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Molecular Identification of Malaria Vector in Gusau Township, Nigeria

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ABSTRACT

There about 460 *Anopheles* species recognized, over 100 can transmit human malaria but only 30–40 commonly transmit parasites of the genus *Plasmodium*, which cause malaria. *Anopheles gambiae* is the principal vector of the most dangerous malaria parasite species in Africa, which is *Plasmodium falciparum*. *Anopheles gambiae* are a complex consisting of eight morphologically indistinguishable species and each of the members of the complex having unique biology, ecology and behaviour and should be studied and differentiated. The research objective was to identify the species of malaria vector in Gusau township Nigeria using molecular biological technique to know the exact malaria vector. Knowledge of the exact malaria vector in a given environment enables formulating a carefully designed tailor-made vector control measure. Indoor and outdoor mosquito samples were collected from selected areas in five wards of Gusau township using standard collection methods, for a period of twelve months. The samples were identified using polymerase chain reaction (PCR) technique and the result showed *Anopheles gambiae* in all sites of sample collection. It is therefore recommended that integrated malaria vector control should be adopted in the mosquito control programme because *Anopheles gambiae* is rugged and can be difficult to control and/or eradicate because of the emerging insecticide resistance and its close association with human host.

Keywords: Gusau township, Malaria vector, *Anopheles gambiae*, Polymerase chain reaction (PCR), Integrated control.

1.0 INTRODUCTION

1.1 Mosquitoes

Mosquitoes are small flying insects belonging to the Order Diptera and Family Culicidae. They have slender, elongated body of the adult is covered with scales as are the veins of the wings. Mosquitoes are also characterized by long, fragile-looking legs and elongated, piercing mouthparts (Britannica, 2023, *Anopheles*, 2023). There are about 112 genera and over 3,700 species of mosquitoes worldwide. Adult mosquitoes live both indoors and outdoors and can bite day and night. Adult mosquitoes live for about 2 to 4 weeks depending on the species, humidity, temperature, and other factors. Female mosquitoes often live longer than male mosquitoes (Harbach and Kitching, 2016).

1.2 Public Health

Mosquitoes are of important in public health because of the bloodsucking habits of the females and some of them are vectors, known to transmit serious viruses, including yellow fever, Zika fever, malaria, filariasis, and dengue (CDC, 2023).

1.2 *Anopheles* mosquitoes

Anopheles, also called marsh mosquitoes was first described and named by Meigen in 1818 (Meigen, (1818). There about 460 recognized species, over 100 can transmit human malaria but only 30–40 commonly transmit parasites of the genus *Plasmodium*, which cause malaria in humans in endemic areas. *Anopheles gambiae* is one of the best known, because of its predominant role in the transmission of the most dangerous malaria parasite species (to humans), which is *Plasmodium falciparum*. Other genera (*Aedes*, *Culex*, *Culiseta*, *Haemagogus*, and *Ochlerotatus*) can also serve as vectors of disease agents, but not human malaria (Harbach, 2008).

This study was aimed at sampling and identifying the malaria vector species using molecular biological technique i.e., polymerase chain reaction (PCR) in Gusau township. Identification of malaria vector to the individual species level gives a clearer picture to understanding the biology and behaviour of the mosquito. This shall enable the making an effective tailor-made vector control and preventive measure.

2.0 MATERIALS AND METHODS

2.1 Study Area

Gusau township is the capital city of Zamfara State and the Local Government Headquarter of Gusau LGA, is located in the northwestern Nigeria, having geographic Coordinates: 12°09′N 6°40′E. The township has an area of 3,364 km² (2,090 sq miles) and a population of 383,162 as of the 2006 census (NPC, 2009).

Figure 1: Map of Zamfara State, Nigeria (Maps, 2023)

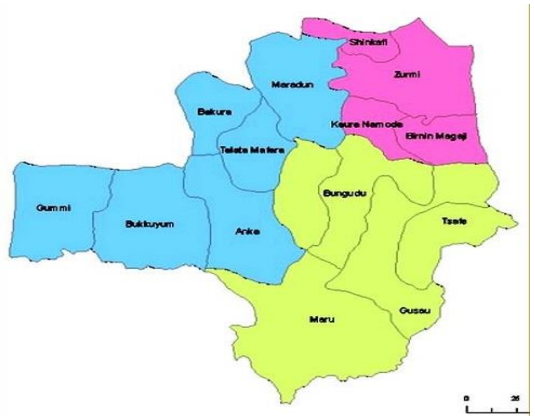

Figure 2: Gusau township and its environ (Maps, 2023)

The climate is described as The Climate is described as a tropical savanna climate (Köppen climate classification , with rainy seasoning commencing in late March and ending in mid to late October (Klimatafel, 2023).

Hausa/ Fulani are the main ethnic groups of the township, although other ethnic groups are also found cohabiting within. Agriculture is the main occupation of the population (Post Offices, 2012 and The World Gazetteer, 2007).

2.2 Consent

Permission to undertake this study was sought from relevant bodies, namely Ministry of Health Gusau. Residents within/around the mosquito collection sites were duly informed about the study and periodic sample collection.

2.3 Mosquito Collection

Indoor mosquitos' collection was undertaken fortnightly with Gusau metropolis between 5.00am and 7.00am from selected locations within five wards of Gusau Local Government Area, which are Shiyar Magaji, Uban Dawaki, Galadima, Mayana and Sarkin Fada, for the period of twelve months. Mosquitoes were collected indoors both indoor and outdoor in a procedure described by WHO, 2022.

2.3.1 Indoor Mosquito Collection

The indoor mosquito collection was done using pyrethrum-based aerosol spray, Baygum® according. A white sheet of cloth measuring 300 x 400cm was used and spread on floor of the room and all windows closed. Using a face mask, the aerosol insecticide was carefully sprayed until the room becomes saturated and allowed to stay for about 30 minutes. The dead mosquitoes on the white sheet of cloth were carefully sorted out, counted, identified using morphological keys according Coettzee (2004), Gilles and Coettzee (1987) and WHO () and were then transferred into marked Eppendorf tubes containing silica gel and small piece of tissue transported to the laboratory for further confirmation.

2.3.2 *Outdoor Mosquito Collection*

Outdoor mosquito Collection: The outdoor mosquito collection was done using CDC trap that were strategically hung between 6.00pm to 6.00am.

On the daybreak, mosquitoes were carefully sorted out, counted, identified using morphological keys according Coettzee (2004) and WHO (2022) and the female *Anopheles* mosquitoes were then transferred into marked Eppendorf tubes containing silica gel and small piece of tissue transported to the laboratory.

The mosquitoes were further identified by an entomologist at the Usmanu Danfodiyo University in the Department of Biological Sciences.

Representative of the female *Anopheles* mosquitoes according the site and the period of collection (month) were used and taken to a DNA laboratory for molecular species identification using polymerase chain reaction technique in a similar protocol of Scott, Brogdon and Collins (1993).

2.2 Polymerase Chain Reaction

2.2.1 *DNA Extraction*

The hind leg of the female *Anopheles* mosquito removed and used to extract its DNA using similar protocol of Collins *et al.* (1987). The individual mosquitoes were picked using clean forceps, placed into the universal container containing 25 microliters (ul) of deionized water and crushed thoroughly using a crusher. Twenty-five (25ul) microliter of sodium acetate, which precipitate the proteins, was added to the mixture and incubated at 90^{o}C in a dry incubator for 10 minutes. The mixture was then centrifuged at 13,000 revolution per minute (rpm) for 2 minutes, DNA pellet formed the sediment the supernatant was discarded. The DNA pellet was re-suspended in 50ul of high-grade PCR master mix containing standard primers (Sigma, UK) for the identification of *Anopheles gambiae* complex similar to those used by Scot *et al.* (1993) and Wilkin (2006).

- **UN-** the plus (+) strand that matches and anneals to the same position of rDNA of all species of *A. gambiae.*
- **GA**- the *A. gambiae s.s.*-specific primer that anneals specifically to *A. gambiae s.s.*
- **AR**- the *A. arabiensis* specific primer.
- **ME**- the *A. melas* and *A. merus* primer that anneals and identifies both *Anopheles* species.
- **QD**- the *A. quadriannulus* specific primer.

The primers contained 20base pairs (bp) long oligonucleotides that lie within the inter-genic spacers (IGS) of the region of ribosomal deoxyribonucleic acid (rDNA). The sequences were described as follows:

UN: 5'-GTC TGC CCC TTC CTC GAT GT-3'
GA: 5'-CTG GTT TGG TCG GCA CGT TT-3'
AR: 5'-AAG TGT CCT TCT CCA TCC TA-3'
ME: 5'-TGA CCA ACC CAC TCC CTT GA-3'
QD: 5'-CAG ACC AAG ATG GTT AGT AT-3'

The following were needed for PCR to commence:

a. The PCR water (or master mix) contained the reaction buffer at pH 7.4 providing most suitable environment for the optimum activity of the DNA polymerase *deoxynucleotide triphosphate* (dNTP), that is the building block containing various bases.
b. DNA polymerase, which catalyzes the synthesis of new DNA material.
c. Magnesium Chloride ($MgCl_2$ – is a divalent cation) which a vital component of DNA tag polymerase.

The mosquito samples along with the PCR master mix were carefully transferred into PCR machine SimpliAmp™ Thermal Cycler (96-well Veriflex) to begin the PCR cycle, by the reaction temperature was increased to 90-95^{o}C for about 5-10 minutes and comprising of the following main phases:

i. **Initialization/Denaturation:** This step involves heating the reaction strongly at 96^{o}C to separate, or denature, the DNA strands providing single-stranded template for the next step.
ii. **Annealing:** In this step, the primers are cooled at 55 – 65^{o}C so it can bind to their complementary sequences on the single-stranded template DNA.
iii. **Extension/elongation:** (72°C): The temperature is raised so that *Taq* polymerase extends the primers, synthesizing new strands of DNA.

a. This cycle repeats 25 - 35 times in a typical PCR reaction.

After the PCR amplification process, each of the master mix (10ul) containing the mosquito DNA samples were placed into the wells on agarose gel using micropipettes and mixed with 2.5ul of ficol dye. The mixture was electrophoresed at 100 volts electric current for one (1) hour in the electrophoresis bath. The gel was then removed and placed onto an ultra-violet (UV) light trans-illuminator system.

3.0 RESULTS

3.1 PCR Identification of Mosquitoes

The amplification of the genes of the mosquito samples using PCR assay showed the chromosomal bands were positioned matching with those of *Anopheles gambiae* on the positive control as seen in figure 2.

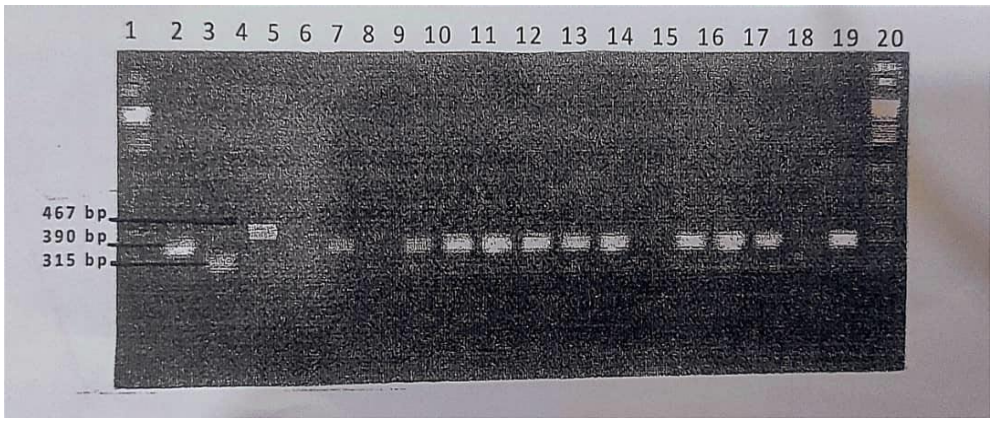

Figure 2: End product of Polymerase Chain Reaction showing amplified genes of *Anopheles gambiae* Giles in lanes7, 9–14 and 15-17.

Guide

Lanes 1 & 20:	Standard DNA ladder	Lane 5:	Negative control
Lanes 2 & 19:	Positive control	Lanes 8 – 13 & 15 – 17:	Positive *An. gambiae*
Lanes 3 & 18:	Positive *An. Arabiensis*	Lane 14:	Negative

4.0 DISCUSSION

4.1 *Anopheles gambiae*

This research has revealed *Anopheles gambiae* Giles in Gusau township (north western, Nigeria) confirming earlier studies of Onyabe and Conn, 2001, Abdullahi *et al*., 2010 and Okorie *et al*., 2011.

Anopheles gambiae are a complex consisting of eight morphologically indistinguishable species namely *An. arabiensis, An. melas, An. merus, An. quadriannulatus, An. gambiae sensu stricto, An. coluzzii* and *An. amharicus.* They also include some of the most important vectors of the deadliest malaria species, which is *Plasmodium falciparum,* which are *An. gambiae* and *An. arabiensis.* The other effective malaria vector species, not among the *An. gambiae* complex is *An. funestus* (Harbach, 2004).

Each species of the complex has unique biology, ecology and behaviour. Knowledge on these differences can help understand how malaria is transmitted, and can aid in designing appropriate control strategies taken into consideration when designing a control program include the susceptibility of malaria vectors to insecticides and the preferred feeding and resting location of adult mosquitoes (Yakob, 2011).

4.2 Challenges of Insecticide Resistance

The challenges being faced in the malaria vector control included among others resistance by the mosquitoes to most of the insecticides, due to many of the following factors: Behavioral changes, changes in the metabolism of the mosquito that help it survive insecticide exposure, genetic changes or adaptations that prevent the insecticide from acting on target(s) within the mosquito or decreased penetration of insecticides into the mosquito body. Insecticidal resistance of *Anopheles gambiae* is rapidly spreading (Soumaila *et al.* 2017) and becoming a big challenge in the malaria vector programmes in the affected areas.

4.2 New Malaria Vector Discovered in NW Nigeria

As the relevant agencies work harder in the malaria vector control a new mosquito vector of malaria has been discovered by the Nigerian Institute of Medical Research, NIMR, which is *Anopheles stenphensi***,** which is also rugged and difficult to eradicate (Dailytrust, 2022). *Anopheles stephensi* is a primary mosquito vector of malaria in urban India and is not included in the same subgenus as *Anopheles gambiae* which is the primary malaria vector in Africa. *A. gambiae* consists of a complex of morphologically identical species of mosquitoes, along with all other major malaria vectors; however, *An. stephensi* has not yet been included in any of these complexes (Harbach, 2007).

5.0 CONCLUSION

Anopheles gambiae has further been confirmed as a major vector of malaria in Gusau township. Its biology and also being in very close association with the human host makes it extremely difficult to control.

6.0 RECOMMENDATIONS

Integrated vector control should be adopted in the control of *Anopheles vector* to include use of insecticide along with use of insecticidal treated mosquito nets. Human activity should be moderated to reduce contact with the mosquitoes. Households and communities should ensure habitat and environment management to reduce the breeding the resting area of the mosquitoes.

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8.0 CONFLICT OF INTEREST

The author hereby declares that there is no conflict of interest.

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