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# Insecticide susceptibility status of human biting mosquitoes in Muheza, Tanzania

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**Abstract**

**Background:** There has been a rapid emergence in insecticide resistance among mosquito population to commonly used public health insecticides. This situation presents a challenge to chemicals that are currently used to control mosquitoes in sub-Saharan African. Furthermore, there is limited information on insecticide susceptibility status of human-biting mosquitoes in some areas of Tanzania. This study aimed to determine insecticide susceptibility status of human biting mosquitoes in a rural area of north-eastern Tanzania.

**Methods:** The study was conducted in two villages in Muheza district, Tanzania. Insecticide susceptibility bioassays were performed according to the World Health Organization standard operating procedures on two to five-day old human biting mosquitoes. The mosquitoes of each species were exposed to four classes of insecticides commonly used for malaria vector control. Mosquito mortality rates (%) were determined after 24 hours post insecticide exposure.

**Results:** Mosquito species tested were *Anopheles gambiae* s.l., *An. funestus*, *Aedes aegypti*, and *Culex quinquefasciatus* species. Real-time PCR have showed that the main sibling species of *An. gambiae complex* and *An. funestus* group were *An. gambiae s. s.* (58.2%) and *An. funestus s. s.* (91.1%), respectively. All mosquitoes, except *Ae. aegypti formosus* were susceptible to pirimiphos-methyl (0.25%). *An. gambiae s. l.* was found to be resistant to permethrin (0.75%) but showed possibility of resistance to DDT (4%) and bendiocarb (0.1%). Our findings have shown that, *An. funestus* was fully susceptible to all insecticide tested.

**Conclusion:** The present study has revealed different levels of insecticide susceptibility status to four classes of commonly used insecticides in the most common mosquito vectors of human diseases in north-eastern Tanzania. The findings of the present study call for integrated vector control interventions.

**Keywords:** insecticide resistance, mosquitoes, Tanzania

## Introduction

Mosquito-borne diseases such as dengue, Rift Valley fever, malaria and lymphatic filariasis remain the major cause of illness and deaths in many parts of the world, particularly in tropical and sub-tropical climates (Goddard, 2008). Chemical insecticides remain the main tool for mosquito control although it is challenged by the development of resistance to commonly used public health insecticides (Gatton *et al.,* 2013; Nkya *et al.,* 2013; David *et al.,* 2013). The emergence and rapid spread of pyrethroid resistance in *Anopheles gambiae* complex populations may be a threat for the sustainability of malaria control programme for both indoors residual spray (IRS) and insecticide treated mosquito nets (ITNs) (Ranson *et al.,* 2011). Resistance has developed to common classes of insecticidal active ingredients used for mosquito vector control, mainly the synthetic-derived pyrethroids (Chareonviriyaphap *et al.,* 2013). Resistance to malathion, fenitrothion, propoxur, DDT and chlorpyriphos in *Culex quinquefasciatus* has been reported in Brazil (González *et al.,* 1999; Bracco *et al.,* 1999). The occurrence of insecticide resistant *Culex* mosquitoes has also been reported in Wete, Tanzania against DDT, deltamethrin, permethrin and lambda-cyhalothrin (Jones *et al.,*2012). In North-western Tanzania, *An. gambiae s.s.* has been reported to be resistant to bendiocarb (Gatton et al.,

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2013; Protopopoff *et al*., 2013; Matowo *et al*., 2015). The use of agricultural pesticides, ITNs and IRS for malaria vector control and the use of acaricides against pests of veterinary importance, is strongly suspected to be responsible for the emergence of resistance in Tanzania (Nkya *et al.,* 2014).

In Tanzania, insecticide resistance until recently was mainly reported in *An. gambiae s.l.* (Kabula *et al.,* 2012, 2013, 2014; Protopopoff *et al.,* 2013; Nkya *et al.,* 2014; Matowo *et al.,* 2010, 2014, 2015) while there is only one report of insecticide resistance of DDT, bendiocarb and pyrethroids such as permethrin, deltamentrin and lambdacyhalothrin in *An. funestus* in the country (Lwetoijera *et al.,* 2014). There is no report in Tanzania of insecticide susceptibility status in *An. funestus* to Pirimiphos-methyl insecticide. Furthermore, there is no report regarding insecticides susceptibility to four classes of insecticides in other human biting mosquitoes in Tanzania including *Cx. quinquefasciatus,* and *Ae. Aegypti.* Therefore, the present study aimed to determine the insecticide susceptibility status of four species of human biting mosquitoes to four classes of commonly used insecticides in north-eastern Tanzania.

## Material and Methods

### *Study site*

This cross-sectional study was conducted between May 2015 and March, 2016 in Muheza District of north-eastern Tanzania. The district covers a geographical area of 4922 km$^2$, stretching from a coastal plain at sea level to the East Usambara Mountains at 1,050 m above sea level. The climate is tropical, with dense rainforest over the Usambara mountain ranges with annual rainfall 1000 - 2000mm. According to the 2012 Population and Housing census, the district has a total of 204,461 people (URT, 2013). The area is endemic for malaria and lymphatic filariasis (Simonsen *et al*., 2014; Derua *et al.*, 2015). Two villages, namely Zeneti (5$^O$13'23.44"S; 38$^O$39'32.6"E) and Mkuzi (5$^O$13'49.9"S; 38$^O$50'19.44"E) were selected for mosquito sampling.

### *Mosquito collection and identification*

Mosquito larvae were collected from the wild in order to obtain an age-standardized sample of the adult population for the insecticide bioassay tests. Mosquito larvae were collected by using a standard dipper in a variety of breeding sites, including rice paddy, rain water collection pools, road potholes, hoof prints around swamps at Zeneti and Mkuzi villages. Collected larvae were sorted to remove larval predators. Culicine and Anopheline larvae were kept in separate larval rearing containers basing on their larval stages. Emerged pupae were sucked from the larval containers using a plastic pipette and placed in plastic cups inside the mosquito cages to prevent emerging adult mosquitoes from escaping. Emerged adult mosquitoes were identified morphologically using identification keys (Edwards, 1941; Gillies & De Meillon, 1968; Huang, 2004). *An. gambiae* s.l. and *An. funestus* were further identified to sibling species by Real-Time Polymerase Chain Reaction (PCR) (Bass *et al.,* 2007).

### *Insecticide susceptibility bioassays*

Insecticide susceptibility bioassays were performed according to standard guidelines (WHO, 2013). Assays were carried out with four insecticides namely, DDT (4%), permethrin (0.75%), bendiocarb (0.1%) and pirimiphos-methyl (0.25%). Each type of insecticides was replicated five times. Of the five replicates, one was a control replicate. Twenty to 25 mosquitoes per tube were hence, a sample size of 100 to 125 mosquitoes was tested for each insecticide. Two to five-day old female mosquitoes were used for bioassay tests. Tested mosquitoes were monitored at different time interval of 10, 15, 20, 30, 40, 50 and 60 minutes. *An. gambiae s. l., An. funestus*, *Ae. aegypti formosus* and *Cx.*

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*quinquefasciatus* mosquitoes were tested against the four (4) insecticides belonging to the major public health insecticide classes.

In the tested mosquitoes, the numbers knocked-down were recorded. *Cx. quinquefasciatus* was exposed for 80 minutes because knockdown was below 80% after the 60-minute exposure to Permethrin (0.75%) and DDT (4%). After exposure time, all mosquitoes were transferred to the holding tubes and provided with 10% glucose solution through a cotton wool. The mortality rates were determined at 24 hours post exposure. In each bioassay, a control experiment using papers impregnated only with insecticide carrier oil, was performed in the same way as in treatment experiments. Susceptibility tests were conducted in the laboratory under 25±2$^{o}$C and 70-80% temperature and humidity, respectively. Dead and surviving mosquitoes at the end of an experiment were kept in separate Eppendorf tubes containing silica gel and labelled (WHO, 2013; Umar *et al.,* 2014).

### *Data analysis*

The knockdown (KD) mosquito data was subjected to Polo Plus probit and logit analysis version 1, 2002-2009 LeOra Software to estimate the KDT50 and KDT95, which is the time taken to knock down 50% and 95% of the exposed mosquitoes, as well as their 95% confidence interval. The 24hr mortality rate (%) was established by counting the number of mosquitoes killed at the end of the holding period (24 hours) divided by the total number of mosquito exposed times 100. The insecticide susceptibility status of tested mosquitoes was assessed based on standard guidelines (WHO, 2013). Mortality range 98-100% indicates susceptible mosquito population, 90-97% suggests possible resistance that need to be confirmed and below 90% indicates existence of resistance. The adult mosquito mortalities in control experiments were less than 5%, therefore no correction made by Abbott's formula (WHO, 2013).

### *Ethical considerations*

The present study was approved by the Medical Research Coordinating Committee (NIMR/HQ/R.8a/Vol.IX/1613) and Kilimanjaro Christian Medical University College Ethics committee (Ethical Clearance No.885).

## Results

### *Mosquito composition*

A total of 2,515 mosquitoes comprising 1520 (60.4%) from Zeneti and 995 (39.6%) from Mkuzi village were tested to four classes of insecticides in order to assess their susceptibility status. A total of 528 *Anopheles* mosquitoes were identified by real-time PCR. Among these, *An. gambiae* s.l. accounted for 91.5% (n=483) and *An. funestus* group was 8.5% (n=45). *An. gambiae* s.l. was composed of An. *gambiae s.s.* (58.2%) and *An. arabiensis* (41.8%). Within the *An. funestus* group, four sibling species were identified which were *An. funestus s.s. (*91.1%), *An. rivulorum* (4.4%), *An. leesoni* and *An. parensis* (Table 1). The none-amplified and the missing samples were excluded in the analysis.

### *Mosquito mortality rate*

The 24hr mortality rate post-exposure to insecticides revealed resistant of *An. gambiae* s.l. to permethrin (0.75%) and bendiocarb (0.1%) with mortalities of 72% and 87%, respectively for mosquitoes collected from Zeneti village. In this village, *An. gambiae* s.l. was susceptible to DDT (4%) and pirimiphos-methyl (0.25%). For mosquitoes collected from Mkuzi village, *An. gambiae s.l.* was also resistant to permethrin (0.75%) with mortality rate of 69%. However, with exposure to DDT (4%), *An. gambiae* s.l. exhibited possible resistance. Unlike, Zeneti village, *An. gambiae* s.l. from

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Mkuzi village was susceptible to bendiocarb (0.1%) and pirimiphos-methyl (0.25%). *An. funestus* from Zeneti village were susceptible to all insecticides tested (permethrin, DDT, bendiocarb, pirimiphos-methyl (Table 2).

**Table 1: *An. gambiae* complex and *An. funestus* group sibling species identification**

| Species complex | Sibling Species | Total (n) | % |
|---|---|---|---|
| *An. gambiae* s.l. | *An. arabiensis* | 202 | 38.3 |
| | *An. gambiae s.s.* | 281 | 53.2 |
| *An. funestus* | *An. funestus s.s.* | 41 | 7.8 |
| | *An. leesoni* | 1 | 0.2 |
| | *An. parensis* | 1 | 0.2 |
| | *An. rivulorum* | 2 | 0.4 |
| **Total** | | **528** | |

In both villages, *Cx. quinquefasciatus* was resistant to three classes of insecticides tested except for pirimiphos-methyl (0.25%). *Ae. aegypti formosus* was susceptible to all insecticides tested at Mkuzi village. *Ae. aegypti formosus* collected from Zeneti exhibited possible resistance to DDT (4%) and pirimiphos-methyl (0.25%).

**Table 2: Insecticide resistance status of mosquito species exposed to four classes of insecticides in two villages**

| Study Site | Species | Permethrin | | DDT | | Bendiocarb | | Pirimiphos methyl | |
|---|---|---|---|---|---|---|---|---|---|
| | | %Mortality | RS[a] | %Mortality | RS[a] | %Mortality | RS[a] | %Mortality | RS[a] |
| Zeneti | *An. gambiae s.l.* | 72 | R | 99 | S | 87 | R | 99 | S |
| | *An. funestus* | 100 | S | 100 | S | 100 | S | 100 | S |
| | *Cx. quinquefasciatus* | 12 | R | 56 | R | 9 | R | 100 | S |
| | *Ae. aegypti formosus* | 100 | S | 96 | PR | 100 | S | 94 | PR |
| Mkuzi | *An. gambiae s.l.* | 69 | R | 96 | PR | 100 | S | 99 | S |
| | *Cx. quinquefasciatus* | 17 | R | 60 | R | 26 | R | 100 | S |
| | *Ae. aegypti formosus* | 100 | S | 100 | S | 100 | S | 100 | S |

**Key:** [a]WHO criteria for assessing susceptibility to insecticides of mosquitoes; RS=Resistance status; R=resistance, S=susceptible and PR= Possible resistance

***Mosquito knockdown times***

DDT (4%) and permethrin (0.75%) were able to knock down 50% of most of the mosquito species (KDT50) exposed within 1 hour. However, the KDT50 for *Cx. quinquefasciatus* was 156.5 minutes when exposed to permethrin (0.75%). The mean-time required to knock down 50% of the mosquitoes exposed to permethrin (0.75%) ranged from 16.8 to 27.7 minutes for *Ae. aegypti formosus* and 17.9 to 156.5 minutes for *Cx. quinquefasciatus* (Table 3).

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**Table 3: Knockdown times (KDTs) of mosquitoes exposed to DDT and Permethrin by site**

| Site | Species | Insecticide (%concentration) | n[b] | KDT50[c] (95%CI) | KDT95[d] (95%CI) |
|---|---|---|---|---|---|
| Zeneti | *An. gambiae s. l.* | Permethrin (0.75%) | 100 | 45.8 (43.0-48.6) | 60.0 (55.5-69.1) |
| | | DDT (4%) | 100 | 37.7 (31.7-42.5) | 52.5 (45.8-74.7) |
| | *An. funestus* | Permethrin (0.75%) | 100 | 30.5 (27.7-33.5) | 68.6 (58.7-85.7) |
| | | DDT (4%) | 100 | 35.8 (34.4-37.1) | 51.2 (48.6-54.9) |
| | *Cx. quinquefasciatus* | Permethrin (0.75%) | 100 | 156.5 (114.4-378.1) | 393.9 (214.4-2295.8) |
| | | DDT (4%) | 100 | *[e] | *[e] |
| | *Ae. aegypti formosus* | Permethrin (0.75%) | 80 | 16.8 (15.4-18.2) | 28.4 (25.2-34.1) |
| | | DDT (4%) | 80 | 56.4 (53.5-59.8) | 104.6 (93.4-122.4) |
| Mkuzi | *An. gambiae s. l.* | Permethrin (0.75%) | 80 | 43.2 (36.3-54.8) | 187.4 (119.5-437.1) |
| | | DDT (4%) | 80 | 37.5 (35.8-39.2) | 59.1 (55.1-64.9) |
| | *Cx. quinquefasciatus* | Permethrin (0.75%) | 89 | 17.9 (12.2-23.7) | 36.1 (26.7-86.0) |
| | | DDT (4%) | 80 | 56.2 (52.8-60.1) | 69.5 (63.9-84.0) |
| | *Ae. aegypti formosus* | Permethrin (0.75%) | 80 | 27.7 (25.5-29.9) | 47.6 (42.8-55.2) |
| | | DDT (4%) | 83 | 44.3 (42.3-46.3) | 74.6 (69.0-82.5) |

**Note:** n[b] = total number of mosquitoes exposed to each insecticide, KDT50[c] = time (min) required to knock down 50% of the mosquitoes exposed to an insecticide, KDT95[d] = means time (min) required to knock down 95% of the mosquitoes exposed to an insecticide, 95%CI = 95% Confidence Interval, *[e] = the estimates of KDT50, KDT95 and their associated 95% Confidence Interval could not be estimated by the Probit model.

## Discussion

The present study has demonstrated the occurrence of mosquito resistance to important mosquito species in north-eastern Tanzania. Our findings are consistent with other previous surveys conducted in the area (Kisinza *et al.,* 2013) and elsewhere in Tanzania (Kabula *et al.,* 2014; Matowo *et al.,* 2014). Various drivers of insecticide resistance in *An. gambiae* s.l. in Tanzania have been identified to include the use of insecticide-treated nets and agricultural pesticides (Nkya *et al.*, 2013). Various countries have also reported permethrin resistance in *An. gambiae* s.l., including Benin, Gambia and Nigeria (Yadouleton *et al.,* 2010; Betson *et al.,* 2013; Umar *et al.,* 2014).

In Mkuzi village, *An. gambiae* s.l. showed possible resistance to DDT (4%). This finding is similar to reports from previous results in Muheza (Kisinza *et al.,* 2013). *An. gambiae* s.l. from Zeneti were susceptible to DDT (4%). This is in contrary to previous findings in Tanzania (Kabula *et al*., 2012, 2013; Kisinza *et al.*, 2013) and Benin (Yadouleton *et al.,* 2010) which reported the species to be resistant to DDT (4%). This is likely to be due to use of agricultural pesticides in the area (Nkya *et al.*, 2013, 2014; Philbert *et al.,* 2014; Reid & McKenzie, 2016).

In the present study, *An. gambiae* s.l. collected from Zeneti were found to be resistant to bendiocarb (0.1%). However, previous studies in Muheza district have shown that the species was susceptible to bendiocarb (0.1%) (Kisinza *et al.,* 2013; Nkya *et al.,* 2014). These findings suggest that, insecticide resistance is rapidly increasing and its occurrence within the same locality may be different due to various environmental factors (Nkya *et al.,* 2013; Philbert *et al.,* 2014). *An. gambiae* s.l. from Mkuzi were susceptible to bendiocarb (0.1%). Similar findings have been reported by previous results in the same area (Kisinza *et al.*, 2013; Nkya *et al*., 2014). However, studies elsewhere in Tanzania and Benin have reported that *An. gambiae s.l.* mosquitoes are resistant to bendiocarb (0.1%) (Matowo *et al.,* 2015; Aïkpon *et al.,* 2013).

In this study, *An. gambiae* s.l. was found to be susceptible to pirimiphos-methyl. Our findings concur with results from previous studies in Muleba, Tanzania (Matowo *et al.,* 2015) and Atacora,

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Benin (Aïkpon *et al.,* 2013). Pirimiphos-methyl formulation has been reported in Zambia and Zanzibar to be highly effective and appropriate insecticide for IRS and can be used for management of insecticide resistance in malaria vectors (Chanda *et al.*, 2013; Haji *et al.,* 2015).

The present study has shown that, *An. funestus* is susceptible to all insecticide tested. *An. funestus* is an important vector of malaria and lymphatic filariasis in north-eastern Tanzania (Derua *et al.,* 2015). This is the first study in Tanzania to assess insecticide susceptibility status of *An. funestus* on pirimiphos-methyl. The susceptibility to insecticide status of *An. funestus* implies that, the existing common classes of insecticides used for public health can still be used for control of *An. funestus.* However, a study in south-eastern Tanzania has reported *An. funestus* to be highly resistant to permethrin (0.25%), bendiocarb (0.1%) and DDT (4%) (Lwetoijera *et al.,* 2014). The underlying reasons for the differences between the findings of these studies could be due to environmental factors including differences in agricultural activities.

The occurrence of insecticide resistance in mosquitoes is dynamic and sometimes focal. Our findings are consistent with previous studies on the *An. funestus* to organophosphates (malathion and fenitrothion) with 100% mortality (Riveron *et al.,* 2015), pirimipho-methyl (0.25%) and DDT (4%) (Choi *et al.,* 2014). In previous studies in Malawi and Kwazulu-Natal, South Africa *An. funestus* showed high resistance to pyrethroid insecticides (Riveron *et al.*, 2015; Hargreaves *et al.,* 2000). In Kwazulu-Natal, *An. funestus* resistance to deltamethrin used for IRS, led to an increase in malaria incidence to over six-folds in during late 1990s (Hargreaves *et al.,* 2000). *An. funestus* resistance to DDT and bendiocarb has been reported in Malawi, Zambia and Zimbabwe (Choi *et al*., 2014; Riveron *et al*., 2015).

Our findings have shown that, *Cx. quinquefasciatus* is highly resistant against majority of classes of insecticides tested. Multiple resistances to deltamethrin, permethrin, malathion, fenitrothion, propoxur, DDT, chlorpyriphos and lambda-cyhalothrin have been reported in *Cx. quinquefasciatus* in Brazil (González *et al.,* 1999; Bracco *et al.,* 1999), Wete Island in Tanzania (Jones *et al.,*2012) and India (Karlekar *et al.,* 2013). In a situation where resistance is very high, other control measures such as environmental management such source reduction and selection of effective compounds can be opted for successful mosquito control. The present study has found that, *Cx. quinquefasciatus* was fully susceptible to pirimiphos-methyl. Our findings concur with those of Ansaria *et al*. (2004) in India and Rowland *et al.* (2013) in Benin.

Interestingly, our study has documented the existence of *Ae. aegypti formosus* a sub-species of *Ae. aegypti* for the first time in the study area. *Aedes aegypti* consists of two sub-species namely *Ae. aegypti aegypti* and *Ae. aegypti formosus*. *Ae. aegypti formosus* showed possible resistance to pirimiphos-methyl. This insecticide is used for agricultural purposes in Tanzania. Our findings concur with those of a study in Brazil where *Ae. aegypti* was reported to be resistant to temephos which is an organophosphate (Lima *et al.,* 2011; Macoris *et al.,* 2014). Temephos resistance may occur due to alterations in the target site of the insecticide; the acetylcholinesterase gene or through elevated levels or differential efficacy of metabolic genes (Lima *et al.,* 2011). Additionally, *Ae. aegypti formosus* exhibited possible resistance to DDT. The findings of the present study are supported by a previous study conducted in Nigeria and Central African Republic, which revealed existence of DDT resistance among *Ae. Aegypti* (Ayorinde *et al.,* 2015; Ngoagouni *et al.,* 2016).

The present study has found that the mean-time required for knocking down 50% and 95% of *Ae. Aegypti formosus* was lowest and that of *Cx. quinquefasciatus* to be highest among the mosquitoes exposed to permethrin (0.75%) and DDT (4%). Longer knockdown times (min) are indications of presence of resistance to a particular insecticide, therefore knockdown resistance has been implicated in the *Cx. quinquefasciatus* population in this study area. Both *Ae. aegypti formosus* and *An. arabiensis* are outdoor biting mosquitoes and might not be controlled by interventions such

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as LLINs and IRs. There is need therefore to opt for other more effective control methods, including larval source management and larviciding for this group of mosquitoes.

The present study has revealed different levels of insecticide susceptibility status to four classes of commonly used insecticides in the most common mosquito vectors of human diseases in north-eastern Tanzania. The study has also provided baseline information on the insecticides susceptibility status of non-malaria mosquito vectors and the current susceptibility status on malaria vectors in the study area. The findings of the present study call for integrated vector control interventions.

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