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RESISTANCE STATUS AND MECHANISM OF *Aedes aegypti* AGAINST THREE CLASSES OF INSECTICIDES IN SALATIGA CITY, INDONESIA

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Abstract

Insecticide resistance is a major problem in dengue vector control in Indonesia. Salatiga City, one of Central Java's dengue-endemic areas, has been using insecticide for vector control for decades. To select the insecticide used, it is necessary to understand the status and mechanism of resistance to various insecticides. This study aimed to determine the status and mechanism of *Aedes aegypti* resistance in Salatiga City. This study used a descriptive method. Larvae were collected from 8 endemic villages in Salatiga City. The larvae were then reared to produce adult mosquitoes. A resistance test was conducted using the WHO-Impregnated paper method on F1 mosquitoes. The insecticides used for the test were malathion 5%, phenytotrione 1% (Organophosphate); Bendiocarb 0.1%, Propoxur 0.1% (Carbamate); Lambda Cyhalothrin 0.03%, Deltamethrin 0.05%, Permethrin 0.25%, and Cypermethrin 0.05.% (Pyretrhoid). To determine the resistance mechanism of the target site mutation, sequencing of the Domain II VGSC and ACE1 genes was carried out. The test results showed that Ae. aegypti from 8 locations were resistant to all insecticides. The highest mortality after 24 hours observation was on organophosphate group. Molecular tests showed that Single Nucleotide Polymorphism had occurred in the S989P and V1016G alleles of the VGSC gene, indicating molecular resistance to Pyretrhoid insecticides. The ACE1 gene sequences showed no mutations in the G119S allele, indicating the possibility of metabolic resistance to organophosphates and carbamates. In case of a dengue outbreak, it is recommended to use organophosphate because it has the highest percentage of mosquito mortality, and only metabolic resistance occurs without target site mutations.

Keywords : resistance, salatiga, *Aedes aegypti*, insecticide

Introduction

Dengue Infection is still a public health problem in the world. Reported by WHO, about 50 million dengue infections worldwide with 75 percent being in Asia (Hsan et al., 2019; Singhi, 2018). In 2015 there were reported at least 451,000 cases of dengue in Southeast Asia (Maula et al., 2018). Indonesia is one of the countries with the highest dengue cases in Southeast Asia. After being first discovered in 1968, dengue fever has spread to all provinces in Indonesia. Over a period of 43 years, the Incidence of Dengue cases increased dramatically from 0.05 per 100,000 in 1968 to 51.48 per 100,000 in 2019. Salatiga City is one of Central Java Province's dengue endemic locations. The incidence of dengue

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fever in Salatiga City fluctuates, with the highest number in 2006 being 38.9/100,000 population. Dengue is a disease transmitted by the *Aedes aegypti* as its main vector. With no effective cures for the treatment of dengue infection yet to be found and vaccines still being developed, vector control is the most effective strategy for controlling the incidence of Dengue. Insecticide as a chemical method is still the most widely used vector control method in Indonesia, including in Salatiga City. The use of insecticides was chosen because of its ability to rapidly lower the *Aedes aegypti* population.

The continuous use of insecticides will create a new problem, namely the emergence of resistance from vector mosquitoes. In Southeast Asia, particularly Malaysia, Thailand, Singapore, and Indonesia, *Aedes aegypti* pesticide resistance has been frequently reported. (Rueda, 2004; Sayono et al., 2016; Siti-Futri et al., 2020). The mechanism of insecticide resistance can occur in four ways: 1. increased metabolic enzyme activity; 2. mutation of the target site; 3. thickening of the cuticle; and 4. changes in vector behavior (Mitchell et al., 2014). Organophosphates, pyrethroids, and carbamates are three classes of insecticides currently used in Indonesia to control dengue vectors. DDT insecticides from the organochlorine group have been banned in Indonesia for vector control, including dengue fever, since 1989 (Elyazar et al., 2011).

The use of insecticides without considering the status of vector mosquito resistance will suppress the number of vulnerable mosquito populations in an area, and on the other hand resistant mosquito populations take over. The effectiveness of pesticides will decrease as the number of resistant mosquitoes increases. Based on these considerations, the selection of the type and class of insecticide used in an area should be based on information about the status and mechanism of resistance of vector mosquitoes.

Dengue disease control programs in Salatiga City have been carried out by various methods, including community mobilization, eradication of mosquito nests, counseling, and the use of insecticides through fogging activities and the use of larvicides. The use of insecticides in Salatiga City has been carried out for decades. In an effort to control dengue vectors, organophosphate insecticides have been used in Salatiga City for several decades, and in the last ten years or so, they have switched to using pyrethroid class insecticides.

Several studies of *Aedes aegypti* resistance in Salatiga City were carried out, among others reported by widiarti (2010) and yanti (2011), that aedes aegypti in Salatiga City are still susceptible to cypermethrin, but have been resistant to malation, bendiocarb, permethrin, deltamethrin, etophenprox, and *Aedes aegypti* larvae are still susceptible to organophosphates. The *Aedes aegypti* resistance research in Salatiga City only reported resistance status information, and did not report the resistance mechanism information.

The effectiveness of chemical control is determined by the accuracy of the selection of insecticides used. Rotation of the active ingredients of the insecticide used is the main key in maintaining the success of lowering the density of the vector. To support these efforts, the latest information is needed on the status and mechanism of resistance of the *Aedes aegypti* mosquito as the main vector in Salatiga City. Resistance vector surveillance can be used as a potential early warning system and can provide data on the development of resistance at a certain location.

Materials and Methods

This study used an analytical descriptive method with a cross-sectional study design. Data collection was carried out in July 2019. The larvae and pupae of *Aedes aegypti* were caught from 8 dengue endemic villages, namely Sidorejo Lor, Ledok, Tegalrejo, Dukuh, Tingkir Lor, Kecandran, Kauman Kidul and Mangunsari.

The larvae and pupae of *Aedes aegypti* were collected from containers that are in the vicinity of residential areas, both inside and outside the house. Furthermore, larvae and pupae were reared at the Institute for Vector and Reservoir Control Research and Development (IVRCRD) Insectarium, Salatiga until adult F1 and F2 mosquitos were acquired for susceptibility testing. The species *Aedes aegypti* was identified using the Rueda's illustrated identification key (2004).

WHO Impregnated Paper Methods

Aedes aegypti was exposed to the specified impregnated paper for the WHO Bioassay. In this study, 0.8 percent malathion and 0.05 percent cypermethrin were used. Mosquitoes ranging in age from 2 to 5 days were aspirated into the WHO test tube. The mosquitos were placed in five tubes, four of which contained impregnated paper containing insecticide and one of which did not. There were 20-25 female mosquitos in each tube. The mosquitos were transferred to a neutral tube after 1 hour of contact using a wet cotton swab soaked in sugar water. After 60 minutes and 24 hours of observation, the knockdown and fatality rates were calculated. The ambient temperature in the test room was 28.1 ° C, with a relative humidity of 60-65 percent.

Molecular Assay

PCR tests were performed to identify KDR gene mutations using specific primers that target the VGSC gene's domain II. The primer sequences were vgscF (5'-GGTGGAACTTCACCGACTTC-3') and vgscR (5'- GGACGCAATCTGGCTTGTTA-3'). The PCR reaction was carried out with the initial step of denaturation at 94oC for 10 minutes, followed by 40 amplification cycles at 94o C for 1 minute, 63o C for 45 seconds, and 72oC for 1 minute, with a final extension at 72oC for 7 minutes. All PCR amplicons were then loaded into a 2% agarose gel electrophoresis using SYBR-safe Invitrogen staining for 60 min at 90 V in a TAE buffer to check the quality of the PCR product.

To detect the presence of mutations at the target site of organophosphate and carbamate class, a PCR test was carried out using primer with ace1 gene targets, namely AceF (5'-CGATAACGAATGGGGAACGACG-3') and AceR (5'-TCAGAGGCTCACCGAACACA -3'). PCR is performed under the following conditions: the initial step of denaturation at 94ºC for 3 minutes, followed by 35 amplification cycles at 94ºC for 1 minute, 58ºC for 1 minute, and 72ºC for 2 minutes, with the final extension step at 72º C for 10 minutes. Purified PCR products were then directly sorted in both directions with the same primer for PCR amplification at the G119S location.

PCR analysis using Applied BiosystemsTM thermal cycler simpliamp (Perkin Elmer, Branchburg, NJ, USA), and DNA sequencing analysis was performed using the Applied Biosystems 3500 series genetic analyzer.

Analysis and interpretation Data

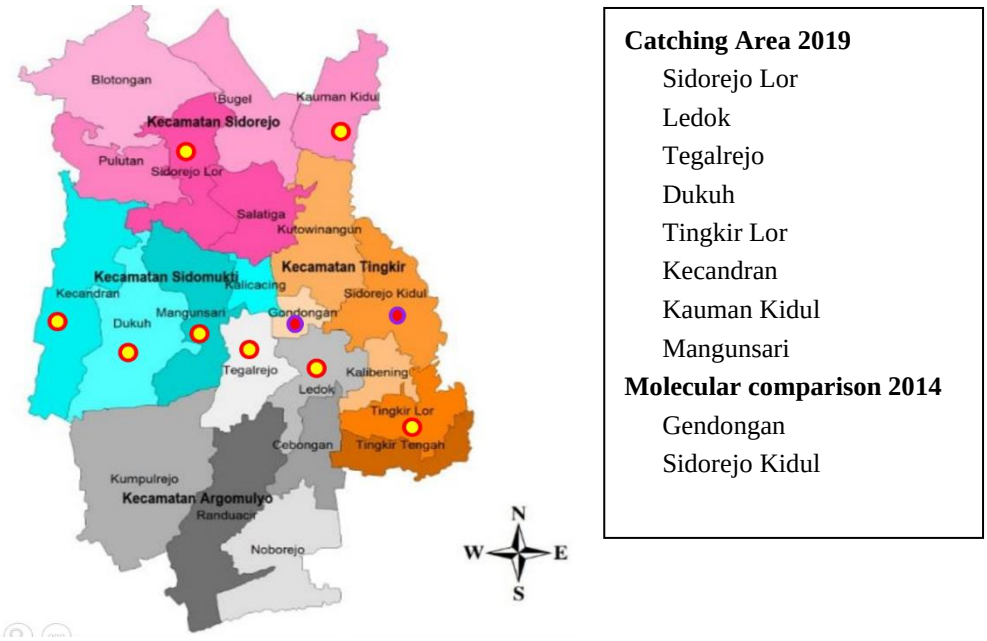
Data analysis was carried out descriptively, by comparing the results of the resistance test of the WHO Impregnated paper method with genetic mutation data at the insecticide target site. Data on the target site mutation of *Aedes aegypti* which were captured in 2014 was used as a comparison

The paper is part of the research on the dengue outbreak in Salatiga City which has received ethical approval from the Health Research Ethics Commission of the Health Research and Development Agency of the Ministry of Health of the Republic of Indonesia with No. LB.02.01/ 2/ KE.022/ 2019.

Result and Discussion

Result

Sampling locations were carried out in 8 villages. The sampling location is based on the Dengue case in 2019 (picture 1).



Picture 1. Location of Aedes aegypti resistance test sampling in Salatiga City, 2019.

Table 1. mosquito deaths based on sample location and insecticide

No	Villages	% Death after 24 hours							
		Organophpsphate		Carbamate		Pyrethroid			
		Malathion (5%)	Fenitro thion (1%)	Bendio carb (0.1%)	Propuxur (0.1%)	Lambda Cyhalothrin (0.03%)	Delta methrin (0.05%)	Permethrin (0.25%)	Cypermethrin (0.05%)
1	Sidorejo Lor	35	60	60	13	56	55	60	57
2	Mangunsari	35	60	17	0	35	57	11	22

3	Tegalrejo	37	53	23	12	40	57	31	48
4	Ledok	34	60	4	0	6	29	7	8
5	Tingkir Lor	60	60	10	7	10	33	4	0
6	Dukuh	59	60	10	5	19	47	11	27
7	Kauman Kidul	60	58	13	0	5	8	7	3
8	Kecandran	56	0	0	0	28	0	0	0
	Mean	47	51.4	17.1	4.63	24.9	35.8	16.4	20.6
	Range	35-60	0-60	0-60	0-13	5-56	0-57	0-60	0-57

The results of the resistance test showed that *Aedes aegypti* from all study sites were resistant to all insecticides from the 3 classess, namely Organophosphate, carbamate and pyretrhoid (table 1). The mosquito population is resistant if the percentage of deaths is below 90%. The highest average mortality was phenitrothion and malathion of the organophosphate class (51.37 and 47%, respectively); percent of carbamate class deaths, namely bendiocarb and propuxur were 17.13% and 4.63%, respectively; percent mortality of pyrethroid-class insecticides, namely lambda cyhalothrin, deltamethrin, permethrin and cypermethrin, was 24.9%; 35.8%; 16.37% and 20.63%, respectively.

Table 2. Molecular assay of ACE1 gene and domain II VGSC Gene of Aedes aegypti in Salatiga City, 2019

No	Villages	SNP on Target site					
		ACE1	VGSC				
		119	989	1011	1014	1016	1023
1	Sidorejo Lor	G119	S989	I1011	L1014	V1016G*	L1023
2	Tegalrejo	G119	S989+	I1011	L1014	V1016G*	L1023
5	Ledok	G119	S989	I1011	L1014	V1016G*	L1023
4	Dukuh	G119	--	I1011	L1014	V1016G*	L1023
3	Tingkir Lor	G119	S989+	I1011	L1014	V1016G*	L1023
6	Kecandran	G119	S989	I1011	L1014	V1016G*	L1023
7	Kauman Kidul	G119	--	I1011	L1014	V1016G*	L1023
8	Mangunsari	G119	--	I1011	L1014	V1016G*	L1023
9	Gendongan x	G119	S989	I1011	L1014	V1016	L1023
10	Sidorejo Kidul x	G119	S989	I1011	L1014	V1016G*	L1023

+ wild type heterozygote

*mutated

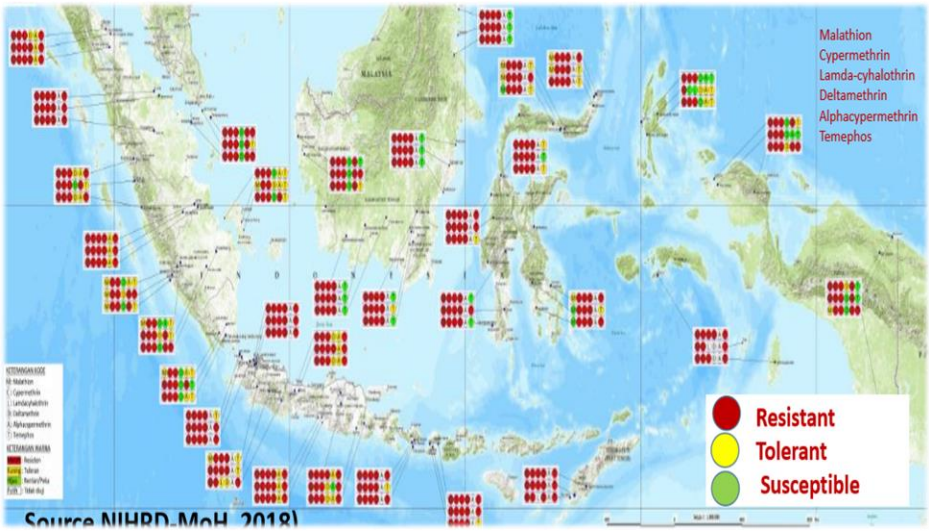
x collected at 2014

Molecular test results on the ACE1 gene showed that samples from all over the village did not find any SNP in codon 119. Allele in codon 119 were wild type, i.e., GGC encodes the Glycine protein, and no nucleotide AGC is found encoding the Serine protein. Molecular tests on domain II of the

VGSC gene, showed that in codon 1011 and codon 1014 no mutation was found. Protein on codon 1011 were Isoleucin, and at codon 1014 were Leucin. Conversely in codon 1016, a 2019 mosquito sample showed a mutation had occurred, with the discovery of SNP i.e. alleles had mutated from valine to Glycine. From Collected samples in 2014 showed that in Sidorejo Kidul area there has been a mutation (V1016G), but the sample from Gendongan village still has no mutation (V1016)

Discussion

The incidence of *Aedes aegypti* resistance to insecticides has been widely reported in Indonesia. Reported by the Indonesian Ministry of Health in 2018, resistance has been found in many locations across the country (figure 2) (National_Institute_of_Health_Research_and_development, 2018).



Picture 2. The Emergence of Various Types Insecticide Resistance Against Aedes aegypti

The results of this study show the same situation as the national data, where *Aedes aegypti* in Salatiga City has been resistant to 8 types of insecticides from 3 classes. These results show a change in the status of resistance in Salatiga City in recent years. Reported by widiarti (2010), *Aedes aegypti* in Salatiga City is susceptible to cypermethrin (pyrethroid), but is already resistant to malathion (organophosphate), bendiocarb and etofenprox (Carbamat), as well as permethrin, and deltamethrin (pyretrhoid)(Widiarti et al., 2011).

Molecular tests in Salatiga City were reported by Brengues in 2003. The Brengues report shows that in 2003 no mutations were found in the VGSC gene(Brengues et al., 2003). Mutations of the VGSC gene are responsible for the occurrence of resistance to pyroethroid class insecticides. Under normal circumstances, the voltage gate opens to send nerve impulses and closes to break them off. The proteins at this gate become the target site of the attachment of pyrethroid insecticides. This attachment interferes with the function of opening and closing so that the nerve impulses become continuous so that the insect becomes tremorous and gives rise to death (Yu et al., 2003). This target site mutation will change the receptor binding site so that the pyrethroid insecticide cannot bind to the target and cause the insecticide to not work properly and cause the mosquito not to die or be resistant (Hemingway et al., 2004; Martins et al., 2009).

Mutations of the VGSC gene in mosquitoes occur in the II-IV domain, and spread in various codons. VGSC gene mutations in anopheles and culex are characterized by changes in the amino acid codon 1014, while in *Aedes* it spreads (Davies & Williamson, 2009). Several studies related to this VGSC gene mutation have been widely carried out in Indonesia. Mutations found in previous studies include S989P and V1016G in Palembang and Jakarta, F1565C, F1023C, S996P in Yogyakarta, F1534C, V1016G in Thailand and S989P, V1016G and F1534C in Central Java (Islami et al., 2018; Plernsub et al., 2016; Sayono et al., 2016; Wuliandari, n.d.).

This study showed the presence of VGSC gene mutations in the V1016G codon as a target for high-frequency pyrethroid insecticide sites. This mutation is caused due to prolonged contact with insecticides, so the susceptible *Ae. aegypti* that is dominant in the population is increasingly depressed, and resistant mosquitoes are growing. The results of this mutation in 2019 showed an increase, where the *Aedes aegypti* sample caught in 2014 showed that there were still mosquitoes that had not mutated (wild type).

Molecular tests on the ACE1 gene showed no mutations found in codon 119. Alleles of the sample were GGC encoding the Glysine protein, and no Serine protein was found. Mutations in codon 119 of the ACE1 gene were responsible for the occurrence of insect resistance to insecticides of the organophosphate and carbamate classes. The WHO Standard test showed *Aedes aegypti* at all study sites had been resistant to organophosphate and carbamate class insecticides, but no mutations were found in the ACE1 gene. This indicates that resistance to organophosphate and carbamate insecticides is not through the mechanism of mutation of the target site, but metabolic.

Some studies show insecticide resistance is not only due to exposure to insecticides used in health programs but also from agriculture and households' activity. Research in Sao Paulo, Brazil in 2007 showed that after 10 years of insecticide use caused adult mosquitoes *Ae. aegypti* with a high level of resistance to pyrethroids and larvae with low resistance to organophosphoria (Maria de Lourdes da Graça Macoris et al., 2007). Gray's experiments showed that theuse of spray insecticides in the household has reduced mortality from 99% to 44%, and residual spray to 50%. The use of residual spray also increases the risk of mutation of the VGSC gene in V1016I, which is responsible for knockdown resistance (kdr) resistance (Maria de Lourdes da Graça Macoris et al., 2007). In Indonesia, housohold pesticides are widely used. The use of mosquito coils is a favorite because it is easy to obtain and cheap, with a price of around Rp. 5.000, - (USD 0.35) per week. This led to its considerable use. Households using mosquito coil in Pangandaran reached 82%, in Salatiga it reached 72%, and in Semarang it reached 64% (Kusumastuti, 2014; Riani et al., 2017; Wigati & Susanti, 2012).

Conclusion

Aedes aegypti in Salatiga has been resistant against 3 classes insecticides that used in Indonesia i.e. organophosphate, carbamat and purethroid.The mechanism of resistance against pyrethroid is mutation of the target site and against organophosphate and carbamate is metabolic. In case of a dengue outbreak, it is recommended to use organophosphate due to the highest percentage of mosquito mortality, and only metabolic resistance occurs without target site mutations. *Aedes aegypti* resistance testing should be carried out at least once a year to determine the rotation of the insecticide used for strengthtening the resistance surveillance.

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Declaration of Interest Statement

The authors declare that they have no conflict of interests.

References

Brengues, C., N.J.Hawkes, Chandre, F., L.Mccarroll, Duchon, S., Guillet, P., MANGUIN, S. ., J.C.MORGAN, HEMINGWAYy, J., & Hemingway, J. (2003). Pyrethroid and DDT cross-resistance in Aedes aegypti is correlated with novel mutations in the voltage-gated sodium channel gene. *Medical and Veterinary Entomology*, 17, 87–94.

Davies, T. G. E., & Williamson, M. S. (2009). Interactions of pyrethroids with voltage gated sodium channel. *Bayer CropScience Journal*, 62(January), 159–178.

Elyazar, I. R. F., Hay, S. I., & Baird, J. K. (2011). Malaria Distribution, Prevalence, Drug Resistance and Control in Indonesia. *Adv Parasitol*, 1–107. https://doi.org/10.1016/B978-0-12-385897-9.00002-1.Malaria

Hemingway, J., Hawkes, N. J., Mccarroll, L., & Ranson, H. (2004). The molecular basis of insecticide resistance in mosquitoes. *Insect Biocheistry and Molecular Biology*, 34, 653–665. https://doi.org/10.1016/j.ibmb.2004.03.018

Hsan, K., Hossain, M. M., Sarwar, M. S., Wilder-Smith, A., & Gozal, D. (2019). Unprecedented rise in dengue outbreaks in Bangladesh. *The Lancet Infectious Diseases*, 19(12), 1287. https://doi.org/10.1016/S1473-3099(19)30616-4

Islami, S., Puspa, A., Hidayati, N., Wibowo, H., & Syafruddin, D. (2018). The role of Voltage-Gated Sodium Channel (VGSC) gene mutations in the resistance of Aedes aegypti L . to pyrethroid permethrin in Palembang and Jakarta, Indonesia. Preprints, March, 1–6. https://doi.org/10.20944/preprints201803.0070.v1

Kusumastuti, N. H. (2014). Use of House Insecticide in Pangandaran Village. Widyariset, 3, 417–424.

Maria de Lourdes da Graça Macoris, Andrighetti, M. T. M., Otrera, V. C. G., Carvalho, L. R. de, Júnior, A. L. C., & Brogdon, W. G. (2007). Association of insecticide use and alteration on Aedes aegypti susceptibility status. Mem Inst Oswaldo Cruz, 102(December), 895–900.

Martins, A. J., Mazzei, R., Andrade, M. De, Gerlinde, J., Linss, B., Peixoto, A. A., & Valle, D. (2009). Voltage-Gated Sodium Channel Polymorphism and Metabolic Resistance in Pyrethroid-Resistant Aedes aegypti from Brazil. *Am.J.Trop.Med.Hyg.*, *81*(June 2014), 108–115.

Maula, A. W., Fuad, A., Utarini, A., & Maula, A. W. (2018). Ten-years trend of dengue research in Indonesia and South-east Asian countries : a bibliometric analysis. *Global Health Action*, 11(1), 1–8. https://doi.org/10.1080/16549716.2018.1504398

Mitchell, S. N., Rigden, D. J., Dowd, A. J., Lu, F., Wilding, C. S., Weetman, D., Dadzie, S., Jenkins, A. M., Regna, K., Boko, P., Djogbenou, L., Muskavitch, M. A. T., Ranson, H., Paine, M. J. I., Mayans, O., & Donnelly, M. J. (2014). Metabolic and Target-Site Mechanisms Combine to Confer Strong DDT Resistance in Anopheles gambiae. PLOS ONE, 9(3), 1–10. https://doi.org/10.1371/journal.pone.0092662

Plernsub, S., Saingamsook, J., Yanola, J., Lumjuan, N., Tippawangkosol, P., Walton, C., & Somboon, P. (2016). Temporal frequency of knockdown resistance mutations, F1534C and V1016G, in Aedes aegypti in Chiang Mai city, Thailand and the impact of the mutations on the efficiency of thermal fogging spray with pyrethroids. *Acta Tropica*, 162, 125–132. https://doi.org/10.1016/j.actatropica.2016.06.019

Riani, D. A., Wahyuningsih, N. E., & Budiharjo, A. (2017). Hubungan Praktik Penggunaan Insektisida Dengan Kejadian Demam Berdarah Dengue Di Semarang. *Jurnal Kesehatan Masyarakat* (e-Journal), 5(5), 592–598.

Rueda, L. M. (2004). Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission. In Zootaxa (1st ed., Vol. 589, Issue 1). Magnolia Press. https://doi.org/10.11646/zootaxa.589.1.1

Sayono, S., Hidayati, A. P. N., Fahri, S., Sumanto, D., Dharmana, E., Hadisaputro, S., Asih, P. B. S., & Syafruddin, D. (2016). Distribution of voltage-gated sodium channel (NAV) alleles among the aedes aegypti populations in central Java province and its aociation with resistance to pyrethroid insecticides. PLoS ONE, 11(3), 1–12. https://doi.org/10.1371/journal.pone.0150577

Singhi, S. C. (2018). Severe Dengue: Developing a Universally Applicable Simple Prediction Model. *Indian Journal of Pediatrics*, 85(6), 413–414. https://doi.org/10.1007/s12098-018-2668-2

Siti-Futri, F. F., Rosilawati, R., Wan, K. L., Cheong, Y. L., Nazni, W. A., & Lee, H. L. (2020). Status of pyrethroid resistance in Aedes (Stegomyia) aegypti (linneaus) from dengue hotspots in Klang valley, Malaysia. Tropical Biomedicine, 37(1).

Widiarti, Heriyanto, B., Boewono, D. T., Widiastuti, U., Mujiono, Lasmiati, & Yuliadi. (2011). The Resistance Map of Dengue Haemorrhargic Fever Vector Aedes aegypti Againts Organophosphates, Carbamates and Pyrethroid Insecticides in Central Java and Yogyakarta Province. Bul. Penelit. Kesehat, 39(4), 176–189.

Wigati, R. A., & Susanti, L. (2012). Hubungan karakteristik pengetahuan, sikap dan perilaku masyarakat dalam menggunakan antinyamuk di Kelurahan Kutowinangun. Buletin Penelitian Kesehatan, 40(3).

Wuliandari, J. R. (n.d.). Frequency of kdr mutations in the voltage-sensitive sodium channel (VSSC) gene in Aedes aegypti from Yogyakarta and implications for Wolbachia-infected mosquito trials. 1–34.

Wuliandari, J. R., Lee, S. F., White, V. L., Tantowijoyo, W., Hoffmann, A. A., & Endersby-Harshman, N. M. (2015). Association between three mutations, F1565C, V1023G and S996P, in the voltage-sensitive sodium channel gene and knockdown resistance in aedes aegypti from yogyakarta, Indonesia. *Insects*, 6(3), 658–685. https://doi.org/10.3390/insects6030658

Yu, F. H., Catterall, W. A., Hodgkin, A., Huxley, A., Hille, B., Catterall, W., Rohl, C., Boeckman, F., Baker, C., Scheuer, T., Catterall, W., Klevit, R., Doyle, D., Cabral, J., Pfuetzner, R., Gulbis, J., Cohen, S., Chait, B., MacKinnon, R., … Vandenberg, J. (2003). Overview of the voltage-gated sodium channel family. *Genome Biology*, 4(3), 207. https://doi.org/10.1186/gb-2003-4-3-207