



INFECTIOUS DISEASES

Increased carriage of macrolide-resistant fecal *E. coli* following mass distribution of azithromycin for trachoma control

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Abstract

Background: Mass drug treatment with azithromycin (MDA) is part of the WHO-endorsed ‘SAFE’ strategy for trachoma control in endemic communities. MDA has been associated with reduced trachoma prevalence and short-term reductions in other bacterial infections, but can also lead to increased circulation of macrolide-resistant bacteria.

Methods: We prospectively monitored macrolide resistance in fecal *E. coli* collected from young children participating in the PRET+ Study in rural Tanzania. MDA was administered in four villages with >10% trachoma prevalence. Four nearby communities with lower trachoma prevalence served as controls. Rectal swabs were collected during cross-sectional surveys performed at baseline, 1, 3 and 6 months after MDA. Fecal *E. coli* isolates were screened for macrolide susceptibility using disc diffusion and minimum inhibitory concentration methods. Cross-sectional and longitudinal differences in resistance prevalence by MDA exposure were compared using t-tests and logistic regression.

Results: There was no difference in the proportion of individuals carrying azithromycin-resistant *E. coli* at baseline (0.21 vs 0.16, $P > 0.05$). Azithromycin resistance carriage prevalence remained stable over follow-up in non-MDA villages but increased sharply in MDA villages (0.61 at 1 month, 0.42 at 3 months and 0.31 at 6 months). MDA exposure was highly associated with azithromycin resistance carriage at 1 month post-MDA (OR 15.27, $P < 0.001$) and subsequent surveys. Younger age and recent diarrhoea were also associated with increased odds of resistance ($P < 0.01$).

Conclusions: MDA resulted in significantly increased prevalence of macrolide resistance in *E. coli*. Although MDA is effective for trachoma elimination, it has costs; it is essential to monitor antimicrobial resistance following MDA.

Key words: Azithromycin, *E. coli*, antimicrobial resistance, children, Africa

Key Messages

- Mass drug treatment with azithromycin (MDA) resulted in a 4-fold increase in carriage of macrolide-resistant strains of *E. coli* in a study of young children in rural Tanzania.
- The increase in resistance prevalence was highest 1 month after treatment and waned over 6 months, but was still elevated over baseline levels.
- Although MDA is effective for trachoma elimination, it has costs; it is essential to monitor antimicrobial resistance following MDA.

Introduction

Trachoma affects an estimated 40 million people worldwide and occurs in areas with poor sanitation and water access.^{1,2} Caused by ocular infection with *Chlamydia trachomatis*, repeated infections result in irreversible blindness. Mass drug treatment with azithromycin (MDA) is part of the World Health Organization (WHO)-endorsed SAFE strategy (Surgery for trichiasis, Antibiotic therapy, Facial cleanliness and Environmental improvements) for trachoma control. Communities with active trachoma prevalence >10% among children 1–9 years are targeted for MDA.³ Azithromycin (AZM) is a broad-spectrum macrolide antibiotic active against several bacterial species. Studies in Nepal, Ethiopia, the Gambia and Tanzania have shown beneficial effects of MDA on childhood illness morbidity and mortality.^{3–8}

A major concern regarding MDA is potential selection for and dissemination of antibiotic resistance among non-target bacteria. We recently reported AZM resistance prevalence of 82% in *Streptococcus pneumoniae* isolates 6 months post-MDA.⁹ Single- and multi-drug resistance has transformed many formerly routine organisms into emerging pathogens of considerable public health concern.^{10,11} Infections by resistant pathogens lead to longer hospital stays, higher costs and greater morbidity and mortality resulting from delayed or ineffective treatment.¹² Newer antimicrobial agents are expensive (thus unavailable) in low-income countries, meaning many infections are effectively untreatable.

Much of antibiotic resistance research has focused on pathogens and clinical isolates from persons with disease, but commensal bacteria can serve as important resistance reservoirs.¹³ Community-based studies of fecal *Escherichia coli* have demonstrated high levels of resistance in diverse settings in Africa, Asia and South America, even in populations with limited antibiotic access.^{14–17} However, cross-sectional studies are unable to assess temporal relationships between antimicrobial administration and resistance selection.

We hypothesized that MDA would result in increased carriage of macrolide-resistant *E. coli* strains by young children in rural Tanzania. We characterized the impact of MDA on the distribution of AZM and erythromycin (ERY) resistance levels and monitored the duration of resistance carriage over 6 months. Additionally, we evaluated individual, household and environmental characteristics as potential risk factors for resistance carriage.

Methods

Study overview and subjects

This study was nested within the PRET+ study, a longitudinal cohort study of the ancillary benefits of MDA for trachoma elimination.^{4,5} Briefly, PRET+ was conducted January–July 2009 in eight villages in Kongwa District, Tanzania. Over two-thirds of communities in Kongwa District were exposed to annual MDA in the preceding 2 years during a large clinical trial; PRET+ villages were not exposed for the preceding 6 years because trachoma prevalence was thought to be below the SAFE eligibility threshold.¹⁸ In the four PRET+ treatment villages, the MDA regimen was a single oral dose of azithromycin (20 mg/kg) administered to all residents except children <6 months old and pregnant women. It was considered unethical to use SAFE-eligible communities as controls, so four villages in the same geographical area with trachoma rates below the intervention threshold were selected for comparison.

Children aged <5 years were the sentinel group for incidence of diarrhoea, malaria and acute lower respiratory infections during the study period. Following a census, 130 households per village with at least one member aged <5 years were randomly selected. Within selected households, one child aged <5 years was randomly selected for the PRET+ child cohort. In each village, 40 children aged <3 years attending the baseline survey were enrolled in the *E. coli* cohort. The *E. coli* cohort was limited to children aged <3 years who were expected to have the greatest burden of diarrhoeal disease.

Data and specimen collection

Cross-sectional disease prevalence surveys occurred prior to MDA and 1, 3 and 6 months post-MDA. Mass treatment occurred 2 days after baseline in each village. At baseline, caregivers of study children were asked whether the child had diarrhoea in the preceding week. At all surveys, caregivers were asked whether the child currently had diarrhoea (defined as three or more liquid or semi-liquid stools in a 24-h period or visible blood in the stool).¹⁹ Study households were visited bi-weekly and administered a standardized questionnaire on illness in the previous 3 days including diarrhoea. Children with symptoms of acute lower respiratory infections were treated with amoxicillin and those with bloody diarrhoea were treated with chloramphenicol. The census collected information on the education of the head of household, household latrine ownership and walking time to water during the dry season. Water source types and household usage practices were assessed June–August 2009. We surveyed the eight dispensaries and medical shops in study villages to identify available antibiotics.

Rectal swabs were collected from the *E. coli* cohort at each survey. Due to the travel and work schedules of many study participants, it was not possible to contact subjects who did not attend the surveys and thus they were considered lost to follow-up. Swabs were placed into Cary-Blair and stored at 4°C once at the study office. Fecal specimens were subsequently transported to the Kongwa District Hospital laboratory for *E. coli* isolation and archiving, then shipped to Johns Hopkins University for further analysis. The time between swab collection and *E. coli* isolation was variable, subject to the availability of electricity and water at Kongwa Hospital.

Laboratory methods

Fecal specimens were streaked on MacConkey agar and grown overnight at 37°C. Up to three lactose-fermenting colonies were inoculated into nutrient agar stabs and grown overnight at 37°C followed by room temperature storage. Indole-positive, citrate-negative isolates were considered *E. coli*. ERY (15 µg) susceptibility was determined by disc diffusion on Mueller-Hinton agar. Zone diameters (ZD) were measured in millimetres. AZM minimum inhibitory concentrations (MIC) were determined using E-test strips. *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 and 29213 were used for quality control testing.

Statistical methods

Breakpoints to define macrolide resistance in *E. coli* have not been established. Initially, we took a conservative

approach to defining resistance: categorizing isolates with no growth inhibition as fully resistant and those with any inhibition as susceptible. Using this definition, cross-sectional resistance prevalence was compared by treatment group and time-point using t-tests; comparisons were made considering all isolates and at the person level (if any of the isolates from a given fecal specimen were resistant, that person was considered resistant). To better characterize the impact of MDA on macrolide resistance, Wilcoxon signed rank tests were used to compare the distributions of AZM MICs and ERY ZDs by treatment group. To identify an epidemiologically relevant resistance cutoff, a receiver operating characteristic (ROC) curve was generated for AZM MIC values using the conservative binary ERY categorization as the reference variable.²⁰

Descriptive analyses compared the distribution of demographic and water characteristics in the MDA and non-MDA villages at each follow-up using Fisher's exact tests and t-tests. Using logistic regression, the odds of conservatively-defined AZM resistance were modelled as a function of MDA treatment group, time-point and treatment group by time-point interaction. The outcome was modelled at the person level assuming a binomial variable representing the number of resistant isolates out of the total number of isolates analysed from each specimen. Generalized estimating equations with exchangeable correlation were used to adjust for village-level clustering, and robust standard errors were calculated to account for residual within-person correlation due to repeated measures.²¹ Additional regression models were considered that accounted for person- and household-level covariates derived from the census, illness history and water usage surveys. Covariates with $P < 0.05$ in the extended models were retained in the full multivariate model. In sensitivity analysis, the full model was re-fit with AZM resistance defined using the cutoff value determined by the ROC analysis.

A first-order Markov model was used to quantify the odds of transitioning to resistance as a function of treatment group and time. This transition model is a logistic regression model for any resistant isolate at the current follow-up as a function of resistance measured at the prior time-point, treatment group, time-point and treatment group by time-point interaction. Additional models were considered that accounted for person- and household-level covariates. Analyses were performed using STATA 12 (StataCorp, College Station, TX, USA).

Ethical review

This study was approved by the Tanzanian National Institute for Medical Research and the Committee for

Human Research of the Johns Hopkins School of Medicine. Study protocols and rationale were explained at community meetings in each PRET+ village; community leaders provided consent for village participation. Parents/guardians of study children gave written, informed consent prior to enrolment.

Results

At baseline, 320 children were enrolled in the *E. coli* cohort and 293, 281 and 257 participants, respectively, returned for 1-, 3- and 6-month follow-up surveys (Figure 1). Overall loss to follow-up between baseline and 6-month surveys was 20%. From 1151 rectal swabs, 2157 *E. coli* isolates were recovered from 318 unique participants; 76 participants had *E. coli* isolated at all four surveys. PRET+ MDA treatment coverage was 91% overall with coverage levels >93% in three of four villages; coverage was similar for the *E. coli* cohort, ranging 80–100% per village.

Demographic and household characteristics of the *E. coli* cohort are shown in Table 1. Mean age, household size, latrine ownership and water storage and retrieval methods were similar in MDA and non-MDA villages. Drinking water source was dropped from the analysis due to collinearity with village of residence. Average schooling for heads of household was greater in the non-MDA group (4.38 vs 3.11 years, $P < 0.001$). MDA village residents were farther from dry-season water sources (67% vs 13% reported walking time of >1 h; $P < 0.001$) and more likely to report boiling and/or filtering drinking water than non-MDA residents (67% vs 32%, $P < 0.001$). For ethical reasons, it was not possible to randomize villages to treatment

assignment; MDA villages were eligible for treatment due to the prevalence of trachoma, and village-level trachoma prevalence may be related to village characteristics such as access to water. In the medical shops and dispensaries, AZM was available only in one MDA village; ERY was available in two MDA and three non-MDA villages.

We evaluated macrolide resistance in both the population of *E. coli* isolates and at the person level (pooling information from strains isolated from the same fecal specimen). Approximately 20% of fecal specimens for which >1 isolate was available had both macrolide-susceptible and -resistant strains. At baseline, there was no difference between treatment groups in the percentage of subjects carrying *E. coli* isolates resistant to either macrolide (AZM: 21% vs 16%, $P = 0.387$; ERY: 23% vs 26%, $P = 0.598$) (Table 2). Isolate-level AZM-resistance prevalence was greater in non-MDA villages (19% vs 10%, $P = 0.004$). At 1 month post-MDA, 61% and 76% of individuals in MDA villages carried AZM- and ERY-resistant isolates, respectively, whereas carriage prevalence changed little from baseline in non-MDA villages. Three months post-MDA, the prevalences of person-level and isolate-level macrolide resistance were lower than at 1 month but still higher in MDA vs non-MDA villages (AZM: 42% vs 16%, $P < 0.001$; ERY: 55% vs 24%, $P < 0.001$). At the 6-month survey, isolate-level resistance was greater in MDA villages than non-MDA villages (AZM: 23% vs 12%, $P = 0.019$, ERY: 32% vs 17%, $P = 0.004$), but not significantly different at the person level.

We compared the distributions of AZM MICs and ERY ZDs in *E. coli* isolates from baseline with subsequent surveys by treatment group (Figure 2). The distributions

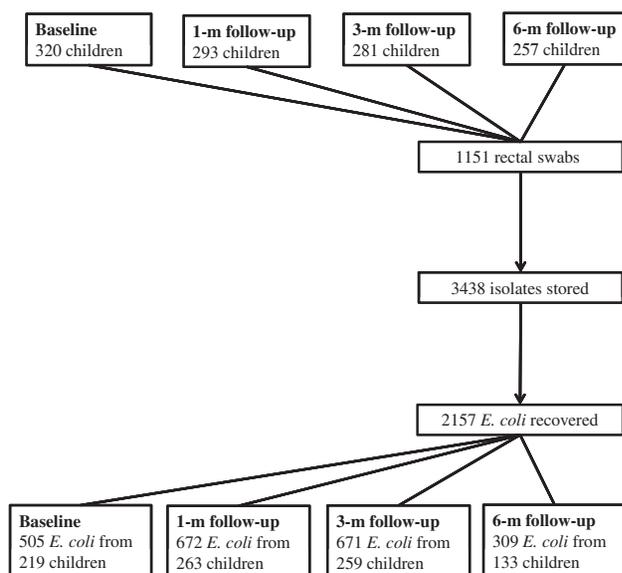


Figure 1. Flow chart of specimen collection and *E. coli* isolation.

Table 1. Study population characteristics in MDA- and non-MDA-exposed groups

Population	MDA	Non-MDA	<i>P</i> -value*
Mean age (years) at baseline	0.98	1.11	0.149
No. of household members	5.41	5.70	0.188
% of households with latrine	68%	73%	0.252
Mean years of education of head of household	3.11	4.38	<0.001
Walking time to dry season water source			
<30 min	13%	42%	<0.001
30 m–1 h	20%	45%	
>1 h	67%	13%	
Drinking water treated	67%	32%	<0.001
Open container used for water storage	6%	10%	0.203
Method of removing water from storage container (poured)	18%	11%	0.098

**P*-values calculated from *t* tests and Fisher's exact tests.

Table 2. Comparison of macrolide resistance prevalence in 2157 *E. coli* isolates from 318 children, by treatment group and time

	Resistance to azithromycin						Resistance to erythromycin					
	Person level			Isolate level			Person level			Isolate level		
	Non-MDA (N)	MDA (N)	P-value*	Non-MDA (N)	MDA (N)	P-value*	Non-MDA (N)	MDA (N)	P-value*	Non-MDA (N)	MDA (N)	P-value*
Baseline	21% (96)	16% (123)	0.387	19% (205)	10% (300)	0.004	23% (96)	26% (123)	0.598	17% (205)	17% (300)	0.981
1 month	19% (134)	61% (129)	<0.001	14% (325)	44% (347)	<0.001	28% (134)	76% (129)	<0.001	20% (325)	63% (347)	<0.001
3 months	16% (126)	42% (133)	<0.001	10% (324)	30% (347)	<0.001	24% (126)	55% (133)	<0.001	16% (324)	43% (347)	<0.001
6 months	20% (50)	31% (83)	0.155	12% (118)	23% (191)	0.019	26% (50)	39% (83)	0.138	17% (118)	32% (191)	0.004

*P-values calculated from two-sample test of proportions.

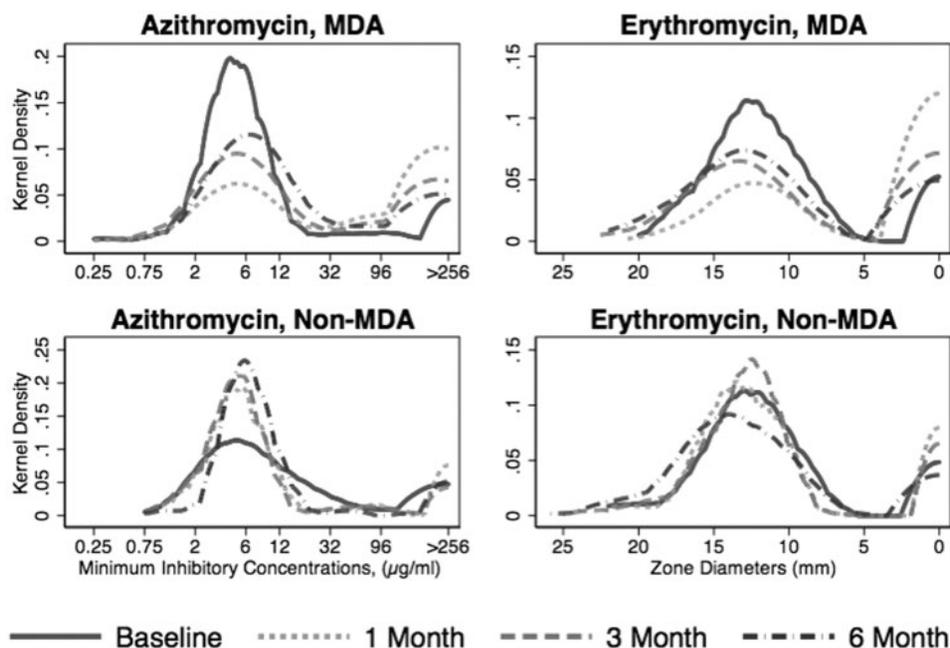


Figure 2. Distribution of macrolide susceptibility seen in 2157 *E. coli* isolates by treatment group and time-point. The x-axis scale of the ERY plots is reversed to facilitate comparison with the AZM distributions. AZM minimum inhibitory concentrations plotted on a linear scale.

appear bimodal, with the primary peak composed of macrolide-susceptible isolates and a smaller peak at the right tail of the distribution representing resistant isolates. In MDA village isolates, the distribution of AZM MICs differed at each follow-up survey compared with baseline ($P < 0.001$) with more of the observations shifted to the right tail. Similarly, the distribution of ERY ZDs from MDA isolates differed from baseline at the 1-month ($P = 0.017$) and 3-month ($P < 0.001$) follow-up surveys.

We generated a ROC curve for different AZM MIC cut-offs using the binary ERY classification as the reference variable (Supplementary Figure S1, available as Supplementary data at *IJE* online). Using a cutoff of MIC $\geq 32 \mu\text{g/ml}$ to classify an isolate as AZM-resistant yielded the greatest percentage of correctly classified isolates (97.4%) with a sensitivity of 95.9% and a specificity of

98.1%. After MDA, we observed a lower proportion of AZM-susceptible isolates (MICs $< 32 \mu\text{g/ml}$); however the median ZDs for susceptible isolates (MICs $< 32 \mu\text{g/ml}$) ranged from 4 to 6 mg/ml regardless of time-point or treatment, indicating that the exposure to MDA did not shift the location of the distribution of susceptible isolates.

The base logistic model, using conservatively defined AZM resistance, yielded similar results to crude prevalence comparisons (Table 3). Residents of MDA villages were more likely to carry AZM-resistant isolates at each follow-up survey, with the largest treatment effect at 1 month post-MDA [odds ratio (OR) 10.38, 95% confidence interval (CI): 6.09, 17.70]. Although this effect decreased over follow-up, the odds of carrying resistant isolates were still elevated above baseline levels 6 months after MDA (OR 4.54, 95% CI: 1.83, 10.90). We tested the association

Table 3. Multivariate logistic regression models to evaluate risk factors for AZM resistance

Potential risk factors (reference group)	Variable definition	Base model OR [95% CI]	Base model + 1 additional covariate OR [95% CI]	Full model OR [95% CI]	Full model AZM ^R ≥32 µg/ml ^a OR [95% CI]
1 month		0.70 [0.41, 1.18]		0.79 [0.53, 1.18]	0.78 [0.48, 1.28]
3 months		0.45 [0.17, 1.18]		0.46 [0.23, 0.94]	0.59 [0.53, 0.66]***
6 months		0.58 [0.49, 0.69]***		0.69 [0.40, 1.19]	0.52 [0.28, 0.98]*
Residence in MDA village		0.46 [0.26, 0.81]**		0.39 [0.21, 0.75]**	0.42 [0.19, 0.90]*
MDA* 1 month		10.38 [6.09, 17.70]***		11.21 [7.13, 17.63]***	15.27 [6.96, 33.50]***
MDA* 3 months		8.80 [2.89, 26.73]***		10.64 [3.79, 29.92]***	8.58 [4.36, 16.87]***
MDA* 6 months		4.54 [1.83, 10.90]***		4.76 [1.52, 14.90]***	6.98 [1.63, 29.96]***
Age (years) of participant at baseline	Continuous (0-2)		0.68 [0.57, 0.80]***	0.70 [0.61, 0.82]	0.80 [0.68, 0.94]**
Years of education of head of household	Continuous (0-12)		0.99 [0.94, 1.05]		
No. of household members	Continuous (2-11)		1.02 [0.96, 1.09]		
No. of children aged <10 years in household	Continuous (1-6)		1.12 [0.96, 1.31]		
Diarrhoea in the past 7 days	Binary		1.77 [1.17, 2.67]**	1.50 [1.11, 2.03]**	1.29 [1.05, 1.58]*
Lattrine in household	Binary		0.84 [0.61, 1.15]		
Distance to water during the dry season (<30 min)	30 min-1 h		1.18 [0.68, 2.05]		
	≥ 1 h		0.95 [0.63, 1.44]		
Treatment of drinking water (none)	Boil and/or filter		1.23 [1.02, 1.47]*	1.18 [0.93, 1.49]	1.10 [0.89, 1.37]
Type of water storage container (closed)	Open		0.63 [0.44, 0.89]**	0.53 [0.38, 0.72]***	0.73 [0.52, 1.02]
Water removal method (dip into container)	Pour from container		1.12 [0.73, 1.73]		
Amoxicillin in the prior 7 days ^b	Binary		1.48 [0.87, 2.51]		
Chloramphenicol in the prior 7 days ^b	Binary		1.27 [0.12, 13.32]		

^aIn primary analysis, AZM resistance defined as MIC >256 µg/ml; less conservative outcome AZM resistance defined as MIC ≥32 µg/ml.

^bExposure to therapeutic antibiotics; results similar for prior 14 days and 30 days.

* <0.05, ** <0.01, *** <0.001.

between individual, household and water-related characteristics with carriage of AZM-resistant isolates. When added to the base model as a single additional covariate, older age and water storage in an open container were associated with reduced odds of AZM resistance; reporting diarrhoea within the prior week (including survey day) and boiling/filtering drinking water were associated with increased odds of resistance. Schooling of the head of household, household composition, latrine ownership, walking time to water source, water retrieval method and recent exposure to therapeutic antibiotics were not associated with carriage of AZM-resistant isolates. In the full multivariate model, older age (OR 0.70, 95% CI: 0.61, 0.82) and water storage in open containers (OR 0.53, 95% CI: 0.38, 0.72) were associated with lower odds of AZM resistance. Diarrhoea in the prior week was associated with increased odds of resistance (OR 1.50, 95% CI: 1.11, 2.03). We refit the full multivariate model using AZM MIC ≥ 32 $\mu\text{g/ml}$ to define resistance. The parameter estimates were similar to the model using the conservative resistance definition; however, estimates for treatment effects at the 1-month and 6-month follow-up surveys increased to 15.27 (95% CI: 6.96, 33.50), and 6.98 (95% CI: 1.63, 29.96), respectively, and the water storage variable was no longer associated with resistance.

To assess the duration of resistance within an individual, we evaluated the relationship between AZM resistance status at the prior survey to current status (Supplementary Table S1, available as Supplementary data at IJE online). The odds of resistance at the current survey were only 5% greater if resistant isolates were carried at the prior survey, regardless of treatment group, age and recent diarrhoea, and this association was not statistically significant (OR 1.05, 95% CI: 0.75, 1.46). The estimate increased with resistance defined as MIC ≥ 32 $\mu\text{g/ml}$ but was not statistically significant (OR 1.20, 95% CI: 0.76, 1.88).

Discussion

Our study suggests that MDA with a single dose of azithromycin resulted in a substantial increase in carriage of macrolide-resistant *E. coli*, with $\geq 60\%$ of exposed individuals carrying resistant strains 1 month post-MDA. Whereas resistance carriage tapered over the follow-up period, macrolide resistance remained elevated over baseline levels 6 months after dosing. To our knowledge, no published studies have investigated the development of macrolide resistance in fecal *E. coli* following mass azithromycin treatment.

Our results align with studies of *S. pneumoniae* post-MDA. In the PRET+ child cohort, prevalence of AZM-resistant *S. pneumoniae* increased from 49% at baseline to

82% at 6 months and was significantly higher in the MDA villages compared with non-MDA villages at all post-treatment surveys.⁹ Following six rounds of MDA in Ethiopia, prevalence of AZM resistance in *S. pneumoniae* was 77% after 6 months; prevalence declined to 31% and 21% 12 and 24 months, respectively, after the last treatment.²² An earlier Australian study similarly observed tapering of AZM-resistant *S. pneumoniae* carriage from 55% at 2–3 weeks to 6% at 6 months following single-dose AZM treatment given to children with trachoma and household contacts.²³ AZM resistance has not been seen in ocular *C. trachomatis* following MDA although macrolide resistance has been observed in urogenital clinical isolates.^{24–27}

Younger age was associated with greater odds of carrying AZM-resistant *E. coli* despite the relatively narrow age range of our study population. Other studies have shown a similar association between younger age and risk of carriage of single- or multi-drug resistant *E. coli*.^{28,29} Younger children may have more exposure to circulating environmental strains via fecal oral routes of transmission. Diarrhoea in the week before the survey was associated with increased odds of resistance carriage. Children experiencing diarrhoea may have acquired resistant *E. coli* from the environment, or may have been infected with resistant organisms. A Tanzanian study of diarrhoeagenic *E. coli* showed high levels of resistance to common antibiotics, although the study did not assess macrolide resistance.³⁰

Whereas MDA was associated with a sustained increase in village-level macrolide resistance, within individuals AZM, resistance status was not stable during follow-up. Increased village-level macrolide resistance may have resulted from transmission events within MDA communities rather than long-term carriage of resistant strains in individuals. It is likely that both human and environmental sources contribute to resistance transmission. Since macrolide resistance prevalence declined over follow-up, there may be a threshold resistance prevalence required to sustain circulation of resistant strains. Sanitation and hygiene interventions in conjunction with MDA programmes could potentially limit transmission of resistant strains, thus speeding the decline of macrolide resistance carriage.

We identified 32 $\mu\text{g/ml}$ as an epidemiologically meaningful cutoff to designate resistance in community-based screening.²⁰ The Clinical Laboratory Standards Institute (CLSI) and other standards organizations do not publish susceptibility cutoffs to define macrolide resistance in Enterobacteriaceae. Some studies reporting macrolide resistance in *E. coli* and *Shigella* spp. do not describe the resistance definition; others used definitions for non-enteric bacteria, potentially resulting in misclassification.^{31–35} Ranges of macrolide MICs reported for *E. coli* vary widely,

from 0.03 µg/ml to >1024 µg/ml.^{14,34,36} Hoge *et al.* report that all AZM-resistant enterotoxigenic *E. coli* and *Salmonella* isolates tested had MICs >64 µg/ml.³⁵ A resistant strain of *S. sonnei* associated with AZM treatment failure had an MIC of 64 µg/ml.³⁷

We sampled study participants four times in 6 months and may have missed interim changes in resistance status. For the 6-month survey, isolates were available principally from three villages (two MDA and one non-MDA village). Due to limited resources, we conducted antimicrobial sensitivity assays on up to three isolates per fecal sample, which may have affected our estimates of the prevalence of resistance carriage. Our logistic regression models were weighted by the number of isolates tested to improve treatment effect size estimates. The variability in *E. coli* isolation times may have affected our ability to detect certain strains; we do not know whether storage duration would differentially affect isolates based on macrolide susceptibility.

Study subjects may have been exposed to macrolide antibiotics apart from MDA. AZM was not widely available for purchase in the PRET+ villages, but ERY was more common. If non-MDA village residents were more likely to purchase macrolide antibiotics, resulting in increased resistance carriage, this would minimize the observed treatment effect. Since the PRET+ study provided free treatment for common illnesses in all villages, study subjects likely utilized PRET+ provided services rather than purchasing treatment.

Our information about water usage patterns was cross-sectional. We did not capture temporal variability in characteristics that may be affected by seasonal water availability. Our water questionnaire was not piloted prior to field use so may not have captured all relevant information. In future work we would like to collect water samples and in-depth information about water and hygiene practices (particularly handwashing, food preparation and water collection) to determine how these factors relate to transmission of resistance.

Trachoma control programmes should be aware that MDA gives rise to macrolide resistance in other bacteria. Evidence suggests that, over time, without additional exposure *E. coli* macrolide resistance levels decay, although the underlying resistance genes may persist. However, many communities participating in trachoma control programmes receive repeated MDAs, sometimes multiple courses within 1 year. AZM is a broad-spectrum drug used in a variety of bacterial infections, so its effectiveness must be preserved. It is critical to monitor macrolide resistance levels in MDA communities. The cost of MDA-associated antibiotic resistance should be weighed against the risk of trachoma, especially in low prevalence communities.

Policy makers should establish decision-making thresholds to consider shifting from MDA to other components of the SAFE strategy, particularly sanitation and hygiene interventions.

Supplementary Data

Supplementary data are available at *IJE* online.

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Conflict of interest: None declared.

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