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MOLECULAR ANALYSIS OF THE KNOCKDOWN RESISTANCE (*KDR*) GENE IN *ANOPHELES GAMBIAE* SENSU LATO AND *ANOPHELES FUNESTUS* COMPLEX POPULATIONS FROM KENYA

BY

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ABSTRACT

Insecticide resistance affects the re-emergence of vector-borne diseases and their control. Resistance to insecticides poses a risk to compromise the role of chemical vector control as a component of the integrated vector management. The *kdr* (knock-down resistance) allele is characterized by a single base pair substitution causing a change from *leu* to *phe* (West African) or *leu* to *ser* (East African) in codon 1014 of the voltage-sensitive sodium channel protein sequence. In Kenya the knockdown resistance gene (*kdr*), a target-site insensitivity based L1014S mutation to both DDT and pyrethroids confers resistance to these insecticides and has been reported in some *Anopheles gambiae* strains.

The use of ITNs is one of the government's priority strategies in combating malaria. The use of ITNs has been implicated by various studies to contribute largely to pyrethroid resistance development. The effectiveness of the ITNs has been shown to be lowered by resistance to pyrethroids by previous studies and therefore it was vital to conduct research to investigate how widespread the *kdr* is in the wild mosquito populations. Such a study helps in designing of malaria management programmes by use of treated nets and helps lower the risks of control failures due to resistance development. The study was therefore aimed at studying the spread of the resistance gene in the major malaria vectors of Kenya and targeted the malaria endemic regions of the country namely Mbita (western Kenya), Mwea (central highlands) and the Kenya coast (Malindi, Kilifi and Kwale areas). Adult mosquitoes were randomly collected by pyrethrin spray catch (PSC) or by aspiration method and preserved in ethanol awaiting further analysis. From each sampling site, a total of 600 specimens were collected and analysed for species composition by use of

morphological keys and further by PCR, molecular form composition by PCR-RFLP for the *An. gambiae* s.s specimens and *kdr*-genotyping by use of PCR.

A PCR-RFLP analysis of the *An. gambiae* s.s molecular forms detected only the S-form and non of the samples was a M-form. An allele specific polymerase chain reaction assay was adopted for screening of knockdown resistance allele (L1014S) in the members of the *An. gambiae* complex and *An. funestus* complex populations. The revised genotyping approach involved a susceptibility assay (*kds*-PCR) and a resistance assay (*rkdr*-PCR), revealed that the *kdr* allele only existed in the heterozygous form (RS). The sensitivity and application of this genotyping approach as compared to an earlier described cocktail *kdr*-PCR (*ckdr*-PCR) in the screening of diverse mosquito species are discussed. In the overall species *kdr* frequency (%RS), the *An. gambiae* s.s had the highest frequency (14%) followed by *An. arabiensis* (8%) and lastly *An. funestus* with 7%. The mean resistance frequency levels were highest in Coast (12.4%), followed by Mbita (8%) and lastly by Mwea with a frequency of 5.75%. A chi-square analysis of association between genotype and site revealed a strong association (X^2_1= 10.3, P< 0.05) indicating that resistance was heterogeneous across the 3 sites.

A resistance homogeneity analysis using the Marascuilo multiple comparison procedure revealed that the frequencies of Coast-Mwea and Coast-Mbita were significantly different (heterogenous) whereas Mwea-Mbita was homogenous. Conformance to HWE tests by chi-square analysis indicated that the population sampled in the 3 sites was not in HW equilibrium. This finding conformed to the evidence of selection pressure as indicated by the *kdr* frequencies, which was attributable to the deviation from the Hardy-Weinberg equilibrium. The study provides data that is useful to the understanding of the knock down resistance patterns

associated with the use of ITNs and indoor spraying of pyrethrin compounds. The use of the revised *kdr* genotyping assay will facilitate feasible future screening of resistance and can be adopted in Government resistance monitoring and management programmes. The limitations of the *kdr*-PCR and suggestions on the improved of such limitations are also discussed.

Key words: *rkdr*-PCR, *kds*-PCR, *Anopheles gambiae*, *Anopheles funestus*, Resistance frequency, genotype.