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A Cross-Sectional Study of the Availability of Azithromycin in Local Pharmacies and Associated Antibiotic Resistance in Communities in Kilosa District, Tanzania

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Abstract. Mass drug administration (MDA) for trachoma control using azithromycin has generated concern for the development of resistant organisms. However, the contribution from azithromycin available in local pharmacies has not been studied. In Kilosa district, Tanzania, MDA stopped over 4 years ago, and this study sought to determine the availability of azithromycin in local pharmacies and correlate it with azithromycin resistance in children born after MDA. A cross-sectional survey was conducted in 644 randomly selected hamlets in Kilosa district, in which the presence of a pharmacy and the availability of azithromycin and erythromycin were determined. In 30 randomly selected hamlets, a random sample of 60 children less than 5 years were tested for azithromycin-resistant *Streptococcus pneumoniae* (*Spn*) and *Escherichia coli* (*Ec*), from nasopharyngeal and rectal swabs, based on disk diffusion criteria. Only 26.6% of hamlets had a pharmacy. Azithromycin and erythromycin were available in 30.8% and 89.1% of pharmacies closest to the hamlets, respectively. In the 30 communities tested for resistance, the overall prevalence of azithromycin-resistant *Spn* isolates was 14%. Six of seven (87%) hamlets where azithromycin was available had resistant *Spn,* compared with 14 of 23 (61%) hamlets without availability. Similarly, six of seven (87%) hamlets where azithromycin was available had resistant *Ec* isolates compared with 21 of 23 (70%) hamlets without availability. However, the differences were not statistically significant ($P = 0.46$ and 0.49, respectively). The availability of azithromycin in pharmacies in the district was limited, and a strong correlation with azithromycin-resistant *Spn* or *Ec* was not observed.

INTRODUCTION

Mass drug administration (MDA) of azithromycin, whereby a single dose (20 mg/kg) is administered annually to all residents in a trachoma-endemic district until the prevalence in children aged 1–9 years falls below 5%, is central to trachoma control.[1] Despite its established efficacy, there is concern for the development of antibiotic resistance, particularly in *Streptococcus pneumoniae* (*Spn*) and *Escherichia coli* (*Ec*).[2,3]

Although resistance of *Chlamydia trachomatis* to azithromycin in the context of trachoma control has not been reported,[4] studies have found a greater than 2-fold rise in the proportion of children colonized with macrolide-resistant *Spn* 6 months following MDA.[5] When azithromycin was used in multiple rounds of MDA in Ethiopia, prevalence of resistance was high in treated children and remained elevated (> 20% prevalence) for at least 2 years after MDA cessation.[6]

However, the contribution of freely available antibiotics in local pharmacies in these settings to the development of resistance has not been studied, and the availability of azithromycin in these pharmacies is relatively unknown.

In Kilosa district, Tanzania, 4 years after the cessation of four consecutive years of MDA of azithromycin, a study found the prevalence of antibiotic resistance in *Spn* isolates to be 14% in children aged less than 5 years; most these children were born after MDA had ceased.[7] Given the persistence of resistance in the district years after cessation of MDA, the aim of this study was to determine the prevalence of local pharmacies in hamlets in the district, the frequency of azithromycin availability in the pharmacies, and to correlate the presence of azithromycin in the pharmacy with the prevalence of resistance in children aged less than 5 years.

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MATERIALS AND METHODS

Ethics statement. This study was approved by the Johns Hopkins School of Medicine Institutional Review Board and the Tanzania National Institute for Medical Research. Written informed consent was provided to all participants, and the study was conducted according to the tenets of the Declaration of Helsinki.

Setting and population. There were 706 hamlets in Kilosa district, Tanzania, ranging in size from 1,000 to 2,000 residents. The hamlets were grouped into larger "villages" that ranged in population size from 3,000 to more than 10,000. Kilosa district had active trachoma and underwent 4 years of annual MDA, until the prevalence of trachoma decreased to less than 5% in 2010 and MDA was no longer deemed necessary. In June 2014, a trachoma surveillance study found that the prevalence of trachoma in children ages 1–9 years was 0.4%.

In November 2014, a complete census of all children between the ages of 1 month to 59.9 months was conducted in 644 randomly selected hamlets, preparatory to a clinical trial.[8] In 30 randomly selected hamlets, a random sample of 60 children between the ages of 1 month to 59.9 months were invited to the baseline survey, or all children were invited if the total was less than 60. The baseline survey included testing for resistance to azithromycin in isolates of *Ec* from rectal swabs and isolates of *Spn* from nasopharyngeal swabs in randomly selected children. Nasopharyngeal and rectal specimens were collected and stored as previously described.[7]

Antimicrobial susceptibility testing. *Streptococcus pneumoniae* was isolated from nasopharyngeal swabs with blood agar containing 5% sheep blood and 5 mg/L gentamicin (Becton Dickinson, Cockeysville, MD) and incubated at 37°C in 5% CO_2 for 18–24 hours. The *Spn* isolates were tested for sensitivity to azithromycin (15 µg disk) using the Kirby–Bauer disk diffusion method on Mueller–Hinton

media with 5% lysed sheep blood. The isolates were classified as "sensitive (> 17 ZD)," "intermediately resistant (≥ 14, ≤ 17 ZD)," or "resistant (< 14 ZD)" using inhibitory zone diameters based on the recommendations of the Clinical and Laboratory Standards Institute.[9] The rectal swabs were cultured for Ec on MacConkey agar overnight at 37°C. Resistance was defined as a zone diameter < 10 mm.

Drug shop/pharmacy surveys. All of the 644 hamlets were surveyed for the presence of a pharmacy or small shop that sells medications. The hamlets were categorized as to whether or not a pharmacy was present in the hamlet. If more than one pharmacy was present in the hamlet, a single pharmacy was selected at random for the survey. Data were collected by a trained interviewer who observed the availability of azithromycin tablets and/or liquid, as well as erythromycin tablets and/or liquid in each pharmacy. In the 30 randomly selected hamlets where the resistance study was undertaken, all pharmacies were queried about the availability of azithromycin and erythromycin. Of note, a prescription is not required to access antibiotics in the local pharmacies. As a quality check, a sample of five pharmacies were revisited to determine the presence or absence of azithromycin, and no discrepancies were found.

Additional data. Using Samsung Galaxy 4 and Nexus 7 tablets (Samsung Electronics, Suwon, South Korea), latitude and longitude data were collected from the built-in global positioning system (GPS) transmitter and satellite-based systems for all households in each hamlet, and an average was used to characterize the location of each hamlet.

Analysis. Data were entered into custom-built Access databases (Microsoft Corp, Redmond, WA) and analyzed with SAS software (SAS, Raleigh, NC). An algorithm based on our mapping was used to model the availability of azithromycin in the hamlet: If a pharmacy was present in a hamlet, data on availability were used for that hamlet. If there was no pharmacy present in a hamlet and there was at least one other hamlet within the same village with a pharmacy, the closest pharmacy's data on availability were used. If there was no pharmacy present in any hamlet in the village, data on availability were used from the nearest hamlet with a pharmacy in another village.

The percentage of hamlets in Kilosa district with a pharmacy was calculated, and the percentage of those pharmacies that had azithromycin or erythromycin determined. These were geo-positioned on a map using Google Earth.

The association of the presence or absence of a pharmacy with azithromycin with the presence, or percentage, of resistant isolates was examined at hamlet level. Significance was tested using a Fisher's exact test. In addition, Fisher's exact test was used to determine if there was any association between the presence of resistant isolates of Spn and Ec, and hamlet location within 10 km of a main road.

RESULTS

Of the 644 hamlets surveyed, 171 (26.6%) had at least one pharmacy. Of these, 28.1% or 41 had at least one form of azithromycin, and 88.9% had erythromycin. Of the 171 pharmacies, there were 44 (25.7%) pharmacies where both drugs were available, 108 (63.2%) where only erythromycin was available, four (2.3%) where only azithromycin was available, and 15 (8.8%) where neither was available. In summary, only 41 of 688 (6%) of hamlets had a pharmacy that carried azithromycin.

The majority of pharmacies, 69% (118/171) were within 10 km of a main road. The higher prevalence of azithromycin availability in pharmacies within 10 km of a main road was not statistically significant (P = 0.58) (Table 1).

Using GPS to locate the closest pharmacy to every hamlet, 30.8% and 89.1% of the 644 hamlets had access to a pharmacy with azithromycin or erythromycin available, respectively, even if the pharmacy was not within the hamlet itself. The randomly selected hamlets had comparable levels of access as the rest of the hamlets to azithromycin (23.3% versus 31.1%) and erythromycin (90.0% versus 89.1%) (Table 2).

In the 30 randomly selected hamlets where the resistance study was undertaken, six of seven (87%) of hamlets where azithromycin was available had resistant Spn as compared with 14 of 23 (61%) hamlets without availability. Similarly, six of seven (87%) hamlets where azithromycin was available had resistant Ec isolates compared with 21 of 23 (70%) hamlets without availability. However, the differences were not statistically significant (P = 0.46 and 0.49, respectively). In addition, there was no statistically significant association between the proportion of isolates that were resistant in the hamlet and hamlet location within 10 km of a main road for both Spn (P = 0.88) and Ec (P = 0.40) (Table 3).

The spatial relationships between hamlets with Spn and Ec isolates resistant to azithromycin, and hamlets with azithromycin availability, which were not part of the resistance study, can be visualized in Figure 1. There is no clear resistance pattern seen when all the pharmacies with azithromycin are included in the map.

DISCUSSION

The contribution of azithromycin availability in local pharmacies to the finding of ongoing resistance after cessation of MDA 4 years prior appears to be small. First, the availability of a pharmacy at all in the local hamlets was relatively small; only 27% of hamlets had pharmacies. Second, the number of pharmacies in which azithromycin was present was even fewer, 28% of the 171 pharmacies. Even after allowing for access to pharmacies in nearby hamlets, less than a third of

TABLE 1
Availability of azithromycin and erythromycin in all 171 pharmacies surveyed, by distance to a main road

Antibiotic	n (%)	Pharmacy ≤ 10 km to main road (N = 118)	Pharmacy > 10 km to main road (N = 53)	P-value
Azithromycin availability				
At least one form available	48/171 (28.1)	35/118 (29.7)	13/53 (24.5)	Fisher's exact test **0.58**
No azithromycin available	123/171 (71.9)	83/118 (70.3)	40/53 (75.5)	
Erythromycin availability				
At least one form available	152/171 (88.9)			
No erythromycin available	19/171 (11.1)			

TABLE 2
Availability of azithromycin and erythromycin in the pharmacy closest to the hamlet

	Kilosa district (excluding randomly selected hamlets) (N = 614)	Randomly selected hamlets (N = 30)	Overall (N = 644)
Percentage of hamlets with azithromycin available	191/614 (31.1)	7/30 (23.3)	198/644 (30.8)
Percentage of hamlets with erythromycin available	547/614 (89.1)	27/30 (90.0)	574/644 (89.1)

the hamlets had access to a pharmacy that carried azithromycin. Finally, the findings suggested that the few hamlets with access to a pharmacy with azithromycin were more likely to have isolates with resistant strains of Spn or Ec, but the finding was not statistically significant. All the pharmacies in the study were geo-mapped in the district according to whether they had azithromycin or not, and the spatial relationship to the hamlets with isolates resistant to azithromycin, but again no pattern was observed.

There are a variety of possible reasons as to why antimicrobial resistance is present in the index hamlets. Following multiple rounds of MDA, investigators have found a slow decline in resistance 4 years after MDA for trachoma control had ended.[6] The fact that Kilosa district had undergone multiple rounds of MDA in the past is the most likely contributor to ongoing resistance in these communities. In addition, erythromycin was readily available in the pharmacies. Cross-resistance between erythromycin and azithromycin can develop and contribute to resistance.[10] It is also possible that the observed resistance developed from a transfer of resistance and housekeeping genes from related pneumococci and/or other species.[11] The contribution of any one of these mechanisms to the level of resistance in the district is uncertain.

The availability of azithromycin and erythromycin in local pharmacies suggests nonprescription use of antibiotics is present in the district. The cost of a course of azithromycin was $5,000 TSH (~$2 USD at time of writing), whereas a course of erythromycin was even less. The technician on duty was not queried to find out the indications for which azithromycin or erythromycin would be provided to a customer, but when asked to see them, the technicians provided them freely. As the hamlets grow and more pharmacies open, the practice of nonprescription use of antibiotics will likely increase.

There is evidence showing that the use of antibiotics, including macrolides, in food animals plays a role in the colonization of antibiotic-resistant bacteria, such as E. coli, in humans through exposure or consumption.[12] In northern Tanzania, antimicrobial use in animals is relatively common, especially among agropastoral groups where there may be little regulation or involvement of veterinary professionals. In addition, the continued consumption of meat and milk from animals during and shortly after antimicrobial treatment is common among certain Tanzanian ethnic groups.[13] However, widespread antibiotic use in animals in Kilosa district is unlikely given that the population is socioeconomically marginalized. Nevertheless, purchase of animals and migration of herds is very common and could lead to the spread of resistant isolates. Improper handling and cooking of animal products, as well as poor infection control and unsanitary conditions can lead to the spread of antimicrobial resistance.[14]

The study had limitations. First, it did not consider the availability of azithromycin in every pharmacy in Kilosa district; as the sample included 644 hamlets of the 706, and there were a few hamlets where there was more than one pharmacy, data were collected from one randomly selected pharmacy. Nonetheless, the sample is representative and large relative to the district, so the relative scarcity of azithromycin is likely a reasonable conclusion. In the random sample of 30 pharmacies, only two hamlets had more than one pharmacy and complete data were collected from all of them. For the resistance component, the status of the hamlet pharmacy is known for those hamlets.

Second, because each pharmacy was visited on only one occasion, it is possible that a pharmacy could have run out of its supply of azithromycin when the survey was conducted. The interviewer asked to see the tablets and liquid to verify that they were present. Based on the packaging, it was apparent that none of the azithromycin was diverted from the National Trachoma Control program functioning elsewhere in the country. However, it is possible that the pharmacies that denied having azithromycin did so to avoid drawing attention, if the drug had been obtained from a non-authorized source.

Third, the estimation of proximity of each pharmacy to a hamlet was based on straight line distance. Although terrain

TABLE 3
Azithromycin resistance based on azithromycin availability and proximity to a main road in 30 randomly selected hamlets

| Presence of resistant isolates in hamlet	Azithromycin available in local pharmacy			Distance from center of hamlet to a main road		
	Yes (N = 7)	No (N = 23)	P-value	≤ 10 km (N = 20)	> 10 km (N = 10)	P-value
Streptococcus pneumoniae						
None	1/7 (14.3)	9/23 (39.1)	Fisher's exact test **0.46**	6/20 (30.0)	4/10 (40.0)	Fisher's exact test **0.88**
> 0 to < 10%	2/7 (28.6)	3/23 (13.0)		4/20 (20.0)	2/10 (20.0)	
≥ 10%	4/7 (57.1)	11/23 (47.8)		10/20 (50.0)	4/10 (40.0)	
Escherichia coli						
None	1/7 (14.3)	2/23 (8.7)	Fisher's exact test **0.49**	2/20 (10.0)	1/10 (10.0)	Fisher's exact test **0.40**
> 0 to < 10%	0/7 (0.0)	5/23 (21.7)		2/20 (10.0)	3/10 (30.0)	
≥ 10%	6/7 (85.7)	16/23 (69.6)		16/20 (80.0)	4/10 (40.0)	
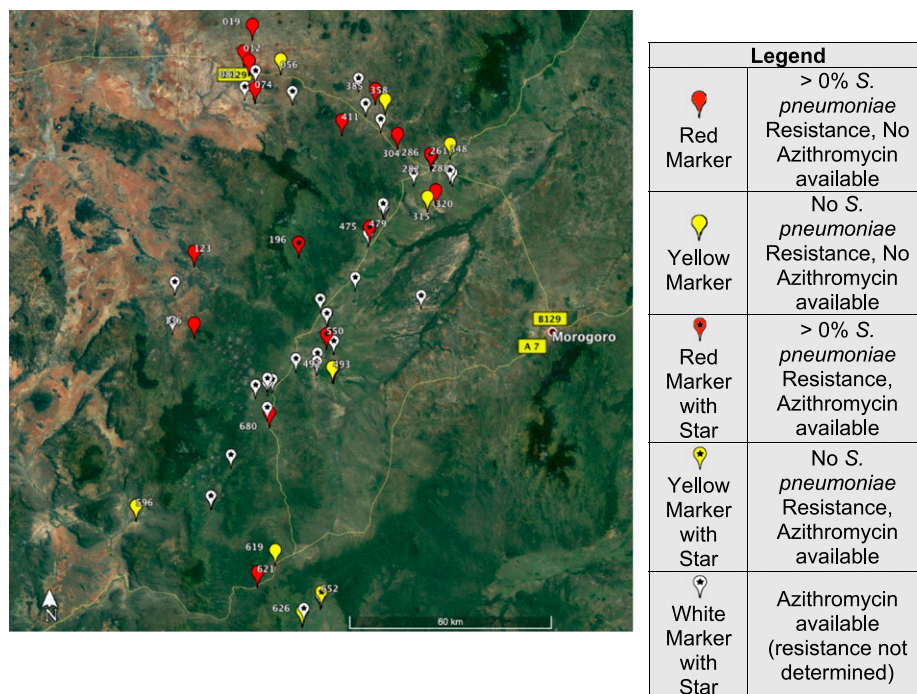
FIGURE 1.   Map of hamlets with *Streptococcus pneumoniae* resistance and azithromycin availability. Map data: Google, Landsat/Copernicus. This figure appears in color at www.ajtmh.org.

was considered, many households were dispersed in a hamlet and a lack of clearly defined roads limited our estimation of each hamlet's closest pharmacy; however, the proximity was confirmed by checking with the field staff in the event of uncertainty. Finally, with 30 hamlets contributing to the resistance data, and few hamlets with access to pharmacies that had azithromycin, we were limited in our power to detect an association between resistance and local azithromycin availability.

The study has strengths to support its findings. Foremost, 644 of 706 hamlets in the district were surveyed, thus providing a comprehensive evaluation of pharmacy coverage, and availability of azithromycin and erythromycin in Kilosa district. The use of GPS data for each hamlet with resistance to azithromycin strengthened the finding that the availability of azithromycin through nonprescription outlets was a modest, at best, contributor to the presence of azithromycin resistance.

In conclusion, this study suggests there was likely limited impact of the availability of azithromycin in pharmacies on the prevalence of azithromycin-resistant bacteria in Kilosa district.

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