Evaluation of the Pyrethroid Resistance based on Voltage-Gated Sodium Channel (VGSC) Mutations in *Aedes aegypti* populations of Colombo, Gampaha and Kandy Districts in Sri Lanka

Tharaka Ranathunge[1], Lahiru Udayanga[2], Sumudu Sarasija[3], Samudra Karunathilaka[4], Shavindhya Nawarathne[5], Haruthra Rathnarajah[6] Fathima Fazla Dulficar[7], Fathima Nafla Shafi[8], Ranil S. Dassanayake[9], Y.I. Nilmini Silva Gunawardene[10]

Many countries focus on chemical based vector control strategies to restrict the disease transmissions, where pyrethroid insecticides are widely used as the first line of defense against *Ae. aegypti.* However, the constant use of insecticides have proven to induce insecticide resistance in mosquitoes. The knockdown resistance (kdr) occurs due to mutations in the Voltage Sensitive Sodium Channel (VSSC) or mutations in the Voltage-Gated Sodium Channel (VGSC), coded by the VSSC gene. Only three kdr mutations namely, the V1016G, S989P, and F1534C have been confirmed as commonly occurring amino acid substitutions among mosquito populations in Southeast Asia. Therefore, to extend this observation, current study was conducted to evaluate the prevalence of V1016G and F1534C mutations among *Ae. aegypti* mosquito populations in three different geographical regions of Sri Lanka. Immature (both pupae and larvae) stages of *Ae. aegypti* mosquitoes were collected from Colombo, Gampaha and Kandy districts from March to December 2018 and samples were transported to the Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya. A total of 855 *Ae. aegypti* larvae were collected from all districts and polymerase chain reaction (PCR) assay for molecular genotyping of mutations was performed for collected all *Ae. aegypti* larvae (III instar), to identify the prevalence of kdr mutations in the three *Ae. aegypti* populations. The frequencies of the resistant and susceptible kdr alleles were determined by using the Hardy–Weinberg Equilibrium for each of the point mutation. The *Ae. aegypti* populations from Colombo, Gampha and Kandy districts showed 40.07% (123/307), 39.58% (114/288) and 19.58% (47/240) of V1016G and F1534C mutations, respectively. The wild type (RR) genotype remained predominant within all the three districts, whereas the homogenous (SS) mutation genotype occurred only in minority. Further, the F1534C was predominant in *Ae. aegypti* populations of all districts. Among the kdr mutation population, heterogeneous genotyping (RS) for both V1016G and F1534C was prominent, while SS genotyping for V1016G mutation was not observed in the Kandy district. The findings clearly denote that long-term insecticide applications and multiple use of pyrethroids has led to the progression of insecticide resistance among local *Ae. aegypti* populations. Therefore, evaluation of the prevalence levels of these kdr mutations highlights the necessity for shifting towards novel vector control strategies.

Keywords: *Ae. aegypti*, kdr, pyrethroid, insecticide resistance, F1534C and V1016G

[1] Molecular Medicine Unit, Faculty of Medicine University of Kelaniya, Sri Lanka
[2] Department of Biosystems, Faculty of Agriculture and Plantation Management, Wayamba University, Sri Lanka
[3] Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka
[4] Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka
[5] Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka
[6] Business Management School (BMS), Colombo, Sri Lanka
[7] Business Management School (BMS), Colombo, Sri Lanka
[8] Business Management School (BMS), Colombo, Sri Lanka
[9] Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka
[10] Molecular Medicine Unit, Faculty of Medicine University of Kelaniya, Sri Lanka, *nilminisg@kln.ac.lk*