



## Involvement of knockdown resistant gene allele in population of *Anopheles gambiae* complex in Bambam and Ture communities of Gombe State

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### Abstract

Lack of information on resistance mechanism is the major hindrance facing malaria vector control implementation. This work focused on insecticide susceptibility and involvement of KDR Allele point mutation of *Anopheles gambiae* population in Bambam and Ture communities of Gombe State. Susceptibility test were carried out on adult mosquitoes using the CDC bottle bioassay, followed with morphological identification. Identified mosquitoes were subjected to PCR analysis to further confirm the member of species complex and the molecular form present in the tested sample. The result showed resistance in population of *A. gambiae* to Organochlorine and Pyrethroid in Ture and Bambam communities. Resistance to permethrin (0-46.3% mortality) was recorded in both studied sites, resistant to lambda-cyhalothrin was recorded (in Ture community; 0-70%), resistance to Deltamethrin were recorded in both sites (56.8%-70%). High resistance of *A. gambiae* to DDT was prevalent in the two communities (28.3% and 16.7% mortality). The knock down resistance (KDR Gene Allele point mutation) result revealed non-involvement of KDR in tested resistance samples. The resistance might be due to increase in the rate of insecticide metabolism by enzymes, which lowered the amount of insecticide reaching the target site.

### INTRODUCTION

Malaria remains the world most important parasitic disease of public health importance (WHO, 2013). It is the major public health problem in Nigeria, contributing a quarter of the malaria burden in Africa (WHO, 2008). In 2013, malaria killed an estimated 584,000 people with over 2 million cases (WHO, 2014). Nigeria

is reported to have the unenviable record of contributing about 25% of the world malaria burden (WHO, 2012).

The major control interventions against malaria vectors include Insecticide-treated mosquito nets (ITNs) and indoor residual spray (IRS) with insecticides. Indoor residual spray has helped to eliminate

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malaria from great parts of Asia, Russia, Europe, and Latin America. Long-lasting insecticidal nets (LLINs) are the preferred form of ITNs for public health distribution programmes.

Malaria vector control is currently very dependant on a single class of insecticide, the pyrethroids. These insecticides are safe and fast acting and are the only class approved for use on insecticide treated materials (Zaim *et al.*, 2000). Pyrethroids are also increasingly deployed in IRS programmes in Africa (WHO, 2009) and are widely used in the control of agricultural pests worldwide. The dramatic increase in reports of Pyrethroid resistance in malaria vectors over the past decade (Santolamazza, 2008) is therefore a great cause for concern. Resistance to organochlorine, organophosphate and carbamate insecticides is conferred by a limited number of mechanisms in all insects analysed to date. These mechanisms predominantly involve either metabolic detoxification of the insecticide before it reaches its target site, or changes in sensitivity of the target site so that it is no longer susceptible to insecticide inhibition. Lack of surveillance research interventions is the greatest challenges facing malaria vector control (Slutsker, 2012). To overcome these challenges from undermining control programmes, correct identification, distribution of *Anopheles* vector and insecticide resistance must be understood. This study was aimed at studying the involvement of knockdown resistant gene allele (KDR point mutation) in the resistant population of *Anopheles gambiae*

## MATERIAL AND METHOD

Gombe is a region located in the North eastern part of Nigeria, one of the country's

36 States, having its capital at Gombe. The state nicknamed "Jewel of excellence" is right within the expansive savannah sharing common borders with the States of Borno, Yobe, Taraba, Adamawa and Bauchi. The State has an area of 20,265km<sup>2</sup>. The study was conducted at two different communities of **Ture** (N09° 49' 09.8", E11° 22' 44.2") and **Bambam** (N09 ° 42'22.7", E11 ° 32' 23.5") of Kaltungo and Balanga Local Government Areas of Gombe State, respectively.

### Collection of Mosquitoes

Larval collections were done from their natural breeding sites (pool, gutters, rice farms, potholes) and raised to adult in Gombe State Malaria Control Insectary. CDC bottle bioassay susceptibility test was conducted on the adult female *Anopheles* species

### Identification of *Anopheles* Samples

#### *Morphological identification*

Morphological identification of the survivor and dead mosquitoes were carried out with the aid of identification keys, followed by preserving individually on silica gel in Eppendorf tube for molecular identification. The following key features were considered for the identification of *Anopheles gambiae s.l.*

Wings: Have dark spots on the wing veins

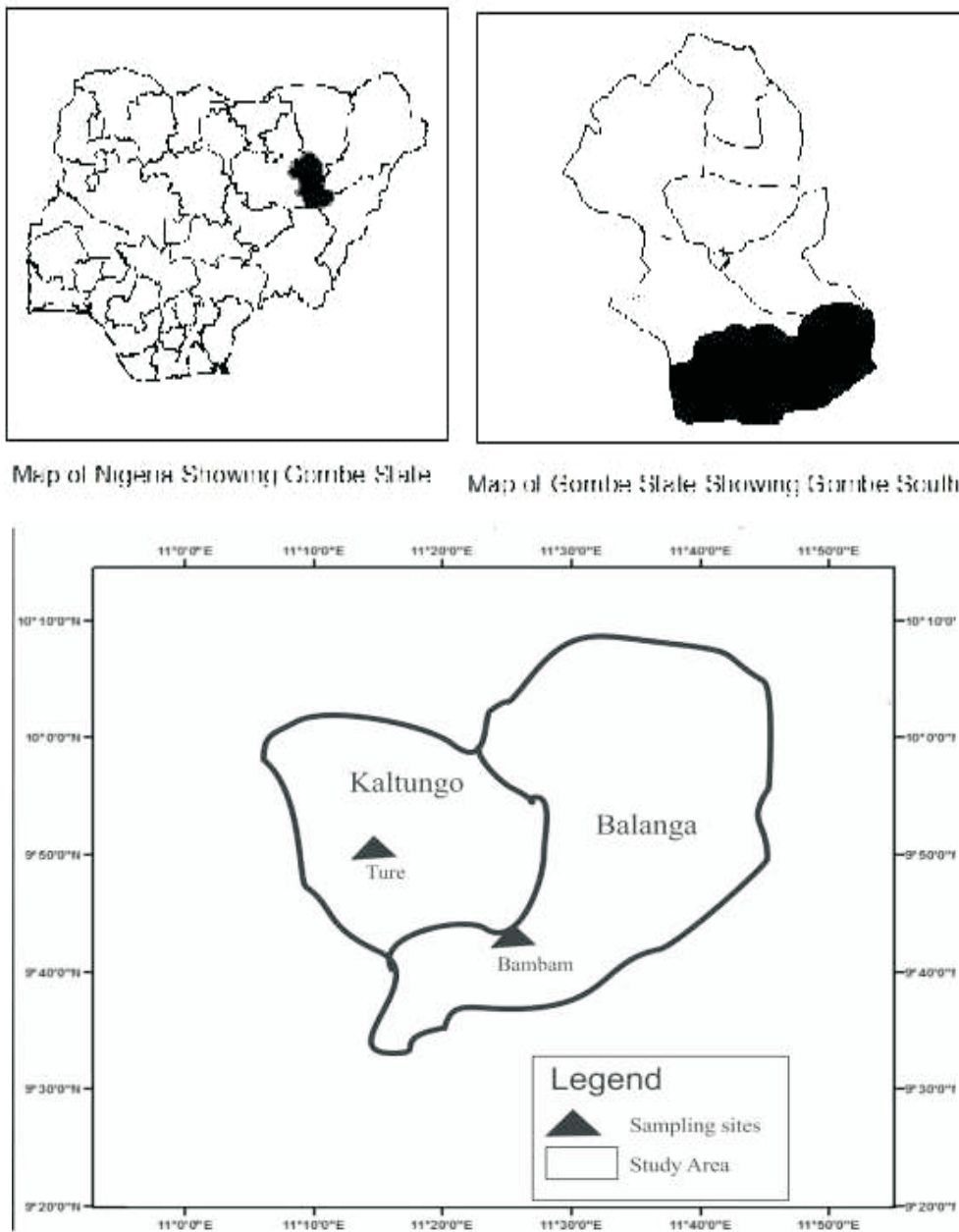
Proboscis/palps: Black spot on the palps

Legs: spotted or speckled legs

Colour: Yellowish brown to brown

#### *Molecular Identification of Anopheles gambiae*

Samples from survivor and dead mosquitoes exposed to insecticide were identified to species level using specie-specific PCR assay. The PCR was performed with universal and species



**Figure1:** Map of Gombe State showing the study communities

specific primers for the *A. gambiae* complex. Molecular identification of *A. gambiae* species complex is based on the species specific nucleotide sequences in the ribosomal DNA intergenic spacers (IGS). The amplified DNA was separated on a 2.0% agarose gel stained with ethidium

bromide and viewed under a Gel documentation machine.

**Extraction of DNA**

The whole preserved mosquito was grinded and washed in 100µl grinding buffer in 1.5ml Eppendorf tube with micro-pestle for

a single mosquito sample. The resultant solution was spun for 20 seconds at 14,000g. It was then cooled at 65 °C for 30 minutes. 13µl KAc (Potassium acetate) was added and gently tapped to mix. It was placed on ice for another 30 minutes followed by centrifuging at 14,000 g for 15 minutes. The supernatant was transferred to a fresh 1.5ml tube. 200µl ice cold of 100% ethanol was added and placed at room temperature for five minutes, followed by centrifuging for 20minutes at 14,000 g. The supernatant was carefully discarded and pellet washed with 200µl ice cold of 70 and 100% ethanol. The pellet was dried on a bench overnight, the dried pellet was suspended in TBE buffer and DNA was stored at -20°C.

### Amplification of DNA

Five sets of primers designed from the DNA sequences of the intergenic spacer (ITS) region of *A. gambiae* complex ribosomal DNA (rDNA) were used in PCR for the member species identification. The sequence details of these primers abbreviated Universal (UN), *gambiae s.l* (GA), *merus* and *melas* (ME), *Arabiensis* (AR) and *quadriannulatus* (QD). The UN primer anneals to the same position on the rDNA sequences of all five species, GA anneals specifically to *A. gambiae s.s.*, ME anneals to both *A. merus* and *melas*, AR to *A. arabiensis* and QD to *A. quadriannulatus*.

### Characterization of kdr point mutation

PCR-based assay was carried out to check for knock-down resistance gene in the survived mosquitoes. PCR genotyping to detect the standard Leu-Phe '*kdr*' allele (conferring knockdown resistance to pyrethroids) was performed using Martinez-Torres *et al.* (1998) technique.

The amplified fragments were analysed by electrophoresis on a 2% ethidium bromide agarose gel and were visualised under ultraviolet light. To check whether the PCR generated the anticipated DNA fragment (amplicon), agarose gel electrophoresis was employed for size separation of the PCR products. The size of PCR product was determined by comparison with a DNA ladder (a molecular weight marker), which contained DNA fragments of known size run on the gel alongside the PCR products.

## RESULTS

### Susceptibility test

In Ture community, all the mosquitoes 174 (100%) assayed showed resistance to all the insecticides without any susceptible strain while 129 (76.3%) resistant and 40 (23.7%) susceptible were discovered in Bambam community. A total of 88 *Anopheles* mosquitoes were exposed to permethrin insecticide, no mortality was recorded in both communities. Another batch of 83 *Anopheles* mosquitoes from both studied communities were exposed to Lambdacyhalothrin insecticide. In Ture community, 70% mortality was recorded while all the *Anopheles* in Bambam community were susceptible to Lambdacyhalothrin (100% mortality). In the same manner, another batch of 84 and 88 *Anopheles* were tested for Deltamethrin and DDT insecticide, respectively, results showed that all the mosquitoes in both communities were resistant to both insecticides with mortality rates ranging from 16.7 - 70%.

### Morphological Identification

A total number of 150 mosquitoes; 72 (48%) and 78 (52%) from Ture and Bambam communities respectively, were randomly pooled from the resistant samples

for morphological identification. Results showed 100% *Anopheles gambiae* s.l. No other species of *Anopheles* was recorded.

**PCR Identification of Sibling species of *Anopheles gambiae* complex**

100 samples were randomly selected from the resistant mosquitoes morphologically identified as *Anopheles gambiae* s.l. (50 from each study community). Species-specific PCR based assay was conducted on them, 25 (25%) amplified while 75 (75%) did not amplify. From those that amplified,

results showed that 25 (25%) belonged to *Anopheles gambiae* s.s. with 11 (22%) and 14 (28%) recorded in Bambam and Ture communities, respectively. No other sibling species of *A. gambiae* complex was recorded.

**Characterization of Kdr Point mutation**

KDR point mutation was absent in both molecular forms of *A. gambiae* in both study communities.

**Table 1:** Morphological identification of mosquitoes within the study site

| Location | <i>Anopheles gambiae</i> | <i>Anopheles funestus</i> | Total   |
|----------|--------------------------|---------------------------|---------|
| Ture     | 72 (48)                  | -                         | 72 (48) |
| Bambam   | 78 (52)                  | -                         | 78 (52) |

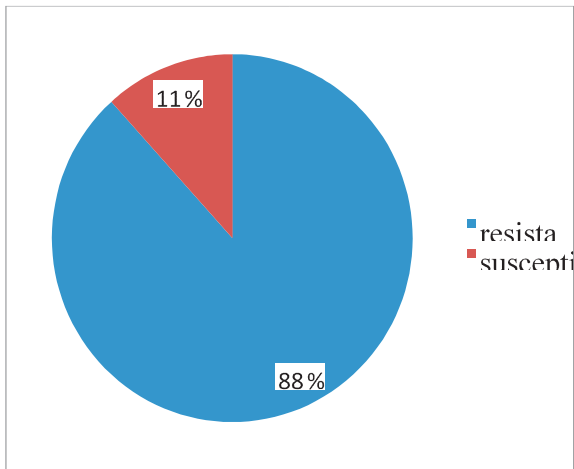
**Table 2:** PCR result of *A. gambiae* complex within the study sites

| Location | Number (%) of identified <i>A. gambiae</i> sibling species |                             |                 |                |
|----------|--|-----------------------------|-----------------|----------------|
|          | <i>Anopheles gambiae</i> s.s                               | <i>Anopheles arabiensis</i> | Total amplified | Non- amplified |
| Ture     | 14 (14)  | -                           | 14              | 36             |
| Bambam   | 11 (11)  | -                           | 11              | 39             |
| Total    | 25 (100)   | -                           | 25              | 75             |

**Table 3:** Resistance Status of *An. gambiae* to Pyrethroid and DDT from Study Sites

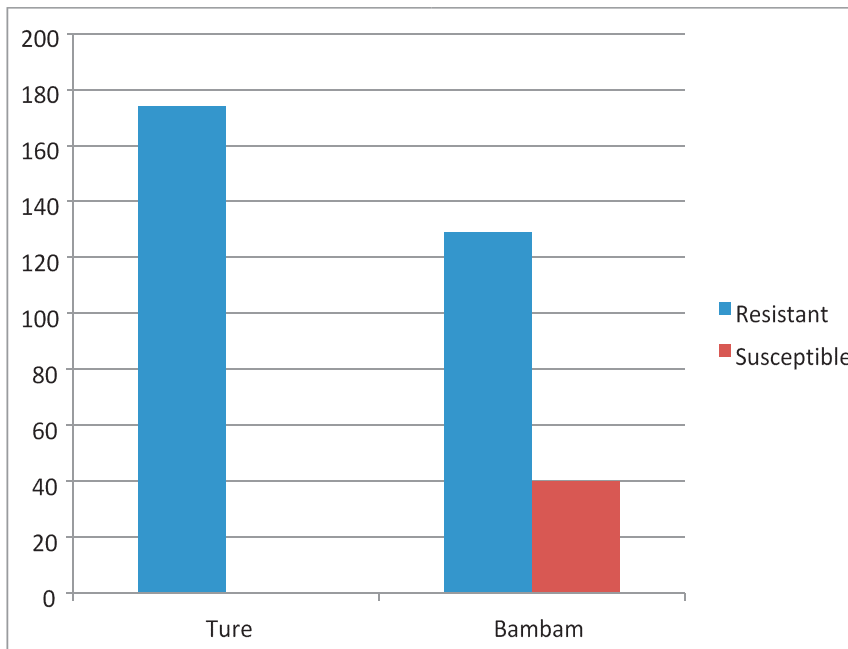
| Study Sites | Deltamethrin      |                  |        | Lambdacyhalothrin |                  |        | Permethrin        |                  |        | DDT               |                  |        |
|-------------|-------------------|------------------|--------|-------------------|------------------|--------|-------------------|------------------|--------|-------------------|------------------|--------|
|             | Number            | Percentage       | Status | Number            | Percentage       | Status | Number            | Percentage       | Status | Number            | Percentage       | Status |
|             | Assayed<br>(N=84) | Mortality<br>(%) |        | Assayed<br>(N=83) | Mortality<br>(%) |        | Assayed<br>(N=88) | Mortality<br>(%) |        | Assayed<br>(N=88) | Mortality<br>(%) |        |
| Ture        | 44                | 25(56.8)         | R      | 43                | 30(70)           | R      | 41                | 19(46.3)         | R      | 46                | 13(28.3)         | R      |
| Bambam      | 40                | 28(70)           | R      | 40                | 40(100)          | S      | 47                | 0(0)             | R      | 42                | 7(16.7)          | R      |

R – Resistant, S- Susceptible



**Figure 1:** Mosquitoes resistant and susceptible to insecticides in the study area

in Ture and Bambam communities in Gombe State. Resistance to permethrin recorded 0 - 46.3% mortality. Permethrin are Pyrethroid class of insecticide commonly used in indoor residual spray and commercial agricultural pest control. Though the effectiveness of Pyrethroid as agricultural pest control is extremely low they easily wear away when exposed to sunlight. That is why most farmers depend on DDT for control intervention. The resistant recorded might be ascribed to excessive usage of the insecticide by uneducated farmers. Though similar research study on resistance carried out on population of *A. gambiae* in Bauchi, Nigeria reported resistance to permethrin



**Figure 2:** Number of resistant and susceptible mosquitoes within study community

**DISCUSSION**

The results of this study demonstrated evidence of resistance in population of *A. gambiae* to Organochlorine and Pyrethroid

insecticide (Umar et al., 2014).

*Anopheles* mosquitoes' resistant to Lambdacyhalothrin recorded in Ture

community (70% mortality) were due to over exposure of the said insecticide to agricultural pest. Full susceptibility of *A. gambiae* population recorded in Bambam community to Lambda-cyhalothrin was due to the under usage of this insecticide. Most farmers used DDT and other formulation for pest control in this community. Resistance to DDT insecticide reported in this research work were due to over-exposure of the insecticide to the insect pest by the farmers. Although DDT had been banned by WHO because it was believed to interfere with animal reproduction system and also build up in the food chain but was heavily relied on in the northern part of Nigeria. The farmers majorly use DDT for commercial agricultural pest control. The essence of inclusion of DDT was to discourage the farmers using the resistance information as excuse. Most control interventions were done by illiterates who do not understand the composition of insecticide being used and the adverse effect on non-targeted species. The co-occurrence of both DDT and Pyrethroids resistance in *A. gambiae* population suggested a similar mechanism modulating for both pyrethroid and DDT resistance since both insecticides act on the same target site.

The low PCR amplification recorded in the samples identified as *A. gambiae* s.l. could be probably due to low DNA extraction and possibly DNA degradation associated with storage or freezing and thawing of DNA. Reports of similar problems are documented (Chris *et al.*, 2007). No kdr point mutation (Leucine-Phenylalanine mutation) was detected in the population despite report of resistance to pyrethroids and DDT in the locations (Awolola *et al.*, 2005). Though similar results were reported

by Awolola *et al.* (2005), where no kdr gene was found in both molecular form of *A. gambiae* in Sudan savannah ecological zone of the country. Hence, the involvement of an additional operative resistance mechanism is possible. The leucine-serine point mutation (kdr-e), high levels of glutathione S-transferase and oxidase activities might be responsible. The activity of metabolic enzymes, though not characterized, might have increased resistance to DDT and pyrethroids. Further studies on identity, distribution and insecticide resistance status of *A. gambiae* in Ture and Bambam communities will provide directions towards implementation of effective insecticide resistance management strategies.

**Conflict of interest:** All authors indicate that there is no any actual or potential conflict of interest that could inappropriately or possibly influence this work after publication.

## REFERENCES

- Awolola, T.S., Oyewole, I.O., Amajoh, C.N., Idowu, E.T., Ajayi, M.B., Oduola, A., Manafa, O.U., Ibrahim, K., Koekemoer, L.L. and Coetzee, M. (2005). Distribution of the molecular M and S forms of *Anopheles gambiae* and pyrethroid knockdown resistance gene in Nigeria. *Acta Tropica*, 95: 204-209.
- Chris, B., Martin, S.W., Craig, S.W., Martin, J.D. and Linda, M. F. (2007). Identification of the main malaria vectors in the *Anopheles gambiae* species complex using a Taq Man real-time PCR assay. *Malaria Journal*, 6: 155.
- Martinez-Torres, D., Chandre, F., Williamson, M.S., Darriet, F., Berge, J.B., Devonshire, A.L., Guillet, P., Pasteur, N. and Pauron, D. (1998). Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae* s.s. *Insect Molecular Biology*, 7: 179-184.
- Santolamazza, F., Calzetta, M., Etang, J., Barrese, E., Dia, I., Caccone, A., Donnelly, M.J.,

- Petrarca, V., Simard, F. and Pinto, J. (2008). Distribution of knockdown resistance mutations in *Anopheles gambiae* molecular forms in west and west-central Africa. *Malaria Journal*, 7(1): 74.
- Slutsker, L. (2012): Challenges in surveillance and response, *Malaria Journal*, 11 (Suppl. 1).
- Umar, A., Kabir B.G.J., Amajoh, C.N., Inyama, P.U., Ordu, D.A., Barde, A.A., Misau, A.A., Sambo, M.L., Babuga, U., Kobi, M. and Jabdo, M.A. (2014). Susceptibility test of female *Anopheles* mosquitoes to ten insecticides for indoor residual spraying (IRS) baseline data collection in North-eastern Nigeria. *Journal of Entomology and Nematology*, 6(7): 98-103.
- World Health Organization (WHO) (2009). Insecticide Resistance Deepens Malaria Crisis in Nigeria. {AFP}: By Susan Nnaji.
- World Health Organization (WHO), (2008). World Malaria Report. In *WHO Global Malaria Programme*. Geneva, Switzerland.
- World Health Organization (WHO), (2012). World Malaria Report. In *WHO Global Malaria Programme*. Geneva, Switzerland.
- World Health Organization (WHO), (2013). World Malaria Report. In *WHO Global Malaria Programme*. Geneva, Switzerland.
- World Health Organization (WHO), (2014). Update on Artemisinin resistance. In *WHO Global Malaria Programme*. Geneva, Switzerland.
- Zaim, M., Aitio, A. and Nakashima, N. (2000). Safety of pyrethroid treated nets. *Medical and Veterinary Entomology*, 14:1-5.