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Temephos resistance in field *Aedes (Stegomyia) albopictus* (Skuse) from Selangor, Malaysia

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Abstract. Larvae of *Aedes albopictus* obtained from dengue endemic areas in Selangor, Malaysia were evaluated for their susceptibility to operational dosage of temephos (1 mg/L). Larval bioassays were carried out in accordance to modified WHO standard methods. Biochemical microassay of enzymes in *Ae. albopictus* was conducted to detect the emergence of insecticide resistance and to define the mechanisms involved in temephos resistance. The 50% mortality lethal time (LT_{50}) for *Ae. albopictus* tested against temephos ranged between 58.65 to 112.50 minutes, with resistance ratio ranging from 0.75 – 1.45. This study addressed the fluctuation of time-related susceptibility status of *Ae. albopictus* towards insecticide. Significant difference on the weekly enzyme levels of non-specific esterases, mixed function oxidases and glutathione S-transferases was detected ($p \leq 0.05$). No significant correlation was found between temephos resistance and enzyme activity ($p > 0.05$). Only glutathione S-transferases displayed high level of activity, indicating that *Ae. albopictus* may be resistant to other groups of insecticide. The insensitive acetylcholinesterase was detected in some field collected *Ae. albopictus* populations, indicating the possibility of emergence of carbamate or other organophosphate resistance in the field populations. Continuous resistance monitoring should be conducted regularly to confirm the efficacy of insecticides for dengue control.

INTRODUCTION

Dengue has remained endemic in Malaysia since the first case was documented in 1901-1902 (Skae, 1902). The disease was notifiable since 1973 and the first major outbreak of dengue was reported in 1962 in Penang (Lam, 1993).

Dengue is a vector-borne disease, with increasing morbidity and mortality in Malaysia over the years. High human population growth in many areas has led to extensive deforestation, irrigation and urbanization. These high population densities and associated environmental modifications have created conditions that favour the proliferation of the dengue vectors.

Aedes (Stegomyia) albopictus (Skuse) is incriminated as one of the dengue vectors in Malaysia (Rebecca, 1987; Lam, 1993; Lee & Inder, 1993). Experimental results in studies on the possibility of transovarian transmission of dengue virus in *Ae. albopictus* has been reported by Lee *et al.* (1997). In the absence of an effective vaccine and specific treatment, dengue control relies only on controlling the mosquito vectors. One of the most commonly used control agents is chemical insecticides. Temephos, the only approved larvicide, is widely used to control container-breeding *Aedes* since 1973 in Malaysia (Seleena *et al.*, 2001).

However, long term and intensive use of insecticides often lead to emergence of resistance. Insecticide resistance is one of the major obstacles in the control of medical and agricultural arthropod pests (Georghiou & Taylor, 1986).

For many years, resistance was detected in an insect population only when it had evolved to the point where it had no obvious impact on a control programme. The early detection and monitoring of resistance is recognized as vital part of resistance management. Resistance management is an area of research that is directed at developing insecticide usage strategies that minimize the rate of evolution of resistance (Ferrari, 1996).

Today, resistance management in the context of integrated pest management has evolved as the favoured approach to prevent, delay or reduce the impact of insecticide resistance (Soderlund *et al.*, 1989). To fully develop this strategy, a thorough knowledge of the mechanism of insecticide resistance is essential.

Biochemical technique is a useful method to detect resistance in insects, and whether individual insects possess a mutant resistance allele (Brown & Brogdon, 1987). However, this technique should be used with the bioassay method to enable more precise monitoring of insecticide resistance.

Because of the long-term use of temephos and its importance as the sole larvicide in dengue control, we evaluated the temephos resistance of *Ae. albopictus* populations obtained from two dengue endemic areas, Taman Samudera and Kg. Banjar by using both bioassay and biochemical microassay to detect the levels of non-specific esterases (EST), mixed function oxidases (MFO), glutathione S-transferases (GST) and insensitivity of acetylcholinesterase (AChE) that may be involved in temephos resistance.

MATERIALS AND METHODS

Mosquito Strain

Six continuous ovitrap surveillance were conducted in 2 dengue endemic areas in Gombak, Selangor, namely Taman Samudera (N03°13.987', E101°41.918') and Kampung (Kg.) Banjar (N03°14.115', E101°42.015'). Ovitraps as described by Lee (1992a) were used in this study.

The ovitrap consists of 300 ml plastic container with straight, slightly tapered sides. The opening measures 7.8 cm in diameter, the base diameter is 6.5 cm and the container is 9.0 cm in height. The outer wall of the container is coated with a layer of black oil paint. An oviposition paddle made from hardboard (10 cm x 2.5 cm x 0.3 cm) was placed diagonally into each ovitrap. Each ovitrap was filled with tap water to a level of 5.5 cm. The ovitraps were placed outdoor in 30 randomly selected houses in each study site. In this study, "outdoor" was referred to outside of the house but confined to the immediate vicinity of the house (Lee, 1992b). All the ovitraps were collected after 5 days and replaced with fresh ovitraps and paddles. The hatched larvae were subsequently identified at 3rd instar. All strains of larvae were colonized until 1st generation (F1) and late 3rd or early 4th instar larvae were used for larval bioassay and biochemical microassay.

Temephos

For larval bioassay testing, operational dose, 1 mg/L of temephos was prepared from 1.1% a.i. sand granule formulation of Abate® temephos purchased from pharmacy.

Larval Bioassay

Larval bioassay was performed by using the WHO method (WHO, 1981) for determining larval susceptibility. The operational dose of temephos, 1 mg/L was prepared by pipetting the appropriate standard insecticide solution into 300 ml drinking paper cups filled with 200 ml distilled water and 25 late 3rd instar larvae were added. Prior to the test, any larvae showing abnormalities were discarded. The water was then topped up to 250 ml using distilled water. The cups were held at room temperature of 27 ± 1 °C and 70 ± 5 % relative humidity. Larval mortality was recorded every 10 minutes for 120 minutes (2 hours) and finally at 24 hours. Three replicates of each concentration were conducted. The control (untreated) consisted of 1 ml of ethanol added to the distilled water.

Biochemical microassay

Four enzymes namely, non-specific esterases, mixed function oxidases, glutathione-S-transferases and insensitive acetylcholinesterase were studied. Non-

specific esterases enzyme microassay was conducted as described by Brogdon *et al.* (1988) and Lee (1990). The activity of oxidases was measured according to Brogdon *et al*. (1997). Glutathione S-transferase enzyme microassay was conducted according to Lee & Chong (1995). Microassay for acetylcholinesterase (AChE) was conducted by using a modification of Ellman's method (Brogdon *et al*., 1988) to detect the insensitivity of AChE in the larvae.

In each microassay, a total of 24 individual third instar larvae were homogenized in 500- 1000 µl buffer solution. The homogenate was centrifuged at 15,000 rpm for 10 minutes at 4°C. By using a micropipette, 50 ml homogenate was transferred to a well in a microtiter plate. A total of 4 replicate aliquots of the homogenate from a single larva were obtained for this assay. The substrate solution was then added into each well and left to stand for 1 minute, followed by addition of indicator solution. The reaction was allowed to continue for 10 minutes. In AChE insensitivity test, an inhibitor, propoxur was added. The absorbance of the reaction mixture was measured spectrophotometricaly using an immunoassay reader (Dynatech, Model MR 5000) to determine the enzyme activity quantitatively.

Data analysis

Bioassay data obtained from a minimum of three replicates were pooled and analyzed. Log-time probit mortality regression and lethal time (LT_{50}) value for *Ae. albopictus* were analyzed by using standard probit analysis (Finney, 1971) as adapted to personal computer used by Raymond (1985). Based on computed lethal time of all strains of *Ae. albopictus* collected from different sites compared to laboratory strain, the resistance ratio was determined as follow:

$$\text{Resistance ratio (RR) of } LT_{50} = \frac{LT_{50} \text{ of field strain}}{LT_{50} \text{ of laboratory strain}}$$

Mean readings of color intensities of EST, MFO and GST microassay for all strain of mosquito larvae obtained in this study were calculated by using the descriptive statistics (SPSS v.10). Based on the mean EST, MFO and GST enzyme level of all strain *Ae. albopictus* collected from field compared to laboratory strain, the resistance ratio was determined as below:

$$\text{Resistance ratio (RR) of enzyme level} = \frac{\text{Mean enzyme level of field strain}}{\text{Mean enzyme levelof laboratory strain}}$$

To study the sensitivity of AChE, the mean OD of AChE activity in 10 mg/L propoxur-inhibited fraction was compared with mean OD of AChE activity without propoxur (control).

All levels of statistical significance were determined at $p = 0.05$ using t-test and one way ANOVA (SPSS v.11.5). The correlation between LT_{50} value of temephos and enzymes levels in field collected *Ae. albopictus* strains was also determined and the significant correlation was determined at $r \geq 0.5$.

RESULTS AND DISCUSSION

Table 1 shows the LT_{50} and resistance ratio (RR) of *Ae. albopictus* obtained from Taman Samudera and Kg. Banjar tested against operational dosage of temephos. In the bioassay with 1 mg/L of temephos, LT_{50} of *Ae. albopictus* obtained from Kg. Banjar (95.64 ± 4.54 minutes) was significantly higher than Taman Samudera (76.81 ± 6.36 minutes) ($p \leq 0.05$), indicating *Ae. albopictus* populations in Kg. Banjar were more resistant to temephos, in comparison to Taman Samudera. Resistance ratio of *Ae. albopictus* from Taman Samudera and Kg. Banjar was 0.75 – 1.20 and 1.12 – 1.45 folds, respectively more than laboratory strain. Complete mortality was observed in all strains of *Ae. albopictus* after 24 hour exposure period. No mortality was observed in untreated control. This study also indicated that there was weekly variation on the resistance status of *Ae. albopictus* against temephos. Lee & Tadano (1994) also reported the variations in gene frequencies in Malaysian field population of *Culex quinquefasciatus* adults. These time-dependent variations were supposedly due to the presence of vast gene pool in the field mosquito populations which

Table 1. LT_{50} and resistance ratio of *Ae. albopictus* collected from Taman Samudera and Kg. Banjar, Selangor tested against operational dosage of temephos (1 mg/L)

Ovitrap Surveillance No.	Taman Samudera		Kg. Banjar	
	LT_{50} (C.L.) (minute)	Resistance Ratio	LT_{50} (C.L.) (minute)	Resistance Ratio
Laboratory Strain	77.81 (74.76 – 80.80)	–	77.81 (74.76 – 80.80)	–
1	65.81 (62.82 – 68.53)	0.85	101.34 (98.39 – 104.68)	1.30
2	NA	NA	89.82 (86.92 – 92.82)	1.15
3	82.92 (80.24 – 85.53)	1.07	82.78 (79.87 – 85.79)	1.06
4	93.61 (90.02 – 97.69)	1.20	86.77 (83.42 – 90.32)	1.12
5	58.65 (54.83 – 62.33)	0.75	100.61 (96.54 – 105.56)	1.29
6	83.08 (80.33 – 85.90)	1.07	112.50 (108.32 – 118.22)	1.45
Mean ± SE for LT_{50}	76.81 ± 6.36[a]	0.99	95.64 ± 4.54[a]	1.23

C.L. = Confidence Limit
NA = Not Available
[a]Significantly different, p = 0.036

continuously provided genetic variability to population at any particular point of time (Lee *et al*., 1998). The resistance ratio was slightly more than 1, indicating the presence of resistance genes in the populations and *Ae. albopictus* are still undergoing the process to develop the resistance mechanism towards temephos, although the emergence of temephos resistance in different *Ae. albopictus* strainshave been reported earlier in Malaysia by Lee *et al*. (1998) and Nazni *et al*. (2000).

To further understand the possible underlying resistance mechanism in the field strains of *Ae. albopictus*, all strains obtained from Taman Samudera and Kg. Banjar were chosen for biochemical characterization. We evaluated the resistance of *Ae. albopictus* populations by using biochemical microassay to detect resistance due to non-specific esterases (EST), mixed function oxidases (MFO), glutathione S-transferases (GST) and insensitivity of acetylcholinesterase (AChE). In this study, resistance ratio values > 1 indicated resistance has been developed or detected; while values $\leq$ 1 were considered susceptible, in comparison to the laboratory strain.

The mean non-specific esterases (EST) activity in population of *Ae. albopictus* collected from Taman Samudera and Kg. Banjar is presented in Table 2. The mean EST activity in *Ae. albopictus* obtained from Taman Samudera and Kg. Banjar ranged from 0.14 – 0.44 and 0.22 – 0.33 α-Na ηmol/min/mg protein, respectively. The resistance ratio of EST in the field collected *Ae. albopictus* in comparison to laboratory strain was in the range of 0.41 – 1.29. *Aedes albopictus* obtained from Taman Samudera showed a wide range in the EST activity, indicating that susceptibility status of the populations was heterogeneous. However, *Ae. albopictus* obtained from Kg. Banjar showed a narrow range in the EST activity with resistance ratio of less than 1, indicating the population was homogenously susceptible.

There was no significant correlation between mean EST activity and LT_{50} value of temephos for *Ae. albopictus* populations in Taman Samudera and Kg. Banjar ($r = -0.007$, $p > 0.05$). Selvi *et al*. (2009) also reported similar finding, in which no correlation was found between temephos resistance and EST activity in larvae and adults of *Ae. albopictus* ($r = 0.012$, $p > 0.05$) and *Ae. aegypti* ($r = -0.469$, $p > 0.05$). On the other hand, temephos resistance has been reported associated with EST activity in *Musca domestica* and *Ae. aegypti* by Kao *et al*. (1985), Yan & Sudderuddin (1978) and Chen *et al*. (2008). The correlation between esterase activity and susceptibility to temephos makes it possible to establish the resistance mechanism in the *Aedes* populations.

Table 2. Mean non-specific esterases activity in population of *Ae. albopictus* collected from Taman Samudera and Kg. Banjar, Selangor, Malaysia

Ovitrap surveillance No.	Mean ± SE (α-Na hmol/min/mg protein)	Resistance ratio	One way ANOVA
Laboratory strain	0.34 ± 0.02	–	
	Taman Samudera		
1	0.15 ± 0.01	0.44	
2	NA	NA	
3	0.18 ± 0.01	0.53	F = 59.52
4	0.16 ± 0.01	0.47	p < 0.05
5	0.44 ± 0.03	1.29	
6	0.14 ± 0.00	0.41	
Mean ± SE	0.21 ± 0.06	0.63 ± 0.17	
	Kg. Banjar		
1	0.29 ± 0.01	0.85	
2	0.33 ± 0.02	0.97	
3	0.26 ± 0.01	0.76	F = 15.56
4	0.32 ± 0.01	0.94	p < 0.05
5	0.22 ± 0.01	0.65	
6	0.22 ± 0.01	0.65	
Mean ± SE	0.27 ± 0.02	0.80 ± 0.06	

NA: Not Available
$p > 0.05$ indicated no significant difference
$p \leq 0.05$ indicated significant difference

Table 3 shows the mean oxidase activity expressed at absorbance 630nm in population of *Ae. albopictus* collected from Taman Samudera and Kg. Banjar. The optical density value of elevated oxidases activity displayed by all field collected *Ae. albopictus* was in the range of 0.10 – 0.24; while resistance ratio was in the range of 0.53 to 1.26 (Table 3). Elevated oxidases activity is usually associated with pyrethroid resistance and has also been reported in *Ae. aegypti* (Paeporn *et al*., 2004), *Ae. albopictus* (Nazni *et al*., 2000), *Cx. quinquefasciatus* (Rodriguez *et al*., 1995), *Cx. pipiens* (Gong *et al*., 2005) and housefly (Kasai & Scott, 2000; Scott & Zhang, 2003; Scott & Kasai, 2004). The narrow range in the elevated oxidases activity indicated that the *Aedes* populations were homogenous; while low values of resistance ratio (mean RR = 0.63 – 0.80) indicated that no resistance mechanism towards pyrethroid was detected in field collected *Ae. albopictus*, indicating the use of pyrethroids as household insecticides and fogging in dengue control programme are still effective in the field.

The mean oxidases activity also did not correlate with LT_{50} values of temephos obtained from bioassay ($r = 0.170$, $p > 0.05$). This indicated oxidases are not playing a role in temephos resistance in this study as reported by Paeporn *et al*. (2003). However, Nazni *et al*. (2000) found that *Ae. aegypti* larvae resistance to malathion and temephos could be due to the presence of oxidases activity.

Mean glutathione S–transferase (GST) activity in population of field collected *Ae. albopictus* is presented in Table 4. The mean GST activity for *Ae. albopictus* collected from Taman Samudera and Kg. Banjar was in the range of 0.09 – 0.14 and 0.09 to 0.13 CDNA mol/min/mg protein, respectively. The resistance ratio for GST activity in field collected *Ae. albopictus* strains ranged between 1.50 – 2.33. The results further indicated that there was no correlation between mean GST activity and LC_{50} values of temephos ($r = 0.050$, $p > 0.05$). This was in contrast to studies reported by Nazni *et al*. (1997) and Ku *et al*. (1994), where GST was another important mechanism for

Table 3. Mean oxidase activity expressed at absorbance 630nm in population of *Ae. albopictus* collected from Taman Samudera and Kg. Banjar, Selangor, Malaysia

Ovitrap surveillance No.	Mean ± SE (Absorbance 630 nm)	Resistance ratio	One way ANOVA
Laboratory strain	0.19 ± 0.01	–	
	Taman Samudera		
1	0.11 ± 0.00	0.58	
2	NA	NA	
3	0.24 ± 0.01	1.26	F = 52.88
4	0.15 ± 0.00	0.79	p < 0.05
5	0.10 ± 0.00	0.53	
6	0.18 ± 0.01	0.95	
Mean ± SE	0.16 ± 0.03	0.82 ± 0.13	
	Kg. Banjar		
1	0.16 ± 0.00	0.84	
2	0.10 ± 0.00	0.53	
3	0.16 ± 0.00	0.84	F = 27.40
4	0.18 ± 0.01	0.95	p < 0.05
5	0.19 ± 0.01	1.00	
6	0.12 ± 0.00	0.63	
Mean ± SE	0.15 ± 0.01	0.80 ± 0.07	

NA: Not Available
$p > 0.05$ indicated no significant difference
$p \leq 0.05$ indicated significant difference

Table 4. Mean glutathione–S–transferase activity in population of *Ae. albopictus* collected from Taman Samudera and Kg. Banjar, Selangor, Malaysia

Ovitrap surveillance No.	Mean ± SE (CDNA- hmol/min/mg protein)	Resistance ratio	One way ANOVA
Laboratory strain	0.06 ± 0.00	–	
	Taman Samudera		
1	0.10 ± 0.00	1.67	
2	NA	NA	
3	0.09 ± 0.00	1.50	F = 999.99
4	0.12 ± 0.00	2.00	p < 0.05
5	0.14 ± 0.00	2.33	
6	0.09 ± 0.00	1.50	
Mean ± SE	0.11 ± 0.01	1.80 ± 0.16	
	Kg. Banjar		
1	0.09 ± 0.00	1.50	
2	0.11 ± 0.00	1.83	
3	0.09 ± 0.00	1.50	F = 999.99
4	0.10 ± 0.00	1.67	p < 0.05
5	0.13 ± 0.00	2.17	
6	0.10 ± 0.00	1.67	
Mean ± SE	0.10 ± 0.01	1.72 ± 0.10	

NA: Not Available
$p > 0.05$ indicated no significant difference
$p \leq 0.05$ indicated significant difference

Table 5. Mean acetylcholinesterase activity in propoxur-inhibited fraction and without propoxur (control) in population of *Ae. albopictus* collected from Taman Samudera and Kg. Banjar, Selangor, Malaysia

Ovitrap surveillance	Mean acetylcholinesterase activity (± SE)		t-test
	ACTH with 10 mg/L propoxur	Control (without propoxur)	
Laboratory Strain	0.11 ± 0.00	0.11 ± 0.00	$p < 0.05$
Taman Samudera			
1	0.11 ± 0.01	0.12 ± 0.01	$p > 0.05$
2	NA	NA	NA
3	0.11 ± 0.01	0.15 ± 0.01	$p < 0.05$
4	0.10 ± 0.00	0.12 ± 0.00	$p < 0.05$
5	0.09 ± 0.00	0.09 ± 0.00	$p < 0.05$
6	0.09 ± 0.00	0.11 ± 0.00	$p < 0.05$
Kg. Banjar			
1	0.11 ± 0.01	0.11 ± 0.01	$p > 0.05$
2	0.08 ± 0.00	0.08 ± 0.00	$p < 0.05$
3	0.10 ± 0.00	0.10 ± 0.00	$p < 0.05$
4	0.11 ± 0.01	0.12 ± 0.00	$p > 0.05$
5	0.11 ± 0.01	0.15 ± 0.01	$p < 0.05$
6	0.10 ± 0.00	0.10 ± 0.00	$p < 0.05$

NA: Not Available
$p > 0.05$ indicated no significant difference
$p \leq 0.05$ indicated significant difference

organophosphates resistance in *Musca domestica* and diamond black moth, respectively. However, the high resistance ratio of GST activity in *Ae. albopictus* also indicated that GST might play an important role in pyrethroid, DDT and other organophosphate resistance, as reported by many researchers (Ranson *et al.*, 2001; Lumujan *et al.*, 2005).

The mean acetylcholinesterase (AChE) activity in 10 mg/L propoxur inhibited-fraction and without propoxur (control) in field collected *Ae. albopictus* ranged from 0.08 – 0.11 and 0.08 – 0.15 at 415 nm (Table 5). Acetylcholinesterase activity (control) in majority of *Ae. albopictus* strains (8 out of 11 strains) obtained from Taman Samudera and Kg. Banjar showed significantly higher activity than AChE activity in 10 mg/L propoxur-inhibited fraction, implying that AChE enzyme in field collected *Ae. albopictus* was still sensitive against propoxur. However, 3 field-collected *Ae. albopictus* strains (Taman Samudera 1, Kg. Banjar 1 and Kg. Banjar 4) showed no significant difference between AChE activity in 10 mg/L propoxur-inhibited fraction and without propoxur, indicating the AChE activity was insensitive against propoxur. No correlation was found between AChE activity in 10 mg/L propoxur inhibited fraction and temephos LT_{50} values in all strains of *Ae. albopictus* ($r = 0.193$, $p > 0.05$), indicating that insensitive AChE did not play a clear role in temephos resistance as reported by Macoris *et al.* (2003) and Saelim *et al.* (2005).

Organophosphate and carbamate insecticides exert their neurotoxic effects by inhibiting the enzyme AChE, thereby prolonging the residence time of acetylcholine at cholinergic synapses and producing hyperexcitation of cholinergic pathways (Soderlund & Bloomquist, 1990). Therefore, the insensitive AChE found in field collected *Ae. albopictus* also indicated the emergence of organophosphate and carbamate resistance in the field. AChE that is less sensitive to inhibition by organophosphate and carbamate also has been documented as a resistance mechanism in mosquito populations by Hemingway *et al.* (1986), Raymond *et al.* (1985) and Cui *et al.* (2006).

Generally, emergence of temephos resistance was detected, but the temephos LT_{50} values of 11 field collected *Ae. albopictus* strains were not associated with any enzyme mechanisms in this study. Our findings contrasted the study conducted by Paeporn *et al.* (2003), in which major enzyme-based resistance mechanism involved in temephos resistance included elevated non-specific esterase, oxidase and insensitive acetylcholinesterase. Given the lack of associations between the degree of temephos resistance and enzymes activities, it is possible that multiple insecticide resistance involving both detoxification mechanisms and target site alteration could be involved. In fact, the occurrence of multiple insecticide resistance mechanisms in mosquitoes is not a new phenomenon and it has been reported worldwide (Corbel *et al.*, 2007; Vontas *et al.*, 2012). Our study shows that susceptibility status of temephos against *Ae. albopictus* was changing from time to time in Taman Samudera and Kg. Banjar, indicating a weekly variation on the susceptibility status of *Ae. albopictus* towards insecticide. This was further confirmed by biochemical microassay, in which significant difference on the weekly enzyme levels of EST, oxidases, GST and insensitive AChE was detected.

Even though no correlation was found between temephos resistance and enzyme activity in this study, however, GST showed high level of activity compared to the laboratory strain. It is likely that *Ae. albopictus* may be resistant to other groups of insecticide (i.e., carbamates, organophosphates and pyrethroids). Furthermore, the insensitivity of AChE was also detected in some field collected *Ae. albopictus*, indicating the possibility of emergence of carbamate or other organophosphate resistance in the field populations. In addition, more study should be conducted to investigate the involvement of other resistance mechanisms in the field *Aedes* populations.

Continuous resistance monitoring should be conducted in all dengue endemic sites in Malaysia regularly to identify the efficacy of insecticides for dengue control and to facilitate the selection of insecticides with the greatest promise for minimizing dengue infections. The susceptibility / resistance tests should be conducted regularly as an integral adjunct of vector management, so that the progressive development of resistance to insecticide in use may be detected before it reaches the point of a control failure. Community awareness, cooperation with public health campaigns to reduce *Aedes* breeding sites, and well-managed rotation of the effective insecticides are recommended strategies for controlling dengue vectors.

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