Susceptibility Status of Anopheles Gambiae S.I. to DDT and Permethrin in Lagos State, Nigeria.

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Abstract

Malaria is of public health importance in Nigeria and may retain its status, if new intervention methods are not introduced or the current ones monitored. This study was carried out in Alimosho, Kosofe, Ibeju-Lekki and Badagry Local Government Areas (LGAs) of Lagos State to assess the susceptibility status of *Anopheles gambiae* s.l., a dominant malaria vector in the localities to DDT 4% and Permethrin 0.75%. *Anopheles* larvae were collected from larva habitats in both LGAs, reared to adults and fed with 10% sucrose solution. Non-blood fed 2-5 days old female mosquitoes were exposed to discriminating doses of DDT and permethrin according to the standard WHO procedures. The knockdown effect was recorded at interval of 10 minutes and mortality scored 24hours after exposure. Species identification was done by morphological characteristics. Results from the study showed that *Anopheles gambiae* s.l. are resistant to DDT (4%) and permethrin (0.75%) with 24hr post exposure mortality ranging from 15.00-24.0% and 4.25-22.0% respectively. The Knockdown Time (KDT_{50}) ranged from 30.8-941.95minutes for DDT and 12.6-85.51minutes for permethrin. KDT95 ranges from 117.49-27524.22 minutes and 117.49-412.1 minutes for DDT and permethrin respectively. All the LGAs showed high level of resistance to DDT and permethrin. The high level of insecticide resistance recorded in this study could be detrimental to the elimination of malaria. Therefore, integrated vector management is needed to effectively carry out to manage the development of insecticide resistance.

Key words: *Anopheles gambiae* s.l. Insecticide resistance, DDT, permethrin, Lagos State

Introduction

Malaria is currently one of the most widespread infectious diseases, the World Health Organization (WHO)reported 584,000 deaths due to malaria in 2014, 90 % of which were from sub-Saharan Africa (WHO, 2014). Application of insecticide-based interventions has played a major role in reducing the global malaria morbidity and mortality. (Kleinschmidt *et al.*, 2018).

In, Nigeria, malaria accounts for 60% of outpatient visits to health facilities, 30% of childhood mortality, 25% mortality of infants under one year and 11% maternal mortality and also DALYs of about 132 billion naira financial loss in treatment costs, prevention and control and loss of man-hours(Umar *et al.*, 2014).

Malaria prevention is heavily dependent on insecticide-based mosquito control interventions such as the use of Long Lasting Insecticide Nets (LLINs) and Indoor Residual Spraying (IRS) –'–(WHO, 2015, 2014). Vector control strategies involve the use of pyrethroid-based LLINs and IRS although bendiocarb and dichlorodiphenyltrichloroethane (DDT) are also used in some areas for IRS (Ranson *et al.*, 2011).

The World Health Organization Pesticide Evaluation Scheme (WHOPES) has mandated that ITNs only be treated with pyrethroid pesticides and IRS programmes use pyrethroids, a handful of organophosphates, carbamates, or DDT (World Health Organization, 2016). The implementation of malaria control strategies, such as the spraying of residual insecticides and the use of insecticide-treated mosquito nets have led to enormous progress in the control of malaria in Sub-Saharan Africa (Chouaïbou *et al.*, 2017).

Resistance to this few approved insecticides in vector control poses a real threat to malaria control. Reports of African vector populations exhibiting resistance to insecticides began in the1950s, and the problem continues to grow today(Reid and McKenzie, 2016). The widespread and indiscriminate use of the insecticides in public health and agriculture has been associated with the development of insecticides resistance (Soko *et al.*, 2015). Resistance to DDT and pyrethriods in *Anopheles* has been reported in different parts of Africa (Boussougou-Sambe *et al.*, 2018; Dabiré *et al.*, 2009; Hakizimana *et al.*, 2016; Messenger *et al.*, 2017; Thiaw *et al.*, 2018; Tmimi *et al.*, 2018). In Nigeria, malaria vector resistance to DDT and pyrethriods have also been previously reported –(Awolola *et al.,* 2007; Ibrahim *et al.*, 2013; Oduola *et al.*, 2010; Opara *et al.*, 2017; Oyewole *et al.*, 2006; Umar *et al.*, 2014). There is therefore need for regular monitoring of the level of resistance to different insecticides approved for control of malaria vectors, for effective and efficient vector control. This study seeks to provide current information on the resistance/susceptibility profile of malaria vectors collected from four different LGAs of Lagos State.

Materials and methods

Study Area

The study was carried out in four (4) Local Government Areas (LGAs) of Lagos State, Nigeria. Alimosho LGA (6°36′38″N 3°17′45″E), the largest local government in Lagos with 1,288,714 inhabitants according to the National Population Census (NPC) of 2006,Kosofe LGA situated at 6°45'N, 3°4'E and 35 meters,Ibeju-Lekki LGA (6º26'34"N, 3º28'29"E), situated within the southern area of Lagos State, just below the Lagos lagoon and Badagry LGA (6°25′N 2°53′E); a coastal town located between Metropolitan Lagos, and the border with Republic of Benin at Seme.please provide map of Lagos showing study area.

Mosquito sample collection

Anopheles mosquitoes immature stages were collected using WHO standard methods between the period of May and August 2018, from suitable larva habitats in the selected 4 Local Government Areas of Lagos State and transferred to the insectary at the Department of Zoology, University of Lagos for emergence. Emerged adult female mosquitoes were fed on sugar 10% solution and used in the bioassays.

Insecticide Susceptibility Test

The insecticide susceptibility tests were carried out using the WHO standard procedures and test kits for adult mosquitoes. It was conducted using glucose-fed but non-blood fed female *Anopheles* mosquitoes. Four replicates of 25 adult female *Anopheles* mosquitoes were exposed to test papers and allowed to stand for 1 hour. The numbers of knocked-down mosquitoes were recorded at 10 min, 15 min, 20 min and subsequently at intervals of ten minutes. After the exposure, mosquitoes were then transferred to recovery tubes and fed with 10% glucose solution and placed on the mesh-screen end of the holding tubes. Mortality was recorded after 24 hours post-exposure(WHO, 2016).

Identification of *Anopheles* mosquitoes

Morphological keys of —(Gillet, 1972; Gillies and Coetzee, 1987) were used as a guide in the morphological identifications of adult *Anopheles* mosquito by wing venation, breakage in wings, palps and proboscis length and hair patterns.

Data Analysis

Insecticide susceptibility was based on the criteria that ≥98% mortality indicates susceptibility, 90–97% mortality indicates possible resistance and <90% mortality indicates confirmed resistance (WHO, 2016). All controls showed no mortality hence there was no need to apply Abbott formula, while (KDTs) knock down times were estimated using a log time probit model with SPSS version 23 for windows.

Results

Adult female mosquitoes morphologically identified as *An. gambiae* s.l were exposed to diagnostic doses of two insecticides, permethrin (0.75%) and DDT (4%), 100 mosquitoes were exposed per insecticide in 4 replicates. The mean knockdown rate of*An. gambiae* s.l against DDT and permethrin at time intervals and 24hours post exposure in Lagos State is shown in Table 1. The knockdown rates showed gradual increase for all the locations. All the three insecticides showed gradual knockdown abilities at various times during the 1 hour exposure. Both DDT and permethrin were unable to knockdown 50% of the mosquitoes at 60 minutes of exposure, the highest mean knockdown rate (23.0±0.0) recorded in this study was in DDT from Ibeju-Lekki while the lowest value of 6.0±0.0 was recorded in DDT in Alimosho and Kosofe. After 24hours post exposure period, mean mortality of An. gambiae exposed to DDT ranges from 15.00± 0.0 to 24.0±0.0 in the four LGAs while mean mortality for permethrin ranges from 4.25± 1.03 to 22.0±0.0

The average Knockdown Time (KDT_{50}) obtained from time-mortality probit regression analysis range from 30.8 to 941.35 minutes, while the KDT_{95} ranged from 117.49 to 27524.22 minutes for DDT. KDT_{50} and KDT_{95} permethrin ranges from 12.6 to 85.5 minutes and 132.3 to 412.1 minutes, respectively (Table 2).

Table 2: Mean knockdown and mean total mortality rates of *Anopheles gambiae* s.l. population from Alimosho, Badagry, Ibeju Lekki and Kosofe LGAs of Lagos State exposure to DDT and permethrin

LGAs	Insecticide	N	10min	15min	20min	30min	40min	50min	60min	Mean mortality at 24 hours
	Control	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Alimosho	DDT	100	0.0±0.0	3.0±0.0	5.0±0.0	5±0.0	6.0±0.0	6.0±0.0	6.0±0.0	15.0±0.0
	Permethrin	100	0.0±0.0	0.50±0.29	2.50±0.29	4.50±0.29	6.50±0.29	6.5±0.29	8.50±0.29	14.50±0.29
Badagry	DDT	100	1.75±0.5	4.5±0.9	6.25±1.0	9.25±1.0	16.50±0.9	18.75±0.5	22.25±0.3	19.75±0.3
	Permethrin	100	10.8±1.9	12.8±1.4	17±0.7	18.8±1.3	19.8±1.7	20.0±1.4	21.8±1.0	21.5±0.3
Ibeju-lekki	DDT	100	2.0±0.0	5.0± 0.0	8.0± 0.0	10.0± 0.0	14.0± 0.0	16.0 ± 0.0	23.0 ± 0.0	24.0±0.0
	Permethrin	100	6.0 ±0.0	7.0± 0.0	9.0±0.0	10.0±0.0	13.0±0.0	18.0±0.0	20.0±0.0	22.0±0.0
Kosofe	DDT	100	0.0±0.0	3.00± 0.0	5.00± 0.0	5.00 ± 0.0	6.00± 0.0	6.00± 0.0	6.00± 0.0	15.00± 0.0
	Permethrin	100	0.0± 0.0	0.0± 0.0	0.25± 0.3	3.75± 1.10	5.50 ± 1.32	8.75± 2.32	12.75± 2.28	4.25± 1.03

Table 2: Log-time probit model used to estimate the KDT50 & KDT95 values and percentage mortality of *Anopheles gambiae* s.l exposed to DDT and permethrin in Alimosho, Badagry, Ibeju Lekki and Kosofe LGAs of Lagos State.

LGAs	Insecticide	N	KDT 50 (min)	KDT 95 (min)	Knockdown After 1hr Exposure(%)	Mortality(%) After 24 hr
Alimosho	DDT	100	168.2	3634.7	15.5	Resistance
	Permethrin	100	85.5	412.1	14.5	Resistance
Badagry	DDT	100	30.8	196.2	19.75	Resistance
	Permethrin	100	12.6	132.3	21.5	Resistance
Ibeju-lekki	DDT	100	31.56	117.49	23.00	Resistance
	Permethrin	100	29.66	213.92	20.0	Resistance
Kosofe	DDT	100	941.35	27524.22	6.00	Resistance
	Permethrin	100	59.36	142.95	12.75	Resistance

Discussion

The development of resistance by malaria vectors to the different classes ofinsecticide threatens the efficiency of the insecticides approved by WHO for usage in malaria control. In this study, the susceptibility status of *Anopheles gambiae* s.l. to DDT and permethrin was investigated in Alimosho, Badagry, Ibeju-Lekki and Kosofe Local Government Areas of Lagos State. Delete high level of resistance was recorded to DDT and permethrin in *An. gambiae* s.l. in all the study areas, previous studies in different parts of Nigeria have confirmed *Anopheles* resistance to DDT and pyrethriods –(Awolola *et al*., 2009; Ibrahim *et al*., 2013, 2014; Oduola *et al*., 2010; Oyewole *et al.*, 2006) but the level of resistance at 24 hours post exposure period was higher than most of pervious reports though similar to report for Bichi in Northern Nigeria (Abdu et al., 2017).Increase in the level of resistance to DDT and permethrin could be related to the widespread use of LLINs, which will in turn lead to selection pressure on local *Anopheles* population and the use of insecticides for other economic purposes such agriculture could have influence resistance in densely populated LGAs of Badagry and Ibeju-Lekki. The level of resistance found in the present study should not be allow to affect the use of insecticides in malaria control especially in the utilization of LLINs, as LLINs does not only protection by killing the vectors but also serve as a barrier for the vector from getting to the persons sleeping under it. The KDT50and KDT95 recorded to DDT and permethrin in this study was higher compare to those of previous reports (Ibrahim *et al*., 2013; Opara *et al*., 2017; Umar *et al*., 2014), indicating the possibility of knockdown resistance mechanism. This can be related to the fact that pyrethroids and organochlorines shares similar mechanism of action by targeting the voltage-gated sodium channel on the insects' neurons"–(Rabi et al., 2015; Silva et al., 2014). Resistance to pyrethriods and organochlorines has also been associated with increased activities of the detoxifying enzymes GSTs and Esterases(Rabi et al., 2015)this has been previously demonstrated in populations of malaria vectors in Nigeria and west Africa, indicating the presence of multiple resistance mechanisms in malaria vectors (Awolola *et al*., 2009; Corbel *et al*., 2007; Dadzie *et al*., 2017; Fagbohun *et al*., 2019).

Integrated and sustainable vector control and insecticide management strategy is needed for effective malaria control. It is also necessary that regular surveillance is carried out to know current resistance status of these vectors which changes with time, to forestall the waste time and resources on an ineffective control method.

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