi; the London School of Hygiene and Tropical Medicine; and the UCSF Committee on Human Research. Oral informed consent was obtained from the guardians of participants. There were no incentives for participation.

RESULTS

The baseline characteristics of clusters and study participants were similar between placebo and azithromycin arms at baseline, as shown in Table 2. The costs for activities related to the MDA varied by zone and overall and are listed in Table 3. The least costly zone for MDA was Namwera, costing $0.63 per dose distributed, slightly cheaper than Mangochi zone at $0.66. The most expensive zone was Monkey Bay, costing $0.94 per dose distributed. The mean cost per dose administered overall zones was $0.74.

A breakdown of costs by broad category is shown in Figure 1. The highest proportion of costs (41%) was for personnel associated with conducting the MDA, followed by transport (30%), overheads (12%), supervision (10%), and training (7%).

TABLE 2
Baseline characteristics of study clusters and participants

	Placebo	Azithromycin
Number of clusters	167	167
Number of children enrolled	42,825	43,105
Number of children per cluster	286 (SD: 133)	285 (SD: 127)
Gender, male (%)	50.0	50.0
Age-group (%)		
1–11 months	18.7	18.6
12–59 months	81.3	81.4

TABLE 3
Itemized costs for MDA by zone

	Cost (USD)					
	Monkey bay	Chilipa	Makanjira	Namwera	Mangochi	Total
Training						
Environmental health officers (phone credit, fuel, and allowance)	34.29	34.29	114.29	85.71	142.86	411.43
Health surveillance assistants' (HSAs) allowance	263.57	771.43	263.57	457.14	708.57	2,464.28
HSAs' transport	57.14	42.86	28.57	57.14	42.86	228.57
Refreshments	34.29	54.29	43.57	67.14	100.00	299.29
Hall hire (health center or teacher training hall)	8.57	17.14	17.14	17.14	20.00	80.00
Personnel						
HSAs' per diem	732.14	964.29	732.14	1,535.71	2,214.29	6,178.57
Volunteers' per diem	439.29	578.57	439.29	921.43	1,328.57	3,707.14
MORDOR field-workers' pay plus allowances	1,121.43	1,191.43	1,178.57	1,755.71	1,645.71	6,892.86
Field-worker accommodation	650.00	1,041.43	640.71	900.00	855.00	4,087.14
Transport						
Vehicle depreciation	660.87	660.87	660.87	904.35	904.35	3,791.33
Fuel	2,421.87	2,305.27	2,043.78	1,721.56	2,752.24	11,244.73
Boat fare	22.86	0.00	28.57	0.00	0.00	51.43
Bicycle fare	4.29	0.00	0.00	0.00	0.00	4.29
Consumables						
Study drug	0.00	0.00	0.00	0.00	0.00	0.00
Waste bags	28.57	28.57	28.57	28.57	28.57	142.86
Overheads						
Office and storage space rent	846.43	846.43	846.43	1,128.57	1,128.57	4,796.43
Office security and upkeep	77.14	77.14	77.14	102.86	102.86	437.14
Office utilities	154.29	154.29	154.29	205.71	205.71	874.29
Supervision						
MORDOR staff per diems, accommodation, and lunch allowances	1,228.57	257.14	930.00	1,796.43	542.86	4,755.00
Total cost	8,785.60	9,025.43	8,227.51	11,685.20	12,723.02	50,446.77
Number of children treated at the 12-month follow-up	9,304	11,369	9,794	18,451	19,197	68,115
Cost per treatment	0.94	0.79	0.84	0.63	0.66	0.74

The mortality rates by zone for placebo- and azithromycin-treated clusters and calculation of the rate difference between treatment arms are shown in Table 4. Study clusters are plotted in Figure 2, indicating the mortality rate difference in azithromycin and placebo arms by zone.

Overall, the number needed to treat with azithromycin MDA to avert one death was 1,213 children, the cost per death averted was $898.47, and the cost per DALY averted was $9.98 (Table 4). The cost per DALY averted would indicate this intervention has the potential to be highly

TABLE 4
Mortality rate by treatment arm and cost-effectiveness by zone

Zone	Monkey Bay	Chilipa	Makanjira	Namwera	Mangochi	Total
Cost/treatment (USD)	0.94	0.79	0.84	0.63	0.66	0.74
Person-years in placebo clusters (thousands)	7.58	12.28	10.08	18.05	18.94	66.93
Deaths in placebo clusters	53	134	89	191	153	620
Mortality rate in placebo clusters (deaths per 1,000 person-years, 95% CI)	6.99 (5.34–9.15)	10.91 (9.21–12.93)	8.83 (7.18–10.87)	10.58 (9.18–12.19)	8.08 (6.89–9.46)	9.26 (8.56–10.02)
Person-years in azithro clusters (thousands)	10.96	11.60	9.33	17.59	17.36	66.84
Deaths in azithro clusters	73	119	87	135	150	564
Mortality rate in azithro clusters (deaths per 1,000 person-years, 95% CI)	6.66 (5.30–8.38)	10.26 (8.57–12.28)	9.32 (7.56–11.50)	7.67 (6.48–9.08)	8.64 (7.36–10.14)	8.44 (7.77–9.16)
Rate difference (deaths per 1,000 person-years)	0.33	0.65	−0.50	2.91	−0.57	0.82
Number needed to treat to avert one death	3,070	1,530	–	344	–	1,213
Cost per death averted (USD)	2,899.24	1,214.42	–	217.98	–	898.47
Cost per DALY averted (USD)	32.21	13.49		2.42		9.98

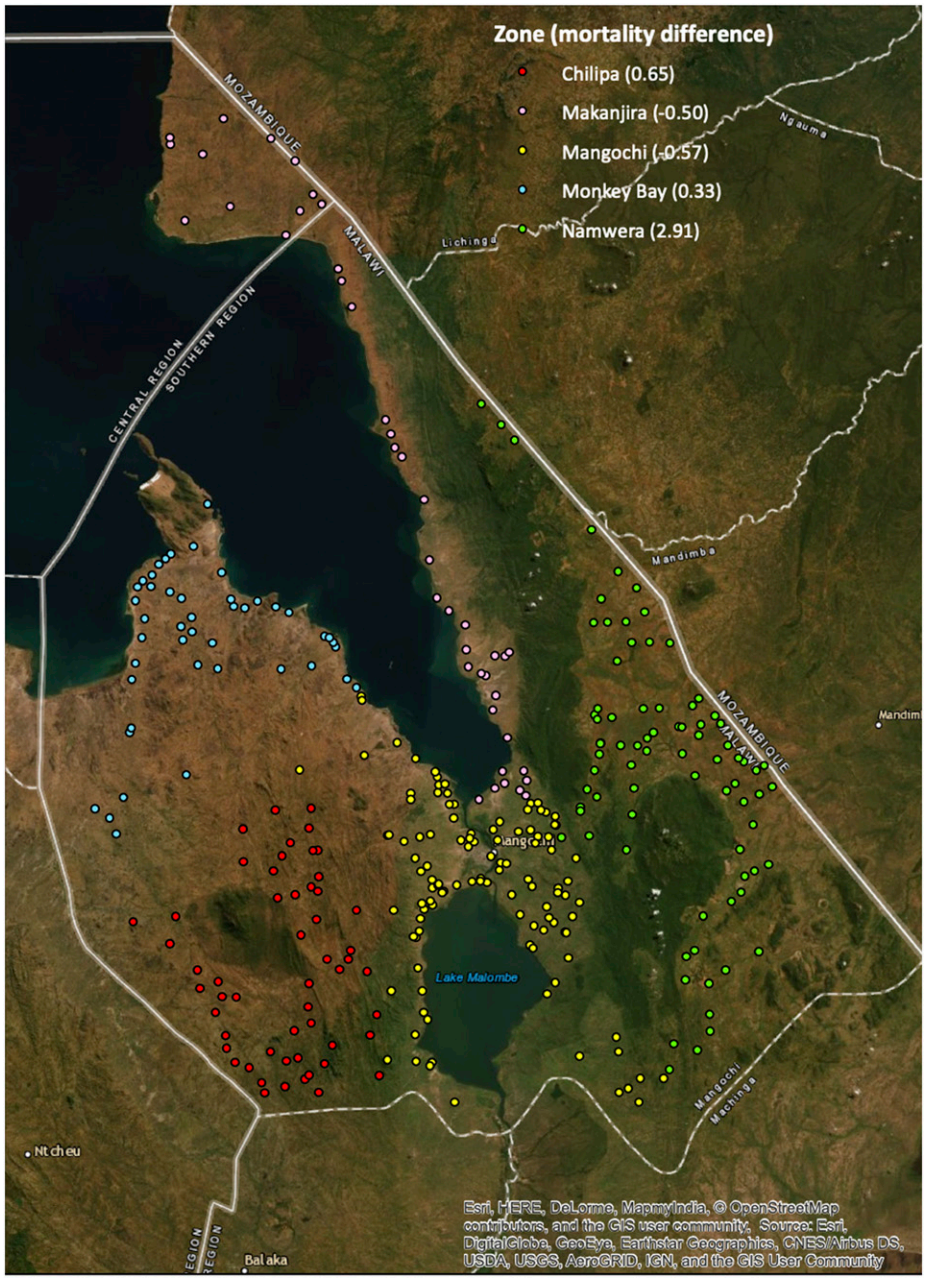

FIGURE 2. Mortality rate difference by zone between placebo- and azithromycin-treated clusters (deaths per 1,000 person-years); negative rate difference indicates higher mortality in azithromycin- than placebo-treated clusters.

cost-effective according to the WHO willingness-to-pay criteria (less than the GDP per capita of $316). The greatest effect was in the Namwera zone, with the number needed to treat as 344, cost per death averted of $217.98, and cost per DALY averted of $2.42. In two zones, Makanjira and Mangochi, the mortality rate estimate was higher in azithromycin- than placebo-treated clusters, so a figure for cost per death averted could not be computed for these zones.

Sensitivity analyses are shown in Table 5. With the addition of the cost of azithromycin, the cost per death averted overall increased to $1,278.59 and the cost per DALY averted to $14.21. With the cost of HSA salaries for the duration of the work also included, the cost per death averted further increased to $1,366.63 and the cost per DALY averted to $15.18.

DISCUSSION

This study estimated the cost-effectiveness of azithromycin MDA in the MORDOR trial in Malawi and compared cost-effectiveness by geographical zone in the intervention district. The cost per treatment distributed overall was $0.74, and the cost per death averted was $898.47. The cost per treatment delivered varied by up to 50% between zones. Cost-effectiveness varied considerably by zone; indeed, in two zones, the mortality rate was higher in azithromycin-treated than placebo-treated clusters. In the zones where mortality

TABLE 5
Sensitivity analyses for cost-effectiveness including cost of azithromycin and HSAs' salaries

Zone	Monkey Bay	Chilipa	Makanjira	Namwera	Mangochi	Total
Including the cost of azithromycin						
Cost/treatment (USD)	1.26	1.11	1.15	0.95	0.98	1.05
Cost per death averted (USD)	3,861.27	1,693.74	–	325.82	–	1,278.59
Cost per DALY averted (USD)	42.90	18.82	–	3.62	–	14.21
Including the cost of azithromycin and HSAs' salaries						
Cost/treatment (USD)	1.32	1.18	1.21	1.01	1.07	1.13
Cost per death averted (USD)	4,054.55	1797.54	–	348.74	–	1,366.63
Cost per DALY averted (USD)	45.05	19.97	–	3.87	–	15.18

HSA = health surveillance assistant.

was lower in azithromycin-treated clusters, cost per death averted varied from $217.98 to $2,899.24.

The MORDOR trial was not powered to identify a mortality difference at any single site, but using the effect estimate of mortality and related cost-effectiveness, under $1,000 per death averted is comparable to recommended interventions for reducing child mortality, such as integrated management of childhood illness[17] and seasonal malaria chemoprophylaxis.[18]

Azithromycin MDA has mostly been conducted by country programs for the control of trachoma, with the estimated costs varying considerably, from $0.50 or less per person treated[19–21] to $1.50 or more.[22,23] Other MDA programs in sub-Saharan Africa report generally similar costs: a systematic review of lymphatic filariasis MDA programs between 2000 and 2014 reported mean financial cost per treatment of $0.46 (adjusted to 2014 USD), mean economic cost excluding donated drug of $0.56, and economic cost including donated drug value of $1.32.[24] Variation in MDA costs by country will be influenced considerably by the costs for staff involved, such as the use of volunteers or health workers. The costs for treatment of children only, as in MORDOR, as opposed to whole community MDA, would be expected to increase as more time will be required to identify eligible individuals. In addition, as fewer treatments are distributed when targeting children only, economies of scale are less, which may also increase the cost per treatment.

The largest component of the cost of the MDA was personnel costs, followed by transport. Personnel costs have been reported as the main driver of azithromycin MDA costs in other studies.[21,22] One of these studies also found transport to contribute the second highest costs[22], but the other study, in relatively accessible areas of the Gambia, found transport to not contribute a major share of costs.[21] It is expected that these costs will vary considerably by geographical location and country.

The two main components of personnel costs were HSAs' and MORDOR field-workers' per diems. Health surveillance assistants collect a monthly salary, the costs of which were included in a sensitivity analysis, and receive per diems for any additional activities required of them beyond their few days rostered work at a nearby health center; this includes government campaigns, such as azithromycin MDA for trachoma, and interventions with other development partners. The per diems vary considerably; those recorded for this study were slightly higher than those paid by the government and significantly lower than those paid by other organizations.

MORDOR field-worker costs were included because of the key role these staff played in the context of the MORDOR trial supporting the HSAs with the MDA, although they were also tasked to update the census and record all treatments distributed. These exact costs would not be required for a government MDA program, although additional support with logistics would be needed from district and national health staff. The time spent during the MDA to complete census is also likely to have slowed the MDA fieldwork and increased costs. The level of supervision included in this analysis was believed to be similar to that which would be performed by EHOs for a government program; the MORDOR trial supervisors' per diems and other support costs similar to the allowance and transport costs required for supervision during a government campaign.

Training costs accounted for 7% of the total MDA costs. These may be reduced slightly with repeated program distributions although some refresher training would usually be recommended. Capital costs for setting up the program were converted to ongoing running costs, for example, by including a daily depreciation cost for vehicles rather than front-loading the initial capital cost. This was performed to provide a simple estimate of the financial costs for a program to provide the intervention.

Interpretation of the findings of this study, providing the first estimates of cost-effectiveness from the MORDOR trial, must consider that it was designed to assess costs from the perspective of the health provider. Costs of donated drug were excluded, although a sensitivity analysis was performed, which indicated a potential increase in cost per treatment of 42% when the value of azithromycin was included and a 53% increase when both azithromycin and HSA salaries were included. Additional economic indicators associated with an analysis from the societal perspective, such as opportunity costs, have not been considered in this study.

The analysis in this study will be relevant to Malawi with its current level of health system and health programs in place, termed intervention mix constrained cost-effectiveness analysis.[25] This evaluates the cost-effectiveness of additional interventions (in this case azithromycin MDA) with respect to the existing set of interventions, including interventions improving child mortality that may or may not have overlapping benefits with azithromycin MDA. Indeed, many health interventions are likely to interact with azithromycin MDA in terms of effect on child health, and therefore, in areas with improved health access and interventions, azithromycin is likely to provide lesser additional benefit.

To attempt to isolate the cost-effectiveness independent of other health interventions in a study area, termed generalized cost-effectiveness analysis, requires the analyst to consider the future effects if all health sector resources could be reallocated[25] and is beyond the scope of this study.

Generalizability of the effects of azithromycin MDA in Malawi as an intervention for child health to other settings may be possible to some extent depending on local child mortality rate, child health interventions, and the as-yet undefined mechanisms by which azithromycin MDA may reduce child mortality.

Future evaluations regarding the cost-effectiveness of azithromycin MDA for reducing child mortality should aim to estimate the cost-effectiveness when using the full economic cost. Further understanding of the mechanism of action is required, and subsequent analyses should assess the costs associated with targeting the intervention in settings where it will be effective. A further consideration is the potential for the development of macrolide resistance and lower effectiveness with continued biannual MDA as a possible consequence. Evidence from the trachoma field indicates that although resistance (in nontarget organisms) develops following azithromycin MDA, this usually decreases to relatively low levels 6 months posttreatment.[26,27] The levels of resistance do vary considerably by country, however, and in Ethiopia, macrolide resistance in carried *Streptococcus pneumoniae* has been reported at 20–30% 12–24 months post-cessation of MDA.[28]

Evidence from the MORDOR trial in Niger indicates macrolide resistance in nasopharyngeal *S. pneumoniae* isolates 6 months after the fourth biannual MDA was higher in azithromycin-treated communities than placebo communities, although still at the relatively low community mean level of 12% macrolide resistance.[29] Despite these low levels of resistance, the effect size appeared to increase over the four intercensal periods of the MORDOR trial, and indeed, with continued treatment for a third year of biannual MDA in the MORDOR II trial in Niger, effectiveness remained similar.[30] This study assessed the cost-effectiveness per round of MDA over the first 2 years of the intervention in Malawi, although there is no evidence that the effect may decrease with subsequent distributions in this setting.

In conclusion, these initial findings from the MORDOR trial indicate that azithromycin MDA for reducing child mortality has the potential to be a highly cost-effective intervention in the Malawian setting, but that there is considerable variation by geographical location. A greater understanding of the reasons for geographical variation in effectiveness would be desirable before wider implementation of the intervention.

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Discussion

The analysis of cost-effectiveness of azithromycin at the MORDOR-Malawi site indicated that the intervention has the potential to be highly cost-effective. An important aspect of this finding is the relatively low cost of azithromycin, and the relatively low cost of delivery through an MDA program. Indeed, if there were any measurable effect of such an intervention on child mortality, it would likely be considered cost-effective by the WHO willingness-to-pay criteria, based on GDP.[167] Combining azithromycin MDA with other NTD drugs or vaccination programs could further reduce MDA costs. Understanding the total direct and indirect costs of the MDA may be important for countries to consider prior to wider implementation. An economic analysis of the intervention on a larger scale than the MORDOR-Malawi study site could be particularly useful for countries considering this approach for reducing child mortality.

Chapter 6: Macrolide resistance

Introduction

A further important consideration regarding implementation of azithromycin MDA for reducing child mortality is the development of antibiotic resistance – and what the consequences of that resistance in the setting of the intervention may be. Macrolide resistance is known to occur following azithromycin MDA for trachoma, although the level of resistance and duration for which it is sustained vary considerably.[85,120] The implications of macrolide resistance for both the efficacy of the intervention and broader clinical impacts, must be considered. The analysis of antibiotic resistance in this study is under review for publication in a peer-reviewed journal and is included in this chapter, below.

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Primary Supervisor	Robin Bailey		

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I coordinated data collection, conducted all data analysis and wrote the manuscript

SECTION E

Student Signature	John Hart
Date	14/06/2021

Supervisor Signature	Robin Bailey
Date	14/06/2021

Title: Prevalence of nasopharyngeal *Streptococcus pneumoniae* carriage and resistance to macrolides in the setting of azithromycin mass drug administration: analysis from a cluster randomized controlled trial in Malawi, 2015-2017

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Abstract

Background

Recent evidence suggests azithromycin mass drug administration (MDA) may reduce child mortality. However, macrolide resistance has generally been reported to develop following whole community MDA for trachoma control but has less commonly been studied in the context of treating children to reduce mortality. Here, we report on macrolide resistance following biannual azithromycin MDA in the MORDOR Study in Malawi.

Methods

In the MORDOR cluster randomised trial in Malawi, children aged 1-59 months were treated with azithromycin 20 mg/kg or placebo between 2015 and 2017. One thousand two hundred children were randomly selected for nasopharyngeal swabs at baseline, 12-months and 24-months, six months after the second and fourth MDA visits, respectively. Samples were processed to culture *S. pneumoniae* and identify resistance to macrolides (the main outcome) and penicillin. Resistance was compared between treatment arms.

Findings

At baseline, carriage of *S. pneumoniae* was >85% in both arms of the study and the proportion of strains resistant to macrolides was 28%. At the 12-month follow-up, macrolide resistance was higher in the azithromycin group compared to placebo: 36.9% (95% CI: 32.5%-41.2%) versus 21.6% (95% CI: 17.7%-25.4%); OR=2.26 (95% CI: 1.46-3.52), p<0.001. At 24 months, macrolide resistance remained higher in the azithromycin group compared to placebo: 43.9% (95% CI: 39.2%-48.5%) versus 32.8% (95% CI: 28.5-37.1); OR=1.60 (95% CI: 1.07-2.40), p=0.02. Penicillin resistance was similar in both arms at all visits.

Interpretation

These findings support previous evidence from trachoma MDA programs and suggest monitoring of macrolide resistance should remain a key component of azithromycin interventions for reducing child mortality.

Funding

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Research in context

Evidence before this study

We searched PubMed on 28 March 2021 using search terms “azithromycin”, “resistance”, and “*Streptococcus pneumoniae*” and either “mass drug administration” or “trachoma”; and the variations “pneumococcus” and “mass treatment”. The search did not include restrictions for language or a start date. We included studies of azithromycin mass drug administration (MDA) that measured the prevalence of carriage of macrolide resistant *S. pneumoniae*. Azithromycin MDA to whole communities for control of trachoma generally produces an increase in macrolide resistance. The level and duration of macrolide resistance appears to be related to frequency of azithromycin distribution and baseline prevalence of resistance. However, very few studies have investigated the effect on macrolide resistance of azithromycin MDA targeted to children only. Data from the MORDOR study sites in Niger and Tanzania, reported low isolation rates of *S. pneumoniae* and varying effects on macrolide resistance. At the Tanzania site, 6 months after the fourth and final biannual MDA, 13.4% of isolates were macrolide resistant in azithromycin communities versus 13.2% in placebo communities. At the Niger site, at the same time point, macrolide resistance was 12.8% in azithromycin communities, and 2.9% in placebo communities.

Added value of this study

In a large cluster-randomized trial in Malawi, biannual azithromycin distributions to children aged 1-59 months significantly increased the proportion of *S. pneumoniae* strains resistant to macrolides. There was no evidence of cross-resistance to penicillin. The evidence from the other MORDOR sites, which had relatively low isolation rates of *S. pneumoniae*, was conflicting as to whether azithromycin MDA targeted to children under 5 years of age increases macrolide resistance. This study provides evidence in a different setting that this intervention to reduce child mortality increases macrolide resistance.

Implications of all the available evidence

Evidence is increasing for a beneficial effect of azithromycin MDA on child mortality and the World Health Organisation has released guidelines for when countries should consider this intervention. Results from the MORDOR Study sites in Tanzania and Niger describe increased macrolide resistance in the setting of azithromycin MDA in the latter, and not in the former. The results of this study emphasise the importance of careful monitoring of macrolide resistance, and further research into its implications in low-income settings, as the intervention is implemented more widely.

Introduction

Mass drug administration (MDA) with azithromycin is an effective strategy for the control of blinding trachoma caused by ocular *Chlamydia trachomatis* infection.[1–4] Azithromycin is an appealing MDA candidate due to its safety profile, long duration of action, and the availability of low cost generic formulations.[5–7] More than one billion doses have been distributed as part of the World Health Organisation (WHO) 'SAFE strategy' for the elimination of trachoma.[8] In addition, there is evidence for broader benefits of azithromycin MDA against the major causes of childhood mortality, including diarrhoea, respiratory infections and malaria.[9–13] In 2009, a cluster randomized trial reported a reduction in child mortality of approximately 50% following azithromycin MDA and recently stronger evidence has been provided by the Mortality Reduction After Oral Azithromycin (MORDOR) Study, which reported a 13.5% reduction in child mortality in azithromycin-treated communities compared to placebo in three African countries.[14,15] Additional data analyses have considered the ideal timing and target population of azithromycin MDA for reducing child mortality and guidelines have recently been produced by the WHO.[16–18] However, the development of macrolide resistance, which has long been shown to occur following azithromycin MDA for trachoma control, is an important concern.[19–22] The development of macrolide resistance in nasopharyngeal *S. pneumoniae* has been reported for the other MORDOR sites. In Niger, macrolide resistance was 12.8% in azithromycin communities at the 24-month follow-up, and 2.9% in placebo communities.[23] At the 24-month follow-up in Tanzania, 13.4% of isolates were macrolide resistant in azithromycin communities versus 13.2% in placebo communities.[24]

The development of macrolide resistance in *C. trachomatis* has not been reported as a serious concern following azithromycin MDA although resistance at extra-ocular sites, especially nasopharyngeal carriage of *Streptococcus pneumoniae*, may be of greater concern. It has previously been estimated that one third of *S. pneumoniae* strains worldwide may be macrolide resistant.[25] As antibiotic resistance in a community appears to correlate with the volume of antibiotic given, an understanding of the effects of azithromycin MDA for reducing child mortality on antibiotic resistance, particularly in *S. pneumoniae*, will be essential for countries to formulate health policies relating to this intervention.[26] In order to investigate antibiotic resistance following azithromycin MDA for reducing child mortality, nasopharyngeal swabs were collected from children in randomly selected communities at the MORDOR Malawi study site. Here we report nasopharyngeal *S. pneumoniae* carriage and macrolide and penicillin resistance before and after biannual azithromycin MDA in children aged 1-59 months in Malawi. The main outcomes are assessed at the individual level,

accounting for clustering by community. This was a pre-conceived cluster randomised controlled trial conducted within MORDOR.

Methods

Trial Design

This study was performed as part of the MORDOR trial in Mangochi District, Malawi. Prior to MORDOR commencing, 30 communities were randomly selected from all eligible communities in Mangochi District for surveillance of morbidity outcomes. Allocation of communities to either morbidity assessment or the MORDOR mortality outcome was performed at the coordinating centre for the study at the University of California, San Francisco (UCSF). The randomisation unit (community) was defined as the catchment area of a Health Surveillance Assistant (HSA), which usually has a total population of approximately 1,000. Communities with a total population greater than 2,000 on a pre-baseline study census were excluded. The randomisation was restricted to six communities in each of the five administrative zones of Mangochi District (Makanjira, Namwera, Chilipa, Monkey Bay and Mangochi zones) for logistical reasons as well as geographical generalisability. Biannual community visits were conducted as for the MORDOR study to record census updates, household GPS coordinates and administration of study drug, which has previously been described in detail.[14]

Participants

Participants were selected from all children aged <5 years identified in the study clusters at the biannual census visits who had slept at the household the previous night. Individuals were removed or added to the cohort at each of the bi-annual follow-up censuses depending on age and residence status. All children aged 1-59 months and weighing ≥3.8 kg were eligible for treatment biannually for a total of 4 distributions. Only children allergic to macrolides or azalides were not offered treatment. At the baseline, 12- and 24-month follow-up visits, a sample of 40 children aged 1-59 months, per community, were randomly selected from all censused children using a function of the data collection app, to undergo nasopharyngeal swabbing (target 1,200 swabs at each visit). Guardians provided written informed consent for tests following discussion with the study nursing staff speaking the local language. Illiterate guardians provided a thumb print to acknowledge consent.

Patient and public involvement

Multiple visits by study supervisors, fieldworkers and nurses were made to study villages for sensitisation, discussion of research conduct and to plan dissemination of findings. Study findings were discussed at community meetings across the study site.

Interventions

Azithromycin 20 mg/kg, or placebo, was administered as an oral suspension biannually for a total of 4 treatments. Both placebo and azithromycin were donated by Pfizer, New York, United States. The placebo contained the vehicle of the oral azithromycin suspension and was labelled identically to azithromycin. Children able to stand received an approximate dose based on their height, measured using a height-dose stick, and smaller children were weighed. The height-dose stick was optimised using local anthropometry data from the study site. Distribution of the drug took place after sample collection was complete and was performed by the HSAs and fieldworkers conducting house-to-house visits. Guardians were asked to inform the HSA of any adverse events that occurred within seven days of receiving the study drug. HSAs subsequently informed the study team.

Outcomes

The primary pre-specified outcome was the proportion of *S. pneumoniae* isolates exhibiting macrolide resistance in children aged 1-59 months at the 12- and 24-month follow-up visits, 6 months after the second and fourth biannual treatment visits, respectively. Pre-specified secondary outcomes were carriage rates and the proportion of *S. pneumoniae* isolates resistant to penicillin at 12- and 24-months. The following pre-specified secondary outcomes assessed in the MORDOR study in Malawi have been published elsewhere: malaria parasitaemia and haemoglobin;[27] cost effectiveness of the intervention for reducing mortality;[6] and cause-specific mortality rates.[28] The samples have not yet been processed to assess outcomes related to: prevalence of macrolide resistance in stool samples; and the fraction of conjunctival swabs yielding ocular chlamydia.

Sample collection

Sample collection took place during the baseline visit (May-July 2015), 12-month follow-up (April-June 2016) and 24-month follow-up (April-June 2017). A nasopharyngeal swab sample was collected via the nasal passage from selected children using a FLOQSwab™ (Copan Diagnostics, California, USA). Sample tubes were labelled with a random number and barcode and scanned using a feature of the custom-built data collection application (Conexus, Salt Lake City, United States) on Android devices to link to census data. Samples were stored in skim milk tryptone glucose glycerine (STGG) media and placed on ice in the field. Samples were then frozen at -80°C at Mangochi District Hospital each afternoon.

Samples were transported regularly, on ice packs, to Blantyre, where microbiological testing took place at Malawi-Liverpool-Wellcome Trust Laboratories.

Microbiological processing

Samples were thawed at room temperature and 10 µl of the transport media was inoculated onto gentamicin blood agar plates using a calibrated loop. An optochin disc (5 µg) was placed near the initial inoculum and plates were incubated for 24 hours at 37°C in 5% carbon dioxide. *S. pneumoniae* was identified as optochin susceptible, alpha-haemolytic colonies. A single, well-isolated colony was re-streaked onto blood agar and grown overnight under the same culture conditions. Azithromycin and penicillin sensitivity of the purified isolate was then tested using the Kirby Bauer method with ISO-blood agar, 15 µg azithromycin discs and 1 µg oxacillin discs. Zones of inhibition were measured and scored the following day according to M100 Clinical and Laboratory Standard Institute guidelines.[29] Isolates were defined as azithromycin resistant if the azithromycin disc zone diameter was ≤13 mm; and penicillin resistant if the oxacillin disc zone diameter was ≤19 mm.

Sample Size

Six hundred children per arm were selected for sampling at each round (40 children from 15 communities in each of the azithromycin and placebo treated communities), standardised across the three MORDOR country sites. In Malawi, using a conservative prediction of pneumococcal carriage rates of 40% in preschool children, we would expect 240 isolates to be cultured in the control arm at each time point.[30] This would provide approximately 80% power to detect a 20% increase in nasopharyngeal pneumococcal macrolide resistance from a baseline of 12% in the azithromycin treated group compared to the placebo group, assuming alpha of 0.05 and an intra-cluster correlation coefficient (ICC) of 0.11 for pneumococcal carriage.[20]

Randomisation and blinding

To ensure blinding, six letters were used by the manufacturer (Pfizer Inc., New York, NY) to label the study drug; three letters corresponding to azithromycin and three to placebo. Communities were randomly assigned to a drug letter using the sample function in R software, version 3.1 (R Foundation for Statistical Computing). Simple randomisation without stratification was performed at the coordinating centre for the study at UCSF. All study staff and participants in Malawi were blind to the treatment code until after all field work and data collection was complete.

Statistical analysis

Prevalence of *S. pneumoniae*, resistance of strains to macrolides, and resistance of strains to penicillin, was assessed by treatment arm at the individual level at the 12- and 24-month follow-up visits using mixed effects logistic regression models, including random effects for randomisation unit and fixed effects for baseline values for *S. pneumoniae* carriage, macrolide resistance and penicillin resistance, respectively. Odds ratios and respective 95% confidence intervals are presented for the proportion of individuals carrying *S. pneumoniae*, and the proportion of *S. pneumoniae* strains resistant to macrolides and penicillin, in the azithromycin arm compared to placebo. In addition, per protocol analyses were performed using similar mixed effects logistic regression models to assess the effect of treatment on *S. pneumoniae* carriage, macrolide resistance and penicillin resistance. The per protocol analyses included only children who received the study drug at the previous visit (i.e. the 6-month treatment round for the 12-month follow-up visit and the 18-month treatment visit for the 24-month follow-up visit). Age and sex were not included as fixed effects as they were balanced at baseline and sensitivity analyses including these covariates showed minimal change in the outcomes. All analyses were conducted using Stata Statistical Software: Release 15 (StataCorp LP).

Mean *S. pneumoniae* carriage, mean proportion of strains resistant to azithromycin, and mean proportion of strains resistant to penicillin at the 12- and 24-month visits, were also assessed at the community level by treatment arm using a generalized linear model. No adjustment was made for baseline prevalence of *S. pneumoniae*, macrolide resistance and penicillin resistance, which were similar between arms. Given the sampling method of 40 children per community, very few siblings were selected, hence family clustering bias would not affect the results.

The baseline prevalence of azithromycin resistance was displayed geographically using QGIS software. Inverse distance weighting interpolation was used to create a smooth surface of estimated macrolide resistance around the 30 clusters where samples were collected.

Study oversight

Ethical approval for this study was obtained from the ethics committees at the College of Medicine, University of Malawi, Blantyre; and the London School of Hygiene and Tropical Medicine, London, UK. ClinicalTrials.gov Identifier: NCT02048007.

Role of the funding source

This work was supported by the Bill and Melinda Gates Foundation. The funder of the study played no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

No serious adverse events attributable to the study drug were reported in the study. At the 12-month visit, 4,131 children were eligible for sampling in the azithromycin arm and 3,851 children in the placebo arm. At 24 months, the respective numbers were 3,416 and 2,880 children. Data from the fifteen communities in each arm are included in all analyses. Treatment coverage over the four rounds of the study was 76.6% in azithromycin communities (12,629 treatments administered to 16,494 eligible children) and 73.5% in placebo communities (11,118 treatments to 15,123 eligible children); detailed breakdown by treatment round is shown in the trial flow (Figure 1). The number of nasopharyngeal swabs included in the analysis from 600 children selected at the baseline, 12- and 24-month follow-up visits in azithromycin communities was: 564, 577 and 538 swabs, respectively; and in placebo communities: 563, 559 and 562 swabs, respectively.

Details of sampled children at the baseline visit are shown in Table 1. Age and sex distributions were similar between azithromycin and placebo communities. These characteristics were similarly balanced at 12 months and 24 months (data not shown). Baseline carriage of *S. pneumoniae* was higher than 85% in both arms of the study. Macrolide resistance was present in 28% of isolates in both arms of the study at baseline (136 of 481 isolates in the placebo arm; and 135 of 489 isolates in the azithromycin arm) and penicillin resistance was present in approximately 45% of isolates (216 of 481 isolates in the placebo arm; and 228 of 489 isolates in the azithromycin arm) (Table 1). Prevalence of macrolide resistance at baseline is shown on the map of Mangochi District (Figure 2). Levels of resistance were higher around Mangochi town and along the main transport and tourist road to Monkey Bay. Resistance was relatively high to the east, towards the Mozambique border, and lower in more remote parts of Makanjira, Namwera, Chilipa and Monkey Bay zones.

In the intention-to-treat analysis, *S. pneumoniae* carriage was similar between groups at the 12- and 24-month follow-up visits (Table 2). The per protocol analysis, also shown in Table 2, gave similar results to the intention-to-treat analysis, thus not providing any evidence for a difference in the effect of the intervention on *S. pneumoniae* carriage, macrolide resistance, or penicillin resistance in those who actually received study drug at the previous MDA round, compared to the effect in the overall study population.

The analysis of the effect of azithromycin at the community level indicated similar results to the individual-level analysis, namely similar *S. pneumoniae* carriage and penicillin resistance between arms, and higher macrolide resistance in the azithromycin arm (Supplementary table 1, appendix p1).

Figure 1: Trial flow for the MORDOR-Malawi *S. pneumoniae* and macrolide resistance study

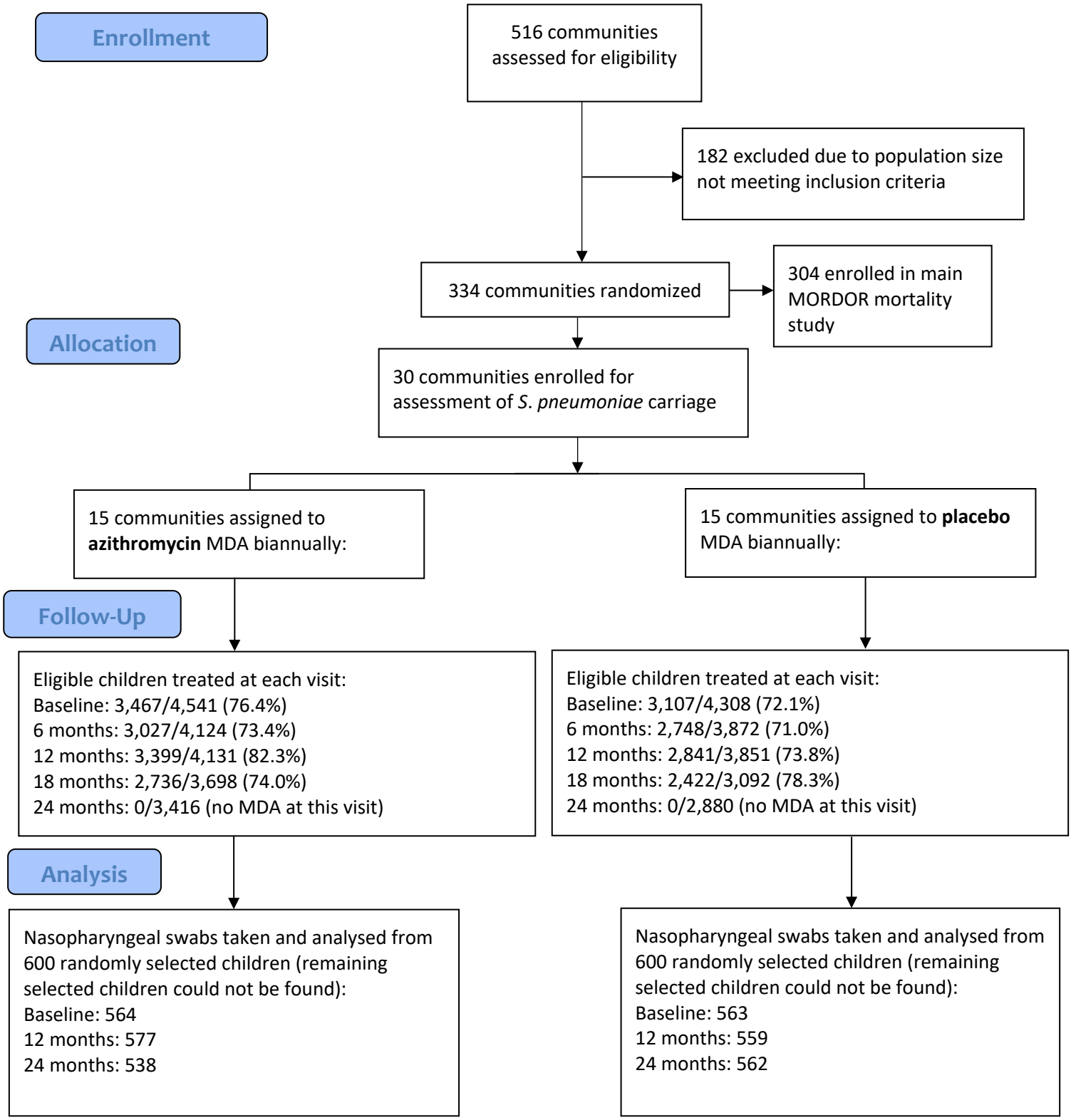

Table 1: Baseline characteristics of children selected for bacteriological sampling in azithromycin and placebo arms

	Placebo arm, *N*=563 *n (%)*	Azithromycin arm, *N*=564 *n (%)*
Sex		
Female	300 (53.3)	284 (50.4)
Age group		
1-11 months	86 (15.4)	101 (18.0)
12-23 months	126 (22.6)	126 (22.5)
24-35 months	116 (20.8)	117 (20.9)
36-47 months	116 (20.8)	113 (20.2)
48-59 months	114 (20.4)	103 (18.4)
S. pneumoniae carriage	481 (85.4)	489 (86.7)
Macrolide resistance	136 (28.3)	135 (27.6)
Penicillin resistance	216 (44.9)	228 (46.6)

Table 2: *S. pneumoniae* carriage, azithromycin resistance and penicillin resistance at the 12-month and 24-month follow-up visits in individuals by intention to treat (ITT) and per-protocol (PP), which included only those who received study drug at the prior visit, six months earlier

	Proportion of individuals carrying *S. pneumoniae*				Proportion of *S. pneumoniae* strains resistant to azithromycin				Proportion of *S. pneumoniae* strains resistant to penicillin			
	n/N	*%* (95% CI)	*OR (95% CI)	*P*-value	n/N	*%* (95% CI)	*OR (95% CI)	*P*-value	n/N	*%* (95% CI)	*OR (95% CI)	*P*-value
12 months												
ITT												
Placebo	450/558	80.6 (77.4-83.9)	1		97/450	21.6 (17.7-25.4)	1		173/450	38.4 (33.9-43.0)	1	
Azithromycin	472/577	81.8 (78.6-85.0)	1.06 (0.69-1.62)	0.79	174/472	36.9 (32.5-41.2)	2.26 (1.46-3.49)	0.0002	200/472	42.4 (37.9-46.8)	1.16 (0.88-1.52)	0.29
PP												
Placebo	327/400	81.8 (77.9-85.6)	1		74/327	22.6 (18.1-27.2)	1		122/327	37.3 (32.0-42.6)	1	
Azithromycin	350/421	83.1 (79.5-86.7)	1.10 (0.68-1.77)	0.71	141/350	40.3 (35.1-45.4)	2.47 (1.56-3.90)	0.0001	150/350	42.9 (37.6-48.1)	1.24 (0.89-1.72)	0.20
24 months												
ITT												
Placebo	457/562	81.3 (78.1-84.5)	1		150/457	32.8 (28.5-37.1)	1		210/457	46.0 (41.4-50.5)	1	
Azithromycin	440/538	81.8 (78.5-85.1)	1.06 (0.67-1.68)	0.80	193/440	43.9 (39.2-48.5)	1.66 (1.15-2.40)	0.0069	173/440	39.3 (34.7-43.9)	0.74 (0.48-1.13)	0.16
PP												
Placebo	319/387	82.4 (78.6-86.2)	1		110/319	34.5 (29.2-39.7)	1		145/319	45.5 (40.0-50.9)	1	
Azithromycin	294/362	81.2 (77.2-85.3)	0.90 (0.50-1.61)	0.72	126/294	42.9 (37.2-48.5)	1.52 (1.05-2.18)	0.025	110/294	37.4 (31.9-43.0)	0.69 (0.44-1.08)	0.11

*OR from mixed effects logistic regression, including randomisation unit as a random effect and baseline values for *S. pneumoniae* carriage, azithromycin resistance, and penicillin resistance, respectively, in the models

Discussion

This study assessed the prevalence of *S. pneumoniae* carriage, macrolide resistance and penicillin resistance in the setting of the MORDOR Study in Malawi. Communities (clusters) were randomly selected to produce data generalisable to the whole MORDOR Malawi study site (Mangochi District). Sample collection took place at baseline, and 12-month and 24-month follow-up visits, each six months after the previous MDA. The results indicate carriage of *S. pneumoniae* was not impacted by up to four biannual rounds of azithromycin MDA in Malawi. Macrolide resistance, however, was higher in *S. pneumoniae* isolates in azithromycin- compared to placebo-treated communities at the 12-month and 24-month follow-up visits. The difference in macrolide resistance between those who received azithromycin compared to placebo at the previous visit, was not greater than that identified in the primary intention-to-treat analysis. This may be expected given the relatively high coverage of study drug (71%-82% coverage per round) and the fact that *S. pneumoniae* strains are frequently passed between individuals in a household or community, particularly by toddlers and older children.[22] There was no increase in penicillin resistance, an important additional consideration due to the well documented potential for development of cross-resistance in bacteria.[31]

Antibiotic resistance studies, mostly conducted in the setting of trachoma control, have generally reported an increase in resistance following azithromycin MDA. However, the extent of the increase in resistance appears to vary considerably, possibly related to background antibiotic use and previous azithromycin MDA. A 1997 study of Australian Aboriginal children aged under 15 years with trachoma, who were treated along with household contacts who were children, showed the carriage of resistant strains of *S. pneumoniae* increased from 1.9% at baseline to 54.5% 2-3 weeks after treatment, 34.5% 2 months after treatment, and 5.9% 6 months after treatment.[21] This suggests resistance wanes with time after treatment. Studies in Nepal and Tanzania have similarly indicated low levels of macrolide resistance 6-12 months after one or three rounds of azithromycin MDA, although *S. pneumoniae* was only isolated from 7-12% of swabs in the Tanzanian study.[32–34] A study in The Gambia, where there is little routine use of macrolides, reported carriage of macrolide resistant *S. pneumoniae* 1 month after 3 biannual rounds of azithromycin MDA of only 1.2%, falling to 0.9% 6 months post-MDA.[35]

Higher levels of macrolide resistance have been reported at more than a year follow-up after several rounds of azithromycin MDA. A study in Ethiopia reported 28.2% of *S. pneumoniae* isolates were macrolide resistant 6 months after 4 biannual MDA rounds, increasing to 76.8% 6 months after the sixth and final biannual MDA. Resistance levels remained high at 30.6% 12 months after the final MDA,

and 20.8% 24 months after, again suggesting resistance decreases over time even from higher levels after multiple rounds of MDA.[36] Background use of macrolides was reportedly low in the study area and resistance in neighbouring control communities was 0.0%-0.9%, although many years of azithromycin MDA have been distributed for trachoma in Ethiopia. A further cluster randomized study in Ethiopia reported an increase in macrolide resistance from 6.3% of strains pre-treatment to 62.3% 3 months after four 3-monthly rounds of azithromycin MDA.[20] Macrolide resistance in control communities was 11.6%.

Coles et al., in 2013, investigated the proportion of resistant isolates at several time points following a single round of azithromycin MDA in Tanzania.[19] Before treatment the proportion of resistant isolates was 2.1%, increasing to 4.5%, 18.3% and 35.4% at 1, 3 and 6 months respectively after MDA. In non-MDA communities, the respective proportions of resistant isolates were 13.1%, 4.4%, 8.2% and 12.4%. The increase in resistance at successive follow-up points in this study is unusual and difficult to explain but could be related to antibiotic use outside of MDA programs – indeed, more than 65% of people in the study communities reported taking drugs to treat suspected infection in the 30 days prior to sampling.

A systematic review of antibiotic resistance in *S. pneumoniae* following azithromycin distribution for trachoma in 2015, identified distinct trends that resistance prevalence was dependent on frequency of azithromycin distribution and baseline prevalence of resistance.[37] Resistance gradually decreased as measurements were taken at longer time periods after the last distribution. It is possible that persistent macrolide resistance could occur above a certain threshold of macrolide use. This could become increasingly important clinically as there may be a time for routine use of macrolides as pneumococcal infections become commonly penicillin resistant. The data presented suggest this is not currently the case and that macrolide resistance is gradually eliminated, presumably due to a fitness cost of carrying macrolide resistance.

Baseline macrolide resistance was relatively high in this study at 28% (as was penicillin resistance at 45%) but the levels are similar to those found in clinical isolates in Blantyre (unpublished data). Macrolides are not used in frontline management of *S. pneumoniae* syndromes in Malawi although trachoma control programs have conducted azithromycin MDA for several years in a minority of districts across Malawi.[38] MDA for trachoma was not conducted in Mangochi but it is possible that some azithromycin may have been used outside of the MDA program and theoretically feasible that, as azithromycin is mainly excreted in the faeces unchanged, there could be environmental

contamination of the waterways that also feed Lake Malawi. Mangochi also has a long and porous border with Mozambique, where antibiotics, particularly amoxicillin with clavulanic acid, azithromycin, and cotrimoxazole, are commonly available over the counter.[39,40] The distribution of macrolide resistance at baseline, shown in Figure 2, would be compatible with antibiotics being brought from Mozambique along the main road to Mangochi town and subsequently along the main transport and tourist road to Monkey Bay. The reasons for relatively high macrolide resistance at the MORDOR Malawi study site are not clear at this stage, although they are in line with levels of resistance seen in clinical isolates.

The levels of resistance reported at the other MORDOR sites (up to 13.4%) are relatively low compared to the Malawi site, the reasons for which are not clear. The isolation rates of *S. pneumoniae* were relatively low at the Niger and Tanzania sites compared to Malawi: 54.7% and 54.0% in placebo and azithromycin communities, respectively, at 24 months at the Niger site, and 11.0% and 15.1% in placebo and azithromycin communities, respectively at 24 months at the Tanzania site. It is feasible that any fitness cost of carrying macrolide resistance could lead to preferential isolation of non-resistant isolates, and that this would be exaggerated if isolation rates were low.

It is not clear what effect macrolide resistance may have on the efficacy of azithromycin MDA for reducing child mortality. It might be assumed that increased resistance would decrease efficacy due to diminished effect against resistant pathogens. However, there is currently no evidence to support this. In fact, the MORDOR Study showed an increase in the effect estimate with each successive follow-up period despite the two sites where the effect was greatest, Niger and Malawi, also reporting an increase in macrolide resistance in the azithromycin communities. An increase in effect could be independent of antibiotic resistance, for example through a cumulative reduction in pathogens with each MDA, or directly related to the resistance, for example if the fitness cost to bacteria of carrying resistance reduced their virulence.[41]

A limitation of this study is that follow-up took place at 12-monthly intervals, six months after the previous MDA. More granular and longer-term follow-up would have been preferable but was not possible due to funding and logistical limitations. It is likely that resistance would have peaked at higher levels in the weeks following MDA and reduced by the time of sampling. However, the study reports persistent resistance of at least six months duration, an important consideration for policy makers and comparable to other studies. Samples were also available at both the mid-point and endpoint of this study, enabling assessment of the change in antibiotic resistance with continued

MDA. The results do not provide evidence that macrolide resistance increases further following four rounds compared to two rounds of biannual azithromycin MDA to children aged 1-59 months only. This study did not investigate the development of resistance in other organisms, which could be of clinical significance as well as serve as a reservoir of resistance genes. Finally, it was outside of the scope of this study to investigate the mechanisms of macrolide resistance via molecular detection of markers of resistance in this setting, which may form part of future research efforts.

The results of this study indicate macrolide resistance in *S. pneumoniae* increases following four biannual rounds of azithromycin MDA to children aged 1-59 months in Malawi. Previous research suggests the development of macrolide resistance varies greatly by population and may increase further and become more persistent with additional rounds of MDA or where there is macrolide use in primary healthcare.[19,36] However, our understanding of the levels of macrolide resistance and their mechanisms in the setting of azithromycin MDA, particularly when targeted solely to children to reduce mortality, remains poor. Prediction of the resistance that would be generated from implementation in other regions and countries if MDA were deployed more widely, would currently be extremely challenging. Further research into azithromycin resistance should remain a key component of studies and interventions with azithromycin MDA for the reduction of child mortality.

Contributors

This study was designed by JDH and RLB. JDH and KK coordinated the fieldwork. LS, HM, SEB and JC coordinated and conducted lab work. JDH analysed the data and all authors contributed to data interpretation. JDH, RLB, KK, JC and SEB had full access to all the data in the study and had final responsibility for the decision to submit for publication. JDH and RLB verified underlying data of the study. JDH wrote the manuscript and all authors revised it and approved the final version.

Declaration of interests

The authors declare no conflicts of interest.

Data sharing

Mothers and guardians of participants in the MORDOR Malawi morbidity study consented to confidential use of the samples and data collected by the study team only hence wider availability of the data is not possible.

The full MORDOR Study protocol is available at: https://clinicaltrials.gov/ct2/show/NCT02047981

Registration

ClinicalTrials.gov Identifier: NCT02048007

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All authors report no conflicts of interest.

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Discussion

The findings of the analysis of macrolide resistance in *S. pneumoniae* in this study support previous research suggesting that macrolide resistance generally increases following azithromycin MDA.[85,120,168] However, whilst antibiotic resistance is a serious concern globally, the impact of macrolide resistance in the setting of azithromycin MDA on the efficacy of the intervention or other clinical outcomes, is not clear. Indeed, as discussed previously, the effect of the intervention tended to increase over the course of the MORDOR study despite the development of macrolide resistance. Again, further research will be required to better define the extent – and in particular the consequences – of macrolide resistance following azithromycin MDA in different settings.

Chapter 7: Discussion

This work investigated factors associated with the effect of azithromycin on child mortality, plus additional key considerations regarding the intervention: macrolide resistance and cost-effectiveness. The findings provide new insight into the effects of azithromycin MDA for reducing child mortality and enable hypothesis generation regarding the populations who may benefit most from the intervention. The findings suggest the child mortality benefit of azithromycin MDA may be due to effects on pneumonia and diarrhoea or HIV/AIDS mortality. Finally, further exploratory analyses identified various indicators associated with the effect of the intervention. Most of the associations were with indicators that are also markers of development and poverty, which may, therefore, underlie the effects that were evident.

Analysis of cause of death has also been conducted for the MORDOR-Niger site, where azithromycin MDA resulted in approximately a third fewer deaths from meningitis and dysentery, and a fifth fewer from malaria and pneumonia.[169] The main differences to the findings in Malawi are the apparent effects on meningitis and malaria mortality. The Niger site used the 2007 version of the WHO questionnaire and an adapted version of a previously derived expert VA algorithm. The Niger algorithm has not been validated in the same manner as the InterVA and SmartVA algorithms but has been reported to perform similarly to physician coding of VA in the Niger setting.[170] Meningitis is not a cause in the SmartVA algorithm due to poor specificity of symptoms for this diagnosis. However, given the common bacterial causes of meningitis and their overlap with pneumonia, an effect of the intervention on meningitis mortality would not be unexpected.

Malaria infection has been reported to decrease following azithromycin MDA in Niger and, indeed, in Malawi the point estimates of the odds ratios of malaria infection in azithromycin- compared to placebo-treated clusters were 0.89 in the ITT analysis and 0.71 in the PP analysis. Whilst the study in Malawi did not identify a reduction in malaria mortality, such an effect would certainly be plausible given the relatively small sample size in this study for assessment of cause-specific mortality, plus the facts that azithromycin has established antimalarial properties and malaria is a leading cause of child mortality in Malawi. No additional evidence for an effect of azithromycin MDA on malaria infection was evident from the Tanzania site,

where clinical malaria was similar between arms and greater odds of decline in malaria rapid diagnostic test positivity in the placebo arm were reported.[171]

Additional morbidity indicators were assessed at the Tanzania site using surveys and measurement of temperature and haemoglobin.[172] Six months post-MDA, there was no evidence for a difference in diarrhoea, fever, or anaemia between treatment arms. There was an indication that cough may have reduced over the course of the study in the azithromycin-treated arm, but not in the placebo arm, although the effect estimate was not significant at the 5% level following adjustment for age and clustering. A reduction in cough would support the finding in this study, using both VA algorithms, of a reduction in pneumonia mortality with the intervention. The lack of evidence for a difference in diarrhoea morbidity between arms at the Tanzania site six months post-MDA does not provide evidence against a shorter-term reduction in diarrhoea morbidity (and thus potentially mortality). In fact, this is supported by a further analysis of the data from all three MORDOR country sites, which suggests that much of the protective effect of azithromycin MDA may occur in the first 3 months post-MDA.[173]

Similar findings to this study, suggesting a greater effect of the intervention in undernourished children, have also been reported for the MORDOR-Niger site.[174] The overall difference in infant mortality in the study was 12.6 fewer deaths per 1,000 person-years in the azithromycin arm (95% CI −18.5 to −6.9). This difference between arms increased to 17.0 fewer deaths (95% CI −28.0 to −7.0) in infants with a z score <-2; and 25.6 fewer deaths (95% CI −42.6 to −9.6) in infants with a z score <-3. Although neither this study nor the Niger study was able to provide strong evidence that nutritional status modified the effect of azithromycin MDA on infant mortality, the similar findings at the two sites and biological plausibility of a greater effect of a broad-spectrum antibiotic in children with either primary malnutrition or chronic illness resulting in low weight, would suggest these vulnerable children may be most likely to benefit from the intervention.

The present study was conducted in a setting that has seen the introduction of a considerable number of interventions to reduce child mortality over the last 20 years. In addition, the presence of the HSA system, which was significantly expanded through the 2000s with support from the Global Fund to Fight AIDS, Tuberculosis, and Malaria, is likely to improve health and reduce child mortality further. The HSAs have a focus on health and sanitation education alongside their association with health centres and conducting health outreach

activities. In such a setting, it may be surprising that azithromycin MDA can produce an additional reduction in child mortality. The trends evident in this study, especially if supported with further research, may be of particular interest for reducing child mortality in the context of reasonably well-organized health systems that have already brought about significant reductions in child mortality. This context is increasingly common as simple effective interventions have reduced child mortality in many low-income settings to closer to 50 per 1,000 live births from levels that were commonly greater than 200 per 1,000 live births in 1990. Further research will be required for us to understand whether azithromycin may be a useful intervention to plug the gap where there is poor coverage of other interventions and child mortality is high; or whether the effects and associations estimated in this study may reflect an additional benefit of the intervention even when child mortality is in the region of 50 per 1,000 live births. Understanding variation in effect in lower mortality settings may be particularly useful for targeting the intervention to at-risk populations.

Porco et al. reported no strong evidence for a greater effect of azithromycin in populations with higher child mortality.[175] However, higher mortality areas are likely to have a greater proportion of deaths due to infectious causes amenable to azithromycin and, indeed, even if the effect is constant, higher mortality areas have the most to gain through absolute numbers of deaths averted. Targeting higher risk groups, for example infants or undernourished children, may be an option to increase the cost-effectiveness of the intervention, or minimize potential risks related to macrolide resistance. However, Tickell et al. present the argument that decisions regarding which populations to target should be based on the absolute numbers of deaths likely to be averted rather than the fluctuating effect sizes in different populations evident from the limited data available to date.[176] In addition, cost savings for the MDA may be very limited when every house may need to be visited to identify children of a particular age or with other characteristics. Analysis of the MORDOR-Niger data indicated that limiting MDA to infants aged 1-5 months would reduce the absolute number of deaths averted 6-fold, and limiting to infants aged 1-11 months would reduce deaths averted 2.5-fold. In addition, it is important to note that no study has compared MDA to children <5 years of age with MDA to a subset of infants only. It is feasible that the effect in younger children may in part be due to herd protective effect, enhanced by treatment of older children as well.[177]

Further to the discussion in Chapter 4 that azithromycin could reduce mortality in low birth weight children, there could be a considerable effect of the intervention in the perinatal or postnatal period. There has been limited research assessing the effects of azithromycin administered to children under 1 month of age although a study of a single dose of azithromycin given to the mother during labour in The Gambia reported significantly reduced GBS, *S. pneumoniae* and *S. aureus* carriage and significantly reduced maternal and infant infections up to two months post-delivery.[178,179] Further studies are underway to investigate the effect of azithromycin administered during childbirth on infant infections in Fiji (ClinicalTrials.gov Identifier: NCT03925480); and administration in the late neonatal period on child mortality in Burkina Faso.[180] Given the findings from MORDOR and this nested study that younger children and underweight children appear to benefit most from the intervention, an effect of azithromycin on mortality in the perinatal or neonatal period is certainly plausible. A concern previously has been the potential for development of infantile hypertrophic pyloric stenosis (IHPS), particularly in small premature neonates, so this would have to be monitored closely with treatment of younger infants. IHPS has been reported to increase more than eight-fold with macrolide treatment in the first two weeks of life, although these cases likely included multiple doses as opposed to the single dose with MDA.[122,181–183]

In late 2020, the WHO released provisional guidelines for azithromycin MDA for reducing child mortality.[184] The guidelines recommend that the intervention be targeted to higher mortality areas and those children aged 1-11 months, and that monitoring continues to assess the effect on macrolide resistance and the mortality benefit. As there is no evidence to date for a mortality effect when only infants are treated, without the potential benefit from treating all children under five years, the results of further studies will be important to further inform the guidelines, particularly whether it should be recommended that younger or older children should also be treated. Further evidence is also required as to whether treatment of individuals when they reach a certain age, for example at immunization visits, could produce the same benefits as MDA. MDA, where all children are treated at the same timepoint, has the potential benefit of maximizing reduction of pathogens in the community. On the other hand, in the context of MDA some infants will not receive a dose with biannual treatment until they are six months of age, after the period of greatest potential benefit, which linking

the azithromycin distribution to immunization would avoid. Understanding the difference in effect between these implementation strategies can only be achieved through further real-world research.

An additional important consideration, alteration of the gut microbiome, has previously been shown to occur following broad spectrum antibiotic administration, with decreased taxonomic richness and diversity persisting for some taxa over six months post-treatment.[185] Evidence from the MORDOR-Niger site indicated gamma diversity did not change significantly but relative abundances of two *Campylobacter* species and 33 other species of gut bacteria were significantly reduced at the 24 month follow-up, 6 months after 4 biannual rounds of MDA.[186] Longitudinal data from children at the MORDOR-Malawi site indicated alpha diversity was not different between treatment arms after two or four rounds of azithromycin MDA.[187] There is currently little understanding of the changes in the microbiome that occur following azithromycin MDA and what the consequences of these might be. Indeed, it is not clear if the changes may be beneficial or deleterious. It is feasible that a decrease in community pathogen load, for example the long-term reduction in *Campylobacter* species at the Niger site, may produce a sustained beneficial effect. There could also be an indirect benefit through a reduction in environmental enteric dysfunction and inflammation, that would improve nutritional status and resilience to further challenges. A study of azithromycin given to infants in India reduced faecal biomarkers of environmental enteropathy (calprotectin, myeloperoxidase, α1-antitrypsin) and the prevalence of bacterial pathogens.[188] However, anthropometric indices as a marker of nutritional status have not always been shown to improve in children following azithromycin MDA.[189,190] A study in The Gambia of azithromycin administered during childbirth reported a beneficial effect on mid upper arm circumference at 1 year of age.[191] This may reflect the greater mortality benefit apparent in younger infants in MORDOR and be related to reduced bacterial colonization and infection of neonates following azithromycin administration to mothers in labour.[178,179]

A limitation of this study is that the sample size was not adequate to provide strong evidence for the effects of azithromycin MDA on different causes of death or on all-cause mortality by the various categories assessed. Therefore, this work aimed to explore biologically plausible factors associated with the effect of the intervention to enable hypothesis generation regarding potential mechanisms of effect. Many variables were analysed and no attempt was

made to adjust for multiple testing in the interests of presenting the unadjusted data at face value. It is possible that random variation in effect estimates has indicated an effect when in truth there is none. However, this work does not purport to provide final answers but rather to give direction to future investigation of the factors identified that may be associated with the effect of azithromycin MDA on child mortality.

In addition, some variables analysed, such as land surface temperature and NDVI, were included as it was believed that an association could be particularly useful in broader assessment of populations likely to benefit. However, given the limited sample size for this study and variation in these indicators, they may be less meaningful than other measures related to accessibility and socioeconomic status of the households.

Following the main findings of the MORDOR study that indicated a 13.5% reduction in mortality in the three-country study, the findings of this study indicate the reduction in mortality may be due to a decrease in pneumonia and diarrhoea or HIV/AIDS deaths. The effect could be explained by the broad spectrum of activity of azithromycin against gut and respiratory organisms, including non-vaccine pneumococcal serotypes and other aetiological causes of pneumonia, sepsis and meningitis. In Malawi, the relatively high ARR in child mortality of 5.3% between 1990 and 2015, would result in a reduction in U5MR from 64 to 28 between 2015 and 2030, close to the SDG target of 25. However, with a reasonably well organized health care system in Malawi and proven interventions such as immunization already at a coverage greater than 80% for the past 10 years, additional interventions or health care improvements are likely to be needed to maintain the ARR.[192] In Malawi and other countries with relatively high child mortality from infectious causes, azithromycin MDA could be a feasible option to reduce child mortality, whilst longer term improvements in health systems are pursued in order to sustainably maintain lower mortality rates.

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Appendix

Appendix 1: Supplemental material to published manuscript on causes of death

Supplemental Table 1: Cause-specific mortality per-protocol for the four main causes of death in the study area *using InterVA*

	Number of cases/person-years	Rate per 1000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value
Pneumonia				
Placebo	71/58,832	1.21 (0.96-1.52)	1	
Azithro	62/58,731	1.06 (0.82-1.35)	0.88 (0.62-1.24)	0.46
Malaria				
Placebo	204/58,832	3.47 (3.02-3.98)	1	
Azithro	202/58,731	3.44 (3.00-3.95)	0.99 (0.80-1.23)	0.92
HIV/AIDS				
Placebo	71/58,832	1.21 (0.96-1.52)	1	
Azithro	56/58,731	0.95 (0.73-1.24)	0.80 (0.54-1.19)	0.27
Diarrhea				
Placebo	37/58,832	0.63 (0.46-0.87)	1	
Azithro	38/58,731	0.65 (0.47-0.89)	1.05 (0.64-1.72)	0.85

**From random effects Poisson model adjusting for clustering at the level of the randomization unit*

Supplemental Table 2: Cause-specific mortality per-protocol for the four main causes of death in the study area *using SmartVA*

	Number of cases/person-years	Rate per 1,000 person-years (95% CI)	Rate Ratio* (95% CI)	*P*-value	Rate after redistribution	Rate ratio after redistribution
Pneumonia						
Placebo	24/58,832	0.41 (0.27-0.61)	1		0.74	1
Azithro	16/58,731	0.27 (0.17-0.44)	0.67 (0.36-1.27)	0.22	0.71	0.96
Malaria						
Placebo	154/58,832	2.62 (2.24-3.07)	1		3.10	1
Azithro	154/58,731	2.62 (2.24-3.07)	1.00 (0.80-1.25)	0.98	3.01	0.97
HIV/AIDS						
Placebo	58/58,832	0.99 (0.76-1.28)	1		1.05	1
Azithro	62/58,731	1.06 (0.82-1.35)	1.07 (0.75-1.53)	0.70	1.06	1.01
Diarrhea						
Placebo	57/58,832	0.97 (0.75-1.26)	1		1.18	1
Azithro	46/58,731	0.78 (0.59-1.05)	0.81 (0.55-1.20)	0.29	0.97	0.82

**From random effects Poisson model adjusting for clustering at the level of the randomization unit*

Supplemental Table 3: Seasonality of cause-specific mortality by intention-to-treat *using InterVA*

	Wet season				Dry season			
	Deaths /person-years	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**	**Deaths /person-years**	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**
Pneumonia								
Placebo	50/38,035	1.31 (1.00-1.73)	1		44/28,900	1.52 (1.13-2.05)	1	
Azithro	32/38,066	0.84 (0.59-1.19)	0.64 (0.41-1.00)	0.05	45/28,772	1.56 (1.17-2.09)	1.02 (0.65-1.61)	0.93
Malaria								
Placebo	158/38,035	4.15 (3.55-4.86)	1		99/28,900	3.43 (2.81-4.17)	1	
Azithro	154/38,066	4.05 (3.45-4.74)	0.97 (0.75-1.26)	0.84	92/28,772	3.20 (2.61-3.92)	0.92 (0.68 - 1.26)	0.62
HIV/AIDS								
Placebo	58/38,035	1.52 (1.18-1.97)	1		45/28,900	1.56 (1.16-2.09)	1	
Azithro	42/38,066	1.10 (0.82-1.49)	0.72 (0.47-1.10)	0.13	29/28,772	1.01 (0.70-1.45)	0.67 (0.40-1.11)	0.12
Diarrhoea								
Placebo	30/38,035	0.79 (0.55-1.13)	1		18/28,900	0.62 (0.39-0.99)	1	
Azithro	27/38,066	0.71 (0.49-1.03)	0.92 (0.51-1.63)	0.76	18/28,772	0.63 (0.39-0.99)	1.02 (0.50-2.09)	0.95

**From univariate Poisson regression*

SupplementalTable 4: Seasonality of cause-specific mortality by intention-to-treat *using SmartVA* (without redistribution of unknown causes of death)

	Wet season				Dry season			
	Deaths /person-years	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**	**Deaths /person-years**	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**
Pneumonia								
Placebo	14/38,035	0.37 (0.22-0.62)	1		21/28,900	0.73 (0.47-1.11)	1	
Azithro	10/38,066	0.26 (0.14-0.49)	0.71 (0.32-1.61)	0.42	10/28,772	0.35 (0.19-0.65)	0.48 (0.23-1.02)	0.06
Malaria								
Placebo	122/38,035	3.21 (2.69-3.83)	1		76/28,900	2.63 (2.10-3.29)	1	
Azithro	102/38,066	2.68 (2.21-3.25)	0.84 (0.64-1.09)	0.18	82/28,772	2.85 (2.30-3.54)	1.08 (0.79 - 1.48)	0.61
HIV/AIDS								
Placebo	40/33,093	1.05 (0.77-1.43)	1		31/25,739	1.07 (0.75-1.53)	1	
Azithro	41/33,086	1.08 (0.79-1.46)	1.02 (0.66-1.58)	0.91	29/25,645	1.01 (0.70-1.45)	0.94 (0.57-1.56)	0.81
Diarrhea								
Placebo	42/38,035	1.10 (0.82-1.49)	1		37/28,900	1.28 (0.93-1.77)	1	
Azithro	27/38,066	0.71 (0.49-1.03)	0.64 (0.40-1.04)	0.07	29/28,772	1.01 (0.70-1.45)	0.79 (0.48-1.28)	0.34

**From univariate Poisson regression*

Supplemental Table 5: Seasonality of cause-specific mortality per-protocol *using InterVA*

	Wet season				Dry season			
	Deaths /person-years	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**	**Deaths /person-years**	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**
Pneumonia								
Placebo	42/33,093	1.27 (0.94-1.72)	1		29/25,739	1.13 (0.78-1.62)	1	
Azithro	26/33,086	0.79 (0.54-1.15)	0.62 (0.38-1.01)	0.06	36/25,645	1.40 (1.01-1.95)	1.24 (0.75-2.06)	0.40
Malaria								
Placebo	133/33,093	4.02 (3.39-4.76)	1		71/25,739	2.76 (2.19-3.48)	1	
Azithro	135/33,086	4.08 (3.45-4.83)	1.01 (0.77-1.33)	0.94	67/25,645	2.61 (2.06-3.32)	0.94 (0.67-1.34)	0.75
HIV/AIDS								
Placebo	46/33,093	1.39 (1.04-1.86)	1		25/25,739	0.97 (0.66-1.44)	1	
Azithro	36/33,086	1.09 (0.78-1.51)	0.77 (0.48-1.24)	0.29	20/25,645	0.78 (0.50-1.21)	0.87 (0.44-1.69)	0.67
Diarrhea								
Placebo	23/33,093	0.70 (0.46-1.05)	1		14/25,739	0.54 (0.32-0.92)	1	
Azithro	26/33,086	0.79 (0.54-1.15)	1.16 (0.62-2.18)	0.65	12/25,645	0.47 (0.27-0.82)	0.87 (0.39-1.93)	0.73

**From univariate Poisson regression*

Supplemental Table 6: Seasonality of cause-specific mortality per protocol *using SmartVA* (without redistribution of unknown causes of death)

	Wet season				Dry season			
	Deaths /person-years	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**	**Deaths /person-years**	**Rate per 1000 person-years (95% CI)**	**Rate Ratio* (95% CI)**	***P*-value**
Pneumonia								
Placebo	13/33,093	0.39 (0.23-0.68)	1		11/25,739	0.43 (0.24-0.77)	1	
Azithro	8/33,086	0.24 (0.12-0.48)	0.62 (0.26-1.49)	0.28	8/25,645	0.31 (0.16-0.62)	0.73 (0.29-1.81)	0.50
Malaria								
Placebo	98/33,093	2.96 (2.43-3.61)	1		56/25,739	2.18 (1.67-2.83)	1	
Azithro	90/33,086	2.72 (2.21-3.34)	0.92 (0.69-1.22)	0.56	64/25,645	2.50 (1.95-3.19)	1.15 (0.80-1.64)	0.45
HIV/AIDS								
Placebo	35/38,035	1.06 (0.76-1.47)	1		23/28,900	0.89 (0.59-1.34)	1	
Azithro	38/38,066	1.15 (0.84-1.58)	1.09 (0.69-1.72)	0.73	24/28,772	0.94 (0.63-1.40)	1.05 (0.59-1.89)	0.87
Diarrhea								
Placebo	33/33,093	1.00 (0.71-1.40)	1		24/25,739	0.93 (0.62-1.39)	1	
Azithro	25/33,086	0.76 (0.51-1.12)	0.76 (0.45-1.27)	0.30	21/25,645	0.82 (0.53-1.26)	0.88 (0.49-1.58)	0.66

**From univariate Poisson regression*

Supplemental Table 7: Sensitivity and specificity of the VA algorithms for predicting the leading causes of child death

	InterVA4		Tariff 2.0	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**
Malaria	44.7	89.2	59.3	92.8
HIV	28.2	95.5	60	96.9
Pneumonia	75	64.3	14.2	98.2
Diarrhea	28.4	98.1	40	95.5

Data from Murray et al., 2014

Appendix 2: Cause-specific mortality by zone (as identified from verbal autopsy using the InterVA algorithm)

Zone	Monkey Bay		Chilipa		Makanjira		Namwera		Mangochi		Total	
Treatment arm	Placebo	Azithro	Placebo	Azithro	Placebo	Azithro	Placebo	Azithro	Placebo	Azithro	Placebo	Azithro
Person-years (1000s)	7.58	10.96	12.28	11.60	10.08	9.33	18.05	17.59	18.94	17.36	66.93	66.84
All-cause mortality												
Total deaths	53	73	134	119	89	87	191	135	153	150	620	564
Total mortality rate (deaths per 1,000 person-years, 95% CI)	6.99 (5.34-9.15)	6.66 (5.30-8.38)	10.91 (9.21-12.93)	10.26 (8.57-12.28)	8.83 (7.18-10.87)	9.32 (7.56-11.50)	10.58 (9.18-12.19)	7.67 (6.48-9.08)	8.08 (6.89-9.46)	8.64 (7.36-10.14)	9.26 (8.56-10.02)	8.44 (7.77-9.16)
Total mortality rate ratio (azithro/placebo, 95% CI)	0.95 (0.67-1.36)		0.94 (0.73-1.20)		1.06 (0.79-1.42)		0.73 (0.58-0.90)		1.07 (0.85-1.34)		0.92 (0.82-1.03)	
Pneumonia												
Pneumonia deaths	6	12	25	13	13	17	25	11	25	24	94	77
Pneumonia mortality rate	0.79 (0.36-1.76)	1.10 (0.62-1.93)	2.04 (1.38-3.01)	1.12 (0.65-1.93)	1.29 (0.75-2.22)	1.82 (1.13-2.93)	1.38 (0.94-2.05)	0.63 (0.35-1.13)	1.32 (0.89-1.95)	1.38 (0.93-2.06)	1.40 (1.15-1.72)	1.15 (0.92-1.44)
Pneumonia mortality rate ratio (azithro/placebo, 95% CI)	1.38 (0.52-3.69)		0.55 (0.28-1.08)		1.41 (0.69-2.91)		0.45 (0.22-0.92)		1.05 (0.60-1.83)		0.82 (0.61-1.11)	
Malaria												
Malaria deaths	19	29	46	55	33	35	94	66	64	60	256	245
Malaria mortality rate	2.51 (1.60-3.93)	2.65 (1.84-3.81)	3.75 (2.81-5.00)	4.74 (3.64-6.18)	3.28 (2.33-4.61)	3.75 (2.69-5.22)	5.21 (4.25-6.37)	3.75 (2.95-4.78)	3.38 (2.64-4.32)	3.46 (2.68-4.45)	3.82 (3.38-4.32)	3.67 (3.23-4.15)
Malaria mortality rate ratio (azithro/placebo, 95% CI)	1.06 (0.59-1.88)		1.27 (0.86-1.87)		1.15 (0.71-1.84)		0.72 (0.53-0.99)		1.02 (0.72-1.46)		0.96 (0.80-1.14)	
HIV/AIDS												
HIV/AIDS deaths	12	7	22	18	19	8	30	17	20	21	103	71
HIV/AIDS mortality rate	1.58 (0.90-2.79)	0.64 (0.30-1.34)	1.79 (1.18-2.72)	1.55 (0.98-2.46)	1.89 (1.20-2.96)	0.86 (0.43-1.71)	1.66 (1.16-2.38)	0.97 (0.60-1.55)	1.06 (0.68-1.64)	1.21 (0.79-1.86)	1.54 (1.27-1.87)	1.06 (0.84-1.34)
HIV/AIDS mortality rate ratio (azithro/placebo, 95% CI)	0.40 (0.16-1.03)		0.87 (0.47-1.62)		0.46 (0.20-1.04)		0.58 (0.32-1.05)		1.15 (0.62-2.11)		0.69 (0.51-0.93)	
Diarrhoea												
Diarrhoea deaths	7	11	11	10	10	3	9	8	11	13	48	45
Diarrhoea mortality rate	0.92 (0.44-1.94)	1.00 (0.56-1.81)	0.90 (0.50-1.62)	0.86 (0.46-1.60)	0.99 (0.53-1.84)	0.32 (0.10-1.00)	0.50 (0.26-0.96)	0.45 (0.23-0.91)	0.58 (0.32-1.05)	0.75 (0.43-1.29)	0.72 (0.54-0.95)	0.67 (0.50-0.90)
Diarrhoea mortality rate ratio (azithro/placebo, 95% CI)	1.09 (0.42-2.81)		0.96 (0.41-2.27)		0.32 (0.09-1.18)		0.91 (0.35-2.36)		1.29 (0.58-2.88)		0.94 (0.63-1.41)	

Appendix 3: Cause-specific mortality by zone (as identified from verbal autopsy using the SmartVA algorithm)

Zone	Monkey Bay		Chilipa		Makanjira		Namwera		Mangochi		Total	
Treatment arm	Placebo	Azithro	Placebo	Azithro	Placebo	Azithro	Placebo	Azithro	Placebo	Azithro	Placebo	Azithro
Person-years (1000s)	7.58	10.96	12.28	11.60	10.08	9.33	18.05	17.59	18.94	17.36	66.93	66.84
All-cause mortality												
Total deaths	53	73	134	119	89	87	191	135	153	150	620	564
Total mortality rate (deaths per 1,000 person-years, 95% CI)	6.99 (5.34-9.15)	6.66 (5.30-8.38)	10.91 (9.21-12.93)	10.26 (8.57-12.28)	8.83 (7.18-10.87)	9.32 (7.56-11.50)	10.58 (9.18-12.19)	7.67 (6.48-9.08)	8.08 (6.89-9.46)	8.64 (7.36-10.14)	9.26 (8.56-10.02)	8.44 (7.77-9.16)
Total mortality rate ratio (azithro/placebo, 95% CI)	0.95 (0.67-1.36)		0.94 (0.73-1.20)		1.06 (0.79-1.42)		0.73 (0.58-0.90)		1.07 (0.85-1.34)		0.92 (0.82-1.03)	
Pneumonia												
Pneumonia deaths	2	4	8	5	2	3	10	2	13	6	35	20
Pneumonia mortality rate	0.26 (0.07-1.05)	0.37 (0.14-0.97)	0.65 (0.33-1.30)	0.43 (0.18-1.04)	0.20 (0.05-0.79)	0.32 (0.10-1.00)	0.55 (0.30-1.03)	0.11 (0.03-0.45)	0.69 (0.40-1.18)	0.35 (0.16-0.77)	0.52 (0.38-0.73)	0.30 (0.19-0.46)
Pneumonia mortality rate ratio (azithro/placebo, 95% CI)	1.38 (0.25-7.56)		0.66 (0.22-2.02)		1.62 (0.27-9.69)		0.21 (0.04-0.94)		0.50 (0.19-1.33)		0.57 (0.33-0.99)	
Malaria												
Malaria deaths	13	18	30	43	40	30	64	44	51	48	198	184
Malaria mortality rate	1.71 (1.00-2.95)	1.64 (1.04-2.61)	2.44 (1.71-3.49)	3.71 (2.75-5.00)	3.97 (2.91-5.41)	3.21 (2.25-4.60)	3.54 (2.77-4.53)	2.50 (1.86-3.36)	2.69 (2.05-3.54)	2.82 (2.13-3.74)	2.96 (2.57-3.40)	2.75 (2.38-3.18)
Malaria mortality rate ratio (azithro/placebo, 95% CI)	0.96 (0.47-1.96)		1.52 (0.95-2.42)		0.81 (0.50-1.30)		0.71 (0.48-1.04)		1.05 (0.71-1.55)		0.93 (0.76-1.14)	
HIV/AIDS												
HIV/AIDS deaths	5	15	13	8	9	5	27	23	17	19	71	70
HIV/AIDS mortality rate	0.66 (0.27-1.58)	1.37 (0.83-2.27)	1.06 (0.61-1.82)	0.69 (0.34-1.38)	0.89 (0.46-1.72)	0.54 (0.22-1.29)	1.50 (1.03-2.18)	1.31 (0.87-1.97)	0.90 (0.56-1.44)	1.09 (0.70-1.72)	1.06 (0.84-1.34)	1.05 (0.83-1.32)
HIV/AIDS mortality rate ratio (azithro/placebo, 95% CI)	2.08 (0.75-5.71)		0.65 (0.27-1.57)		0.60 (0.20-1.79)		0.87 (0.50-1.52)		1.22 (0.63-2.35)		0.99 (0.71-1.37)	
Diarrhoea												
Diarrhoea deaths	11	11	15	8	12	7	19	13	22	17	79	56
Diarrhoea mortality rate	1.45 (0.80-2.62)	1.00 (0.56-1.81)	1.22 (0.74-2.03)	0.69 (0.34-1.38)	1.19 (0.68-2.10)	0.75 (0.36-1.57)	1.05 (0.67-1.65)	0.74 (0.43-1.27)	1.16 (0.76-1.76)	0.98 (0.61-1.58)	1.18 (0.95-1.47)	0.84 (0.64-1.09)
Diarrhoea mortality rate ratio (azithro/placebo, 95% CI)	0.69 (0.30-1.60)		0.56 (0.24-1.33)		0.63 (0.25-1.60)		0.70 (0.35-1.42)		0.84 (0.45-1.59)		0.71 (0.50-1.00)	

Appendix 4: Supplemental material to published manuscript on malaria

Supplemental Table 1: Individual level gametocytemia and gametocyte density (gametocytes/µl) in gametocytemic children analysed by intention-to-treat (unadjusted)

	Prevalence of gametocytemia				Gametocyte density (gametocytes/µl)			
	Placebo		Azithromycin		Placebo		Azithromycin	
Study phase	**N**	**Mean (95%CI)**	**N**	**Mean (95%CI)**	**N**	**Mean (95%CI)**	**N**	**Mean (95%CI)**
Baseline	565	6.0% (4.1-8.0%)	559	8.1% (5.8-10.3%)	34	18.0 (1.7-34.2)	45	6.4 (3.7-9.1)
12 months	548	5.1% (3.3-7.0%)	551	4.9% (3.1-6.7%)	28	6.5 (4.0-8.9)	27	5.8 (2.1-9.5)
24 months	559	3.4% (1.9-4.9%)	544	3.3% (1.8-4.8%)	19	12.6 (1.0-24.2)	18	3.4 (1.6-5.1)

Prevalence of gametocytemia: *P* = 0.35 comparing treatment arms at months 12 and 24 in mixed effects logistic regression model including fixed effects for age, mean baseline community prevalence of gametocytemia and study phase and nested random effects for individuals within communities [odds ratio in azithromycin compared to placebo-treated communities: 0.76 (95%CI 0.43 to 1.35); ICC = 0.04 (95% CI 0.01 to 0.20)]

Gametocyte density: *P* = 0.22 comparing treatment arms at months 12 and 24in mixed effects linear regression model including fixed effects for age, mean baseline community prevalence of gametocytemia and study phase and nested random effects for individuals within communities [3.3 gametocytes/µl lower in azithromycin-treated communities (95%CI -8.7 to 2.0); ICC < 0.01]

Supplemental Table 2: Individual level prevalence of gametocytemia and gametocyte density (gametocytes/µl) in gametocytemic children analysed per-protocol, including only those who received treatment at the previous phase (unadjusted)

	Prevalence of gametocytemia				Gametocyte density (gametocytes/µl)			
	Placebo		Azithromycin		Placebo		Azithromycin	
Study phase	**N**	**Mean (95%CI)**	**N**	**Mean (95%CI)**	**N**	**Mean (95%CI)**	**N**	**Mean (95%CI)**
12 months	391	5.1% (2.9-7.3%)	404	5.9% (3.6-8.3%)	20	6.4 (3.6-9.1)	24	6.0 (1.8-10.2)
24 months	384	3.1% (1.4-4.9%)	367	3.0% (1.2-4.7%)	12	5.8 (2.5-9.0)	11	2.8 (1.7-3.9)

Prevalence of gametocytemia: *P* = 0.61 comparing treatment arms at months 12 and 24 in mixed effects logistic regression model including fixed effects for age, mean baseline community prevalence of gametocytemia and study phase and nested random effects for individuals within communities [odds ratio in azithromycin compared to placebo-treated communities: 0.86 (95%CI 0.47 to 1.54); ICC = 0.04 (95% CI <0.01 to 0.35)]

Gametocyte density: *P* = 0.50 comparing treatment arms at months 12 and 24 in mixed effects linear regression model including fixed effects for age, mean baseline community prevalence of gametocytemia and study phase and nested random effects for individuals within communities [1.2 gametocytes/µl lower in azithromycin-treated communities (95%CI -4.9 to 2.4); ICC 0.04]

Supplemental Table 3: Hemoglobin in parasite-positive and parasite-negative children

	Placebo communities			Azithromycin communities		
Malaria parasitemia	**N**	**Mean Hb (g/dl) (95%CI)**	***P*-value***	**N**	**Mean Hb (g/dl) (95%CI)**	***P*-value***
Baseline						
Negative	404	10.8 (10.7-11.0)	<0.0001	386	10.8 (10.7-11.0)	<0.0001
Positive	160	9.6 (9.4-9.9)		173	10.0 (9.7-10.2)	
12 months						
Negative	359	10.8 (10.6-11.0)	<0.0001	345	10.8 (10.7-11.0)	<0.0001
Positive	188	9.9 (9.7-10.1)		204	9.9 (9.7-10.2)	
24 months						
Negative	394	11.1 (11.0-11.3)	<0.0001	397	11.1 (11.0-11.2)	<0.0001
Positive	164	10.1 (9.9-10.3)		147	10.1 (9.8-10.3)	

**P* values from Student's t-test comparing mean Hb in malaria infected and non-infected individuals by study phase and treatment group.

Appendix 5: Supplemental material to macrolide resistance manuscript under review

Supplementary table 1: Mean *S. pneumoniae* carriage, mean azithromycin resistance, and mean penicillin resistance at the 12-month and 24-month follow-up visits, assessed at the community level by treatment arm

	Mean *S. pneumoniae* carriage			Mean proportion of *S. pneumoniae* strains resistant to azithromycin			Mean proportion of *S. pneumoniae* strains resistant to penicillin		
	Proportion (95% CI)	*OR (95%CI)	*P*-value	Proportion (95% CI)	*OR (95%CI)	*P*-value	Proportion (95% CI)	*OR (95%CI)	*P*-value
12 months									
Placebo	79.7 (73.7-85.7)	1		21.0 (15.9-26.2)	1		37.6 (32.2-43.0)	1	
Azithromycin	81.6 (77.1-86.0)	1.12 (0.73-1.72)	0.59	38.2 (29.3-47.1)	2.32 (1.50-3.59)	<0.001	43.2 (37.3-49.2)	1.27 (0.94-1.71)	0.12
24 months									
Placebo	81.7 (76.6-86.7)	1		33.0 (26.9-39.0)	1		45.3 (36.3-54.3)	1	
Azithromycin	82.1 (76.4-87.9)	1.03 (0.65-1.64)	0.90	43.7 (35.7-51.7)	1.58 (1.08-2.31)	0.019	38.3 (31.2-45.4)	0.75 (0.49-1.15)	0.19

*OR from generalized linear models comparing community means