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Anopheles gambiae s.l. Susceptibility Status to New Generation Insecticides (Chlorfenapyr and Clothianidin) in Selected Local Government Areas of Kebbi State, Nigeria

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Abstract

Female *Anopheles* mosquitoes are the vectors of *Plasmodium* parasites that cause malaria, which is responsible for the highest infant and maternal mortality, especially among pregnant mothers globally. However, the vectors have been reported to exhibit resistance to most pyrethroid insecticides. It was against this background that the use of new-generation (slow-acting) insecticides such as Chlorfenapyr and Clothianidin came to the limelight. This study was conducted to assess the susceptibility status of *Anopheles gambiae s.l.* to new generation insecticides in the study areas. Serial dilution of Chlorfenapyr powder was made (100µg/ml). Stock solution (40µg/ml) of Clothianidin was prepared by mixing 4mg (4,000µg) of technical grade Clothianidin with 100ml of acetone/MERO solution. The Chlorfenapyr test showed that adults *An. gambiae s.l.* were knockdown slowly between 0 and 60 minutes. However, 100% mortality was observed within 24, 48 and 72 hours, and the result of Clothianidin test indicated 100% susceptibility within 24 hours against mosquitoes in the LGAs. There is therefore an urgent need for the use of Chlorfenapyr and Clothianidin for indoor residual spray and the treatment of mosquito nets in Kebbi communities in order to curtail the level of malaria transmission in the LGAs.

Introduction

Mosquito-borne diseases remain a global public health problem and effective vaccines and treatments are often unavailable (Akogbeto *et al* 2005; Ranson *et al* 2011; Knox *et al* 2014; Balarabe *et al* 2015; Kleinschmidt *et al* 2018). Nevertheless, these diseases are preventable, as avoiding mosquito bites and/or controlling potential vectors with insecticides remain a primary way of disease reduction within the framework of Integrated Mosquito Management (IMM) (AMCA 2019). However, control failures may be due to Insecticide Resistance (IR), improper insecticide application practices, and/or other factors (variation in mosquitoes' susceptibility to different insecticides, implementation of disease-specific vector control programmes, changes in mosquitoes' behaviours, etc.), and this is under-reported partly due to limited resources (Corbel *et al* 2019). It is important that mosquitoes are routinely and effectively monitored for IR in order to make informed decisions, such that specific reasons for mosquito control failures or inefficiencies would be understood and corrected (AMCA 2019; Dusfour *et al* 2019). Despite what is known about IR, few Vector Control Programmes (VCPs) monitor it as part of their plans to protect the public from mosquito-borne diseases (Dusfour *et al* 2019). Insecticide resistance results from multigenerational selection through exposure to sublethal doses of insecticides (Stephanie *et al* 2020). In any insect population, some individuals have alleles for IR, possibly due to interactions with plant allelochemicals (Després *et al* 2007; Stephanie *et al* 2020). Alleles for IR are selected when the insect population is exposed to insecticides, which ultimately fixes the alleles in the insect population and leads to chemical-based control failure. In the development of new active substances, it is important to establish diagnostic concentrations to determine the initial

susceptibility of the vectors to enable monitoring of IR, in order to guide national malaria control programs in the introduction and replacement of the insecticide-based vector control.

Therefore, this study was carried out to assess *Anopheles gambiae s.l.* susceptibility status to new generation insecticides (Chlorfenapyr and Clothianidin) in selected Local Government Areas of Kebbi State, Nigeria.

Materials and methods

Study area

Kebbi State is in north-western Nigeria with its capital at Birnin Kebbi. The state was created out from a part of Sokoto State in 1991. It has a latitude of 11.4942°N and longitude of 4.2333°E. Kebbi State is bordered by Sokoto State, Niger State, Zamfara State, Dosso Region in Niger and Benin Republics. It has a total area of 36,800km^2 (14,200 sq mi). Kebbi State is mainly populated by the Hausa and Zarma people, with some members of Fulani, Lelna, Bussawa, Dukawa, Dakarkari, Kambari, Gungawa and Kamuku ethnic communities. Kebbi State is one of the major producers of rice in Nigeria.

Mosquito larvae collection

The characteristics of the various larval collection sites are shown in Table 1. In all breeding sites, dipping method was used as recommended by WHO (2012, 2014, 2015, 2017, 2018a and 2018b). Dipping is normally used to sample from relatively large water bodies such as swamps, ditches, streams and rice fields. The dipper was lowered gently at an angle of about 45 degrees to minimize disruption. The larvae collected were transferred into white plastic buckets and taken to the Biological Sciences Laboratory of Federal University Birnin Kebbi. Using pipettes and forceps, excess water from the white plastic buckets were emptied. The larvae were then filtered to remove unwanted debris and all larvae were later transferred to rearing containers. The number of dips and times were recorded. The average number of dips was 3-5 dips per site visited. Emergent adult mosquitoes were aspirated and transferred to rearing cages under temperature and relative humidity of 27-29°C and 70-80%, respectively. Mosquitoes were fed with 10% sugar solution for 3 days before they were used for the tests.

CDC bottle bioassay chlorfenapyr susceptibility test against female *Anopheles gambiae s.l.*

A serial dilution of the powdered chlorfenapyr insecticide (100µg/ml) was prepared. Five CDC bottles were coated with 1ml each of this concentration. These bottles were covered and kept in the dark for 24 hrs. A total of 20 of 2-5-day old female *Anopheles* mosquitoes each were introduced into five coated bottles and one uncoated bottle (control). These were monitored for a period of 60 minutes and later 24, 48 and 72 hours for all the LGAs to establish resistant status.

CDC bottle bioassay clothianidin susceptibility test against female *Anopheles gambiae s.l.*

Stock solution (40µg/ml) of clothianidin was prepared by mixing 4mg (4,000µg) of technical grade clothianidin with 100ml of acetone/MERO solution. Then 1ml each of the clothianidin working solution (4µg/ml) was used to coat four bottles of 250ml volume according to the standard VectorLink bottle assay (USAID/PMI/VectorLink, 2019, 2020 and 2021).

The coated bottles underwent dark period of 24 hrs after which 25 each of the female *Anopheles* mosquito spp. (2-4-day old) were exposed to four replicates of the coated bottles and one uncoated (control).

Figure 1. Map of Kebbi State with selected Local Government Areas for the study (Source: NPC (National Population Commission, 2006)

Table 1: Mosquito larvae collection sites Kebbi State, June-September, 2021

LGA	Coordinates of Breeding Sites	Characteristics of Breeding Sites	Property
ARGUNGU	Latitude: 12.7510252° N Longitude: 4.5372338°E	Exposure to Sunlight: Presence of Vegetation: Water Characteristics:	Temporary Partially shaded Submerse Clear
AUGIE	Latitude: 12.74832°N Longitude: 4.52873°E	Exposure to Sunlight: Presence of Vegetation: Water Characteristics:	Semi-permanent Partially shaded Submerse Turbid
BUNZA	Latitude: 12.1059691°N Longitude: 4.0245305°E	Exposure to Sunlight: Presence of Vegetation: Water Characteristics:	Semi-permanent ditch Sunlit Submerge Turbid
FAKAI	Latitude: 11.55533°N Longitude: 4.98315°E	Exposure to Sunlight: Presence of Vegetation: Water Characteristics:	Temporary ditch Partially shaded Submerse Turbid
GWANDU	Latitude: 12.603596°N Longitude: 4.4369327°E	Exposure to Sunlight: Presence of Vegetation: Water Characteristics:	Semi-permanent Partially shaded Submerse Clear
JEGA	Latitude: 12.2247583°N Longitude: 4.384435°E	Exposure to Sunlight: Presence of Vegetation: Water Characteristics:	Temporary Partially shaded Submerse Clear
KALGO	Latitude: 12.603596°N Longitude: 4.4369327°E	Exposure to Sunlight: Presence of Vegetation: Water Characteristics:	Semi-permanent Partially shaded Submerse Clear
MAIYAMA	Latitude: 12.3433459°N Longitude: 4.2051474°E	Exposure to Sunlight: Presence of Vegetation: Water Characteristics:	Temporary Sunlit Submerse Clear
SURU	Latitude: 11.6506734°N Longitude: 4.0586266°E	Exposure to Sunlight: Presence of Vegetation: Water Characteristics:	Semi-permanent Sunlit Emergent Turbid

These were monitored for a period of 30 minutes, 60 minutes and later 24 hours holding period for all the LGAs to establish resistant status.

Data analyses

Data collected were analyzed using simple percentage and presented graphically using Microsoft Excel Graphics 2014.

Results

Chlorfenapyr susceptibility test

Mosquitoes demonstrated varying degrees of tolerance to chlorfenapyr (100µg concentration) tests at the end of 60 minutes. The mosquitoes showed resistance with a knockdown range of 59-82%. However, 100% mortality of mosquitoes from 24 hours to 72 hours occurred across all the nine Local Government Areas (Figure 2).

Clothianidin susceptibility test

Susceptibility test for clothianidin (4µg concentration) indicated an increase in percentage mortality of mosquitoes with the increase in diagnostic time of exposure. However, the percentage mortality from 24 hours revealed 100% mortality of mosquitoes in all the Local Government Areas (Figure 3).

Discussion

The chlorfenapyr test showed an interesting result by the fact that, adult *An. gambiae s.l.* were found to have knockdown slowly by chlorfenapyr at the dose of 100µg between 0-60 minutes. However, 100% mortality was observed from 24, 48 and 72 hours across all the nine LGAs. The result obtained was not unexpected because chlorfenapyr is a slow acting toxin that acts by disrupting

respiratory pathways and proton gradients through the uncoupling of oxidative phosphorylation in mitochondria (Black *et al* 1994). Being a broad-spectrum insecticide, it exhibits both contact and stomach toxicity when ingested, therefore, it has a unique mode of action compared to other insecticides currently used for public health. These findings agreed with that of N'Guessan *et al* (2007) who also reported that chlorfenapyr is a broad-spectrum insecticide, which exhibits contact and stomach toxicity when inhaled.

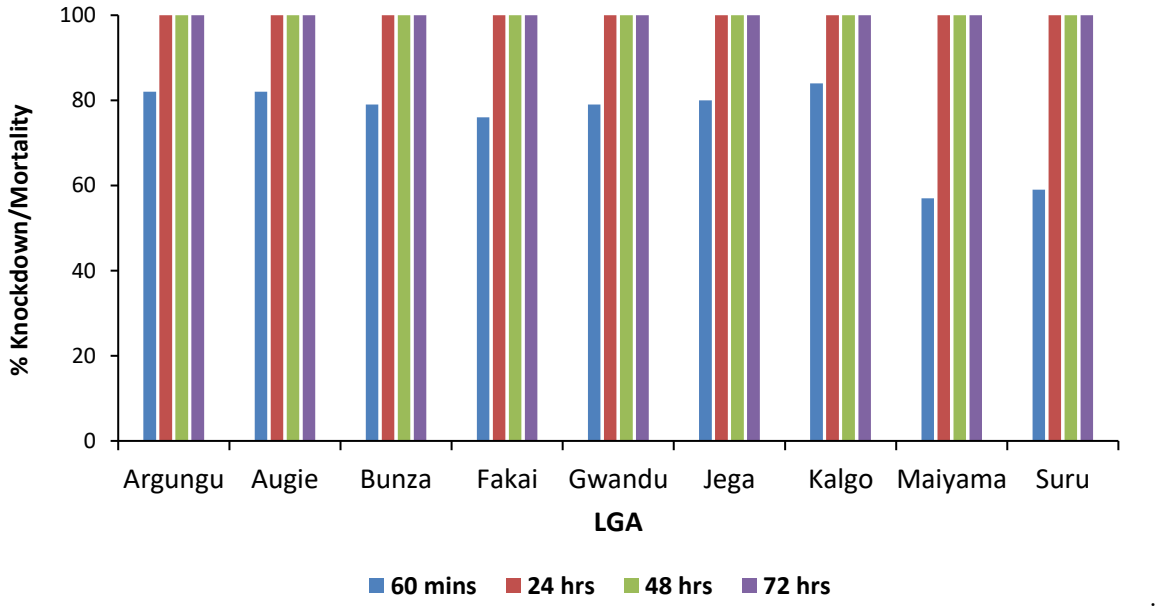

Figure 2: Chlorfenapyr susceptibility status in Kebbi State

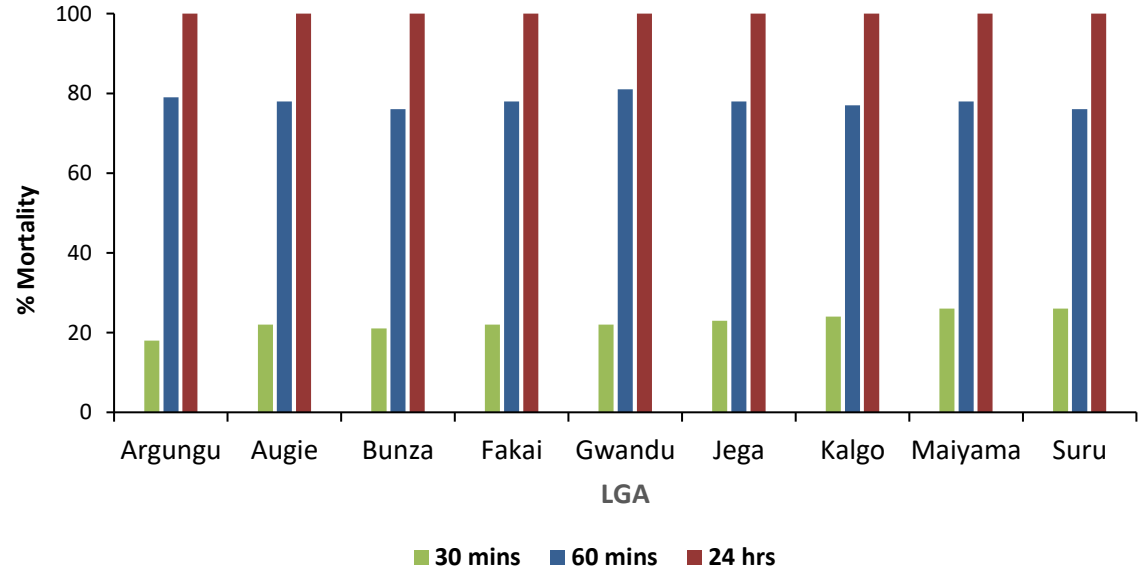

Figure 3. Clothianidin susceptibility status in Kebbi State

Clothianidin is a neonicotinoid insecticide which is chemically similar to nicotine. It acts on the central nervous system of insects as an agonist of acetylcholine and stimulates nicotine acetylcholine receptors. Clothianidin tests on *An. gambiae s.l.* across the nine LGAs within the diagnostic times of 24 hours indicated 100% susceptibility. Results from these tests demonstrated that clothianidin is also a relatively slow-acting insecticide, compared to pyrethroids, organophosphates and pyrroles tested in this study, but was found to show an interesting result in providing effective control against *An. gambiae s.l.* that have developed resistance to many pyrethroids due to the widespread usage of the chemicals. Pyrethroids resistance can be caused by genetic mutations, changes in the mosquito's physiology, or behavioural changes. These observations are in line with that of Ngufor *et al* (2017) and Oxborough *et al* (2019) who reported that

clothianidin is a neonicotinoid insecticide, which acts on the central nervous system of insects and is slow-acting compared to pyrethroid insecticides.

Conclusion

The chlorfenapyr test showed that adults *An. gambiae s.l.* were found to have knockdown slowly between 0 and 60 minutes. However, 100% mortality was observed within 24, 48, and 72 hours across all nine LGAs, while the clothianidin test indicated 100% susceptibility within 24 hours against mosquitoes in all nine selected LGAs of Kebbi State. Since insecticide resistance in malaria vector population is worldwide in distribution and cuts across all classes of insecticides recommended for public health, there is therefore an urgent need for the employment of chlorfenapyr and clothianidin for indoor residual spray, which should be used for the treatment of mosquito nets in Kebbi communities in order to curtail the level of malaria transmission in the LGAs.

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Conflict of interest

Authors have declared that no competing interests exist.

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