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RESEARCH **Open Access**

Evidence of Metabolic Resistance in Pyrethroid Resistant *Aedes aegypti* Population from Lagos, Nigeria

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Abstract

Background: Pyrethroid resistance in *Aedes aegypti* is of major concern to the control of several arboviral infections. The major mechanisms of Pyrethroid resistance in mosquitoes are target-site insensitivity and elevation in the activity of detoxification enzymes. In this study, we assessed the susceptibility status of *Aedes aegypti* population from Lagos to Pyrethroid and the impact of metabolic enzymes on resistance development.

Methods: Larvae of *Aedes aegypti* were collected from different habitats in Lagos Mainland Local Government Area, Lagos state. Adult mosquitoes of 2-5 days were exposed to diagnostic dose of permethrin and Deltamethrin using the CDC method. Synergist assay was done with pre-exposure of samples to PBO before exposure to insecticide. Esterase and GST activities were measured using standard protocol. Regression Probit was used to compute the KDT_{50} and KDT_{95}. Analysis of variance was used to compare the difference in mean of enzymes activities.

Results: *Aedes aegypti* population from the study location is resistant to permethrin (33%) and Deltamethrin (80%) within the diagnostic time. PBO pre-exposure increases percentage knockdown from 33% to 82% and 80% to 87% for permethrin and Deltamethrin respectively. The activity of GST was higher ($P<0.05$) in permethrin exposed mosquitoes in comparison with unexposed.

Conclusion: A robust insecticide resistance management (IRM) plan in Lagos should take into consideration strategies for addressing the effects of metabolic enzymes in resistance development .

Keywords: Metabolic, Resistance, Pyrethroid, Resistant, *Aedes aegypti*

1.0 INTRODUCTION

Mosquitoes are one of the most abundant classes of insects belonging to the genera Anopheles, Culex, Aedes and Monsonia. Of the 4 genera, Aedes are the most aggressive and invasive mosquito species with worldwide distribution [1]. *Aedes aegypti* is the most synanthropic species of the Culicidae, always cohabiting with humans. It is preferentially diurnal and tends to be most active at dawn and dusk, thus avoiding the hottest periods of the day [2]. It is known as the yellow fever mosquito and it poses significant public health concern because of its ability to transmit various viral pathogens. They are readily recognized by the lyre-shaped silver markings on the lateral edges of the scutum with conspicuous patterns on the thorax formed by black, white or silvery scales [3].

They are primarily found breeding in containers with high larval density mainly in peri-domestic environments [4]. Aedes mosquitoes are capable of transmitting various infectious disease of public health importance including dengue fever, chikungunya fever, yellow fever, filariasis, Japanese encephalitis, rift valley fever, Zika virus and other viral encephalitis [3].

Control of Mosquito borne diseases had largely employed vector control strategies than use of drugs and vaccines. This control has largely been done through the use of insecticides with proper understanding of vector ecology, epidemiology and implementation management [5]. Mosquito control strategies focus primarily on the use of pyrethroid insecticide as first line defense [6]. Although evidence based research had clearly stated the effectiveness of pyrethroids in Long Lasting Insecticide Nets (LLIN) and Indoor Residual Spraying (IRS) [7], development of resistance to Dichlorodiphenyltrichloro-ethane (DDT), pyrethroid and carbamates has been reported in mosquitoes [8]. Also, the development of resistance to pyrethroids based on selection pressure had proven to threaten the effectiveness of Aedes control [6].

The most common mechanisms of pyrethroid resistance that have been identified in *Aedes aegypti* include mutations in the voltage sensitive sodium channel gene (VSSC gene) and metabolic-mediated insecticide resistance [6]. Enzymes involved in the metabolic-mediated detoxification (monooxygenases, glutathione-S -transferases and esterases) have been reported to be related to pyrethroid resistance but many specific contributory enzymes are not completely studied [6]. In a bid to ensure the success of malaria control strategies, studies have been conducted on different approaches to managing resistance development. Several evidences had supported the use of Piperonyl Butoxide incorporation with pyrethroids to eliminate detoxifying enzymes mediated resistance to pyrethroid on mosquito population [9]. Hence investigating insecticides resistance mechanisms especially metabolic resistance in Aedes mosquito is pertinent. The aim of this study was to investigate the role of metabolic enzyme against pyrethroid among Aedes species in Lagos, Nigeria.

2.0 METHODOLOGY

2.1 Sample Collection

Immature stages of *Ae. aegypti* larvae were collected from different habitats in Lagos Mainland Local Government Area, Lagos State with the aid of larval ladle and gently poured into small transparent plastic bowls. A strainer was used to sieve and pool the third and fourth instar larvae in order to have sufficient adult emergence of the same age. The bowls were scrutinized for presence of unwanted organisms or predators. Aedes mosquito larvae collected were transported in well labelled plastic bottles to the insectary in the Entomology unit of the Nigeria Institute of Medical Research, Lagos, where they were maintained and reared at a temperature of 26 ± 3°C and 74 ± 4 % relative humidity to adult stage [10]

2.2 Morphological and Molecular Identification

Morphological identification of mosquitoes was done as described by [10], with the aid of a dissecting microscope. Genomic DNA was extracted from individual mosquito according to the method described by [11]. The following primers [AUF (5'-TCAAAATTA AGGGTAGTGGT-3'), AUR (5' GACTTCAACTGGCT TGAACT-3' and AEG (5' GAC ACC GAG GCG CCC ATT GC -3')] were used to amplify at 157 base pair for *Ae. aegypti* DNA fragment [12]. The PCR condition for all assay include an initial denaturation of 94°C for 5 minutes, then 35 cycles 94°C for 30secs, 56°C for 40secs, 72°C for 1 minutes and final extension at 72°C for 1 minutes.

The PCR reactions were carried out in a total volume of 20 ul containing 12.5 µl of PCR master mix containing 1 × PCR buffer, 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.4 µM of each primer, one unit of Taq polymerase (Solis BioDyne) and 1 µl of genomic DNA. 10ul of PCR product was loaded into each sample well on a 1.5% agarose gel visualised by ethidium bromide stains under Ultra Violet light (UV light).

2.3 Insecticide and PBO Synergist Bioassay

Susceptibility and synergistic tests were performed using Center for Disease Control and prevention (CDC) method [13]. Two to three days old 20 female mosquitoes in three replicates were exposed to Deltamethrin (12.5 µg/ml) and Permethrin (21.5 µg/ml). Each test consisted of 3 experimental bottles and a negative control bottle (1ml of acetone). After 30 minutes, the numbers of live and dead mosquitoes in each bottle were recorded. The resistant mosquitoes were knocked down under -20°C freezer before enzyme analysis.

2.4 Microplate Metabolic Enzyme Activity Assay

Mosquitoes resistant to both Permethrin and Deltamethrin were homogenized in 200µl of cold distilled water in a 1.5mL centrifuge tube. The homogenate was centrifuged at 14,000 rpm for 20 seconds and supernatant was stored at -20°C [14]. Glutathione S-transferase (GSTs) and esterase were analyzed on each resistant female mosquito and on the susceptible strain. Mean absorbance value was converted to enzymes activity and was calculated in nmol/mg/ml protein

2. 4.1 Glutathione S-transferase Assay

Two replicates of 10µl of mosquito homogenate were placed in separate well of the microtitre plate, 200µl of the GSH/CDNB working solution was then added. Three blank plates containing 10µl distill water and 200µl of the GSH/CDNB working solution were used as control. The test was then left at room temperature for 20 minutes and the absorbance value was read at 340 nm.

2.4.2 Esterase Assay

For non-specific esterase activity, 50µl aliquot of the homogenate was placed in a well of a 96-well flat bottom microtiter plate, before adding 50µl of freshly prepared substrate solution of (60µg/ml α-naphthyl acetate in PBS) and the mixture was incubated for 60 seconds at 27°C. this was followed by addition of 50µl aliquot of coupling reagent (3 mg/ml Fast Blue B salt in 3.5% SDS), incubated for 10 minutes and the reaction was terminated by the addition of 50 µl of 10% acetic acid and was measured spectrometrically at A450 nm using a Bio-Rad Benchmark Microplate Reader (Bio-Rad, Hercules, CA) [15].

2.4.3 Protein Assay

Total protein was measured for each mosquito using Biuret test [10]. All measurements were done in duplicate.

2.4.4 Data Analysis

Susceptibility status was based on the criteria that 98–100% mortality of mosquito implies susceptibility, 90–97% mortality indicates possible resistance and <90% mortality implies resistance. Regression probit was used to compute the KDT_{50} and KDT_{95}. Chi-square test was used to compare percentage mortality between insecticide only and PBO plus insecticide. Analysis of t-Test was used to compute statistical difference in the enzyme activities exposed to different insecticides. Data were computed using Microsoft Excel version 2016 and IBM SPSS Statistics 23.0. P was considered significant at $P< 0.05$.

3.0 RESULTS

The mosquitoes collected were not all Aedes. We were able to separate them on the basis of moderate size, with a pattern of scales on the head, chest, legs, and abdomen and were easily distinguished from other members of the genus due to the white lyre shape on the dorsal side of the thorax. Resistance to Permethrin (33%) and Deltamethrin (80%) was recorded in *Ae. aegypti*

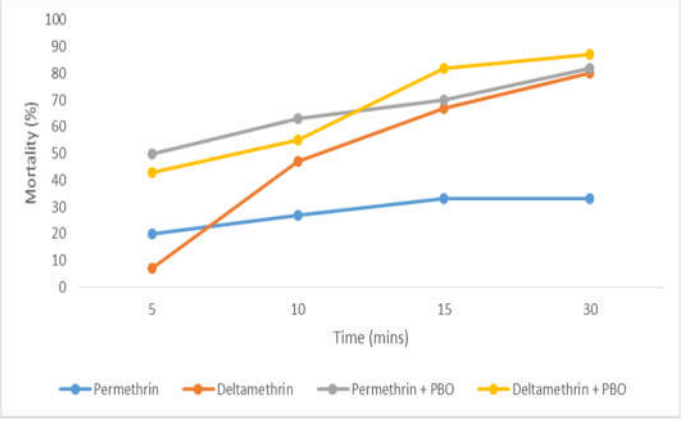

Figure 1. Percentage mortality for *Ae aegypti* mosquitoes population exposed to permethrin, deltamethrin, permethrin + PBO and deltamethrin + PBO in Lagos Mainland, Lagos

population from the study site (Figure 1).

After pre-exposure to PBO synergist, the percentage mortality to Permethrin increased drastically from 33% to 82 %, whereas, percentage mortality to Deltamethrin increased from 80% to 88% (Figure 1). Knockdown time in the PBO synergized population was also low when compared with the non-synergized assay. KDT_{50} for Permethrin exposed population and population pre-exposed to PBO were 700secs and 306secs respectively, while KDT_{50} for Deltamethrin exposed population and population pre-exposed to PBO were 712secs and 417secs respectively (Table 1).

Table 1. Knockdown Time (min) of *Ae. aegypti* Population Exposed to Permethrin and Deltamethrin, permethrin + PBO and Deltamethrin + PBO in Lagos Mainland, Lagos.

Insecticide	Number exposed	KDT_{50}(95Cl)	KDT_{95}(95Cl)
Permethrin	60	11.40(8.98-14.21)	95.15(54.58-285.99)
Deltamethrin	60	11.52(8.89-14.64)	115.19(61.11-435.92)
PBO+Permethrin	60	5.06(1.82-7.55)	36.09(55.62-2195.68)
PBO+Deltamethrin	60	6.57(4.45-8.36)	53.98(34.1-135.5)

There was no significant difference in esterase activity of *Ae. aegypti* obtained from study site when exposed to Permethrin and Deltamethrin when compared with unexposed (P>0.05; Table 2). However, GST activities was significantly expressed among permethrin exposed *Ae. aegypti* population when compared with unexposed (P<0.05), but no significant difference in GST activity among Deltamethrin exposed population (Table 3).

Table 2. Esterase Enzyme Activity in *Ae. aegpyti* Population from Lagos Mainland, Lagos Exposed to Permethrin and Deltamethrin

Insecticide	Mean±SD	P-value
Permethrin	0.0027±0.0014[a]	
Deltamethrin	0.0017±0.0011[a]	0.107
Control	0.0046±0.0044[a]	

Values with same superscript are statistically similar at P < 0.05

Table 3. Glutathione-S-transferase Enzyme Activity in *Ae. aegpyti* Population from Lagos Mainland, Lagos Exposed to Permethrin and Deltamethrin

Insecticide	Mean±SD	P-value
Permethrin	0.0008±0.0025[a]	0.002
Deltamethrin	0.0004±0.0006[a]	
Control	0.0002±0.0004[b]	

Values with same superscript are statistically similar at P < 0.05

4.0 DISCUSSION

Insecticide resistance is one of the main challenges that have negatively impacted vector control and has stalled the progress recorded in the control of several diseases of public health importance. It is one of the main areas that attention should be fixed on, if vector control objectives is to be achieved. Previous studies have also reported susceptibility of *Ae. aegypti* to permethrin in urban areas of Nigeria and Senegal [16, 17].

Other studies have reported resistance of populations of *Ae. aegypti* in other parts of the world [18, 19]. Hence, this study was initiated to assess the resistance status of *Ae. aegypti* to Pyrethroid insecticides and examine the impact of metabolic enzymes in resistance development. This study demonstrates the occurrence of resistance of *Ae. Aegypti* populations to pyrethroid insecticides in Lagos, Nigeria. The findings from the study showed that *Ae. aegypti* population from the study site are resistant to diagnostic dose of both permethrin and Deltamethrin. This is in consonance with the findings of smith *et al.,* [20] and Oduola *et al.,* [21] where resistance of *Ae. Aegypti* to pyrethroids was recorded. Contrarily, susceptibility of *Ae. Aegypti* to pyrethroids has been recorded in some other studies [22]. This incidence with contrary findings in different areas may be due to uncontrolled applications of insecticide in certain areas like Lagos. This increases the amount of insecticide in the environment and consequently impact on the adaptation of mosquito population for survival. This poses serious threat to vector control interventions .

In this study, the use of PBO with Permethrin and Deltamethrin decreased knockdown time (KDT), suggesting that the use of pyrethroids in combination with PBO will be effective in reducing knock down time of resistant population of mosquitoes. This has also been reported by

LeClair *et al.,* [23]. Previous study had revealed that mosquitoes with elevated activities of GST and monooxygenases were resistant to Pyrethroids, Malathion and Propoxur [24]. In our studies, we observed significant difference in the activity of glutathione transferase (GST) in *Ae. Aegypti* study population when exposed to Permethrin ($P<0.05$) but there was no significant difference in GST activity for Deltamethrin in comparison with unexposed ($P>0.05$) under the same laboratory condition. We also observed that there was no statistical significant difference in esterase activity of *Ae. aegypti* obtained from study site when exposed to Permethrin and Deltamethrin when compared with unexposed ($P>0.05$). Therefore, monitoring of resistance in mosquito population is paramount to the success of vector control interventions. The development of resistance by Aedes population in Lagos needs continuous surveillance so as to aid the formulation of robust insecticide resistance management strategy.

The observed resistance to Pyrethroid among *Ae. aegypti* is alarming but increasing susceptibility of *Ae aegypti* to PBO synergized pyrethroids had increased the hope of vector control in Lagos, Nigeria. It is of utmost importance to continue surveillance of insecticide resistance in mosquitoes and also determine the underlying resistance mechanisms in resistant population. This will help in implementation of effective evidence-based control measures.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Authors Contribution

KOA conceived and designed the study, performed data collection, contributed to data analysis and manuscript writing; **AOA** contributed to study design, data collection and manuscript writing; **TAO, STA** contributed to study design and writing of the manuscript; **AKO,** contributed to data analysis tools and performed data analysis; **RTJ, LO** contributed to data collections, insecticide exposure and enzyme assay on the samples; **IKF** contributed to data collection, statistical tools and writing of manuscript; **AIO** contributed to data collection and manuscript writing

References

1. Ahmed UA, Sani ZA. Studies of Mosquitoes in Hadejia Emirate, Jigawa State, Nigeria. In Proceedings of the Academic Conference on Positioning Sub-sahara Africa for Development in the New Development. 2016; 9(1):1-12
2. Carvalho FD, Moreira LA. Why is Aedes aegypti Linnaeus so Successful as a Species?. Neotrop Entomol 2017; 46: 243–255. https://doi.org/10.1007/s13744-017-0520-4
3. Ukpai, Onyinye Mkpola, and Chukwuebuka Mathias Ekedo. "Insecticide susceptibility status of Aedes aegypti in umudike, Ikwuano lga Abia State, Nigeria." Animal Research International 2018 ;15:3.
4. Rathor HR. The role of vectors in emerging and re-emerging diseases in the Eastern Mediterranean Region. EMHJ-Eastern Mediterranean Health Journal. 1996;2 (1): 61-67.
5. Wilson, AL, Courtenay O. Kelly-Hope LA, Scott TW, Takken W, Torr SJ, Lindsay, SW. The importance of vector control for the control and elimination of vector-borne diseases. PLoS neglected tropical diseases. 2020; 14(1), e0007831.
6. Amelia-Yap ZH, Chen CD, Sofian-Azirun M, Low VL. Pyrethroid resistance in the dengue vector Aedes aegypti in Southeast Asia: present situation and prospects for management. Parasites & vectors. 2018;11(1):1-7.
7. World Health Organization. Conditions for deployment of mosquito nets treated with a pyrethroid and piperonyl butoxide: recommendations. World Health Organization; 2017.
8. Ibrahim SS, Mukhtar MM, Datti JA, Irving H, Kusimo MO, Tchapga W, Lawal N, Sambo FI, Wondji CS. Temporal escalation of pyrethroid resistance in the major malaria vector Anopheles coluzzii from Sahelo-Sudanian Region of northern Nigeria. Scientific reports. 2019; 14(1):1-19.
9. Akoton R, Tchigossou GM, Djègbè I, Yessoufou A, Atoyebi MS, Tossou E, Zeukeng F, Boko P, Irving H, Adéoti R, Riveron J. Experimental huts trial of the efficacy of pyrethroids/piperonyl butoxide (PBO) net treatments for controlling multi-resistant populations of Anopheles funestus ss in Kpomè, Southern Benin. Wellcome Open Research. 2018;3.
10. Fagbohun IK, Idowu ET, Olakiigbe AK, Oyeniyi AT, Otubanjo OA, Awolola TS. Metabolic resistance mechanism in Aedes aegypti from Lagos State, Nigeria. The Journal of Basic and Applied Zoology. 2020;81(1):1-7
11. Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C, Kaiser A. Mosquitoes and their control. Springer Science & Business Media; 2010 Aug 18

12. Collins FH, Mendez MA, Rasmussen MO, Mehaffey PC, Besansky NJ, Finnerty V. A ribosomal RNA gene probe differentiates member species of the Anopheles gambiae complex. The American journal of tropical medicine and hygiene. 1987;37(1):37-41.

13. Das B, Swain S, Patra A, Das M, Tripathy HK, Mohapatra N, Kar SK, Hazra RK. Development and evaluation of a single step multiplex PCR to differentiate the aquatic stages of morphologically similar Aedes (subgenus: Stegomyia) species. Tropical Medicine & International Health. 2012; 17(2):235-43.

14. Brogdon W, Chan A. Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay. USA: CDC Atlanta. 2010.

15. Hemingway, J. Insecticide resistance mechanisms (Field and laboratory manual), (pp. 1–39). Geneva: World Health Organisation.1998

16. Mardihusodo SJ. Application of non-specific esterase enzyme microassays to detect potential insecticide resistance of Aedes aegypti adults in Yogyakarta, Indonesia. Berkala ilmu kedokteran. 1996; 28(4):167-71

17. Dia I, Diagne CT, Ba Y, Diallo D, Konate L, Diallo M. Insecticide susceptibility of Aedes aegypti populations from Senegal and Cape Verde Archipelago. Parasites & vectors. 2012;5(1):238.

18. Kemabonta KA, Anikwe JC, Adaezeobiora IB. Bioefficacy of Skaeter Abate and Spintor on Anopheles gambiae and Aedes aegypti mosquitoes from insecticides resistance areas of Lagos and Oyo State, Nigeria. J. Agric. Healthcare. 2013;3: 3

19. Prapanthadara LN. Promtet S. Koothath P, Somboon,W, Suwonkard L, and Hemmingway J. Mechanisms of DDT andPermethrin resistance in Aedes aegypti from Chiang Mai, Thailand. Dengue Bull.2002; 26: 185–189.

20. Srisawat RN, Komalamusra C, Apiwathnaon P., Paeporn S, Roytrakah Y, Rongonyam, and Eshita Y. Field collected permethrin- resistant Aedes aegypti from central Thailand ccontain point mutation in the domain llS6 of the sodium channel gene (kdr). Southeast J. Trop. Med. Public Health . 2012; 43: 1380–1386.

21. Smith, L. B., Kasai, S., & Scott, J. G. (2016). Pyrethroid resistance in Aedes aegypti and Aedes albopictus: Important mosquito vectors of human diseases. Pesticide biochemistry and physiology. 2016; 133: 1-12.

22. Oduola AO, Obembe A, Adelaja OJ, Ande AT. Surveillance and insecticide susceptibility status of culicine mosquitoes in selected communities utilizing long-lasting insecticidal nets in Kwara State, Nigeria. Animal Research International. 2016;13(3):2483-91.

23. Ponlawat A, Scott JG, Harrington LC. Insecticide susceptibility of Aedes aegypti and Aedes albopictus across Thailand. Journal of Medical Entomology. 2005; 42(5): 821-5.

24. LeClair C, Cronery J, Kessy E, Tomás EV, Kulwa Y, Mosha FW, Rowland M, Protopopoff N, Charlwood JD. 'Repel all biters': an enhanced collection of endophilic Anopheles gambiae and Anopheles arabiensis in CDC light-traps, from the Kagera Region of Tanzania, in the presence of a combination mosquito net impregnated with piperonyl butoxide and permethrin. Malaria journal. 2017; 16(1):1-8.

25. Surendran SN, Jayadas TT, Sivabalakrishnan K, Santhirasegaram S, Karvannan K, Weerarathne TC, Karunaratne SP, Ramasamy R. Development of the major arboviral vector Aedes aegypti in urban drain-water and associated pyrethroid insecticide resistance is a potential global health challenge. Parasites & vectors. 2019;12 (1):337