# MOLECULAR ANALYSIS OF MECHANISMS AND IDENTIFICATION OF FACTORS OF PYRETHROID RESISTANCE IN *ANOPHELES GAMBIAE SENSU LATO* IN SOUTHWESTERN NIGERIA AND SOUTHERN BENIN REPUBLIC

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## ABSTRACT

The development of resistance to insecticides by *Anopheles* mosquitoes continues to threaten the success of malaria control programmes in West Africa. Local data on mechanisms and factors causing resistance in the region are scanty. This study was designed to investigate the environmental factors and mechanisms implicated in resistance to pyrethroids by *Anopheles gambiae* in southwestern Nigeria and southern Benin Republic.

Larvae of *Anopheles* mosquito were collected in 2007 from 19 localities in the six states of southwestern Nigeria and 18 localities in the seven divisions of southern Benin and reared to adults. These were identified morphologically and with Polymerase Chain Reactions (PCR). They were also bioassayed for susceptibility to pyrethroids. Molecular characterisation of pyrethroid resistant phenotypes was carried out using PCR and microarray analyses of the expressed genes. Dissolved Oxygen (DO) and pH were determined using a digital multipurpose meter while physical appearances of breeding sites were assessed visually. Xenobiotic factors such as Spilled Engine Oil (SEO) and agricultural pesticides that might contribute to the emergence of resistance in *Anopheles* populations were examined through bioassay. Associations between pyrethroid resistance with environmental factors and molecular profiles of *Anopheles* were evaluated using Chi square.

*A. gambiae* complex genotyped in Nigeria comprised of 73.6 % *A. arabiensis* and 26.3 % *A. gambiae sensu stricto*; while those genotyped in Benin were 92.9 % *A. gambiae s.s.* and 7.0 % *A. melas*. Pyrethroid resistance in Nigeria and Benin were recorded in 68.4 % and 94.4 % of the localities examined respectively. Breeding sites contaminated with SEO (B-SEO) or Pesticide Residues (B-PR) had low DO (B-SEO = 13.4 ± 1.5 mg/l, B-PR=12.2 ± 1.7 mg/l), the Non-contaminated Breeding sites (B-NC) had higher levels of DO (B-NC=33.1 ± 2.3) and mainly produced pyrethroid-susceptible *Anopheles* ($p<0.05$). Significant variations in pH were not recorded. Differences in habitation by resistant-*Anopheles* in breeding sites contaminated with SEO or pesticide residues were observed. *A. gambiae* found around the two agricultural sites (Houeyiho, Benin and Ajibode, Nigeria) exposed to synthetic pesticides showed significant levels of pyrethroid resistance with mortality rates of 70.0 % and 89.7% respectively. *A. gambiae* larvae survived at

SEO concentrations below 11.8x10-3 µL/cm$^2$. Ninety six percent of larval mortality resulted from direct cuticle contact with SEO whereas only four percentage mortality was from larval suffocation. A cross resistance phenomenon was recorded with SEO and pyrethroids. *A. gambiae* showed the presence of elevated frequencies of knock down resistance West (*kdr*-W) mutations in Benin samples (*kdr*-W ranged from 0.6 to 0.9) and absence of *kdr*-W in Nigeria samples. Two detoxification genes (*CYP6P3* and *CYP6M2*) were up-regulated in resistant-*Anopheles*. Additional detoxification genes specific to agricultural and SEO sites were also over-expressed in the resistant populations.

There was an association between residual synthetic pesticides, spilled engine oil and emergence of pyrethroid-resistance in *A. gambiae* in Nigeria and Benin Republic. The diversified profile of identified metabolic genes reflected the influence of a range of xenobiotics on selection of resistance in mosquitoes.

**Keywords:** *Anopheles*, Pyrethroid resistance, Xenobiotics.

**Word count: 487**

## DEDICATION

This project is dedicated to GOD almighty, who has being my source of inspiration and sustenance. To my home institution, colleagues, family members and friends for their constant support in the course of this programme.

## CERTIFICATION

This is to certify that this work was carried out by MR. Jean Rousseau DJOUAKA-FOLEFACK in Cell Biology and Genetics Unit of Department of Zoology, University of Ibadan, Ibadan, Nigeria.

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