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Research Note

First molecular genotyping of A302S mutation in the gamma aminobutyric acid (GABA) receptor in *Aedes albopictus* from Malaysia

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Abstract. Given the lack of molecular evidence in altered target-site insecticide resistance mechanism in *Aedes albopictus* (Skuse) worldwide, the present study aims to detect the presence of A302S mutation in the gene encoding the gamma aminobutyric acid receptor resistant to dieldrin (*Rdl*) in *Ae. albopictus* for the first time from its native range of South East Asia, namely Malaysia. World Health Organization (WHO) adult susceptibility bioassay indicated a relatively low level of dieldrin resistance (two-fold) in *Ae. albopictus* from Petaling Jaya, Selangor. However, PCR-RFLP and direct sequencing methods revealed the presence of the A302S mutation with the predomination of heterozygous genotype (40 out of 82 individuals), followed by the resistant genotype with 11 individuals. This study represents the first field-evolved instance of A302S mutation in Malaysian insect species.

Gamma aminobutyric acid (GABA) receptor is the target site of cyclodiene insecticide (i.e., dieldrin). It has been documented that an amino substitution, alanine to serine at codon 302 (A302S) confers resistance to dieldrin (known as *Rdl* gene) (Hemingway *et al*., 2004). As far as mosquitoes are concerned, the *Rdl* gene has been first identified in *Aedes aegypti* (Linnaeus) in 1993 (Thompson *et al*., 1993). Thereafter, the identifications of A302S and other mutation, A302G have been recently described in other species of mosquitoes such as *Ae. albopictus*, *Culex quinquefasciatus* (Say) (Tantely *et al*., 2010) and *Anopheles* mosquitoes (Asih *et al*., 2012).

While most of the studies focused on *Ae. aegypti*, there has been a dearth of information regarding the molecular basis of insecticide resistance (i.e., target site alterations) in *Ae. albopictus* from different parts of the world, including Malaysia. To the best of our knowledge, the A302S mutation in the GABA receptor only has been reported in *Ae. albopictus* from La Réunion Island, French (Tantely *et al*., 2010). To fill this void in the literature, the present study was carried out to (1) quantify the dieldrin resistance status of *Ae. albopictus* and thereby attempting to (2) determine the prevalence of dieldrin resistance allele, for the first time from its native range of Southeast Asia, namely Malaysia.

Mosquito larvae were collected from residential areas in Petaling Jaya, Selangor, Malaysia, using ovitrap method. Three to five days old female mosquitoes from first generation (F1) reared larvae, were used for adult susceptibility bioassay using WHO (1981) guideline. A total of 15 sucrose-fed

female mosquitoes were exposed to 4% dieldrin impregnated paper for 1 hour and the test was performed in triplicates. A cumulative knockdown was recorded every minute within the exposure period and mortality was recorded at 24 h post-treatment.

To characterize the dieldrin resistance mechanism, the survived individuals (nine out of 45) from dieldrin bioassay tests were subjected to dieldrin resistance gene detection. Given the low numbers of survivors were obtained, additional 73 non-treated individuals were included to determine the frequency of dieldrin resistance allele. A PCR-RFLP test developed by Tantely *et al.* (2010) was used to characterize the resistance and susceptible alleles. An approximately 200 base pairs fragment of *Rdl* gene was amplified by mqGABAdir (5'-TGT ACG TTC GAT GGG TTA T-3') and mqGABArev (5'-CAT GAC GAA GCA TGT GCC TA-3') primers. The PCR conditions included an initial denaturation of 94°C for 5 min, followed by 30 cycles of 94°C for 30 s (denaturation), 52°C for 30 s (annealing), 72°C for 1 min (extension) and a final extension at 72°C for 5 min. The PCR fragments were digested with 1 µL of BstAPI restriction endonuclease (New England Biolabs, UK) at 60°C for 2 h and fractionated on a 2% agarose gel prestained with SYBR Safe (Invitrogen, CA). A subset of 20 individuals was subjected to direct sequencing.

The susceptibility status of *Ae. albopictus* towards dieldrin is presented in Table 1. The knockdown times at 50% (KT_{50}) and 90% (KT_{90}) were 111.22 min and 253.74 min, respectively. The field strain showed a 2.04-fold resistance to dieldrin compared to that of the laboratory susceptible strain, an indicative of low resistance (resistance ratio < 5) (Mazzarri & Georghiou, 1995). Additionally, 80.00% mortality after 24 h post-treatment was recorded. The present results are comparable to the previous report, where the *Ae. albopictus* from a pig farm in Tanjung Sepat, Selangor exhibited 1.82-fold resistance ratio and complete mortality at 24 h post-treatment (Chen *et al.*, 2013).

Given that only 80.00% mortality was observed, this suggests the possibility of resistance that needs to be further confirmed (WHO, 2009). In this context, the nine survived individuals from bioassay tests were then further subjected to *Rdl* gene detection. The results are in concordance with the resistant phenotype exhibited by bioassay tests; three individuals carried the homozygous resistant genotype and six individuals carried the heterozygous genotype.

In this study, a larger sample size was used to determine the prevalence of *Rdl* gene in specimens from this study site. Both PCR-

Table 1. Susceptibility status of *Aedes albopictus* against 4% dieldrin

Strain	KT_{50} (min) (95% CL)	KT_{90} (min) (95% CL)	RR_{50}	Mortality (%)
Laboratory	54.44 (52.20-57.54)	77.56 (70.61-89.51)	–	100.00 ± 0.00
Field	111.22 (93.30-146.55)	253.74 (181.13-426.23)	2.04	80.0 ± 3.85

KT = knockdown time; CL = confidence limit; RR = resistance ratio; adult mortality percentage was recorded 24 h after an initial exposure period of 1 h

Table 2. Genotypes and frequency of *Rdl* alleles in *Aedes albopictus*

Sample size	Genotype			Allele Frequency		HW (*P*-value)*
	SS	RS	RR	S	R	
82	31	40	11	0.62	0.38	0.82

HW = Hardy-Weinberg test. *The exact probability for rejecting Hardy-Weinberg equilibrium

RFLP and sequencing methods exhibited similar results and confirmed the presence of alanine-serine *Rdl* mutation in the wild population of *Ae. albopictus* in Petaling Jaya, Selangor. Overall, the SS genotype was found in 31 individuals (37.8%) from a total sample size of 82. The RS genotype was detected and was most predominant with 40 individuals (48.8%), followed by the RR genotype with 11 individuals (13.4%). The genotype frequency from this population conformed to the Hardy-Weinberg expectations at the 95% confidence level ($P > 0.05$). The resistance *Rdl* frequency was 0.38. Dieldrin has been introduced in Malaysia since 1980 until 1994. The predomination of RS genotype found in the present study is unexpected because dieldrin has been banned for 20 years, however, the resistant phenotype and genotype still remain in Malaysian population. Similar observation was also reported in *Cx. quinquefasciatus* and *Ae. albopictus* from French, although dieldrin has been banned for over 15 years in that country (Tantely *et al*., 2010).

This first appearance of altered target-site in GABA receptor in Malaysian *Ae. albopictus* provides an early warning to public health stakeholders in Malaysia regarding the evolution of insecticide resistance after the extensive use of dieldrin in the past. Although low dieldrin resistance level was detected from this study, however this is an indication of the long-term persistence of dieldrin resistance phenotype and genotype in Malaysian population. Hence, this knowledge has to be considered in formulating effective prevention and control programmes in Malaysia.

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