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Molecular Identification and Insecticide Resistance Status of *Culex* mosquitoes collected from blocked drainages in Lagos State, Nigeria

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Background: *Culex* mosquitoes are important vectors of several human pathogens causing infections such as lymphatic filariasis and several viruses. Poor and blocked drainage system can lead to impediment in water flow, leading to the artificial creation of larval habitats for *Culex* mosquitoes. *Culex* mosquitoes has the ability to breed in organically polluted water bodies and exhibit high resistance to insecticides. Therefore, this study assessed the species and insecticides susceptibility status of Culex breeding in blocked drainages in Lagos State.

Methods: *Culex* mosquito larvae were collected from blocked drainages in three Local Government Areas (LGAs) of Lagos State, Nigeria, using standard WHO technique. The physicochemical parameters of the larval habitats were also recorded. Collected mosquito larvae were raised to adult, 2-3 days old. Glucose fed adults female mosquitoes were exposed to permethrin (0.75%) and DDT (4.0%) WHO insecticide test papers. Morphological identification was carried out using standard keys and molecular identification of *Culex pipiens* sub-species and *kdr* genotyping was carried out using PCR

Results: High level of resistance was recorded with mortality rate after 24 hours for DDT ranging from 20% to 32% while permethrin ranges from 14% to 36%. The pH of the all the *Culex* mosquito larva habitats ranges from 7.38±0.11 to 7.62±0.29, while TDS ranges from 592.6±79.1 to 655±68.1. A total of 1113 *Culex pipiens* mosquitoes that were identify morphologically, some were selected for molecular identification using PCR assays, out of which 96.2% were identified as *Culex p. quinquefasciatus* while 3.7% were unidentified. Knockdown mutation (L1014F) was not detected in DDT and pyrethroids resistant *Cx. quinquefasciatus* in this study.

Conclusion: Unplanned urbanization, inadequate water supply and inefficient solid waste and sewage management practices can result in the creation artificial larval habitats for *Culex* mosquitoes leading to potential outbreak of *Culex* mosquito borne diseases. The resistance to DDT and permethrin insecticides in *Cx. quinquefasciatus* in Lagos State may represent a threat towards the efficacy of ITNs and other forms of vector control such as indoor residual spraying in the future.

Keywords: *Culex* mosquito, Insecticide resistance, Lagos State

1.0 INTRODUCTION

Culex mosquitoes are vectors of several pathogens causing infectious diseases of public health importance. These includes lymphatic filarial nematodes and arboviral infections. Blocked and improperly maintained drainages have been reported to serves as habitats for the *Culex* species and other disease vectors [1,2]. The drainage of surface water in Lagos State is facilitated by network of gutters and canals that empties into the Lagos lagoon [2], but increasing human population in the State as result of continuous rural-urban migration led to the creation of urban slums in most parts of the State. Anthropogenic activities including disposal of solid and domestic wastes in most parts of the State has resulted in the blockage of the drainage systems, preventing the continuous flow of water thereby leading to the creation of breeding sites of mosquitoes immature stages [2,3]. *Culex* mosquito immature stages had been reported to breed in varieties of habitat, and can breed in polluted waters [4]. The frontline tool in vector control strategy are insecticides based, but development and spread of resistance to public health insecticides has been a major challenge to this mosquito control method. Resistance to different classes of public health insecticides for mosquito control have been reported in Nigeria and other parts of Africa [5–9]. To determine the possibility of *Culex* mosquito-borne diseases outbreak in Lagos State, there is need to accurately identified the *Culex* species present in the State, determine the physiochemical parameters of immature stages habitats and evaluate their susceptibility to WHO approved insecticides. Therefore, this study was designed to identify the *Culex* species breeding in blocked drainages in three selected Local Government Areas (LGAs) in Lagos State, determine the physicochemical properties of their breeding sites and assess their susceptibility to DDT and permethrin.

2.0 METHODOLOGY

2.1 Study Area

The study was carried out in three selected Local Government Areas in Lagos state. The areas covered comprises of Ikorodu (6°35'N, 3°37'E and 37 meters above sea level). It is essentially a subsistent agricultural dominated community, although considerable animal husbandry and trading are also undertaken. Lagos Island situated at 6° 28 'N, 3°21' E and 44 meters above sea level, the area is a largely a congested residential area with inadequate sanitation and low quality housing facility. Yaba situated at 6°31'N, 3°24'E and 39 meters above sea level, this area is located and situated in Lagos Mainland sub-locality. It is mostly surrounded by swampy areas with a local fishing community beside the Lagoon boundary.

2.2 Study Design

Larval collections were carried out in all selected three local government areas of Lagos state. Larval survey was carried out on foot to locate mosquito breeding sites. *Culex* larvae were identified by their angular position on the surface of water. Larvae were collected with a 350 ml dipper and transferred into a 5litre plastic container and transferred to the laboratory for emergence. Once in the laboratory, the water with the mosquito juveniles were transferred into another plastic container to a depth of 2 cm and each container labelled according to the site. All emerged mosquitoes were fed on 10% sugar solution imbibed in cotton wool.

2.3 Mosquito Identification

Emerged adult mosquitoes were examined to confirm they belonged to the genus *Culex* using morphological features such as the palps, proboscis, scutellum, abdomen and siphon. The differentiation of *Culex* mosquito into various species was done with a dissecting microscope using the keys of Gillies and Smith [10] as a guide in identification.

2.4 Insecticide Susceptibility and Resistance Tests

The tests were performed using WHO test kits and method for accessing insecticide susceptibility and resistance [11]. Non-blood fed, two to three days old mosquitoes were exposed to insecticide-treated filter papers in groups of 25. Each experiment consisted of at least three replicates. The mosquitoes were exposed for an hour with the bioassay cylinders in a vertical position. Knockdown rates of mosquitoes were recorded at 10 minutes interval for one hour and the final mortality noted after 24 hours. After the one-hour exposure, the mosquitoes were transferred into tubes with untreated papers and allowed a 24-hour recovery period after which mortality was recorded. All the bioassays were accompanied by negative control tests where mosquitoes were exposed to untreated papers only. The mosquitoes were supplied with a 10% sugar meal during the recovery period.

2.5 Physiochemical Properties of Water sample in the Larval Habitats

The pH, temperature, conductivity and total dissolved solid (TDS) of the *Culex* mosquitoes larval habitats from

each of the three selected locations were recorded on field using a portable water quality meter

2.6 Molecular Identification of *Culex* mosquitoes

Mosquito DNA extraction was carried out according to the method described by Collins *et al.*,[12]. Three primers, ACEquin (5'-CCTTCTTGAATGGCTGTGGCA-3'), ACEpip (GGAAACAACGACGTATGTACT-3' and B1246s (5'TGGAGCCTCCTCTTCACGG-3') were used to amplify a 274 bp and 610 bp diagnostic fragment of the entire extracted DNAs according to [13]. This was done to identify two member of *Culex pipiens* complex which are; *Culex quinquefasciatus* and *Culex pipiens*. Amplified fragments were analysed in 1.5% agarose gel and were visualised by Ethidium bromite stains under Ultra Violet light(UV light).

2.7 Detection of Kdr (L1014F) Mutation

Detection of kdr mutations was performed as described by Martinez-Torres *et al.*,[14]. Four primers, Agd1 (5'-ATAGATTCCCCGACCATG-3'), Agdl2 (5'AGACAAGGAT GATGAACC-3'), Agdl3 (5'-AATTTGCATTACTTACGACA-3') and Agdl4 (5'-CTGTAGTGATAGGAAATTTA-3') were used for the PCR assay and two PCR reactions were run in parallel. The amplification condition consisted of one cycle of denaturation at 95˚C for 15 minutes, followed by 29 cycles at 94˚C for one minute, 49˚C for two minutes, 72˚C for two minutes each, and one cycle of final extension at 72 °C for 10 minutes according to Sarkar *et al.*,[15]. In one reaction, the primers Agd1, Agd2, Agd3, and Agd4 were combine. The PCR reactions was conducted by adding 12.5 µL volume of PCR master mix containing 1× PCR buffer which is made up of 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM of each primer, one unit of Taq polymerase and 1 µl of genomic DNA into each 20uL tube. 10ul of PCR product with 1uL of loading buffer and 10ul of Standard markers per gel were loaded into each well of the prepare agarose gel. Amplified fragments were analyzed in 1.5% agarose gel and were visualized by Ethidium bromite stains under Ultra Violet light(UV light).

2.8 Data Analysis

Insecticide Susceptibility was based on the criteria that 98–100% mortality indicates susceptibility, 80–97% mortality implies potential resistance that needs to be confirmed via biochemical assays and <80% mortality implies resistance [16]. All controls showed no mortality, thus there was no need for the use of the Abbott's formula. Knockdown times (KDT_{50} and KDT_{95}) were estimated using a log time probit model with SPSS version 16.0 for windows (SPSS Corporation USA).

3.0 RESULTS

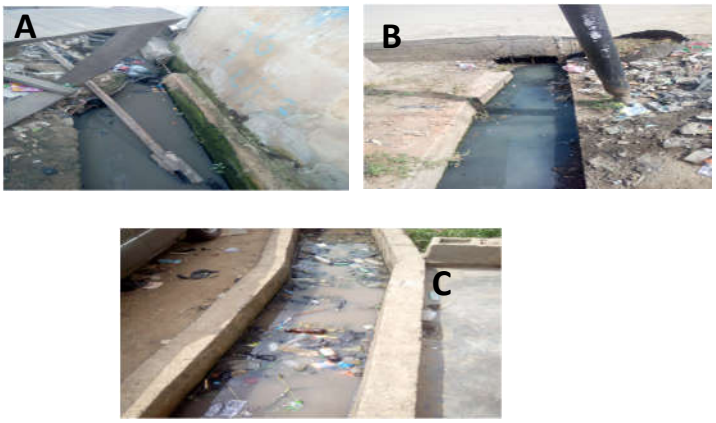
RESULTS

Table 1 shows the physiochemical parameters of the larva habitats in selected LGAs in Lagos, the pH for *Culex* mosquitoes larval habitats are slightly basic while the conductivity ranges from 364 to 652.2. Plate 1A-C shows the blocked drainages in the three surveyed LGAs of Lagos State. The progressive percentage Knockdown of *Cx. quinquefasciatus* collected from the sampled LGAs exposed to DDT and permethrin is shown in Table 2 . The percentage and mean (±SD) mortality against DDT (4%) and Permethrin of *Culex* mosquitoes collected in three local government areas are shown in Table 3. Mortality results from exposures to different insecticides showed that *Culex* mosquitoes resistance to DDT ranged between 24 and 32 while Permethrin resistance ranged between 14- 18 mortality rate.

A total number of 159 *Culex pipiens* complex mosquitoes that were identified morphologically were selected for molecular identification using PCR assays, out of which 153 (96.2%) were identified as predominantly *Culex quinquefasciatus* while 6 (3.7%) were unidentified (Plate 2). No amplification was detected for *kdr* mutation genotyping using specific allele PCR (AS-PCR) in *Culex quinquefasciatus* from the selected LGAs.

Table 1: Physiochemical parameters of *Culex* larval habitats in the sampled areas of Lagos State

Location	PH (mean±SD)	Temp (mean± SD)	Conductivity (mean±SD)	TDS (mean±SD)
Lagos Island	7.38±0.11	30.9±0.24	564±85.3	592.6±79.1
Ikorodu	7.62±0.29	29.74±0.57	656.2±73.6	655±68.1
Yaba	7.58±0.24	30.08±0.58	606.2±79.4	641.7±57.2



Plates 1A-C: Blocked drainages and *Culex* breeding sites in (A) Yaba, (B)Ikorodu, (C) Lagos Island

Table 2: The Progressive percentage knockdown of *Culex quinquefasciatus* exposed to DDT and permethrin in the samples LGAs of Lagos State.

Site	Insecticides	0min (%) KD	10mins (%) KD	15mins (%) KD	20mins (%) KD	30mins (%) KD	40mins (%) KD	50min (%) KD	60mins (%) KD
Lagos Island	DDT (4%)	0	12	12	20	20	20	20	20
	Permethrin (0.75%)	0	0	0	0	0	2	2	2
Ikorodu	DDT (4%)	0	4	4	4	4	4	12	16
	Permethrin (0.75%)	0	2	6	10	10	10	10	10
Yaba	DDT (4%)	0	12	12	20	20	20	20	20
	Permethrin (0.75%)	0	2	2	2	2	2	2	2

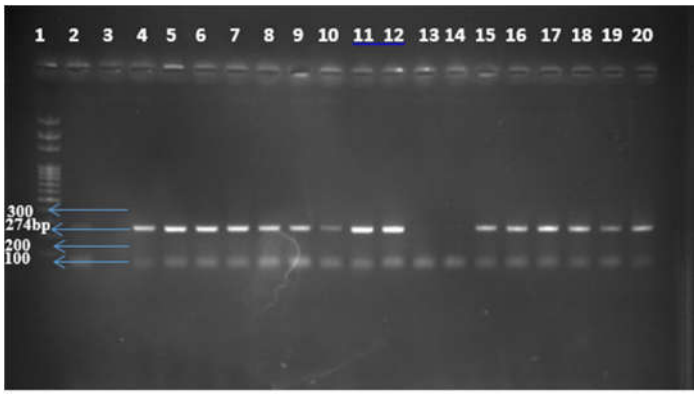


Plate 2: UV light illuminated agarose gel showing Specific Allele PCR (AS-PCR) for Molecular identifi (DNA bands) in *Culex pipiens* complex.

Lane 1: Standard ladder (100bp)

Lane 2: Positive control (*Culex quinquefasciatus* with DNA Template)

Lane 3: Negative control (with no DNA Template) Lane 4: Identified specie of *Culex quinquefasciatus* (4,5,6,7,8,9,10,11,12,15,16,17,1819,20 on 274bp)Lane 5: Unidentified species of the *Culex pipiens* complex (13 and 14 not shown on 274b)

Table 3: Insecticide resistant status of *Culex* mosquitoes to DDT and Permethrin in the Lagos State

Location	Insecticide	No Exposed	Kdt_{50}	Kdt_{95}	Mortality (%)	Status
Lagos Island	DDT (4%)	100	3313.2	15848156.76	32	R
	Permethrin (0.75)	100	351.12	1576.6	14	R
Ikorodu	DDT (4%)	100	835.7	32474.6	24	R
	Permethrin (0.75)	100	3915.58	1313145.46	18	R
Yaba	DDT (4%)	100	252.9	7539.874	32	R
	Permethrin (0.75)	100	-	-	14	R

Note: 98-100% mortality indicate susceptibility, 80_97% mortality implies mortality and <80% mortality implies resistance (WHO, 2013). R– Resistant

4.0 DISCUSSION

Culex mosquitoes collected from blocked drainages in this study were mainly composed of *Cx. pipiens quinquefasciatus*; a major biting nuisance especially in urban areas and vectors of several arboviral infections and filariasis. The presence of Culex species has been reported indifferent parts of Nigeria [3,14,17,18]. Temperature, conductivity and pH of *Cx. quinquefasciatus* larval habitats recorded in this study was higher than what was previously reported for different mosquito genera in Ondo State, Nigeria [19,20]. The higher level of the physicochemical parameters of Culex larval habitat in this study could be related to the urban nature of Lagos State, leading to increased anthropogenic activities including artificial creation of larval habitats and generation of more organic waste that find their way into these habitats due to lack of proper disposal. Culex species have associated with organically polluted larval habitats [4,7].

High level of resistance to DDT and permethrin was recorded in *Cx. quinquefasciatus* in this study, similarly previous studies in Lagos State and other parts of Nigeria have reported resistance to DDT and pyrethriods [5,6]. KDT_{50} and KDT_{95} recorded in this study was similar to that of a previous report from Lagos State [5] but higher

than what was reported in Kwara State [6]. Insecticides resistance recorded in *Cx. quinquefasciatus* in this study could affect the effectiveness and consequently the utilization of main insecticides-based control measures including LLINs and IRS. In this study *kdr* mutation (L1014F) was not detected in DDT and permethrin resistant *Cx. quinquefasciatus* similar to a previous report in Kosofe, Lagos State [5] . Although, some researcher reported identified L1014F mutation in Culex in some parts of Africa have [8,9,21].

Insecticides resistant *Cx. quinquefasciatus* were found to be dominant *Culex* species found in Lagos blocked drainages. These mosquitoes are not only biting nuisance but also importance vectors of arboviral infections and lymphatic filariasis. Therefore, there is need for regular sanitation, clearing of solid wastes from drainages and the discouraging the disposal of household wastes in the open drains

Authors Contributions

AVO performed data collection and analysis. **IET** conceived, designed and supervised the work. **FIK** contributed to study design, data analysis and revised the manuscript. **OTA, JTR** contributed to data analysis tools and revision of the manuscript. **OOA** contributed to study design and co-supervised the work

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