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Full Length Research Paper

An evaluation of the dynamics of insecticide resistance and the rate of *kdr* metamorphosis of the primary malaria vector *Anopheles arabiensis* in rural villages of Lower Moshi, North Eastern Tanzania

***Mizengo H. Kikwete, Bernard O. Pinda and John Membe**

Department of Medical Parasitology, Faculty of Medicine, University of Dar es Salaam, Dar es Salaam, Tanzania.
Corresponding Authors. Email: dr_kikwete22@yahoo.com

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The major foci of pyrethroid resistance in 1990 to 2010 were in West and Central African populations of *Anopheles gambiae s.s.* Pyrethroid resistance in *Anopheles arabiensis* has been reported in several countries of East and Central Africa. Four cross-sectional surveys of *A. arabiensis* in Lower Moshi were conducted in 2009, 2011, 2012 and 2013 to determine levels of resistance to pyrethroids, organochlorines, organophosphates and carbamates using World Health Organization (WHO) standard diagnostic dosages. Mosquitoes were identified to species level and genotyped for both L1014F and L1014S mutations by hydrolysis probe assays. *A. arabiensis* remains the dominant malaria vector in the area. Full susceptibility to dichlorodiphenyltrichloroethane (DDT), organophosphates and carbamates was recorded in all eight villages. Following the current WHO guidelines, resistance to permethrin and lambdacyhalothrin was observed in 2009 with mortality rates ranging from 80 to 90% and from 66 to 97% for lambdacyhalothrin. Reduced susceptibility to deltamethrin was observed (87 to 97% mortality). The percentage mortality to permethrin, deltamethrin, lambdacyhalothrin was less than 90% in 2013 in all villages except in one village where mortality rate for deltamethrin was found to be 99%. These results clearly demonstrate the presence of pyrethroid resistance in *A. arabiensis* in Lower Moshi. The L1014F resistant allele was detected in one mosquito out of 642 that were screened for *kdr* mutation (allele frequency of 0.08%). The lack of DDT resistance coupled with previous studies showing very low frequency *kdr* suggests that enzyme-based mechanisms are responsible for resistance in *A. arabiensis*. Further studies are needed to investigate operational impact of observed resistance on malaria vector control interventions in the area.

Key words: *Anopheles*, resistance, mortality, dynamics, mutation, insecticide.

INTRODUCTION

Vector control is an important part of the global malaria control strategy that offers the greatest potential for the reduction of the disease burden (Townson et al., 2005). It mostly relies on the use of insecticide treated materials such as bed nets and indoor residual spraying with residual insecticides. In the framework of malaria elimination

programmes (Feachem and Sabot, 2008), the use of long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) are being scaled up in many endemic countries.

According to World Malaria Report of 2013, the number of ITNs delivered to malaria-endemic countries in sub-Saharan Africa increased from 6 million in 2004 to 145 million in 2010. Such increase remained flat for two years (2011 to 2012), however 200 million ITNs have been financed by donors for the year 2014, the number that is close to the number of ITNs required annually to protect all populations at risk of malaria in Sub-Saharan Africa. Likewise, the proportion of the population at risk that was protected by IRS in Africa fell from 11% in 2010 to 8% in 2012.

In 2011, the Tanzanian National Malaria Control Programme, in the framework of Universal Coverage Campaign (UCC, 2011), scaled-up the use of LLINs, with a free mass distribution of Olyset nets®. This was to ensure that all sleeping places were covered by an LLIN and to increase usage to 80% in the general population. According to World Malaria Report of 2013, the percentage of households that owned at least one ITNs in Tanzania was 91% and the percentage of population that was potentially protected by ITNs was 95%. Concomitantly, the number of people that were protected by IRS in Tanzania was more than six million. Deployment of IRS in Tanzania has been targeted in the Lake zone; areas of high malaria prevalence and unstable transmission. In 2007 an IRS campaign began in Muleba and Karagwe Districts of Kagera Region, which were experiencing malaria outbreaks at that time. The IRS was expanded to cover all of the two remaining regions of the Lake Zone: Mwanza and Mara in 2010 and early 2011.

The scaling-up of these vector control interventions has resulted in significant reduction in malaria transmission in many areas. The use of ITNs has been shown to reduce malaria illness, severe disease, and death due to malaria in endemic regions. For example, in Zanzibar where ten-fold decrease in *Plasmodium falciparum* prevalence in children under five years was recorded following mass distribution of LLINs (Bhattarai et al., 2007). Across tropical Africa the use of ITNs has achieved considerable success. The use of ITNs has also increased community protection where those without bed nets but live near to bed net clusters are protected from malaria (Howard et al., 2000; Binka et al., 1998). Like ITNs, deployment of IRS has achieved considerable success. The study that was conducted by Lee et al. (2010) for example, showed a steep decline in malaria incidence in Bioko Island.

Unfortunately, intensive use of pyrethroids in IRS and ITNs and in agriculture depending on location, has led to the development of pyrethroid resistance in many major malaria vectors world-wide. This resistance is threatening the sustainability of both programmes (Ranson et al., 2011) in particular ITNs as pyrethroids are the only class of insecticides approved for use on ITNs. They have been shown to be of very low mammalian toxicity but are toxic to insects and knock them down (kill them), even at very low doses (Zaim et al., 2000). Insecticides sprayed on house walls or impregnated in ITNs work in part, by repelling mosquitoes and in part by killing them (Lines, 1996). This latter effect imposes a significant selection pressure for resistant mosquito populations leading to increase in insecticide resistance as observed in Niger (Czeher et al., 2008) and Burundi (Protopopoff et al., 2008).

Over the past two decades, pyrethroid resistance has become widespread in *A. gambiae s.s*, in West African countries. However there is increasing reports of pyrethroid resistance in other parts of Africa including East Africa, particularly in Kenya (Kamau et al., 2007; Ochomo et al., 2013), Uganda (Ramphul et al., 2009; Verhaeghen et al., 2006, 2010; Morgan et al., 2010; Okia et al., 2013; Mawejje et al., 2013).

Previous study in Lower Moshi, North-Eastern Tanzania showed that *A. arabiensis* was predominant in the area (Mahande et al., 2012). In 2004 to 2005, longitudinal susceptibility testing of *A. arabiensis* in Lower Moshi revealed resistance (based on WHO criteria of 2013, WHO, 2013) of this vector population to permethrin, with percentage mortality ranging from 84 to 87% (Pan African Malaria Vectors Research Consortium (PAMVERC Table 1). However, the observed resistance to permethrin was based on mosquito collections from two villages of Mabogini and Rau Kati which are situated within the rice irrigation scheme. The West African type of *kdr* mutation (L1014F) had also already been detected in *A. arabiensis* in one of the villages, Msitu wa tembo by Kulkarni et al. (2006). However, the current species composition, resistance to other pyrethroids and the frequency of *kdr* mutation at present remained unknown. To address these issues the current study was carried out over eight villages of Lower Moshi from 2009 to 2013. The study will contribute to achieving one of the Global Plan for Insecticide Resistance Management in Malaria Vectors (GPIRM) goals by providing knowledge on insecticide resistance and underlying mechanisms which is important for insecticide resistance management strategies.

MATERIALS AND METHODS

Study area

The study was carried out in Lower Moshi, (700 meters altitude) on the Southern slope of Mount Kilimanjaro, North-Eastern Tanzania.

Table 1. Knockdown times and the mortality rates of the *A. arabiensis* field populations to various insecticides in 2004-2005.

Insecticide	Locality	No. tested	KdT50 (CI)	Mortality (%)
0.75% Permethrin	Mabogini	1405	19.3 (18.4-20.10	83.8
	Rau Kati	150	19.6 (18.2-21.0)	86.7
0.05% Deltamethrin	Mtakuja	200	15.2 (14.4-16.0)	97.5
	Mabogini	275	15.2 (14.9-15.6)	99.6
	Rau Kati	200	14.8 (13.7-15.9)	100
4% DDT	Chekereni	100	53.9 (49.8-61.3)	100
	Mabogini	150	40.7 (37.4-44.9)	100
	Rau Kati	100	38.7 (36.7-40.8)	100
0.1% Propoxur	Mabogini	100	28.1 (26.8-29.2)	100
0.1%Fenitrothion	Mabogini	100	-	100
	Chekereni	200	-	100
	Rau Kati	200	-	100
	Mtakuja	200	-	100
Malathion	Mabogini	200	39.3 (37.4-41.3)	100
	Rau Kati	300	33.1 (32.3-33.9)	100

KdT_{50} refers to time to knockdown 50% of tested mosquitoes.

The villages surveyed were Mabogini (37° 21′ E, 3° 24′ S), Rau Kati (37° 23′ E, 3° 25′ S), Chekereni (37° 23′ E, 3° 27′ S), Kileo (37° 33′ E, 3° 28′ S), Kikavu (37° 17′ E, 3° 35′ S), Mserekia (37° 21′ E, 3° 30′ S), Mtakuja (37° 22′ E, 3° 28′ S), and Msitu wa Tembo (37° 17′ E, 3° 33′ S). Figure 1 shows a map of the study area. The villages fall into two agro-ecosystems, rice irrigation and savannah where subsistence crops such as maize, banana are grown. Mabogini, Rau Kati and Chekereni are adjacent to each other, situated within the rice irrigation scheme. There are two rice growing seasons, the main season from June to October and the second season from September to February. Irrigated rice fields provide a breeding site for malaria vectors in the area (Matowo et al., 2010). Irrigation of rice fields during the early growing season coincide with peak *Anopheles* populations (Matowo et al., 2010). Mserekia, Mtakuja, Msitu wa tembo, Kileo and Kikavu are found in savannah agro-ecosystems. Mserekia, Mtakuja are adjacent to each other and they are close to Tanganyika Planting Company (TPC) sugar plantations. Msitu wa tembo, Kileo and Kikavu are isolated, not adjacent to the rice irrigation scheme. The densities of *Anopheles* mosquitoes in savannah villages are high only during the rainy seasons since they breed in temporary stagnant water pools following the rains. Livestock in this area are mainly cattle, goats, sheep and poultry. Farmers apply pesticides of different classes including pyrethroids not only to control rice and other crop pests but also as acaricides to control animal pests. Rainfall occurs from March to May, with a second rainy season during October to December. Between the two rainy seasons are a hot, dry season from January to February and a cool, dry season from June to September.

Mosquito collections

The adult *Anopheles* mosquitoes were collected during the longer rainy season, from April to June in 2009, 2011, 2012 and 2013 in the study villages by indoor resting catches, using mouth aspirators with torches. In each village, there were at least four sentinel sites from which the mosquitoes were consistently collected. The houses were mainly occupied by animals, mainly cattle, goats and sheep. Sampling was done by a team of four field assistants who surveyed the sentinel houses three times per week. The same team visited all sentinel houses from all villages where aspiration of mosquitoes was carried out between 06.00 and 9.00 h as described in the WHO protocol (WHO, 1975). The captured mosquitoes were placed in paper cups with a wet cotton wool at the top to maintain the relative humidity and transported to field insectary in a cool box. In the insectary, the mosquitoes were sorted based on morphology and the *Anopheles* female mosquitoes were kept under insectary condition for testing in the next day.

Diagnostic resistance tests

Diagnostic tests were conducted on batches of the collected semi-gravid *A. gambiae* complex specimens using WHO susceptibility test kits for adult mosquitoes (WHO, 1998). We used wild mosquitotes as it was very difficult to obtain larvae in some villages. Batches of 25 semi-fed mosquitoes of unknown age from field collections were exposed for one hour to 4% DDT, 0.05% deltamethrin, 0.05% lambdacyhalothrin, 0.75% permethrin, 0.1% propoxur, 0.5% malathion,0.1% bendiocarb and 0.1% fenitrothion according to WHO procedures (WHO, 1998). The tests were done in Mabogini field station in the early morning when humidity was high (60 to 80% RH) and low temperatures (22 to 28°C). The number of mosquitoes knocked down was recorded after 5, 10, 15, 20, 30, 40, 50 and 60 min. A mosquito was considered knocked down if it lay on its side on the floor of the exposure tube and was unable to fly. Mosquitoes were then transferred into holding tubes,

and provided with cotton wool soaked in 10% glucose and mortality recorded 24 h post-exposure. Mosquitoes were kept in a cool shaded room, with a wet towel placed over the containers. For each village, tests were replicated at least four times. The tests were also carried out on laboratory reared susceptible strain (Kisumu). When the control mortality was between 5 and 20%, the mean observed mortality was corrected using Abbott's formula (Abbott, 1925). At least one hundred and twenty five mosquitoes were enough for one test. The number of mosquitoes that were tested per site was according to the availability of mosquitoes during that season. Testing was not done in all villages in all years following low numbers of mosquitoes in some villages.

Species identification and *kdr* genotyping

Only the mosquitoes that survived pyrethroid exposure (642 mosquitoes) from 2013 collections were genotyped for species identification and *kdr* mutations. Of these, 430 survived 0.75% permethrin exposure, 116 survived 0.05% deltamethrin exposure and 96 survived 0.05% lambdacyhalothrin exposure. The mosquitoes were obtained from seven villages of Lower Moshi. Genomic DNA extracted from mosquito legs using 10% chelex-100 was stored at -20°C until use. Real time polymerase chain reaction (PCR) using hydrolysis probe 3plex assay described by Bass et al. (2008), with a minor modification were used to determine mosquito species within the *A. gambiae* complex. The Uni-F, UNI-R and the HEX and FAM probes were supplied by Applied Biosystems and were already mixed in one assay. Locked nucleic acid (LNA) probe was provided by Sigma, Germany. Briefly, 1 µl genomic DNA was used as template DNA.

The PCR thermocycling conditions consisted of an initial denaturation phase of 95°C for 10 min followed by an annealing phase consisting of 45 cycles of 95°C for 25 s and 67°C for 45 s. The PCR was run in a Stratagene thermocycler using MxPRO software (Aligent technologies, Stratagene, USA). The *kdr* locus was genotyped by hydrolysis probe assays as described by Bass et al. (2007), with minor modifications using TaqMan minor groove binding (MGB) probes and primers (Applied Biosystems, UK) and SensiMix DNA kit (Quantace). The minor modifications were based on the cycling conditions and the number of cycles to obtain good amplification. The PCR cycling conditions included an initial denaturation at 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 63°C for 45 s. The PCR was run in a Stratagene thermocycler using MxPRO software (Aligent technologies, Stratagene, USA).

Data analysis

Percentage mortalities 24 h post exposure was used to assess the status of susceptibility/resistance to insecticides. The KdT_{50} and their 95% confidence interval values were calculated for each insecticide using probit analysis in statistical package for social sciences (SPSS) 18.0 for windows. KdT_{50} refers to time to knock-down 50% of tested mosquitoes. The WHO (2013) criteria were used to evaluate the resistance/susceptibility status of the mosquito tested. Species identification and *kdr* genotyping results were analyzed using MXPro software (Agilent technologies, Stratagene, USA).

Ethical consideration

The ethical clearance was given by Kilimanjaro Christian Medical College (KCMC) Research Ethics Committee. Oral consent was given to the community leaders and the households from which the mosquitoes were collected.

RESULTS

Species identification

All 642 mosquitoes that were identified to species level were collected during the 2013 survey and they had survived pyrethroid exposure. Of these, 118 were from Chekereni village, 100 from Mtakuja, 168 from Mserakia, 104 from Mabogini, 87 from Rau Kati, 32 from Kikavu and 33 from Kileo. 98.8% (634) were *A. arabiensis*. All mosquitoes from Chekereni, Rau Kati and Kileo villages were found to be *A. arabiensis*. Only 3, 0.6, 1.9 and 6.3% from Mtakuja, Mserekia, Mabogini, Kikavu were found to be of other species of the anophelines than *A. arabiensis* and no *A. gambiae s.s* was found in any of the study sites. Overall, 98.8% of the mosquitoes (634) were *A. arabiensis* (Table 1).

Diagnostic resistance tests

Following WHO criteria (WHO, 2013) resistance of *A. arabiensis* to permethrin was recorded in all villages in 2009 except Mabogini village where reduced susceptibility to permethrin resistance was recorded with mortality rate of 90%. Lower mortalities were recorded in all villages in the year 2011, 2012 and 2013 (Table 2), indicating an increase in permethrin resistance in this mosquito population. The missing mortality data in the table is due the fact that it was difficult to collect a sufficient number of mosquitoes for testing in those localities. Using the same criteria, reduced susceptibility of *A. arabiensis* to deltamethrin was also observed in some areas while in other areas resistance was recorded in 2009, with percentage mortality ranging from 87 to 97%. Lower percent mortalities were recorded in 2011, 2012 and 2013, indicating *A. arabiensis* has become resistant to deltamethrin in all villages except Kikavu, where mortality to deltamethrin in 2013 was 99% (Table 3). Further investigation is needed to verify susceptibility of *A. arabiensis* to delamethrin in Kikavu village. The missing mortality data in the table is due the fact that it was difficult to collect a sufficient number of mosquitoes for testing in those localities. For lambdacyhalothrin, the same trend was observed with mortality rates ranging from 66 to 97% in 2009. However the mortalities de-creased in subsequent years, and by 2013, resistance to lambdacyhalothrin was recorded in all villages (Table 5. An. arabiensis from all study sites remains susceptible to DDT, organophosphates and carbamates (Table 6). The missing mortality data in tables is due to the fact that it was difficult to collect a sufficient number of mosquitoes for testing in those localities. Trends of susceptibility status of *Anopheles arabiensis* populations from different villages of Lower Moshi to pyrethroids are summarized in Figure 2. The missing mortality data in the table is due to the fact that it was difficult to collect a sufficient number of mosquitoes for testing in those localities. Trends of

Table 2. Composition of the Anopheles mosquitoes that survived pyrethroid insecticide exposure among villages

Village	Number tested	Species (%)		
		An. arabiensis	***An.gambiaes.s***	**Other anophelines**
Chekereni	118	118(100)	0(0)	0(0)
Mtakuja	100	97(97)	0(0)	3(0.46)
Mserekie	168	167(99.4)	0(0)	1(0.15)
Mabogini	104	102(98.1)	0(0)	2(0.31)
Rau Kati	87	87(100)	0(0)	0(0)
Kikavu	32	30(93.8)	0(0)	2(0.31)
Kileo	33	33(100)	0(0)	0(0)
Total	**642**	634**(98.8)**	0**(0)**	8**(1.2)**

susceptibility status of *An. arabiensis* populations from different villages of Lower Moshi to pyrethroids are summarized in figure 2.

Knockdown

The time to knockdown 50% of tested mosquitoes (KdT_{50}) obtained from time-mortality regression using probit analysis was used to calculate resistance ratios between the wild population and the susceptible reference strain (Kisumu). In 2009, the KdT_{50} for permethrin was found to be 33 to 40 min in all villages except Kileo which was 14 min. This increased to 39 to 61 min in 2013. The KdT_{50} for deltamethrin ranged from 21 to 31 min in all villages in 2009 except Mabogini where a KdT_{50} of 43 min was recorded. In 2013, KdT_{50} had increased to 30 to 40 min in all villages.

For lambdacyhalothrin, KdT_{50} of 20 to 69 min was recorded in 2009. By 2013, the KdT_{50} was found to be ≥ 60 min in most of the localities. The resistance ratio at KdT_{50} ($KdT_{50}R$) for permethrin ranged from 2.4 to 3.1 min in 2009; 2.7 to 3.4 min in 2011 and 2.6 to 4.1 min in2013. For deltamethrin, it ranged from 1.5 to 3.1 min in 2009; 2009; 1.1 to 3.9 min in 2011 and 1.6 to 2.2 min in 2013. The resistance ratio for lambdacyhalothrin ranged from 1.1 to 3.3 min in 2009; 2.7 to 3.9 min in 2011 and 3.0 to 4.7 min in 2013. In general, there was subsequent increase in knockdown times for all pyrethroids. However there was a decrease in knockdown time for deltamethrin from 2011 to 2013.

Knockdown resistance (*kdr*) mutation

Of the 642 *Anopheles* mosquitoes that survived pyrethroid exposure and genotyped for *kdr* mutation, only 1 mosquito (*A. arabiensis*) from Mtakuja village was heterozygous (RS) for West African (L1014F) *kdr*

DISCUSSION

The findings of this study suggest that *A. arabiensis* is still the predominant malaria vector in the study area where it constitutes 98.8% of the vector population. The previous study by Mahande et al. (2012) reported the occurence of both *A. arabiensis* (99.8%) and *A. gambiae s.s* (0.2%). The absence of *A. gambiae s.s* in this study could be due to *A. gambiae s.s* species shift with decrease of abundance of *A. gambaie* towards the more zoophilic sibling species *A. arabiensis* following scaling-up of ITNs as reported in Kenya (Mathias et al., 2011) and Tanzania (Kitau et al., 2012). Earlier study by Ijumba et al. (2002) had reported predominance of *A. arabiensis* in different agro-ecosystems in the area, with few secondary vectors including *A. funestus, A. pharoensis* and *A. coustani*. These secondary species could constitute 1.2% of the mosquitoes that were identified as othermutation, with allelic frequency of 0.08%. The mosquito had survived 1 h exposure to 0.75% permethrin. The remaining mosquitoes were homozygous for the wild type (SS). anophelines in this study that is, neither *A. arabiensis* nor *A. gambiae s.s*.

A. arabiensis from all villages of Lower Moshi demonstrated resistance to all pyrethroids tested. In 2013 *A. arabiensis* in Lower Moshi showed less than 90% mortality to permethrin and lambdacyhalothrin which according to WHO (2013), reflects resistance. The percentage mortality for deltamethrin was also less than 90% in all villages except Kikavu where the percentage mortality was found to be 99%. The reason why *A. arabiensis* in Kikavu resistance to deltamethrin in 2011 reverted to susceptibility in 2013 remains unknown. This guarantees further study in the near future to investigate the reasons for this trend, including decrease in knockdown times (KdT_{50}) for deltamethrin exposure in all localities from 2010 to 2013. The KdT_{50} of *A. arabiensis* exposed to permethrin, deltamethrin and lambdacyhalothrin increased dramatically in all villages from 2009 to 2011. The observed pyrethroid resistance is probably caused by selection pressure exerted by

Table 3. Knockdown times and the mortality rates of the *A. arabiensis* field populations from various localities exposed to 0.05% permethrin for 1 h.

Village	Mortality (%, number tested)				KdT_{50} (95%CI)				$KdT_{50}R$		
	2009	2011	2012	2013	2009	2011	2012	2013	2009	2011	2013
MsituwaTembo	80 (106)	63 (123)	-	-	40 (37-44)	38 (36-39)	-	-	2.9	2.7	-
Chekereni	-	81 (101)	-	28 (100)	-	45 (42-49)	-	49 (54-52)	-	-	3.3
Mtakuja	-	-	-	61 (100)	44 (41-47)	-	-	59 (57-62)	-	-	3.9
Mserekie	82 (101)	-	-	47 (100)	35 (33-38)	-	-	50 (47-51)	3.1	-	3.3
Mabogini	90 (111)	76 (490)	46 (203)	84 (198)	33 (31-36)	43 (42-44)	56 (54-59)	39 (38-41)	2.5	3.1	2.6
Rau Kati	67 (102)	67 (207)	73 (203)	48 (100)	39 (35-41)	48 (45-51)	41 (39-42)	61 (57-66)	2.4	3.4	4.1
Kikavu	87 (100)	74 (99)	-	77 (100)	36 (35-38)	47 (45-49)	-	47 (45-50)	2.8	3.4	3.1
Kileo	81 (101)	85 (111)	-	67 (100)	14 (11-17)	41 (37-44)	-	47 (45-49)	2.6	2.9	3.1
Susceptible strain	100 (100)	100 (100)	-	100 (100)	14 (11-17)	14 (13-15)	-	15 (14-17)			

KdT_{50} refers to time to knockdown 50% of tested mosquitoes. $KdT_{50}R$ refers to KdT50 of tested wild population divided by KdT50 of the 'Kisumu' susceptible strain.

Table 4. Knockdown times and the mortality rates of the *A. arabiensis* field populations from various localities exposed to 0.05% deltamethrin for 1 h.

Village	Mortality (%, number tested)				KdT_{50} (95%CI)				$KdT_{50}R$		
	2009	2011	2012	2013	2009	2011	2012	2013	2009	2011	2013
MsituwaTembo	87 (407)	63 (123)	-	-	31 (28-34)	55(53-57)	-	-	2.2	3.9	-
Chekereni	-	81 (101)	-	58 (100)	-	54(53-56)	-	36 (34-37)	-	3.9	2.0
Mtakuja	-	-	-	70 (100)	-	-	-	40 (38-41)	-	-	2.2
Mabogini	90 (113)	83 (405)	-	85 (100)	43 (42-45)	45 (44-46)	-	30 (26-34)	3.1	2.8	1.6
Rau Kati	95 (102)	67 (207)	-	78 (100)	25 (13-44)	43 (42-44)	-	30 (24-36)	1.8	3.1	1.6
Kikavu	97 (104)	74 (99)	-	99 (102)	21 (20-22)	53 (51-57)	-	36 (33-38)	1.5	3.8	2.0
Kileo	91 (102)	85 (111)	-	-	25 (17-34)	16 (13-19)	-	-	1.8	1.1	-
Susceptible strain	99 (113)	100 (100)	-	100 (100)	14 (13-14)	16 (13-19)	-	18 (17-18)	-	-	-

KdT_{50} refers to time to knockdown 50% of tested mosquitoes. $KdT_{50}R$ refers to KdT50 of tested wild population divided by KdT50 of the 'Kisumu' susceptible strain.

agricultural and public health use of insecticides. Malaria vector control in this area relies exclusively on insecticide-treated nets (ITNs) impregnated with pyrethroids for protection against host-seeking mosquitoes. The study that was conducted in several sentinel sites for insecticide resistance monitoring and surveillance in Tanzania (Kabula et al., 2012) revealed decreased susceptibility of the *Anopheles* popula-

Table 5. Knockdown times and the mortality rates of the *A. arabiensis* field populations from various localities exposed to 0.05% lambdacyhalothrin for 1 h.

Village	Mortality (%, number tested)				KdT_{50} (95%CI)				$KdT_{50}R$		
	2009	2011	2012	2013	2009	2011	2012	2013	2009	2011	2013
MsituwaTembo	66(191)	52(331)	-	-	49 (46-52)	57(55-59)	-	-	2.3	2.7	-
Chekereni	-	48(99)	-	71(100)	-	81(72-101)	-	58(56-60)	-	3.9	3.9
Mtakuja	78(97)	-	-	31(104)	49 (47-51)	-	-	>60min	2.3	-	-
Mserekie	84(97)	-	-	60(100)	69 (64-77)	-	-	67(63-74)	3.3	-	4.5
Mabogini	97(77)	58(508)	68(202)	74(100)	20 (17-22)	69(66-71)	73(68-81)	70(65-80)	1.0	3.3	4.7
Rau Kati	77(160)	52(108)	80(196)	85(100)	42 (40-43)	72(67-81)	57(56-59)	46(44-49)	2.0	3.4	3.0
Kikavu	97(102)	-	-	75(100)	40 (39-42)	-	-	65(61-70)	1.9	-	4.3
Kileo	81(104)	-	-	-	38 (36-40)	-	-	-	1.8	-	-
Susceptible strain	100(107)	100(100)	-	100(100)	21(20-22)	24(21-27)	-	15(13-18)			

KdT_{50} refers to time to knockdown 50% of tested mosquitoes. $KdT_{50}R$ refers to KdT50 of tested wild population divided by KdT50 of the 'Kisumu' susceptible strain.

tions to some insecticides in some study sites especially those with large-scale agricultural production, including Lower Moshi area. Therefore the potential contribution of agricultural insecticid use to resistance development should be considered.

An earlier study by Ngowi et al. (2007) revealed agricultural use of DDT and dieldrin (organochlorines) in Lower Moshi and pyrethroid-based insecticides such as permethrin and deltamethrin for public health and veterinary purposes. In this area, *A. arabiensis* commonly take blood meals from cattle (Mahande et al., 2007), hence a potential for selection pressure through frequent contact with pyrethroid-treated cattle. The increasing level of pyrethroid resistance in this *A. arabiensis* could result from increased selection pressure on this mosquito population following scaling-up of pyrethroid-based ITNs from 2004 through discounted vouchers issued at antenatal clinics (Mushi et al., 2003); between 2009 and 2010 through a national mass campaign (under five years children campaign) where 8.7 million long-lasting insecticidal nets (LLINs) were distributed free of charge to families with children under five years of age (Bonner et al., 2011); and in 2011 through a Universal Coverage Campaign (UCC, 2011).

In Kenya, the localized use of permethrin-impregnated nets in Kisumu did increase the permethrin tolerance of the local population of *A. gambiae* (Vulule et al., 1994, 1999). However, there was no evidence that it reduced the efficacy of permethrin impregnated nets as a malaria control measure. An increase in the level of resistance has recently been observed in other areas of Kenya as a result of ITN programs (Stump et al., 2004). The present level of pyrethroid resistance and *kdr* frequency in Lower Moshi is unlikely to impair the effectiveness of permethrin treated nets. A recent field trial in Moshi showed that while commonly used ITNs killed relatively few host-seeking *A. arabiensis,* the nets continue to provide personal protection through the strong excito-repellent activity of permethrin (Mosha et al., 2008). The bed nets also form a protective barrier around people sleeping under them hence protecting them against mosquito bites. Permethrin incorporated Olyset LLINs have been scaled up in Tanzania as national malaria control policy (UCC, 2011) and if the increased coverage is selected further for permethrin resistance, the effectiveness of the LLIN strategy may ultimately be undermined.

The previous study (Kulkarni et al., 2006) detected two out of 642 individuals from Msitu wa tembo village that were heterozygous for the L1014F *kdr* genotype (allele frequency = 0.16%). The same mosquito population from Msitu wa tembo village gave 100% mortality to DDT in 2011, suggesting that the *kdr* frequency in the area is still very low. Detection of L1014F mutation in one of 100 mosquitoes genotyped from Mtakuja village (allele frequency = 0.5%), about thirty kilometres from Msitu wa tembo village (where exactly the same type of mutation was detected in 2006) implies that the *kdr* mutation is spreading among the villages of Lower Moshi. With such low frequency, *kdr* is too rare to be important at this

Table 6. Knockdown times and the mortality rates of the *An. arabiensis* field populations from various localities to DDT, carbamates and organophosphates.

Insecticide	Village	Mortality (%, number tested)				KdT50 (95% CI)			
		2009	2011	2012	2013	2009	2011	2012	2013
4% DDT	MsituwaTembo	100 (93)	100 (103)	-	-	30 (22-39)	42 (39-45)	-	-
	Chekereni	-	100 (110)	-	100 (100)	-	36 (30-42)	-	39 (38-41)
	Mabogini	100 (465)	100 (280)	100 (203)	99 (100)	31 (29-33)	38 (34-41)	-	38 (35-42)
	Rau Kati	100 (280)	100 (210)	99 (204)	100 (100)	40 (38-42)	37 (33-41)	-	41 (39-44)
	Kikavu	100 (104)	99 (99)	-	100 (24)	34 (33-36)	45 (43-46)	-	42 (40-45)
	Kileo	100 (103)	99 (126)	-	-	35 (30-40)	35 (34-37)	-	-
	Mserekie	-			100 (100)				44 (42-45)
0.1% Propoxur	MsituwaTembo	-	100 (137)	-	-	-	22 (20-24)		-
	Rau Kati	-	99 (151)	-	-	-	23 (22-24)	-	-
	Chekereni	-	100 (50)	-	-	-	23 (22-24)	-	-
	Mabogini	-	100 (195)	100 (202)		-	-	-	-
0.1% Fenitrothion	Chekereni	-	100 (51)	-		-	-	-	
	Rau Kati	-	-	99 (181)				-	-
	Mabogini	-	-	98 (201)	100 (100)		38 (37-39)	-	26 (25-27)
0.1% Bendiocarb	Chekereni	-	-	-	-	-	-	-	-
	Rau Kati	-	-	98 (203)	100 (100)	-	39 (38-40)	-	38 (34-41)
	Mabogini	-	-	-	100 (100)	-	-	-	38 (35-42)
0.5% Malathion	Chekereni	-	-	-	100 (110)	-	-	-	31 (23-39)
	Rau Kati	-	-	-	100 (100)	-	-	-	38 (34-41)

KdT_{50} refers to time to knockdown 50% of tested mosquitoes. $KdT_{50}R$ refers to KdT50 of tested wild population divided by KdT50 of the 'Kisumu' susceptible strain.

time.

It should be noted that we acknowledge the fact that the bioassays were conducted on semi-gravid wild mosquitoes instead of unfed female mosquitoes of known age. This might contribute to the limitation of the study, however the mosquitoes were exposed to diagnostic concentrations of the insecticides for 1 h as per WHO recommendations, giving us confidence that pyrethroid resistance had developed in *A. arabiensis* in this part of Tanzania. The study would have been more robust if we used of 3 to 5 day old unfed female mosquitoes.

The absence of DDT resistance in the study area and the presence of pyrethroid resistance suggest insecticide detoxification by enzymes to be the more important mechanism for pyrethroid resistance in Lower Moshi. It is important to study the impact of this pyrethroid resistance on the efficacy of LLINs and IRS in the field.

Conclusion

The findings of this study suggest the presence of pyrethroid resistance in Lower Moshi population of *A. arabiensis*. Development of pyrethroid resistance in Lower Moshi was most likely due to a combination of factors based on agricultural and public health use of insecticides. Malaria incidence is relatively low in the study area and ITNs have recently been shown to be effective in

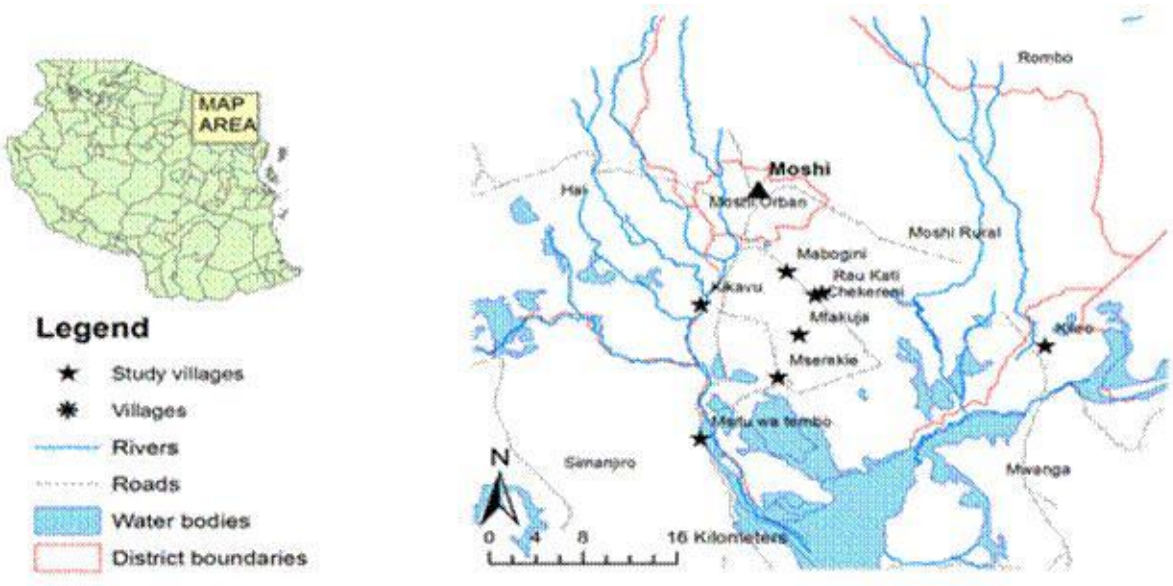

Figure 1. Map of Tanzania showing the study area.

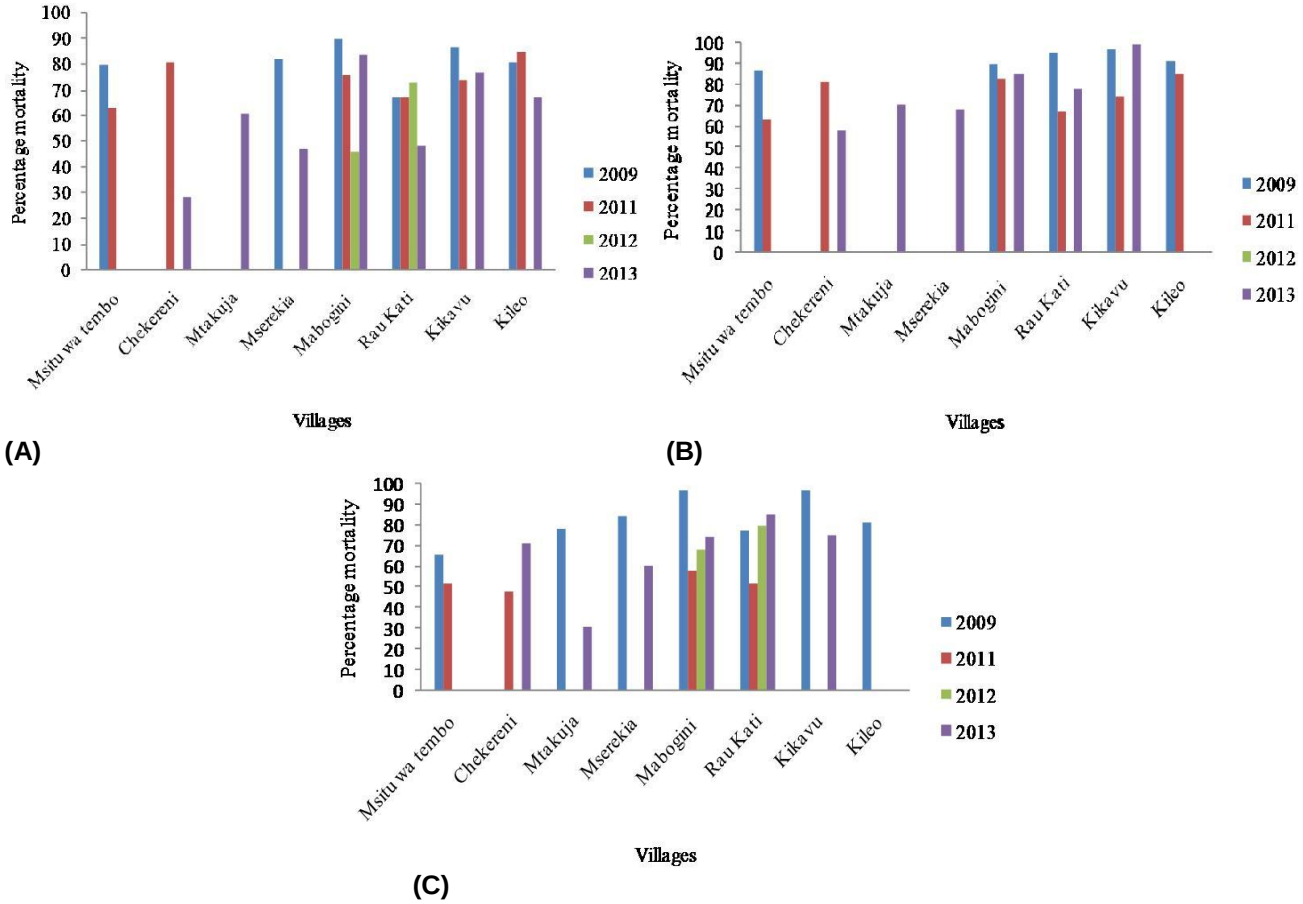

Figure 2. (A) Trends of susceptibility status of *Anopheles arabiensis* populations from different villages of Lower Moshi to permethrin; (B) Trends of susceptibility status of Anopheles arabiensis populations from different villages of Lower Moshi to deltamethrin; (C) Trends of susceptibility status of Anopheles arabiensis populations from different villages of Lower Moshi to permethrin.

reducing biting success of *A. arabiensis*. With high coverage ITNs following the national universal coverage campaign, it is likely that pyretihroid resistance will spread to other areas, particularly areas of pyrethroid IRS hence the danger of malaria resurgence.

The Olyset-LLINs, treated with permethrin have been scaled-up in the country, reaching universal coverage in 2011. More studies need to be carried out, particularly in areas of IRS, and assessment made of its impact on malaria control programs that utilize pyrethroids.

Conflict of Interest

The authors declare that they have no conflict of interests.

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