Artikel 22. Molecular Surveillance of Pyrethroid Resistance - 1976-4083-1-SM

by Pak Sayono

Submission date: 27-Apr-2020 08:42PM (UTC+0700)
Submission ID: 1309114284
File name: ular_Surveillance_of_Pyrethroid_Resistance_-_1976-4083-1-SM.docx (590.69K)
Word count: 1930
Character count: 10846

Molecular Surveillance of Pyrethroid Resistance of Dengue Vector [*Aedesaegypti*] and its Implication To Public Health

Sayono[1,a)], Ulfa Nurullita[2,b)], Irfanul Chakim[3,c)]

[1,2,3]*Faculty of Public Health, University of Muhammadiyah Semarang*

[a)] Corresponding author: say.epid@unimus.ac.id

Abstract. Resistance of *Aedesaegypti* mosquito to pyrehtoid compounds have been a common problem in controlling this disease among the tropical countries, including Indonesia. Knockdown resistance alleles of voltage-gated sodium channel gene have been proposed as an effective marker for early detection of this problem. This study aimed to understand the pyrethroid resistance status of *Aedesaegypti* population among Dengue endemic areas in Central Java Province. The house hold larval surveys were conducted in Temanggung, Kendal and Jepara districts and Semarang municipal based on the Dengue cases. Mosquito larvae were reared to be 3-5 days old imago stage in entomologic laboratory, and then subjected to molecular experiment for detection of kdr alleles of domain II VGSC gene. This study found two single nucleotide polymorphisms, namely S989P and V1016G. These SNPs indicated that *Aedesaegypti* population has developed resistant to pyrethroid compounds. Generally, we found the high percentage of those SNPs, namely 26 and 93 percents. It is an important data input for public health officer in planning of Dengue prevention.

INTRODUCTION

Dengue hemmoragic fever (DBD) has been worldwide problem caused by flaviridae family of dengue virus resulting in a million cases of mortality and morbidity worldwide [1]. The absence of dengue treatment and preliminary protection, i.e. vaccine, to reduce its environmental mass impact have made significant disruption for dengue control program [2]. As presented data by Indonesian government, stagnant case number of dengue for at least 4 last years elucidated its consistency [3]. In other hands, the occurrence of dengue vector resistance to various insecticides, as dengue control manifestation, made another list of prevention obstacle [4].

Resistance of *Aedesaegypti* mosquito to pyrehtoid compounds have been a common problem in controlling this disease among the tropical countries especially in Indonesia. The causes were known as intensive vector control using insecticide andbecoming merely prevention program attempted by local health department. The report of our previous study explaining the history of insecticide use, i.e. mosquito coils or sprays to protect mosquito bites, have played important role in stimulating resistant competence against various insecticide compounds [4]. There are plenty of report tried to address vector resistance to various insecticide known to be phyretroid compounds, that are α-cypermethrin, deltamethrin and permethrin, that spread throughout the world,including Indonesia [5-12].

Many studies have been conducted trying to understand the base mechanisme of insecticide exposure affected genetical changes and led to resistance status mosquito acquires. The most common target site mechanism conferring phyrethroid resistance is linked to single nucleotide polymorphisms (SNPs) on the voltage-gated sodium channel (VGSC), collectively reffered as knock down resistance (kdr) alleles. It has been proposed as an effective marker for early detection of this problem [7,12,13-19]. Our prior study has discovered a nationwide distribution of S989P and V1016G of VGSC genes and found highly resistance status as well as strongly correlated with phyretroid compounds [4]. This finding suggests the need of routine molecular surveillance of molecular bases resulting in resistant phenotype could give important facts for early detection strategy.

In our present stage of research, we were trying to reveal the need of molecular surveillance. We have found that Central Java isolates been resistant based on S989P and V1016G markers. This finding was sthrengthening our prior study and also provide ongoing data for national and local health department to consider it as an effective approach.

MATERIAL AND METHODS

Study sites and mosquito collection

This study was conducted in four region of high endemic of dengue, which is proven by local health data, in Central Java province, Indonesia. Semarang, Kendal and Jepara district are located in northern coastal area of Central Java, Which is commonly urban, while temanggung is inland and suburban.

Mosquito was derived from household clusters that are near with dengue cases. The cases played as a core for their neighboring houses subsequently we survey in- and outside containers of their house for approximately 100 m arround the cases. We collected larva of each region and housed in a different hutch as well as reared it to second generation. The second generation was reared by strandard treatment thereafter blood feed 2 days post emergence, as WHO standard protocol, and then store it in a plastic microtube.

DNA extraction and PCR amplification

Whole body of each mosquito was homogenized individually in 1.5 ml Eppendorfmicrotubes containing 50 μl of grinding buffer. We used Chelex ion exchanger to extract the DNA from mosquito segment as previously described [16]. Aedesaegyptinatrium voltage-gated channel (AaNav) gene ecompassing domains IIS6 with predicted length of 437 bp was amplified using single-step polymerase chain reaction (PCR) with specific primer pairs [14] (AaNavF20_kdr): 5'-ACAATGTGGATCGCTTCCC-3'- (AaNav_R21_kdr): 5'-TCACAAAAGCAAGGCTAAG-3'. The PCR reaction mixture consisted of 25 μl containing of 5 μl template DNA; 50 mMKCl; 10 mMtris-HCl, PH 8.3; 1.5 mM Mgcl2; 200 mMdNTP; 1 taq polymerase and a primer pairs (20 pM each). The reaction was performed in thermocycler machine for 5 minutes at 95○C for initial denaturation, followed by 40 cycles of 30 s at 95○C for denaturation, 30 s at 58○C for annealing, and 30 s at 72○C for elongation and eventually 72○C polymerase extension, according to the KAPA kits instruction (KAPABIOSYSTEMS, Boston, MA, USA). Electrophoresis of 5 5 μlaliquots of the PCR product in 2% agarose gels used to successful polymerase amplification. The purified amplicons were sequenced using an ABI Prism TM Dye BigDye terminator cycle sequencing ready kit (Applied biosystem, Foster City, USA). In an automatic sequencer through fluorescent DNA capillary electrophoresis (ABI 3130X1) at the Eijkman institute, Jakarta, Indonesia. The sequence obtained was analyzed using an aligment editor program (Biological Sequence Aligment Editor, BioEdit, Ver 7.0.9, IbisBioSciences Carlsbad, USA). Descriptive statistical analysis were perfomed with SPSS software.

RESULT

After DNA extraction and amplification, the samples was sequenced due to point out the mutational changes of AaNav gene IIS6 region. There are three types of three base element, the minimum amount of base element to express an amino acid, Homozygous-susceptible and Homozygous-resistant as well as Heterozygous (Figure 1).Homozygous-susceptible express susceptible strain of mosquitoes, inversely to homozygous-resistant, while heterozygous produce double invisible bases indicating indistintcness between resistan or susceptible. We found they occurred in almost all of the study sites and further used for allelic frequency. There are several kodon that represent AaNav resistant to phyretroid, i.e 989, 1011, 1014 and 1016, the number of the bases accordingly based on Drosophila (Figure 2). However, there are only 989 and 1016 known to affect resistance status of Aedesaegypti.

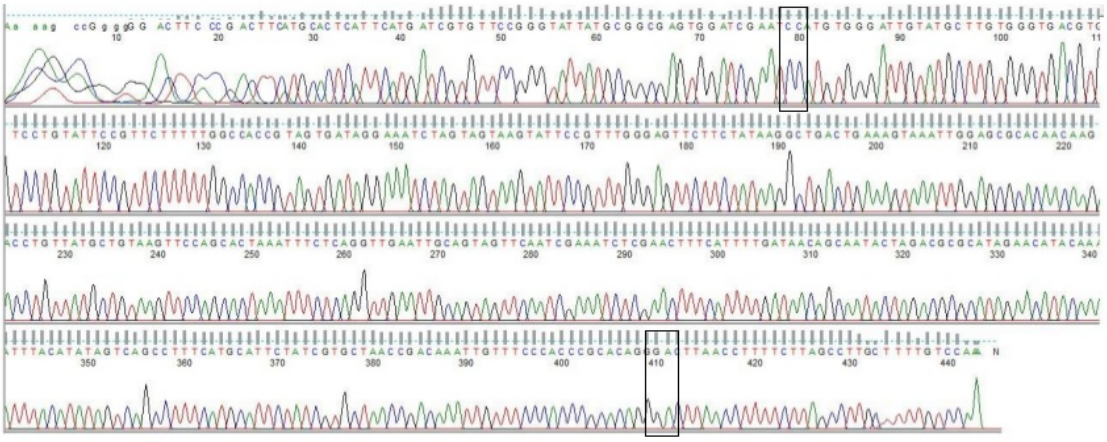

Figure 1, Chromatogram curve of domain II VGSC gene sequence. This figure clearly show the peaks of each base along the gene sequent, indicating the homozygous or heterozygous mutation.

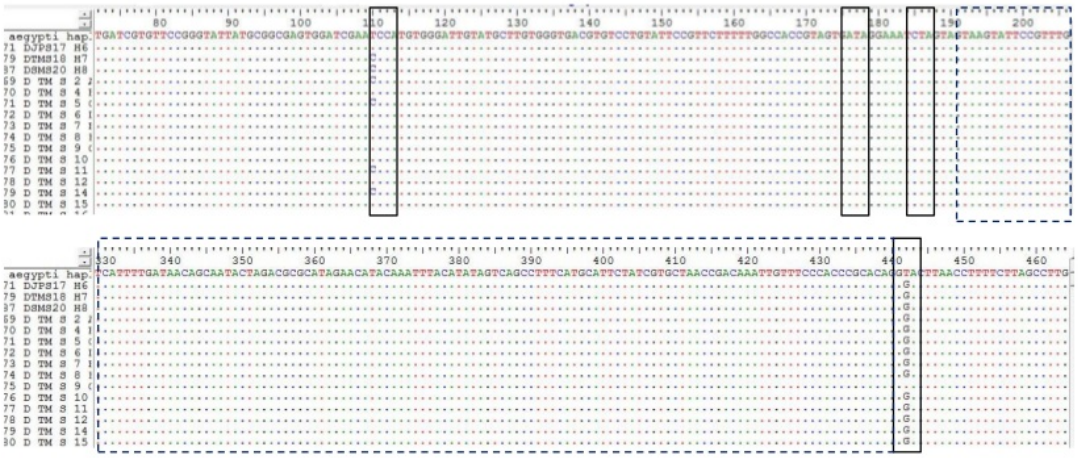

Figure 2, SNPs site in the domain II of VGSC gene sequence. There are five important sites in this sequence, namely bases number 110, 176, 185, 191-440, and 441. Those sites represent codon 989, 1011, 1014, intron, and codon 1016. All of DNA samples showed that there were no bases substitution in codon 1011, 1014 and intron. I1011M is common SNP of this gene among the *Aedesaegypti* population in Latin America, but never found in other regions. While the 1014 is usually found in Anopheles mosquito with Leucine to Phenylalanie amino acid changing, and also never found in Aedes.

To decide if our isolates have a high resistance status molecularly, we provide allelic frequency analysis in our study. The base subtitution of codon 989 is known by TCC (wild-type) to CCC (resistant-type) which likewise changing serine to proline, it called *Non-synonymous mutation*. There are 26 of TCC, 18 of homozygous or mixed, and 3 of CCC. The overall alleles frequency of serine is 0.74, while proilne 0.26, indicates susceptible domination in this codon.

Inversely, there are extremely high status of resistance in the codon 1016 of AaNav. The subtitutional bases are GTA to GGA which revamping valine to glycine. Forty samples were belonged to GGA (resistant-type), two samples of susceptible and heterozygous. The alleles frequency showed 0.93 are glycine and valine for the rest.

Table 1. Genotype and kdr allele frequency of codon 989 Nav gene of *Ae. aegypti* in Central Java

Population	n	TCC	TCC/ CCC	CCC	Genotype frequency			Allele frequency	
					S/S	S/P	P/P	S	P
Semarang city	13	6	4	3	0.46	0.31	0.23	0.62	0.38
Kendal	3	1	2	0	0.33	0.67	0.00	0.67	0.33
Jepara	11	7	4	0	0.64	0.36	0.00	0.82	0.18
Temanggung	20	12	8	0	0.60	0.40	0.00	0.80	0.20
Total	44	26	18	3	0.55	0.38	0.06	0.74	0.26

Table 2. Genotype and kdr allele frequency of codon 1016 Nav gene of *Ae. aegypti* in Central Java

Population	n	GTA	GTA/ GGA	GGA	Genotype frequency			Allele frequency	
					V/V	V/G	G/G	V	G
Semarang city	13	2	1	10	0.15	0.08	0.77	0.19	0.81
Kendal	3	0	0	3	0.00	0.00	1.00	0.00	1.00
Jepara	8	0	0	8	0.00	0.00	1.00	0.00	1.00
Temanggung	20	0	1	19	0.00	0.05	0.95	0.03	0.98
Total	44	2	2	40	0.05	0.05	0.91	0.07	0.93

DISCUSSION

To date, dengue vector control program using insecticides have been widely implicated by national and local health department of Indonesia. Fogging, Abate, mosquito-net, have been the base line approach to prevent vector-borne-related-disease. The frequently use of household insecticide to prevent mosquito bites seen to be cultural behavior of Indonesian society. Unfortunately, Aedesaegypti has been live together with humans in their containers and exposed daily to insecticides. Therefore, constant exposure to insecticide from various sources resulting in a rapid selection for resistance to phyretroid. Taken together, a mass genetical damage is unavoidable.

Molecullar study has been initiated from the last decadeby researchers, trying to connect the basic mechanism of biological changes caused by specific exposure with field eradication technique. There are plenty of papers revealed the synonymous mutation of kdr alleles[7,12,13-19], especially 989 and 1016 in Aedesaegypti [13,14-15], that have a strong connection with susceptibility status of mosquito population. Our prior study supported the previous effort to elucidate their connection [4]. Now, we repeatedly reported the important of molecular surveillance for prevention strategy and early detection to every decision making. We found that 0.74 of mosquito was susceptible molecularly in codon 989 and inversely 0.97 resistant types detected in codon 1016 which is similar with our prior result. Taken together,altoughat codon 989 is not entirely resistant, nevertheless almost all of codon 1016 contains resistant genotype. It is in line with phenotype status when RR95 to α-cypermethrin ranging 14.5-125.5 [4], despite a highly resistance status found, susceptible strain still exist. It indicates that different rates of resistant SNPs occurred between codon 989 and 1016 are as a consequence of rapid mutational steps from susceptible to resistant. This study gave important additional information that our molecular surveillance strongly suggest early detection program for planning prevention strategy.

In conclusion, vector resistance is an common instance widely spread overseas, including Indonesia. The abundance of papers reporting Aedesaegypti resistant to various insecticides and correlating them with VGSC genes (S989P and V1016G) for several decades have exhorted public health sector to implement routine molecular surveillance. It is enhanced by our latest study that includes a wide range of geographical areas of Central Java.

ACKNOWLEDGEMENT

The authors wish to thank Prof. Amin soebandrio, MD, PhD Clin. Microbiol, director of the Eijkman institute for molecular biology for his kindness in permitting us to conduct the laboratory study; Prof. Din Syafruddin, PhD as malarial laboratory director for his material and non-material support; the directorate for Research and Public

Artikel 22. Molecular Surveillance of Pyrethroid Resistance - 1976-4083-1-SM

ORIGINALITY REPORT

15%	14%	3%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	garuda.ristekdikti.go.id Internet Source	10%
2	Submitted to University of Huddersfield Student Paper	1%
3	malariajournal.biomedcentral.com Internet Source	1%
4	Sayono, A Permatasari, D Sumanto. " The Effectiveness of (Wall.) Benth Root Extract Against Temephos-resistant Larvae ", IOP Conference Series: Earth and Environmental Science, 2019 Publication	1%
5	www.ejbiotechnology.info Internet Source	1%
6	real-d.mtak.hu Internet Source	1%
7	cmbl.biomedcentral.com Internet Source	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		