Joint International Tropical Medicine Meeting
JITMM VIRTUAL 2021
Poster No. 1

TARGET-SITE MUTATION OF *RDL* GENE AND INSECTICIDE SUSCEPTIBILITY IN FIPRONIL- AND PYRETHROID- RESISTANT *AEDES AEGYPTI* MOSQUITOES FROM NAKHON PATHOM AND KANCHANABURI PROVINCES

Presenter: Dr. Jakkrawarn Chompoosri

INTRODUCTION

Long-term and intensive applications of various insecticides in mosquito control have inevitably led to the development of resistance mechanisms such as resistance to pyrethroids and dieldrin. Mechanisms of pyrethroid resistance in *Ae. aegypti* mosquitoes, the vector of dengue and Zika, are reported in several dengue risk areas across Thailand (Stenhouse *et al*., 2013; Plernsub *et al*., 2016). *Rdl* (Resistant to dieldrin) gene is the target site for dieldrin and fipronil. *Rdl* mutation by amino acid substitution from alanine (GCA) to serine (TCA) at position 302, A302S, was detected in a cyclodiene-resistant strain of *Ae. aegypti* (Thompson *et al*., 1993). However, A302S mutation in *Ae. aegypti* has not yet been reported in Thailand.

OBJECTIVE

To investigate the presence of *Rdl* mutation and insecticide susceptibility in genetically pyrethroid-resistant *Ae. aegypti* mosquitoes from Nakhon Pathom and Kanchanaburi provinces

METHODS

Two study areas located in Nakhon Pathom (13°44´9´´N 100°7´21´´E) and Kanchanaburi provinces (13° 49´ 12´´N 99° 16´ 48´´E) were chosen for the collection of *Ae. aegypti* in 2020 based on dengue case reports from 2015 to 2019 and reports of high pyrethroid resistance levels in *Ae. aegypti* . The mosquito larvae were collected by droppers in 17.4% (47/270 households) and 49.2% (94/191 households) of total households in the study areas of Nakhon Pathom and Kanchanaburi provinces, respectively. The pyrethroid-resistant *Ae. aegypti* females were exposed to 1.00% fenitrothion (organophosphate), 0.336% fenobucarb (carbamate), 0.14% fipronil (phenylpyrazole), 0.22% Cypermethrin (pyrethroid) and 0.05% Deltamethrin (pyrethroid) by WHO susceptibility test (Fig. 1) (WHO, 2016).

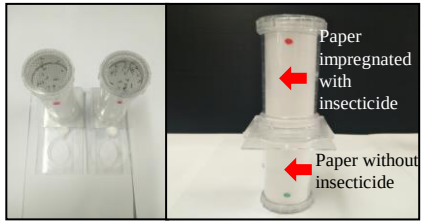

Figure 1 WHO susceptibility test

Genomic DNA extracted from individual fipronil- and pyrethroid-resistant *Ae. aegypti* females was amplified by PCR (Chung *et al*., 2019) and then subjected to DNA sequencing.

RESULTS

The mosquitoes were resistant to the tested pyrethroids with the mean mortality rates of between 2.00±2.30% and 6.00±2.30%. The 0.14% fipronil provided 94.00±5.20% and 96.00±3.30% mortality rates in the mosquitoes from Nakhon Pathom and Kanchanaburi provinces, respectively. However, 1.00% fenitrothion and 0.34% fenobucarb gave a 100% mortality rate in the mosquitoes from all 2 provinces. The mean mortality rates of mosquitoes were significantly different among insecticides (p<0.05) (Table 1). The A302S mutation was detected in the mosquitoes from Nakhon Pathom and Kanchanaburi provinces (Fig. 2) at mutation frequencies of 0.33 (2/6 sequences) and 0.25 (1/4 sequences), respectively.

Table 1 Insecticide susceptibility in *Ae. aegypti* mosquitoes from 2 provinces

Insecticides	Mean mortality rates in *Ae. aegypti* mosquitoes (%) (x̄± SD)	
	Nakhon Pathom	Kanchanaburi
Acetone (negative control)	0±0	0±0
1.0% Fenitrothion (organophosphate)	100±0[a]	100±0[a]
0.336% Fenobucarb (carbamate)	100±0[a]	100±0[a]
0.14% Fipronil (phenylpyrazole)	94.00±5.20[b]	96.00±3.30[b]
0.22% Cypermethrin (pyrethroid)	4.00±3.30[c]	6.00±2.30[c]
0.05% Deltamethrin (pyrethroid)	2.00±2.30[c]	5.00±2.00[c]

Note: Mortality less than 90%: Confirmation of existence of resistant genes in the mosquitoes
Means followed by the different letters in each column are statistically significant (p<0.05).

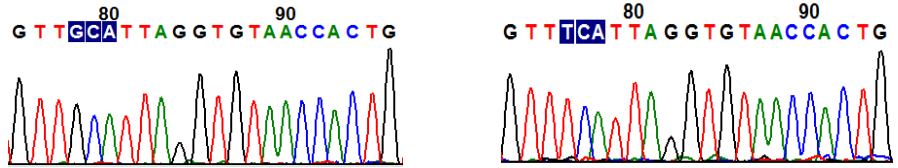

Figure 2 DNA sequencing electropherogram of A302S mutation. (2A) Wild type (2B) Mutant

CONCLUSION

The target-site mutation of *Rdl* gene, A302S, was present in the fipronil- and pyrethroid-resistant *Ae. aegypti* mosquitoes from all 2 provinces and this is the first report in Thailand. In this study, the mosquitoes were completely susceptible to 1.00% fenitrothion and 0.336% fenobucarb. It suggests that those insecticides should be used sparingly and integrated with the safe mosquito control measures. The data on insecticide susceptibility in *Ae. aegypti* mosquitoes were reported to Tambon Health Promoting Hospitals in the study areas for effective mosquito control.