

Research Article

Resistance of *Aedes* as a Vectors Potential for Dengue Hemorrhagic Fever (DHF) in Semarang City, Indonesia

Martini ^{1*}, Retno Hestiningih ¹, Bagoes Widjanarko ¹, Susiana Purwantisari ²

¹ Faculty of Public Health, Diponegoro University, Semarang 50275, Indonesia

² Faculty of Mathematics and Natural Sciences, Diponegoro University, Semarang 50275, Indonesia

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**Corresponding author:*

E-mail: tinihen65@yahoo.co.id

ABSTRACT

Dengue Hemorrhagic Fever (DHF) is a significant health problem in Semarang, Indonesia. A Certain type of insecticides has been widely applied beginning in the last decade as an effort to control the mosquito vectors of dengue virus. This practice could lead to increased resistance in mosquito populations toward these types of insecticides. This research aimed to describe the resistance status of *Aedes* sp. populations in Semarang. A cross-sectional design study was conducted in 2015, with *Aedes* sp. populations sampled in several villages of Semarang. Field strains of *Aedes* sp. eggs were collected using ovitraps and larvae from entomological surveys. Mosquito adults were raised from the collected eggs and larvae under standard conditions and used for biochemical assays. *ELISA* examined *Aedes* sp. resistance status. The results showed that there was monooxygenase activity in *Aedes* sp. populations. In quantitative terms, 77.8% of mosquito samples showed an Optical Density (OD) more than the cut-off point (0.165). Resistance to synthetic pyrethroid insecticide in *Aedes* sp. mosquitoes population in Semarang may be caused by the mechanism of monooxygenase detoxification enzymes in particular. This case indicated that *Aedes* sp. has a high capacity as a vector to transmit the dengue virus in Semarang.

Keywords: *Aedes* sp., vector capacity, dengue, Dengue Hemorrhagic Fever (DHF)

Introduction

In 2010, Indonesia had the highest number of dengue fever cases among the ASEAN countries, with a total of 156,086 cases, with 1,358 deaths due to this disease. In 2011, the number of cases decreased to 49,486, with 403 deaths; yet, in 2012, it increased to 65,725 cases with an incidence rate (IR) of 27.67 (cases/100,000 persons), higher than the national target IR of 20.0. The Intergovernmental Panel on Climate Change (IPCC) has predicted that shortly, dengue fever incidence should increase [1].

Dengue fever occurrences in the Central Java Province of Indonesia increased during 2011 – 2013. In 2011, the IR was 15.26 (cases/100,000), with 46 deaths or 4.948 dengue fever occurrences causing death to 46 people (CFR = 0.76%). In

2013, there was an IR of 45.53, and 15,144 dengue fever occurrences causing death to 183 people (CFR = 1.21%) [2].

The dengue fever IR of Semarang is the highest in Central Java. Semarang's dengue fever IR from 2006 – 2013 was higher than the IR in Central Java and nationwide. In 2013, for instance, Semarang's IR was two times higher than Central Java, recorded as 2,364 occurrences. Compared to the previous year, there was an 89.11% increase from the 1,250 occurrences in 2012. Based on the area of occurrences, the dengue fever IR of the Tembalang Sub-district which was recorded as 218.20, which ranked the highest in Semarang after being in the 3rd position in 2012. In 2014, Tembalang Sub-district also had the most top IR at 110.55 [3]. The increase and spread of dengue oc-

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currences were likely caused by the high mobility of the population, urban development, climate change, changes in the density and distribution of population and other epidemiological factors that still require further research.

In 2014, the dengue fever IR Incidence was 92.43, or 31.13% lower than the previous year, and CFR was 1.66%. There were no DBD occurrences recorded in 17 or 9.6% of the sub-districts in 2014. The dengue fever IR of Tembalang sub-district, 166.89, was the highest in Semarang [3].

There seem to be no differences in the proportion of dengue fever patients based on sex. For instance, in 2014, the number of dengue fever patients was 819 male patients or 50.31% and 809 female patients (49.69%). Toddlers and youngsters are the vulnerable populations to dengue fever. The most occurrences were observed in children 5 – 9 years old, 436 occurrences or 27% of the total, and the fewest occurrences were observed in persons > 60 years old (0.3%) [3].

Semarang, the capital of Central Java province, has varied topography such as hills, plains, and coastal areas, with land slope ranging between 0 – 40% (steep) and is positioned between 0.75 to 348.00 meters above sea level. It has a total area of 373.67 km² and comprises 1.15% of the total land area of Central Java province. Semarang has 16 sub-districts and 177 villages. Mijen sub-district (57.55 km²) and Gunungpati sub-district (54.11 km²) are the two sub-districts with the largest areas and are mostly covered with rice fields and plantations.

Semarang City has a relatively high average air temperature, with the average relative humidity of about 75% [3]. Rainfall distribution throughout the year was with a total average of 9,891 mm per year. Average temperature measured by Climatology Station ranges from 29.9°C to 32.9°C. Average relative humidity by month was 61% at the minimum and 83% at the maximum [3]. High rainfall amounts affect the availability of water for *Aedes sp.* larvae survival, and thus the numbers of mosquitoes available as potential disease vectors transmitting dengue virus from sick individuals to healthy [4].

The success of controlling the dengue fever mosquito vector control depends on its susceptibility to insecticides [5]. Long-term exposure to insecticides may cause lower sensitivity (higher resistance) of vectors to insecticide [6]. Marcombe

(2012) stated that there are several factors causing resistance such as practices in planting, vector control interventions, and urbanization [7]. Resistance to malathion has been reported by 1980 in *Anopheles* [8], *Culex* [9], and *Aedes* [10, 11]. The resistance of *Aedes aegypti* to DDT was first observed in Puerto Rico in 1960 [12], followed reports of resistance occurrences to 24 other kinds of insecticides in locations worldwide. *Aedes albopictus* has been reported to be resistant to six kinds of insecticides. Other reports also support that *Ae. aegypti* populations have become resistant to cypermethrin in Brazil [13, 14]. In Indonesia, *Ae. aegypti*'s resistance to pyrethroid insecticides, namely deltamethrin and permethrin, has been reported in Bandung, Palembang and Surabaya [15], and also Semarang.

This research focused on tests for biochemical resistance, i.e., the activity of monooxygenase enzymes, which has been reported to be related to the mechanism causing the resistance of mosquitos to pyrethroid insecticides. Increasing the activities of monooxygenase enzymes in resistant insects is thought to allow them to reduce lethal dosages to sub-lethal dosage levels. Compared to a bioassay method, biochemical resistance test results are obtained faster, enabling earlier detection of resistance and determination of the possibility of cross-reactions with other insecticides [16].

Material and Methods

The research was conducted in 2015 in all administrative villages in Semarang. Mosquito identification to species was confirmed in Laboratory of Entomology Faculty of Public Health Diponegoro University and resistance tests were conducted in Laboratory of Research and Development of Vectoral Disease Banjarnegara District, Indonesia.

The present study was observational research supported by the cross-sectional survey approach. This study was preceded by conducting spot surveys in the study area. The variables measured were vector density, vectors species, resistance status based on the biochemical test, and distribution of resistance status based on endemicity areas in the villages in Semarang. The samples of the research were mosquitoes captured through their eggs, caught by using ovitraps. Ovitrap were placed in 15–20 houses in each village in Semarang, indoor and outdoor the houses. Ovitrap used

this study to collect the mosquitoes' eggs. Ovitrap was made from a plastic bottle and used an attractant. The solution of attractant was hay infusion with a concentration of 10%. Besides that, mosquitoes were also trapped by larvae surveys similarly the ovitrap method. Vector density was measured through the ovitraps, then tested biochemically for the resistance level of the vectors to pyrethroid. The mosquitoes observed in the resistance test were the eggs obtained from the ovitrap survey and larval survey, which were then reared to mosquitoes. The vector resistance status was tested using biochemical assays.

The procedure of biochemical assay referred to the research before [17]. Mosquito samples were homogenized individually in 200 phosphate buffers (pH 7.4). As many as 20 μL aliquots of the homogenate of each mosquito were included in the well microplate, then added 80 phosphate buffer. In each well the 200 μL substrate TMB and 25 H_2O_2 3% solutions were added, then incubated for 2 hours at room temperature. The response color produced at each well was measured using the microassay reader (a wavelength of 630 nm). Cut off point the Optical Density (OD) to determine the resistant mosquitoes status was > 0.165 . The data obtained from the laboratory test were analyzed with descriptive statistics.

Results and Discussion

The survey was conducted in 43 villages in Semarang which consist of endemic areas (33 villages), sporadic areas (9 villages), and potential areas (1 village). In each village, 10 – 15 houses were observed. When the research was conducted it was seen that high density of vectors was observed based on entomological indicators exceeded the standards for ABJ, House Index (HI), Container Index (CI) and Breteau Index (BI). The larva-free index was recorded low at 30 – 50.6% or HI 49.4 – 70% (Table 1).

Vector density was related to the available breeding media in the research locations. During a larval survey in 43 samples of villages, in endemic, sporadic, and potential areas, a clear description of *Aedes sp.* vector breeding media. The highest number of larva were found in standing water in outdoor bathtubs; besides this, loggerheads (a place to store water) and buckets were the most common containers where larva was found. Mosquitoes can also breed in media that are not

Table 1. Vector density based on entomological indicators in Semarang

Area of endemicity	ABJ (%)	HI (%)	CI (%)	BI (100 houses)
Endemic	45.8	54.2	24.8	76.9
Sporadic	50.6	49.4	23.3	59.5
Potential	30.0	70.0	29.1	70.0

used to store water for drinking or other uses, such as coconut shells, cans, wastebaskets, old tires, flower vases, bird baths, dispensers, refrigerators, and unmaintained pools.

The results confirm that vector density in Semarang is very high, in accord with other studies measuring vector density in Semarang. Mosquito density was measured by various indices like ABJ or HI, CI, and BI, measured as 30 – 50.6%, 49.4 – 71.1%, 23.3 – 29.2%, and 59.5 – 76.9 (per 100 houses), respectively. Dengue virus vector control has been used, yet the result is not satisfactory since it has not been implemented optimally by the community. The community has to be aware that it is community-based prevention, meaning that the control is conducted by the community for the community itself to control health problems in the environment. Since community awareness is considered low, some approaches need to be done creatively.

Pre-adult *Aedes sp.* obtained from rearing the larva sampled during the larval survey and ovitraps were used in resistance test. Adult mosquitoes used in the resistance test were 278 mosquitoes and *Ae. aegypti* has the most number (96.8%). Samples of adult mosquitoes obtained from pre-adult egg or larval stages represented positive containers in each target village. The number of *Ae. albopictus* obtained was very limited because of the dominance of *Ae. aegypti* in the ovitrap survey and larval survey results. From 278 mosquitoes tested, 77.8% were resistant to pyrethroid and 22.2% were susceptible. *Ae. aegypti* resistance occurs in both female and male genders, while *Ae. albopictus* was more resistant in the group of female mosquitoes (100%) than in the group of males (Table 2). If compared based on the percent resistance, more *Ae. albopictus* are resistant compared to *Ae. aegypti*. The OD was used to determine the resistant mosquitoes status. *Ae. aegypti* called resistance if OD showed more than 0.165 based on the biochemical test. This study

Table 2. *Aedes sp.* resistance status to pyrethroid-based on biochemical test

Species		Susceptible		Resistant		Total	%
		f	%	f	%		
<i>Ae. aegypti</i>	Male	35	26.1	99	73.9	134	48.7
	Female	27	20.3	106	79.7	133	48.4
<i>Ae. albopictus</i>	Male	0	0.0	6	100.0	6	2.2
	Female	1	50.0	1	50.0	2	0.7

just used the biochemical analysis for pyrethroid insecticide. We have not yet tested toward organophosphat insecticide, and also WHO diagnostic by using impregnated paper.

Attempts to the eradication of mosquito larvae by removing sources of standing water are more important than chemical control. Extensive usage of insecticides results in vector resistance and making it more challenging to eradicate dengue fever. Although others have replaced the use of ineffective insecticide, resistant mosquito populations remain, however. According to others research extensive and, long-term insecticide usage toward targeted species might result in the selection of populations capable of developing resistance faster. In other words, susceptible insects will become less prevalent than resistant ones [18, 19]. This will result in the insect population which is dominated by insects that are resistant to applied insecticides.

There were many studies affirming the resistance of some species. Shinta *et al.* (2008) reported that the population of *Ae. aegypti* in five areas of Greater Jakarta and Bogor are resistant to 0.8% malathion [20]. Studies in Mampang Prapatan and Tanjung Priok also report on resistant mosquitos [21]. Resistance to malathion was also found in Purbalingga, Kendal, Grobogan, and Semarang [3]. This study also affirms that 77.8% of 278 *Aedes sp.* sampled were resistant. Resistance tests on dengue virus mosquito vectors should be done before the application of certain insecticides.

Resistant mosquitos tested by the biochemical test can be identified from monooxygenase enzymes. An increase of monooxygenase enzyme activity can be an indicator that the mosquito is resistant to insecticides especially pyrethroids. Resistance occurs because of the enzyme activity that blocks insecticide from reaching the target site.

Monooxygenases are some of the enzymes involved in the metabolism of xenobiotic (toxic compounds) in insects, which might confer insect resistance to insecticides. The impact of monooxygenase enzymes in insect resistance to synthetic pyrethroid compounds has been studied in various species of insect vectors, such as *Anopheles gambiae*, which is resistant to permethrin and deltamethrin in Africa, and *Ae. aegypti*, which is resistant to permethrin and deltamethrin in Mexico and Thailand [22].

Ae. aegypti resistance to lambda-cyhalothrin 0.05%, and 0.05% permethrin was reported in 2010 [5] and to permethrin, 0.25% was reported in 2013 in Semarang [3]. If the insects have proven to be resistant, it is necessary to use an insecticide rotation. The use of the same type of insecticides on an ongoing basis will not effectively control the resistance of mosquito populations. Substitution for insecticides types and methods for vector control should be carried out within a maximum of 2 – 3 years or 4 – 6 application times [15].

Monooxygenase enzyme is also known as cytochrome P450. The enzyme has an important role in metabolism, especially of endogenous substances like hormones, fatty acids, and steroids. Besides that, the enzyme also has a role in catabolic and anabolic reactions of poisonous substances (xenobiotic) like pesticides and other poisons in plants. Monooxygenase enzymes can perform the detoxification process of insecticides of pyrethroid types and are also involved in bioactivation reactions and insecticide detoxification from organophosphate types [23]. The increased activity of the enzyme in resistant *Aedes sp.* is probably caused by exposure to synthetic pyrethroid insecticide groups either used by the program (Semarang Department of Health) or community.

Semarang had high larval density based on House Index (HI) = 49.4 – 71.1%, Container Index

=23.3 – 29.1%, and Breteau Index = 59.5 – 76.9. The index means that Semarang has a high risk of transmission of the dengue virus. *Ae. albopictus* is commonly found in Semarang, especially in areas used for farming. The resistance of *Aedes sp.* to pyrethroid was 77.8% from 278 mosquitos tested biochemically, based on monooxygenase enzyme observation. *Ae. aegypti* and *Ae. albopictus* have proven resistant to pyrethroid. Dengue fever prevention education for the community is still needed, especially during the transmission season or at the beginning of the rainy season, with emphasis on bionomic vector aspects, not only *Ae. aegypti* but also *Ae. albopictus*.

Conclusion

Semarang had high larval density based on House Index (HI) = 49.4 – 71.1%, Container Index = 23.3 – 29.1%, and Breteau Index = 59.5 - 76.9. The index means that Semarang has a high risk of transmission of dengue virus. *Ae. albopictus* is commonly found in Semarang, especially in areas used for farming. The resistance of *Aedes sp.* to pyrethroid was 77.8% from 278 mosquitos tested biochemically, based on monooxygenase enzyme observation. *Ae. aegypti* and *Ae. albopictus* have proven resistant to pyrethroid. Dengue fever prevention education for the community is still needed, especially during the transmission season or at the beginning of the rainy season, with emphasis on bionomic vector aspects, not only *Ae. aegypti* but also *Ae. albopictus*.

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