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Comparison of CDC Bottle Bioassay Test with WHO Standard Method for Assessment of *Aedes Aegypti* Susceptibility to Carbamates and Organophosphates Insecticides in Semarang, Indonesia

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Abstract: Dengue hemorrhagic fever (DHF) is still a public health problem globally, including Semarang, one of the dengue-endemic areas in Indonesia. Vector control using insecticides is the main choice. The continuous use of insecticides poses a threat of resistance. The WHO method has been used for insecticide evaluation for decades. Since 2019 Indonesia has adopted the CDC Bottle Bioassay method for resistance testing and the WHO method, which is still being used. This study aimed to compare resistance tests using the CDC Bottle Bioassay method and the WHO Impregnated paper. *Aedes aegypti* larvae and pupae were collected from 3 villages in Semarang City. The larvae are then rearing into adult mosquitoes for resistance testing. The WHO method test was carried out using the insecticide cypermethrin (0.05%) from the pyrethroid group and malathion (0.5%) from the organophosphate group. The CDC method test was carried out using cypermethrin 1X (10µg/bottle) + synergist Piperonyl Butoxide (PBO), as well as 1X malathion (50µg/bottle) and synergist SSS-tributylphosphorotrithioate (DEF). Molecular tests were carried out by sequencing the VGSC and ACE1 genes. The resistance test to cypermethrin showed that the two methods showed the same results, namely resistance. The mortality rate using the WHO method in the villages of Patemon, Terboyo Wetan and, Kandri is 62.4% respectively; 30.0%; and 75.3%, while using the CDC method is 90%; 55.5%, and 84.7% and after the addition of PBO it became 96%; 71% and 93.3%. The status of resistance to malathion using the two methods showed different results. The WHO method's mortality rate was 91.7%, respectively; 86.7% and 81.7%, while using the CDC method of 98.3%, 96.7%, and 98.3%. The resistance mechanisms detected were metabolic and target site mutations.

Keywords: cypermethrin, malathion, Comparison CDC, WHO.

疾病控制中心瓶生物测定试验与世界卫生组织在印度尼西亚三宝垄评估埃及伊蚊对氨基甲酸酯和有机磷杀虫剂敏感性的标准方法的比较

摘要: 登革出血热仍然是全球的公共卫生问题, 包括印度尼西亚登革热流行地区之一的三宝垄。使用杀虫剂控制病媒是主要选择。杀虫剂的持续使用构成了抗药性的威胁。几十年来, 世界卫生组织的方法一直用于杀虫剂评估。自2019年以来, 印度尼西亚采用了疾病控制中心的瓶子生物测定法进行耐药性检测和世界卫生组织的方法, 该方法仍在使用中。本研究

Received: March 7, 2021 / Revised: April 11, 2021 / Accepted: May 14, 2021 / Published: June 28, 2021

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旨在比较使用疾病控制中心瓶生物测定方法和世界卫生组织浸渍纸的耐药性测试。从三宝垄市的3个村庄收集了埃及伊蚊的幼虫和蛹。然后将幼虫培育成成年蚊子进行抗药性测试。使用来自拟除虫菊酯组的杀虫剂氯氰菊酯 (0.05%) 和来自有机磷组的马拉硫磷 (0.5%) 进行世界卫生组织方法测试。疾病控制中心的方法测试使用氯氰菊酯 1 折叠集中 (10 微克/瓶) + 增效剂胡椒基丁醚, 以及 1 折叠集中马拉硫磷 (50 微克/瓶) 和增效剂 $\Delta\Delta\Delta$ -三硫代磷酸酯进行。通过对电压门控钠通道和血管紧张素转化酶1基因进行测序来进行分子测试。对氯氰菊酯的耐药性试验表明, 两种方法显示出相同的结果, 即耐药。百事通、特博约维坦和坎德里村采用世界卫生组织方法的死亡率分别为62.4%; 30.0%; 和75.3%, 而使用疾病控制中心的方法是90%; 55.5%和84.7%, 加入胡椒基丁醚后变为96%; 71%和93.3%。两种方法对马拉硫磷的抗性状况显示出不同的结果。世界卫生组织方法的死亡率分别为91.7%; 86.7% 和81.7%, 而使用疾病控制中心方法的分别为98.3%、96.7%和98.3%。检测到的耐药机制是代谢和目标位点突变。

关键词: 氯氰菊酯、马拉硫磷、疾病控制比较中心、世界卫生组织。

1. Introduction

Dengue is one of the important public health problems in the world. Recent studies estimate that 50 million dengue infections are reported annually, and 75% occur in Asia [1], [2]. Southeast Asia is an area with quite high dengue cases, with at least 451,000 cases reported in 2015 [3]. Indonesia is known as the highest dengue country in Southeast Asia. The disease was first discovered in Indonesia in 1968. Since then, dengue has expanded in all provinces, with annual case incidence increased significantly after the past 43 years, i.e. from 0.05/100,000 in 1968 to 51.48/100,000 population in 2019. Semarang City is one of the dengue-endemic areas in Central Java Province, Indonesia. The dengue incidence rate was reported quite high (26.9 / 100,000 population) in this city in 2019 [4]. While vaccines are still under development and research, vector control is the best way to prevent and control dengue. Currently, insecticides, either thermal fogging or larvicides, are still the main vector control method used in Indonesia, including Semarang. The insecticide was chosen because of its ability to control the population of *Aedes aegypti* quickly. In contrast, the application of other methods in dengue vector control is still not as expected.

However, the continuous use of insecticides has created a serious problem, i.e., the occurrence of insecticide resistance against dengue vectors, particularly *Ae. aegypti*. Resistance of *Ae. aegypti* to insecticides has been widely reported in Southeast Asia, including Malaysia [5], Thailand [5], Singapore [6], and Indonesia [7]. There are four ways in the process of developing insecticide resistance, including 1. Increased metabolic enzyme activity; 2. Target site mutation; 3. Thickening of the cuticle, and; 4. Changes

in vector behavior. Metabolic resistance and target site mutation is played a major role in the occurrence of insecticide resistance [8]. Organophosphates, pyrethroids, and carbamates are 3 classes of insecticides currently used in dengue vector control in Indonesia. While the DDT, a member of an organochlorine class, has been banned for vector control, including dengue in Indonesia, since 1989 [9].

The continuous use of insecticides will cause the number of susceptible mosquito populations and resistant mosquito populations will become dominant. The increase in the frequency of resistant mosquitoes will cause the efficacy of insecticides to decrease, and at one point, it is no longer effective. When a type of insecticide is no longer effective in the mosquito population, it is necessary to replace the type or class of insecticide with a still effective type [10]. Therefore, resistance management becomes a very important issue of effective vector control. Clear information on resistance mechanisms and careful and detailed monitoring of resistance is the key to successful resistance management. Understanding the mechanisms of resistance allows us to determine the type of insecticide to be used appropriately. Regular resistance vector surveillance will provide information on the development of resistance in an area and can be used as an early warning system.

Currently, many resistance tests in Indonesia are conducted using the WHO impregnated paper method. This method has been used for more than 30 years, but its application has some limitations, including 1) cost; 2) the test must be carried out using mosquitoes of the same age (homogenous); 3) testing is limited to the type of insecticide on the available impregnated paper; 4) the mechanism of resistance that occurs is unknown.

To overcome the limitations of the WHO method, the US-CDC has developed a resistance test method using the Bottle Bioassay [11].

The CDC Bottle bioassay has been adopted as a new insecticide resistance test method in Indonesia since 2019 [12]. Currently, insecticide resistance testing can be carried out using the WHO and CDC protocols based on the resistance monitoring guidelines issued by the Indonesian Ministry of Health in 2018. Until recently, the CDC method has only been used to test *Anopheles* mosquitoes. The susceptibility test for *A. aegypti* mosquitoes using the CDC method has never been carried out in Indonesia so that the effectiveness and problems in its application are not known. This study aims to compare the susceptibility test of *Ae. aegypti* to organophosphate and pyrethroid insecticides using the WHO susceptibility test and CDC bottle bioassay test. This study hypothesizes that there is no difference in the resistance status of *A. aegypti* to organophosphate and pyrethroid insecticides using the WHO Impregnated Paper and CDC Bottle Bioassay methods. The molecular assay will be used to strengthen the analysis of the test results of these two methods.

2. Methods

2.1. Mosquitoes Collection

Aedes aegypti larvae and pupae were captured from three sub-districts in Semarang City, namely Patemon, Terboyo Wetan, and Kandri. These three villages were selected based on endemicity criteria and variations in the population's geographical height and economic activities. Kandri and Patemon are high areas (>350 MASL), and Terboyo Wetan is coastal areas (<10MASL). Kandri and Patemon are the fields and traders, while Terboyo Wetan is the fishing area. Sample collection and testing were carried out in September 2019-February 2020.

Larva and pupa of *A. aegypti* were collected from containers in residential areas, both indoors and outdoors the house. Furthermore, the mosquitoes were kept in the IVRCRD Insectarium in Salatiga until the adult mosquitoes F1 and F2 were obtained for resistance testing. Identification of the *A. aegypti* species was carried out using the pictorial identification key of Rueda [6]. Furthermore, a susceptibility test was carried out using the WHO protocol and the CDC protocol to the insecticide malathion from the organophosphate group and cypermethrin from the pyrethroid group.

Malathion was chosen because it has been widely used in Indonesia for quite a long time since the chemical control of *A. aegypti* around the 80s. Cypermethrin was chosen because it is type two pyrethroid and the cheapest, so it is widely used as an active ingredient in insecticides in public health.

2.2. WHO Impregnated Paper Methods

The WHO Bioassay method was carried out by making insect contact with the selected impregnated paper. In this study, 0.8% malathion and 0.05% cypermethrin were used. Mosquitoes age 2-5 days were transferred into the WHO test tube using an aspirator. The mosquitoes were put in five tubes, four test tubes with impregnated paper with insecticide, and one control tube without insecticide. 20 to 25 female mosquitoes were fed with sugar in each tube, 2-5 days old. After contact for one hour, the mosquitoes were transferred to a neutral tube with a cotton swab moistened with sugar water. The knockdown and mortality rates were recorded after 60 minutes and 24 hours, respectively. The environmental conditions of the test room are 28 ± 1 °C, and the relative humidity is 60-65%.

2.3. CDC Bottle Bioassay Methods

The principle of testing the CDC protocol is to contact the mosquitoes into a bottle coated with the test insecticide, then observe the mortality rate. The test step was to coat four bottles of Wheaton 250 ml with the test insecticide dissolved in ethanol, and 1 control bottle was coated with ethanol. The insecticides used were malathion 50 µg / bottle and cypermethrin 10 µg / bottle.

Furthermore, the selected *Ae. aegypti* mosquitoes were inserted into each bottle. *A. aegypti* adult female as many as 10-25 mosquitoes. Observations were made within 120 minutes, with resistance status determined by looking at the percent mortality at the diagnostic time of 30 minutes (diagnostic time). If the test results show that the mosquito status is resistant, proceed with the test using a synergist. One bottle of Wheaton is coated using a synergist. According to the CDC test protocol above, the test mosquitoes were put in a synergistic bottle for one hour then transferred into the test bottle. Mortality rates with synergists and without synergists were compared to see the activity of metabolic enzymes according to the synergist used. In this study, the synergists used were Piperonyl Butoxide (PBO) to bind the monooxygenase enzyme and SSS-tributylphosphorotrithioate (DEF) to bind the esterase enzyme.

2.4. PCR Detection of Species and the KDR and ACE-1 Mutations

To identify *KDR* mutation, PCR was conducted using specific primers targeting domain II of the VGSC, *vgscF*(5'-GGTGGAACTTCACCGACTTC-3') and *vgscR* (5'-GGACGCAATCTGGCTTGTTA-3'). PCR reaction was performed with the initial denaturation step at 94°C for 10 min, followed by 40 cycles of amplification at 94° C for 1 min, 63° C for 45 s, and 72°C for 1 with a final elongation at 72°C for 7 min. All PCR amplification products were then loaded onto a 2% agarose gel electrophoresis following SYBR

safe Invitrogen staining and run for 60 min at 90 V in TAE buffer to check the quality of PCR products.

PCR was performed using the SimpliAmp™ Applied Biosystems thermal cycler (Perkin Elmer, Branchburg, NJ, USA) to detect target site mutations. The primers used for the ACE1 gene PCR were AceF (5'-CGATAACGAATGGGGAACG-3') and AceR (5'-TCAGAGGCTCACCGAACACA -3'). PCR was conducted under the following condition: an initial step of denaturation at 94°C for 3 min, followed by 35 cycles of amplification at 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min, with a final elongation step at 72°C for 10 min. PCR products were purified and then directly sequenced in both directions with the same primer for PCR amplification at the position of G119S.

The sequencing analysis was then conducted by the Applied Biosystems 3500 series genetic analyzer.

2.5. Analysis and Interpretation Data

The results of vulnerability testing using the CDC and WHO methods will be compared and analyzed descriptively. The two tests were compared on the susceptibility status to each type of insecticide and the

comparison of the level of resistance to insecticides with and without synergists. Resistance status was determined according to WHO guidelines: 1) Resistance: mortality <90%; 2) Tolerant: 90-97% mortality; and 3) Susceptible: mortality ≥98%. The results of susceptibility testing using the WHO and CDC methods were then compared with molecular tests.

3. Results and Discussion

3.1. DBD Situation in Semarang City and Research Locations

Semarang City is one of the dengue-endemic areas in Central Java Province, Indonesia. The incidence rate of dengue fever in Semarang City fluctuates. The highest recorded incidence rate was in 2010 at 368 / 100,000 population. Furthermore, it continued to decline until 2018 by 6 / 100,000 population, then increased again in 2019 by 26 / 100,000 population (Fig. 1).

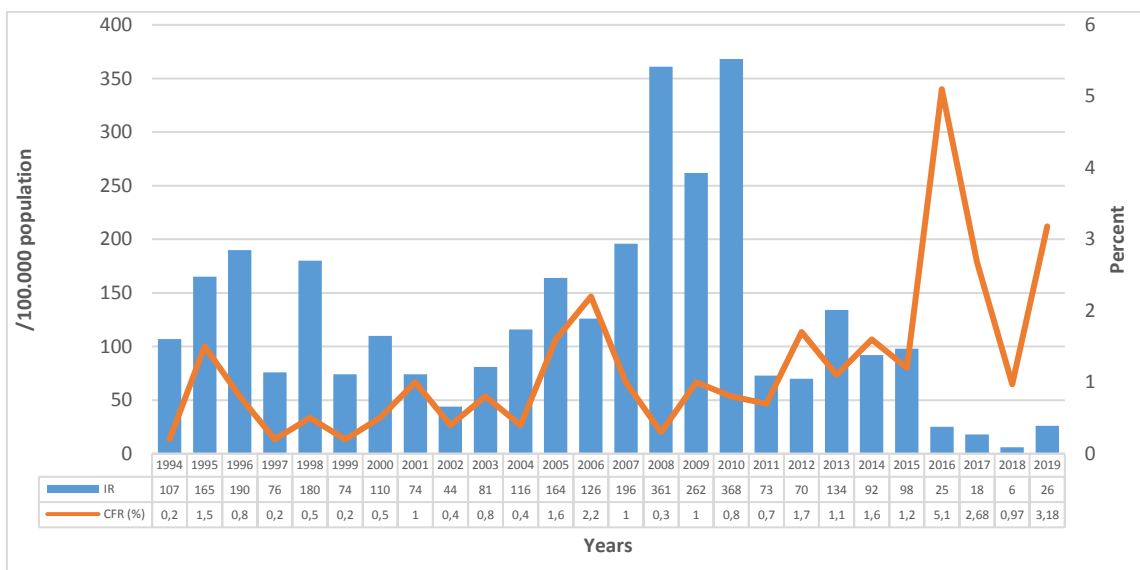


Fig. 1 Incidence rate and case fatality rate DHF in Semarang (1994-2019)

Based on the 2017 entomological survey, stegomyia indices in Semarang City show the numbers: House Index (HI) ranges from 6-44%, Container Index (CI) ranges from 4-26%, and Breteau Index (BI) ranges from 8-54% [13]. Based on Indonesia's Environmental Health Quality Standards for Vectors, the lowest free larvae index is 95%. This free larva index is the opposite of the house index, so the maximum house index allowed is 5% [14].

In the three study locations, namely Patemon, Terboyo Wetan, and Kandri, the DHF conditions showed variations. The incidence rate of the research location shows that in Patemon IR 2016-2019 was 38.5; 59.6; 0, and 18.43 (Table 1). Terboyo Wetan and Kandri Villages are not endemic areas with an incidence rate of 0, except in Kandri in 2019 IR of

0.8/100,000 population. Kandri and Patemon are in high areas with an altitude of > 250 MASL, and the lowest is a coastal area with an altitude of <10 MASL (Fig. 1).

Table 1 Incidence rate of resistance test larva sampling locations in Semarang city (2016-2019)

No.	Sub-District	Year			
		2016	2017	2018	2019
1	Patemon	38,5	59,65	0	18,43
2	Terboyo Wetan	0	0	0	0
3	Kandri	0	0	0	0,80



Fig. 2 Map of the location for catching larvae and pupae of resistance study in Semarang city in 2019

Table 2 Comparison of the resistance test of the CDC Bottle Bioassay method and the WHO Impregnated Paper

Location	% death (Malathion)		% death (Cypermethrin)		
	WHO-standard (after 24h)	CDC Bottle (after 30m)	WHO-standard (after 24h)	CDC Bottle	+ PBO
Patemon	91,7 ⁺⁺	98,3 ⁺⁺⁺	62,4 ⁺	90 ⁺⁺	96 ⁺⁺
Terboyo Wetan	86,7 ⁺	96,7 ⁺⁺	30 ⁺	55,0 ⁺	71 ⁺
Kandri	81,7 ⁺	98,3 ⁺⁺⁺	75,3 ⁺	84,7 ⁺	93,3 ⁺⁺

⁺Resistant
⁺⁺Tolerant
⁺⁺⁺Susceptible

3.2. WHO and CDC Method Insecticide Test Results

3.2.1. Aedes Aegypti Susceptibility Test to Insecticides in the Cypermethrin and Malathion Class

The susceptibility test using the WHO method against the insecticide cypermethrin in table 2 shows the percentage of deaths in 3 locations all have values below 90%. Based on WHO criteria, mortality below 90% means that mosquitoes are resistant to cypermethrin. Test using the CDC method is based on 30-minute observations of mosquitoes in all test locations that are resistant. After the addition of the PBO synergist, there was an increase in the percentage of deaths. The status increased to be tolerant in Patemon and, Kandri, while in Terboyo Wetan, it remained resistant.

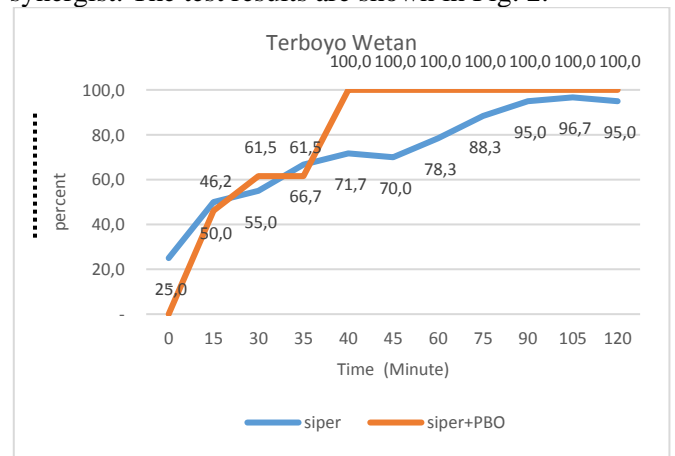
The susceptibility test using WHO against malathion in Patemon, Terboyo Wetan, and Kandri showed a mortality rate of 91.7%, 86.7%, and 81.7%. Using the CDC method, the mortality rate was 98.3%, 96.7%, and 93.3%. Because the numbers on the test with the CDC method indicate susceptible status, the test is not continued using the synergist DEF.

3.2.2. Resistance Mechanism Based on the CDC Bottle Bioassay Test Using a Synergist

A synergist is a chemical that functions to bind enzymes that play a role in detoxifying insecticides. The addition of a synergist in the resistance test using the CDC Bottle will provide information about enzymes that play a role in insecticide resistance. If

resistant mosquitoes are exposed to a synergist, there is an increase in the percentage of deaths or change to become susceptible; enzyme activity certainly plays a role in the incidence of resistance. Enzymes that play a role in detoxifying pyrethroid insecticides are Esterase, and the binding synergist is PBO. The enzyme that plays a role in detoxifying organophosphate insecticides is monooxygenase, and the binding synergist is DEF.

In this study, the test mosquitoes were resistant to cypermethrin and susceptible to malathion (Table 2). Based on the results of these tests, tests were carried out with the addition of PBO synergists in the resistance test to the insecticide cypermethrin. Because the tested mosquitoes were still susceptible to malathion, the test was not carried out using the DEF synergist. The test results are shown in Fig. 2.



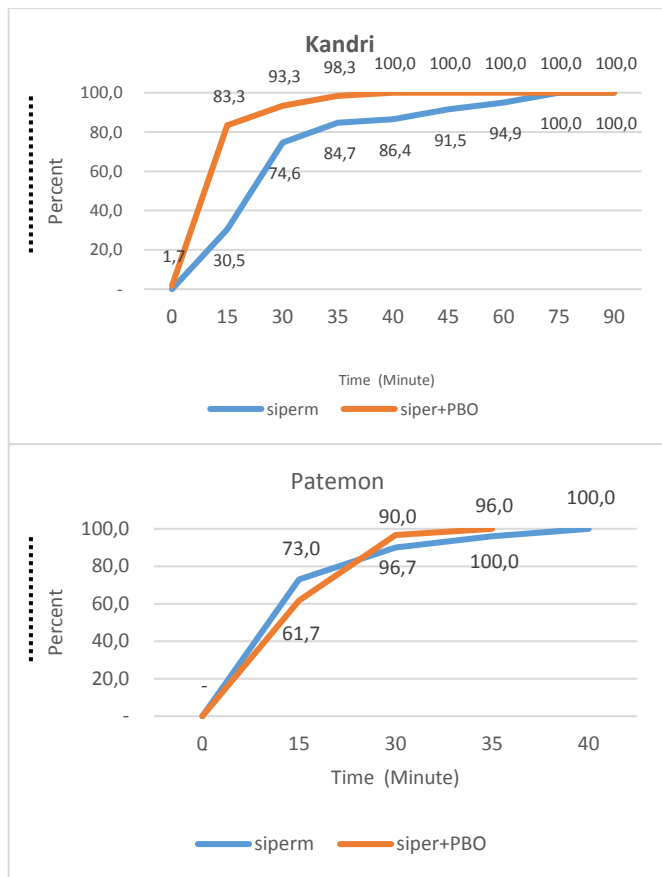


Fig. 2 Test results using synergist of PBO and insecticide cypermethrin against *Aedes aegypti* mosquitoes in Semarang City

Fig. 2 shows the occurrence of *Aedes aegypti* resistance mechanisms that occurred in three research locations. In the Patemon village, the cypermethrin and PBO test charts coincide, which means that the resistance that occurs is the mutation of the target site. In Kandri, the main resistance was a target-site mutation. Metabolic resistance was detected but was weak. In Terboyo Wetan, two mechanisms occur, namely mutation of the target-site and metabolic.

3.3. The Results of Resistance Testing Using the VGSC and ACE1 Gene Molecular Methods Were to Determine the Presence of Mutations in the Target Gene for Organophosphate Insecticides in *Aedes Aegypti*

The target site for Pyrethroid insecticides is the Voltage-gated Sodium Channel (VGSC) in insect nerve cells. Mutations in the VGSC gene will impact insecticide sensitivity, and cause mosquitoes to become resistant. The mutations associated with resistance to pyrethroids and organochlorines were most commonly reported in codons S989P and V1016G. In contrast to pyrethroids, Organophosphate and Carbamate insecticides have a target site on acetylcholinesterase, especially on the ACE1 gene in the nerve synapse of mosquitoes. Mutations in the ACE1 gene will reduce sensitivity to Organophosphate and Carbamate insecticides. The mutation associated with resistance is codon G119S. Molecular tests by looking at mutations in the target site genes can be used to confirm the target

site resistance mechanism. Molecular tests on 19 individual *Aedes aegypti* sequences from 3 research locations, showed mutations in codon S989P occurred in 11 samples (58%).

Location	Alel	Number	%
VGSC			
Alel S989P:	S	8	42,1
	SP	0	0
	P	11	57,9
Alel V1016G	V	1	5,3
	VG	3	15,8
	G	15	78,9
ACE 1			
Alel G119S	G	16	100
	GS	0	0
	S	0	0

Table 3 shows that mutations have occurred in the VGSC domain II gene, particularly in the S989P V1016G allele. In the S989P allele, mutations were found in 57.9% and wild type in 42.1%. In allele 1016, mutation was found in 78.9%, heterozygous was found in 15.8% and wild type was found in only 5.3%.

4. Discussion

4.1. The Use of Program and Household Insecticides and Their Problems in Controlling Dengue Vectors in Indonesia

Insecticides are a mainstay in the eradication of mosquitoes both by health programs and by the community. The chemical control program for DHF vector disease in Indonesia is carried out with the target of adult mosquitoes and larvae [15]. Insecticides used in Indonesia are from the organophosphate, carbamate and pyrethroid groups. In Indonesia, the insecticide malathion from the organophosphate group has been used since the beginning of chemical control. The insecticide DDT from the organochlorine class that has been used in malaria control has been banned in Indonesia since 1989. In Semarang City, chemical control of DHF vectors has been using malathion for decades. Since 1999 pyrethroid synthetic based insecticides have been used interchangeably with organophosphate active ingredients (Sayono, 2016). The pyrethroid synthetics used in the city of Semarang include permethrin, deltamethrin, λ -cyhalothrin, α -cypermethrin and d.d.Transifenotrin. Organophosphate compounds including malathion and temephos [16]. Exposure to insecticides at the study site originated from relatively low fogging activities by the Health Office. In Patemon Village, the last fogging was carried out in 2016 for four times, while in Terboyo Wetan, and Kandri in the last four years there has been no fogging. Household insecticide exposure is quite high. all mosquito coils and sprays use pyrethroid compounds. The use of this household insecticide is in

accordance with previous research reports. The use of household insecticides in Semarang City was reported in 2016 at 56.5% and in 2017 it was 64% [17], [18]. Household insecticides have pyrethroid synthetic active ingredients.

4.2. *Aedes aegypti* Resistance Test Method to Insecticides Used in Indonesia

Aedes aegypti mosquito resistance test to insecticides using the WHO standard method has been used in Indonesia for decades. This WHO method provides information about the status and level of resistance of mosquitoes in a population. Some of the weaknesses of this method are that it cannot provide information on the resistance mechanism that occurs and the type of insecticide tested is limited to the availability of impregnated paper [11]. Another problem faced by district-level health institutions is that they do not have the facilities and the ability to rearing the mosquitoes that are to be tested. Based on this, the CDC has developed a test method that can cover the various shortcomings of the WHO method.

Since 2019, Indonesia has included the CDC Bottle bioassay method for testing disease vector mosquito resistance in Indonesia [19]. The CDC Bottle Bioassay method in Indonesia is mostly used for testing *Anopheles* mosquito resistance in supporting the malaria elimination program. Testing of *A. aegypti* mosquito resistance as a DHF vector has never been carried out in a DHF control program. This study is the first report on vulnerability testing using the CDC Bottle Bioassay method by comparing with the WHO Impregnated paper method in Indonesia. One of the advantages of this CDC method is the use of a synergist. This synergist can provide an indication of the resistance mechanism without having to go through further tests, namely molecular tests or ELISA. Information about this resistance mechanism is very important in determining the selection of insecticides to be used, especially in populations that have been resistant to all insecticide classes. This information is also important for resistance surveillance.

Reports of *A. aegypti* resistance using the WHO method in the city of Semarang have been reported in several studies. Reports of Sayono, Putranto, Widiarti and Widiastuti show that *A. aegypti* in Semarang City is resistant to insecticides in the pyrethroid and organophosphate groups [7], [20], [21]. *Aedes aegypti* resistance to insecticides using the WHO method has also been reported to occur in other areas in Southeast Asia such as Malaysia, Thailand and Singapore [5], [6], [22].

4.3. The Results of *Aedes Aegypti* Resistance Test to Program Insecticides Using the WHO Method, the CDC Method and the Molecular Method in the City of Semarang

The results of this study indicate that using the WHO and CDC methods, *Aedes aegypti* in Semarang City is already resistant to cypermethrin. Tests using the WHO method showed *A. aegypti* in 3 study locations was resistant to cypermethrin, while using the CDC method in Terboyo Wetan and, Kandri it was resistant while in Patemon the status was tolerant. After the addition of the PBO synergy, there was an increase in the percentage of deaths but still unable to change the status to be vulnerable. The graph of the percentage of deaths (Fig. 2) shows that in the village of Kandri the resistance that occurred was due to the mutation of the target site, while in the poorest villages there was multiple mechanism resistance, but the role of the monooxygenase enzyme was weak. It is seen that the increase in mortality does not change the status to be susceptible at 30 minutes. This result is consistent with the molecular test for the VGSC gene. The VGSC domain II gene sequences showed that mutations were quite high in codon S989P (57.9%) and V1016G (78.9%).

The susceptibility test to malathion showed different resistance states. Tests using WHO standards showed *A. aegypti* in the Patemon village was tolerant (91.7%), while in the Terboyo Wetan (86.7%) and kandri (81.7%) villages were resistant. The CDC Test Method shows different results. Mosquitoes in Terboyo Wetan village are tolerant of malation (96.7%), while those in Patemon and Kandri villages are vulnerable (98.3%). The test against Malation was discontinued using the DEF synergist because mosquitoes were susceptible. The results of vulnerability testing in 3 regions showed the same pattern. The 3 research locations had various conditions. Kandri and Patemon are high areas with an altitude of 360 MASL, while Terboyo Wetan is a coastal area with an altitude of <10MASL. From the history of endemicity, Patemon is an endemic village, while Kandri and terboyo Wetan are non-endemic areas.

The status of resistance in Semarang City is in accordance with the presence of insecticide exposure that has occurred. The use of household insecticides is quite massive in Indonesia. The use of household insecticides was reported in Pangandaran at 82%, in the City of Salatiga at 72% and in Semarang at 93%. The active ingredients of household insecticides used were synthetic pyrethroids [23], [24], [25].

Several studies comparing the WHO and CDC methods in other countries using different species of mosquitoes have shown mixed results. Owusu (2015) reported on the susceptibility test of the *A. aegypti* mosquito ROCK strain using WHO and CDC. The susceptibility test to malathion, permethrin and DDT showed the same results, namely resistance. The susceptibility test to Lambda Cyhalotrin showed different results, where using the WHO method *A. aegypti* was susceptible, while using the CDC method

the status was resistant [26]. This difference in results is probably due to previous studies using colony *A. aegypti* mosquitoes from the laboratory, whereas this study used mosquitoes caught in the field.

Vatandoost [26] in Iran reported a similarity in the resistance status of *An. stephensii* mosquitoes to DDT, bendiocarb and deltamethrin using the WHO and CDC methods, but there were differences in LT50 results between the two methods. The susceptibility of *Anopheles Gambiae* mosquitoes using the WHO and CDC methods was 98.33% and 97.95%, respectively [26]. A susceptibility study by Fonseca-González [27] using *An. nuneztovary* mosquito against phenytotriol showed that using the WHO method was still susceptible, but using the CDC method the mortality rate was only 20%.

4.4. The Potential Use of the CDC Method to Increase the Effectiveness of Testing the Resistance of *Aedes Aegypti* to Insecticides in Indonesia

This study is the first report on the use of the CDC method in testing the resistance of DHF vectors to insecticide programs in Indonesia. With the adoption of the CDC method by the Indonesian government, this difference in susceptibility testing status has the potential to cause problems in resistance surveillance and the selection of insecticides used. This CDC method has good potential for use in vulnerability testing and resistance surveillance. In addition to information on resistance mechanisms based on the use of synergists, this method has advantages over the WHO method, namely: 1) the test time is only 120 minutes; 2) can use insecticides that are available in the market, rather than the WHO method which is limited by the available impregnated paper; 3) No requirement for test mosquito homogeneity, so that it can be done in the field; 4) The number of flexible test mosquitoes can be carried out for several days depending on the number of mosquitoes caught and the results are calculated cumulatively [19]. With these various advantages, this method can be carried out by health workers in remote areas without having to have a sophisticated insectarium.

This study has a weakness, namely it does not use standard Diagnostic dose (DD) and Diagnostic time (DT) using local strain mosquitoes in Indonesia. In this study, using DD and DT listed in the guidebook issued by WHO. In the guideline for resistance testing using the CDC Bottle Bioassay method, it is recommended to determine the DD and DT standards for each regional [19]. This study is part of the research on the effect of population genetics and the intensity of insecticide exposure on *Aedes aegypti* resistance in Semarang City, which has received Ethical approval. from the Ethic Health Research Commission, Faculty of Public Health, Diponegoro University with number: 169 / EA / KEPK-FKM / 2019.

The findings of this study provide input for the dengue vector resistance surveillance program. Based on theory, the CDC Bottle bioassay method should be used to complement the WHO method with its various advantages. The purpose of the WHO method is to determine the status of vector resistance to insecticides, while the CDC method has the aim of knowing the status and mechanism of resistance. Based on these objectives, the two methods should have similar results. The results of this study indicate that the simultaneous use of the CDC and WHO methods for resistance testing still requires more in-depth guidance. The differences in the resistance status of *Aedes aegypti* to malathion that occurred in this study will lead to unreliable data, which will lead to errors in the management of insecticide rotation based on class. Another weakness in using the CDC method is that some insecticide active ingredients are still not available with a standard diagnostic dose and diagnostic time. The future challenge in using the CDC method in Indonesia is determining the standard diagnostic dose and diagnostic time of insecticides on the market, using the local Indonesian strain *Aedes aegypti* mosquito.

5. Conclusion and Recommendation

The use of WHO, CDC and molecular methods in testing insecticide susceptibility to *Aedes aegypti* did not show differences in susceptibility status to cypermethrin, but there were differences in malathion. CDC Bottle bioassay has many advantages to be used for resistance testing, especially in the flexibility of use in the field and synergist use. With this difference in resistance status, further study is needed for the simultaneous application of the CDC and WHO methods in the surveillance system for *Aedes aegypti* resistance in Indonesia.

The prospect of using CDC will increase the sensitivity and effectiveness of testing which is expected to provide better recommendations than using the WHO method. The use of molecular data is needed to confirm the potential for permanent resistance to target gene mutations.

Acknowledgement

The authors would like to thank and give appreciation to the Director of IVRCRD, NIHRD-MoH Salatiga. We also thanks all staff of IVRCRD, NIHRD-MoH for all great support during this study. This study supported by IVRCRD, NIHRD-MoH in the fiscal year 2019.

References

- [1] HSAN K., HOSSAIN M. M., SARWAR M. S., WILDER-SMITH A., and GOZAL D. Unprecedented Rise in Dengue Outbreaks in Bangladesh. *The Lancet Infectious Diseases*, 2019, 19(12): 1287. [http://dx.doi.org/10.1016/S1473-3099\(19\)30616-4](http://dx.doi.org/10.1016/S1473-3099(19)30616-4)

- [2] SINGHI S. C. Severe Dengue: Developing a Universally Applicable Simple Prediction Model. *Indian Journal of Pediatrics*, 2018, 85(6): 413–414. <https://doi.org/10.1007/s12098-018-2668-2>
- [3] MAULA A. W., FUAD A., UTARINI A., and MAULA A. W. Ten-Years Trend of Dengue Research in Indonesia and South-East Asian Countries : A Bibliometric Analysis. *Global Health Action*, 2018, 11(1): 1–8. <https://doi.org/10.1080/16549716.2018.1504398>
- [4] SEMARANG CITY HEALTH OFFICE. *HEWS DBD Kota Semarang*, 2019. <https://indohef-award.com/innovation-detail/80>
- [5] SITI-FUTRI F. F., ROSILAWATI R., WAN K. L., CHEONG Y. L., NAZNI W. A., and LEE H. L. Status of Pyrethroid Resistance in *Aedes* (*Stegomyia*) *Aegypti* (Linnaeus) from Dengue Hotspots in Klang Valley, Malaysia. *Tropical Biomedicine*, 2020, 37(1): 201–209. <https://pubmed.ncbi.nlm.nih.gov/33612731/>
- [6] RUEDA L. M. *Pictorial Keys for the Identification of Mosquitoes (Diptera: Culicidae) Associated with Dengue Virus Transmission. 1st ed.* Magnolia Press, Auckland, New Zealand, 2004. <http://biotaxa.org/Zootaxa/article/view/zootaxa.589.1.1>
- [7] SAYONO S., PUSPA A., HIDAYATI N., FAHRI S., SUMANTO D., DHARMANA E., HADISAPUTRO S., ASIH P. B. S., and SYAFRUDDIN D. Distribution of Voltage-Gated Sodium Channel (Na_v) Alleles among the *Aedes Aegypti* Populations In Central Java Province and Its Association with Resistance to Pyrethroid Insecticides. *Public Library of Science One*, 2016: 1–12. <http://dx.doi.org/10.1371/journal.pone.0150577>
- [8] 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., MAYANG O., and DONNELLY M. J. Metabolic and Target-Site Mechanisms Combine to Confer Strong DDT Resistance in *Anopheles Gambiae*. *Public Library of Science One*, 2014, 9(3): 1–10. <https://doi.org/10.1371/journal.pone.0092662>
- [9] ELYAZAR I. R. F., HAY S. I., and BAIRD J. K. Malaria Distribution, Prevalence, Drug Resistance and Control in Indonesia. *Advances in Parasitology*, 2011, 74: 41–175. <https://doi.org/10.1016/b978-0-12-385897-9.00002-1>
- [10] SPARKS T. C., CROSTHWAITTE A. J., NAUEN R., BANBA S., CORDOVA D., EARLEY F., EBBINGHAUS-KINTSCHER U., FUJIOKA S., HIRAO A., KARMON D., KENNEDY R., NAKAO T., POPHAM H. J. R., SALGADO V., WATSON G. B., WEDEL B. J., and WESSELS F. J. Insecticides, Biologics and Nematicides : Updates to IRAC's Mode of Action Classification - a Tool for Resistance Management. *Pesticide Biochemistry and Physiology*, 2020, 167: 1–10. <https://doi.org/10.1016/j.pestbp.2020.104587>
- [11] AIZOUN N., OSSE R., AZONDEKON R., ALIA R., OUSSOU O., GNANGUENON V., AIKPON R., PADONOU G. G., and AKOGBÉTO M. Comparison of the Standard WHO Susceptibility Tests and the CDC Bottle Bioassay for the Determination of Insecticide Susceptibility in Malaria Vectors and their Correlation with Biochemical and Molecular Biology Assays in Benin, West Africa. *Parasites and Vectors*, 2013, 6(1): 1–10. <https://doi.org/10.1186/1756-3305-6-147>
- [12] MINISTRY OF HEALTH OF INDONESIA. *Guidelines for Monitoring Vector Resistance to Insecticides*. Ministry of health of Indonesia, Jakarta, Indonesia, 2018.
- [13] BALAI BESAR LITBANG VEKTOR DAN RESERVOIR PENYAKIT. *Final Report of Gerdu Semarang 2017*, 2017. <http://www.b2p2vrp.litbang.kemkes.go.id/>
- [14] MINISTRY OF HEALTH OF INDONESIA. Environmental health quality standards and health requirements for vector and reservoir of diseases. Jakarta: 2017.
- [15] MINISTRY OF HEALTH OF INDONESIA. *Guidelines for the Use of Insecticides (Pesticides) in Vector Control*. Ministry of health of Indonesia, Jakarta, Indonesia, 2012.
- [16] SEMARANG CITY HEALTH OFFICE. *Disease Prevention and Control Program Reports*. Semarang City Health Office, Semarang, Indonesia, 2017.
- [17] SAYONO S., PUSPA A., HIDAYATI N., FAHRI S., SUMANTO D., DHARMANA E., HADISAPUTRO S., ASIH P. B. S., and SYAFRUDDIN D. Distribution of Voltage-Gated Sodium Channel (Na_v) Alleles among the *Aedes Aegypti* Populations In Central Java Province and Its Association with Resistance to Pyrethroid Insecticides. *Public Library of Science One*, 2016: 1–12. <http://dx.doi.org/10.1371/journal.pone.0150577>
- [18] SETIYANINGSIH R., WIDIARTI W., and LASMIATI L. Efikasi Larvasida Temephos Terhadap *Aedes Aegypti* Resisten Pada Berbagai Kontainer. *Vektora*, 2015, 7(1): 23–28. <https://media.neliti.com/media/publications/126816-ID-none.pdf>
- [19] BROGDON W. G., & ADELIN C. *Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay*. US-CDC Atlanta, Atlanta, USA, 2018.
- [20] WIDIASTUTI D., SUNARYO, PRAMESTUTI N., and MARTINI. Aktivitas Enzim Monooksigenase Pada Populasi Nyamuk *Aedes Aegypti* di Kecamatan Tembalang, Kota Semarang. *Aspirator*, 2015, 7(1): 1–6. <http://dx.doi.org/10.22435/aspikator.v7i1.3696.1-6>
- [21] MARTINI, HESTININGSIH R., WIDJANARKO B., and PURWANTISARI S. Resistance of *Aedes* as a Vectors Potential for Dengue Hemorrhagic Fever (DHF) in Semarang City, Indonesia. *Journal of Tropical Life Science*, 2019, 9(1): 89–94. <http://dx.doi.org/10.11594/jtls.09.01.12>
- [22] SATHANTRIPHOP S., PAEPORN P., YA-UMPHAN P., MUKKHUN P., THANISPONG K., CHANSANG C., BANGS M. J., CHAREONVIRIYAPHAP T., and TAINCHUM K. Behavioral Action of Deltamethrin and Cypermethrin in Pyrethroid-Resistant *Aedes Aegypti* (Diptera: Culicidae): Implications for Control Strategies in Thailand. *Journal of Medical Entomology*, 2020;57(4):1157–67. <https://doi.org/10.1093/jme/tjaa019>
- [23] SUKANINGTYAS R., UDIJONO A., and MARTINI M. Praktik Penggunaan Insektisida Rumah Tangga di Are Buffer Pelabuhan Tanjung Emas Wilayah Kerja Kantor Kesehatan Pelabuhan Kelas II Semarang. *Jurnal Kesehatan Masyarakat*, 2020, 8(6): 746–751. <https://ejournal3.undip.ac.id/index.php/jkm/article/view/28194>
- [24] WIGATI R. A., & SUSANTI L. Hubungan Karakteristik Pengetahuan, Sikap dan Perilaku Masyarakat dalam Menggunakan Antinyamuk di Kelurahan Kutowinangun. *Bul Penelit Kesehat*, 2012, 40(3): 130–141. <http://ejournal.litbang.kemkes.go.id/index.php/BPK/article/vi>

ew/2895

[25] KUSUMASTUTI N. H. Use of House Insecticide in Pangandaran Village. *Widyariset*, 2014, 3: 417–424. <http://dx.doi.org/10.14203/widyariset.17.3.2014.417-424>

[26] OWUSU H. F., JANČÁRYOVÁ D., MALONE D., and MÜLLER P. Comparability between Insecticide Resistance Bioassays for Mosquito Vectors: Time to Review Current Methodology? *Parasites and Vectors*, 2015, 8(1): 1–11. <https://doi.org/10.1186/s13071-015-0971-6>

[27] FONSECA-GONZÁLEZ I., CÁRDENAS R., QUIÑONES M. L., MCALLISTER J., and BROGDON W. G. Pyrethroid and Organophosphates Resistance in Anopheles (N.) Nuneztovari Gabaldón Populations from Malaria Endemic Areas in Colombia. *Parasitology Research*, 2009, 105(5). <https://doi.org/10.1007/s00436-009-1570-2>

参考文献:

[1] HSAN K., HOSSAIN M. M., SARWAR M. S., WILDER-SMITH A., 和 GOZAL D. 孟加拉国登革热疫情史无前例地增加。柳叶刀传染病, 2019, 19(12): 1287. [http://dx.doi.org/10.1016/S1473-3099\(19\)30616-4](http://dx.doi.org/10.1016/S1473-3099(19)30616-4)

[2] SINGHI S. C. 严重登革热：开发普遍适用的简单预测模型。印度儿科学杂志, 2018, 85(6): 413–414. <https://doi.org/10.1007/s12098-018-2668-2>

[3] MAULA A. W., FUAD A., UTARINI A., 和 MAULA A. W. 印度尼西亚和东南亚国家登革热研究的十年趋势：文献计量分析。全球卫生行动, 2018, 11(1): 1–8. <https://doi.org/10.1080/16549716.2018.1504398>

[4] 三宝壟市卫生局. 三宝壟市登革出血热健康预警系统, 2019. <https://indohcf-award.com/innovation-detail/80>

[5] SITI-FUTRI F. F., ROSILAWATI R., WAN K. L., CHEONG Y. L., NAZNI W. A., 和 LEE H. L. 来自马来西亚巴生谷登革热热点的埃及伊蚊（剑鞘）的拟除虫菊酯抗性现状。热带生物医学, 2020, 37(1): 201–209. <https://pubmed.ncbi.nlm.nih.gov/33612731/>

[6] RUEDA L. M. 用于识别与登革热病毒传播相关的蚊子（双翅目：蚊科）的图解键。第一版。玉兰出版社，奥克兰，新西兰，2004.

<http://biotaxa.org/Zootaxa/article/view/zootaxa.589.1.1>

[7] SAYONO S., PUSPA A., HIDAYATI N., FAHRI S., SUMANTO D., DHARMANA E., HADISAPUTRO S., ASIH P. B. S., 和 SYAFRUDDIN D. 中爪哇省埃及伊蚊种群中电压门控钠通道等位基因的分布及其与拟除虫菊酯类杀虫剂抗性的关系。公共科学图书馆一, 2016: 1–12. <http://dx.doi.org/10.1371/journal.pone.0150577>

[8] 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., 和 DONNELLY M. J. 代谢和目标位点机制相结合，赋予冈比亚按蚊强大的二氯-二苯基-三氯乙烷抗性。公共科学图书馆一, 2014, 9(3): 1–10. <https://doi.org/10.1371/journal.pone.0092662>

[9] ELYAZAR I. R. F., HAY S. I., 和 BAIRD J. K.

印度尼西亚的疟疾分布、流行、耐药性和控制。寄生虫学进展, 2011, 74: 41–175. <https://doi.org/10.1016/b978-0-12-385897-9.00002-1>

[10] SPARKS T. C., CROSSTHWAITE A. J., NAUEN R., BANBA S., CORDOVA D., EARLEY F., EBBINGHAUS-KINTSCHER U., FUJIOKA S., HIRAO A., KARMON D., KENNEDY R., NAKAO T., POPHAM H. J. R., SALGADO V., WATSON G. B., WEDEL B. J., 和 WESSELS F. J. 杀虫剂、生物制剂和杀线虫剂：更新问题、规则和结论 - 抗性管理工具。农药生物化学与生理学, 2020, 167: 1–10. <https://doi.org/10.1016/j.pestbp.2020.104587>

[11] AIZOUN N., OSSE R., AZONDEKON R., ALIA R., OUSSOU O., GNANGUENON V., AIKPON R., PADONOU G. G., 和 AKOGBÉTO M. 世界卫生组织标准药敏试验和疾病控制瓶生物测定中心测定疟疾载体中杀虫剂药敏性的比较，以及它们与西非贝宁的生化 and 分子生物学测定的相关性。寄生虫和载体, 2013, 6(1): 1–10. <https://doi.org/10.1186/1756-3305-6-147>

[12] 印度尼西亚卫生部. 监测病媒对杀虫剂抗性的指南。印度尼西亚卫生部，印度尼西亚雅加达, 2018.

[13] 研发载体和疾病库中心. 格都三宝壟 2017 最终报告, 2017. <http://www.b2p2vrp.litbang.kemkes.go.id/>

[14] 印度尼西亚卫生部. 病媒和环境的质量标准和卫生要求。雅加达: 2017.

[15] 印度尼西亚卫生部. 在病媒控制中使用杀虫剂（农药）的指南。印度尼西亚卫生部，印度尼西亚雅加达, 2012.

[16] 三宝壟市卫生局. 疾病预防和控制计划报告。三宝壟市卫生办公室，三宝壟，印度尼西亚, 2017.

[17] SAYONO S., PUSPA A., HIDAYATI N., FAHRI S., SUMANTO D., DHARMANA E., HADISAPUTRO S., ASIH P. B. S., 和 SYAFRUDDIN D. 中爪哇省埃及伊蚊种群中电压门控钠通道等位基因的分布及其与拟除虫菊酯类杀虫剂抗性的关系。公共科学图书馆一, 2016: 1–12. <http://dx.doi.org/10.1371/journal.pone.0150577>

[18] SETIYANINGSIH R., WIDIARTI W., 和 LASMIATI L. 泰美福斯杀幼虫剂在各种容器中对埃及伊蚊的效果。向量, 2015, 7(1): 23–28. <https://media.neliti.com/media/publications/126816-ID-none.pdf>

[19] BROGDON W. G., 和 ADELIN C. 使用疾病控制瓶生物测定中心评估载体中杀虫剂抗性的指南。美国疾病预防控制中心亚特兰大，亚特兰大，美国, 2018.

[20] WIDIASTUTI D., SUNARYO, PRAMESTUTI N., 和 MARTINI. 三宝壟市丹巴朗区埃及伊蚊种群的单加氧酶活性。吸气器, 2015, 7(1): 1–6. <http://dx.doi.org/10.22435/aspirator.v7i1.3696.1-6>

[21] MARTINI, HESTININGSIH R., WIDJANARKO B., 和 PURWANTISARI S. 在印度尼西亚三宝壟市，抗伊蚊作为登革出血热的潜在

载体。热带生命科学杂志, 2019, 9(1): 89-94.
<http://dx.doi.org/10.11594/jtls.09.01.12>

[22] SATHANTRIPHOP S., PAEPORN P., YA-UMPHAN P., MUKKHUN P., THANISPONG K., CHANSANG C., BANGS M. J., CHAREONVIRIYAPHAP T., 和 TAINCHUM K.

溴氰菊酯和氯氰菊酯对拟除虫菊酯抗性埃及伊蚊（双翅目：蚊科）的行为作用：对泰国控制策略的影响。医学昆虫学杂志, 2020;57(4):1157-67.

<https://doi.org/10.1093/jme/tjaa019>

[23] SUKANINGTYAS R, UDIJONO A, 和 MARTINI M. 在三宝壟港卫生办公室第二类丹戎艾玛斯港缓冲区工作区使用家用杀虫剂的做法。公共卫生杂志, 2020, 8(6): 746-751.

<https://ejournal3.undip.ac.id/index.php/jkm/article/view/28194>

[24] WIGATI R. A., 和 SUSANTI L. 库托维南贡村社区使用驱蚊剂的知识、态度和行为的关系特征。健康研究员公牛, 2012, 40(3): 130-141.

<http://ejournal.litbang.kemkes.go.id/index.php/BPK/article/view/2895>

[25] KUSUMASTUTI N. H. 潘甘达兰村使用房屋杀虫剂。研究员, 2014, 3: 417-424.

<http://dx.doi.org/10.14203/widyariset.17.3.2014.417-424>

[26] OWUSU H. F., JANČÁRYOVÁ D., MALONE D., 和 MÜLLER P.

蚊子载体杀虫剂抗性生物测定之间的可比性：是时候回顾当前的方法了吗？寄生虫和载体, 2015, 8(1): 1-11.

<https://doi.org/10.1186/s13071-015-0971-6>

[27] FONSECA-GONZÁLEZ I., CÁRDENAS R., QUIÑONES M. L., MCALLISTER J., 和 BROGDON W. G.

来自哥伦比亚疟疾流行地区的按蚊努涅斯托瓦里·加巴尔东种群的拟除虫菊酯和有机磷抗性。寄生虫学研究, 2009, 105(5).

<https://doi.org/10.1007/s00436-009-1570-2>